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EPIDEMIOLOGY FACETS OF HARMFUL ORGANISMS IN CROPPING SYSTEMS

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Content

GEOGRAPHIC DISTRIBUTION AND EPIDEMIOLOGY OF DISEASES AND PESTS

ECOLOGICAL APPROACH OF MODERN WEED CONTROL SYSTEMS

Geographic distribution and epidemiology of diseases and pests

FLOWER VISITORS ON SPRING OILSEED RAPE IN DIFFERENT CROPPING SYSTEMS

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Abstract

Spring oilseed rape (*Brassica napus* var. *oleifera* subvar. *annua*) is predominantly autogamous and visits of insect pollinators are not essential for the final seed yield. However, the pollinators can have positive effects, such as a reduction of the flowering period, a reduction of raceme production, acceleration of ripening and an increase of seed weight. This study considers (i) the effect of different fertilising systems on the number of flowers and the food resource in flowers of summer oilseed rape; (ii) the effect of flower number and food resources on the abundance of flower visitors.

Field experiments were carried out in July 2003 to compare the density of pollinators foraging on the different cropping systems of the summer oilseed rape. The number of pollinating insects was counted twice a week on sunny days between 11:00 and 15:00 when the temperature was above 16 °C. Flowers were counted at the same time on an area of 1 m^2 on each plot. The amount of nectar present in the flowers was measured by inserting a 1 µl capillary into the flower corolla tube. The number of pollen grains was counted from the samples after dissolving the flower tissues.

The results showed that the most important pollinators were honey bees (*Apis mellifera*), collecting both pollen and nectar. The rape fields were less visited by wild bees, different flies, including syrphus flies (*Syrphidae*), and butterflies. The food resource available for flower visitors did not depend on the fertilising system. The density of bees depended only on the flower density of the field.

Key words: oilseed rape, *Brassica napus*, pollinators, fertilising systems, nectar, pollen, marginal value theorem.

Introduction

Oilseed rape (*Brassica napus* var. *oleifera* subvar. *annua*) is an oil crop with an increasing worldwide cultivation. The crop is commonly considered as a self-pollinating species, but according to many authors (Treu, Emberlin, 2000), 5—41% cross-pollination has been reported under field conditions. The amount of cross-pollination differs in relation to the prevailing environmental conditions (Becker et al., 1992), the varieties cultivated (Delaplane, Mayer, 2000), and the availability of insect pollinators (Australian Government 2002).

Oilseed rape has entomophilous flowers capable of both self- and cross-pollination. There are six stamens in the rape flower, four projecting above the stigma, and two shorter than the style (McGregor, 1976). The anthers begin to dehisce before the corolla is fully expanded and continue to do so until the end of anthesis. Anthers of the two short stamens dehisce inwards, while anthers of the long stamens dehisce outward but, at the end of anthesis, they curve toward the flower centre so that the pollen covering the tops and side of the anthers becomes directed towards the stigma (Free, 1993). Anthers of oilseed rape release pollen when the flowers are shaken, whether by wind or insects (Delaplane, Mayer, 2000; Free, 1993). Insects' visits are necessary to transfer pollen from the short stamens and to facilitate transfer from the long stamens to stigmas. Wind can aid pollination of oilseed rape crops, either by shaking the flowers so that pollen is transferred from their own anthers to their own stigmas or by transferring pollen from one plant to another (Free, 1993).

In the case of cross-pollination, more pollen can reach the stigmas, specially the pollen from short stamens (Free, 1993). Cross-pollination with pollen from short stamens is significantly superior to that from long stamens, and gives a 14% greater weight of seed per pod (Free, 1993; Steffan-Dewenter, 2003). As in the field, pollen from both long and short stamens is used in pollination, the benefit from cross-pollination is not as effective as the existence of pollen only from short stamens.

On oilseed rape supplemental pollination may increase the set of early flowers (Delaplane, Mayer, 2000), therefore the plant would produce fewer flowers (Free, 1993), the flowering period and vegetative growth would shorten (McGregor, 1976; Free, 1993). It increases the number of seeds per pod, the number of seeds per plant (Steffan-Dewenter, 2003), the evenness of ripening, declining thus the seed loss by harvesting (McGregor, 1976; Free, 1993). Altogether the seed yield of oilseed rape could be higher by up to 25% (Delaplane, Mayer, 2000).

Honey bees (*Apis mellifera*) are the most effective pollinators of oilseed rape, but according to many authors bumble bees (*Bombus sp.*), solitary bees and also some non-hymenopterans (flies, butterflies, true bugs) may have a positive effect in pollinating the rape (McGregor, 1976; Free, 1993; Delaplane, Mayer, 2000; Pierre et al., 2003). Honey bees, to a smaller extent also one bumble bee species (*Bombus terrestris*) and some solitary bee species (*Megachile rotundata, Osmia rufa*), can be brought to the fields on the purpose of supplemental pollination. For efficient pollination of rape, the recommended number of honey bee hives is 2.5—5 per hectare (McGregor, 1976). The number of hives needed is quite alternating and depends on the number of other pollinators present in the area as well as the density of oilseed rape plants and flowers in the field.

In order to attract bees on the flowers, plants provide food (nectar and pollen) for them. Nectar consists of water (30—94%, depending on plant species) and sugars, the other components like amino acids, lipids, minerals etc. are in minority (Faegri, van der Pijl, 1979). Bees use nectar mainly as an energy source (Rasheed, Harder, 1997; Pernal, Currie, 2001). Pollen provides them with their only natural source of protein, which is needed for larval development,

and fulfils other dietary requirements for lipids, sterols, vitamins, and minerals (Herbert, 1992; Cook *et al.*, 2003). Pollen is essential not only for the larvae but also for the worker bees to build up their body tissues within the first days after emergence (Hrassnigg, Crailsheim, 1998). Pollens from different plant species differ in amino acid composition, concentration or both, and pollens with high proportions of essential amino acids are assumed to be of greater nutritional value (Cook *et al.*, 2003).

There are several reasons for oilseed rape being highly attractive to bees. (1) Rape flowers produce nectar in abundance and bees visit the fields from a distance of 3.5—4 km from their hives and neglect fruit trees in favour of rape (Free, 1993). The sugar concentration of the nectar is quite high and can show great variety. According to J.B. Free (1993), the amount of nectar produced per 24 h and the sugar concentration are related. Different authors have found that rape flowers secrete a total average of 3.6 mg of nectar with 29% sugar concentration, 0.6 mg with 33% concentration, and 2.4 mg with 38% concentration. Pierre and co-workers (1999) have reported that for the cultivar 'Samourai' the sugar concentrations in the nectar are the highest at the beginning of the flowering period and decrease towards the end. (2) There are high rates of amino acids most essential for bees (Isoleucine, Leucine, and Valine) in the pollen of oilseed rape (Cook *et al.*, 2003). (3) As rape flowers are cruciferous, the food resource is easily available, and (4) their flowering lasts 22—45 days (McGregor, 1976). (5) The flowering period of rape begins at the time when there are few other cultivated food plants available for bees. Honey bees and bumble bees do not switch easily between different food plants, because it takes too much energy to learn new handling skills for other flower types (Free, 1970; Heinrich 1976; Teräs, 1985; Teräs, Pohtio, 1995).

Worker bees and many other insects (flies, butterflies, etc.) are able to discriminate between closely related cultivars or plant phenological stages (Laloi et al., 2000; Gardener, Gillman, 2002) due to the different concentrations and amino acid composition of nectar (Laloi et al., 1999; Gardener, Gillman, 2001a, b). Differently fertilised rape plants should be in different conditions and it should affect food resource in flowers as well as nectar or pollen odours or taste (Gardener, Gillman, 2002). Bees are not the only flower visitors, flies, syrphus flies, butterflies and some bugs also visit rape flowers. The number of flower visitors probably depends on the amount of food available in the field. According to the hypothesis we tested (1) whether different fertilising systems affect the number of flowers on summer oilseed rape, the food resource in flowers, and (2) the effect of the number of flowers and food resource on the number of flower visitors.

Materials and Methods

Field experiments were carried out in July 2003 to compare the density of pollinators on differently fertilised experimental plots with the summer oilseed rape cultivar 'Mascot'. The observation period lasted over three weeks. The Eerika experimental field in Eerika consisted of 1×10-m plots, where 10 different trial variants had four replications. In one control variant (0) no fertilisers and no insecticides and in the other control variant (0+) no fertilisers but the insecticide Fastac (June 29) were used. The eight variants in the trial were sprayed with the insecticide Fastac (June 29) and fertilised with the granular combined fertiliser OptiCropNPK 21-08-12+S+Mg+B+Ca. Each of the trial variants was additionally fertilised with a different microfertiliser: (New Rape (New), HydroPlus™ Micro Manganese (Mn), HydroPlus™ Micro Rape (Micro), Sulphur F3000 (S), HydroPlus™ Micro Copper (Cu), HydroPlus™ Micro Boron (B), Hydromag 300 (Mg) (Hyd), HydroPlus™ Micro Molybdenum (Mo)).

The number of pollinating insects was counted on sunny days between 11:00 and 15:00 when the temperature was above 16 °C. Flowers were counted at the same time from an of area 1 m^2 on each plot. For determining nectar standing crop in flowers, 50 flowers were gathered from each trial variant on the observation days (altogether 500 flowers). The amount of nectar present in the flowers (nectar standing crop) was measured by inserting a 1-µl capillary (DRUMMOND MICROCAPS) into the flower corolla tube. The nectar volume was thereafter calculated from the nectar column length in the tube (the microcapillary tube's length is 32 mm). For determining the number of pollen grains, 12 flowers were gathered randomly twice a week from each plot during the flowering period. The flower tissues were dissolved with CH3COOH (60%) and thereafter washed with distilled water. The number of pollen grains was counted under a light microscope from samples (Fuchs-Rosenthal chamber). From these samples the number of pollen grains per flower was calculated. ANOVA, Kruskal-Wallis test and linear regression were used for analysing the data.

Results and Discussion

The average number of flowers of the fertilised plots differed significantly from the average number of flowers on the unfertilised control plots ($F(1; 198) = 19.6$, $p < 0.001$). On most of the fertilised plots the rape plants had significantly more flowers than on the unfertilised plots (Fig. 1). On the unfertilised plots the rape plants were stunted and with few branches. In comparison with different microfertilisers, the flower number was significantly different between some variants: in the case of copper and sulphur it was lower than on plots fertilised with Hydromag that contains magnesium. Magnesium seemed to be a limiting factor for flowering in the field.

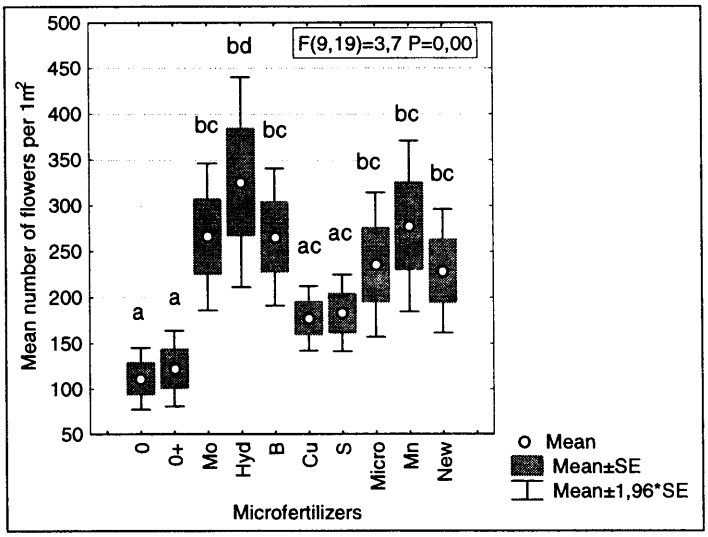

Fig. 1. Effect of different microfertilisers on flower number on 1 m^2.
Letters upon boxes indicate statistically significant differences

In total 1,558 insects were counted, 846 (54%) of which were bees (*Apoidea*), 707 (45%) dipterans (*Diptera*), 3 butterflies (*Lepidoptera*), and 2 bugs (*Hemiptera*) (Fig. 2). Of *Apoidea*, 76% were honey bees and 24% wild bees. The abundance of honey bees was significantly higher in the middle of the flowering period than at the beginning and end of the period (H (4; 200) = 91.4, p = 0.00). The importance of dipterans was statistically different during the observation period (H (4; 200) = 112, p = 0.00), however, only one observation day differed significantly from others. The abundance of wild bees was stable and did not differ significantly over the period (H (4; 200) = 5.57, p = 0.23). The number of *Apoidea* differed significantly between control and fertilised plots (Fig. 3), but no difference was found when comparing different microfertilisers (H (7; N = 160) = 2.4, p = 0.9). The number of *Diptera* did not differ significantly between control plots and fertilised plots, but no difference was found when comparing different microfertilisers (H (7; N = 160) = 5.4, p = 0.6).

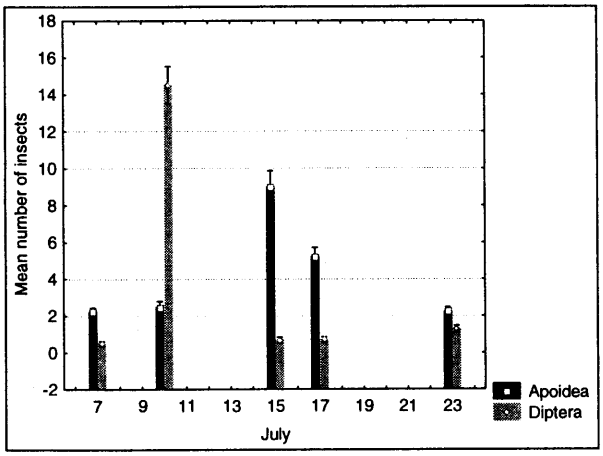

Fig. 2. The mean number of bees and dipterans during the flowering period of oilseed rape. Shown are: mean and std error (bars)

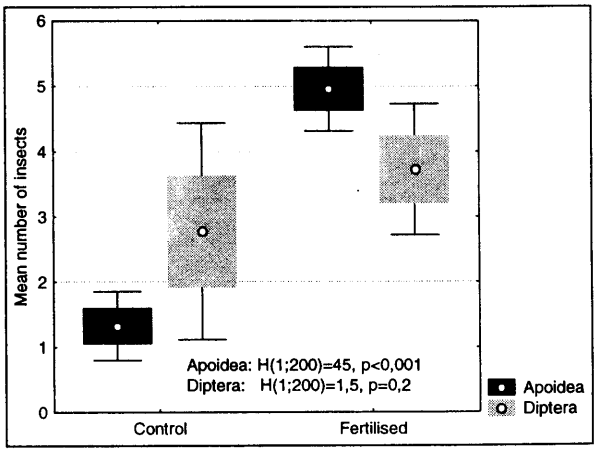

Fig. 3. Effect of different microfertilisers on the number of *Apoidea* and *Diptera*. Shown are: mean, std error (box), and std error*1.96 (bars)

The nectar standing crop in flowers did not differ significantly neither between fertilised and unfertilised plots nor in the case of different microfertilisers (Fig. 4). Also the number of pollen grains in flowers did not differ significantly between different experimental plots (Fig. 5). The flowers from plots fertilised with sulphur contained more pollen than the flowers collected from most of the other trial plots, although statistically significant difference was not found.

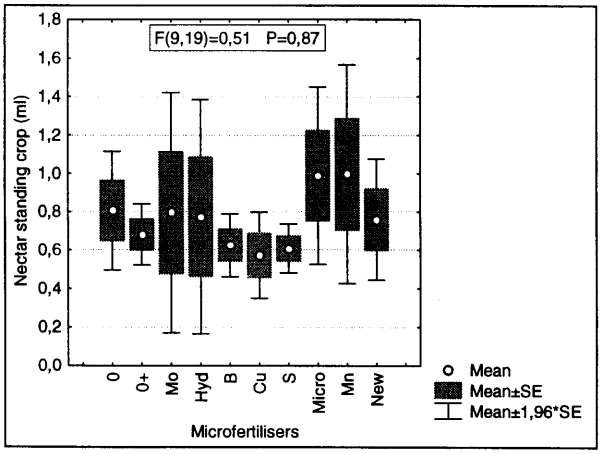

Fig. 4. Effect of different microfertilisers on nectar standing crop per flower

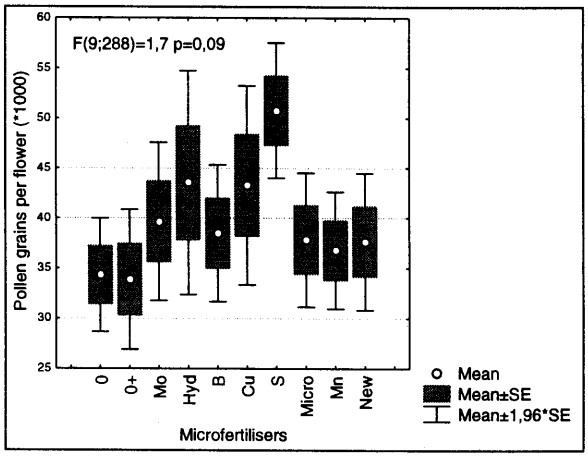

Fig. 5. Number of pollen grains in oilseed rape flowers on differently fertilised plots

We analysed the effect of flower number on bees and dipterans and found positive correlation between the numbers of flowers and bees ($r = 0.61$, $p < 0.001$), no correlation was found between the numbers of flowers and dipterans ($r = -0.08$, $p = 0.2$) (Fig. 6). The data show that the foraging behaviour of bees is dependent on the food patch density, whereas the foraging behaviour of dipterans does not depend on that. Dense food patches attract more bees than sparse patches.

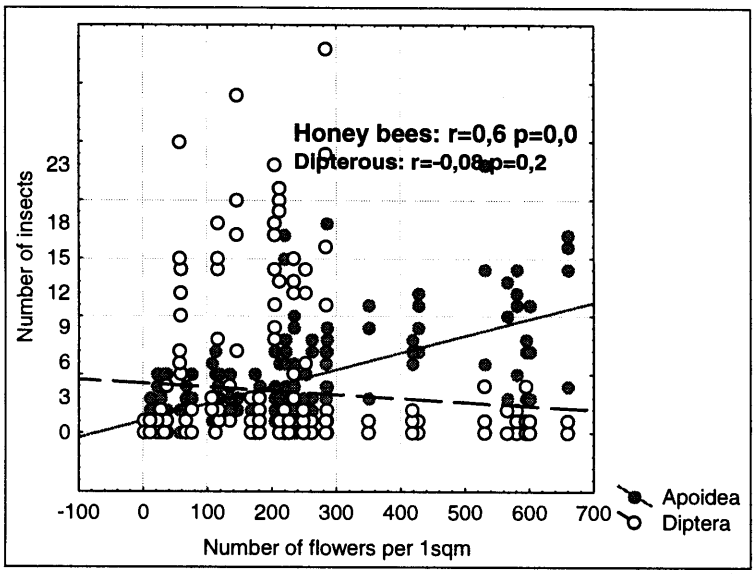

Fig. 6. Number of bees and flies in correlation with flower numbers

Oilseed rape is a crop that produces much nectar. The nectar glands are situated at the bottom of the flower. Bees, the most abundant pollinators of rape, can reach the nectar easily but dipterans cannot. Probably the high number of dipterans on one observation day could have been caused by the preceding three rainy days, when flowers were fulfilled with low concentrated nectar and flies with a short proboscis could reach it easily. The foraging behaviour of dipterans is not as well studied as the behaviour of bees. Syrphus flies do not visit flowers according to their abundance in the habitat and their flower choice is not connected with the previous choices. Their flower visits may be related to the differing nutritional value of pollens (Cowgill et al., 1993).

Oilseed rape is highly attractive to bees. According to Delaplane and Mayer (2000), 64% of bee visitors are honey bees and 36% different wild bees. Of wild bees, both bumble bees and solitary bees successfully transfer rape pollen from anthers to stigmas. Although we saw many species of bumble bees and solitary bees in the field, their number was still quite low.

Butterflies usually visit perennial plants, not annuals (Corbet, 2000). According to S.A. Corbet's observations, the only annual plant species butterflies visited was radish (*Raphanus sativus*). Presumably, few annuals contain enough nectar to make a visit profitable and, at the same time, they are useful for butterflies with their combination of a long tongue with a moderate wing loading. Pierids (*Pieris sp.*), combining a relatively long tongue with a moderately low wing loading, can include in their diet deep solitary flowers such as even brassicaceous (*Brassica sp.*) arable weeds (Corbet, 2000).

Pollen is mostly the part of bees' food that is considered as the source of amino acids. The oilseed rape pollen is rich in three most essential amino acids (Cook *et al.*, 2003). Although the principal ingredients of nectar are sugars, there are also nitrogenous substances found in floral nectar. Amino acids occur in the nectar at millimolar concentration (Gardener, Gillman, 2002). Day-to-day environmental variations are factors influencing the metabolic processes of nectar production and may lead to changes in overall concentration of the nectar components. Physiological processes such as water relations may influence nectar concentration at the production age, and evaporation may influence concentration afterwards. Longer-term environmental variables operate within a growing season, for example soil nutrients are most likely to alter the nectar composition by a variety of mechanisms (Gardener, Gillman, 2001a). According to Gardener and Gillman (2001b), most of the amino acids do not respond significantly to the soil treatment with fertilisers. The responses may be both positive and negative. The amino acids that did respond to fertiliser treatment were glutamine, proline, and GABA. The higher content of amino acids in nectar may derive certain benefits to pollinators (heavier eggs for example). There has still few research been made on the effect of fertilisers on the pollen and nectar amino acid composition and concentration. The differences in pollen or nectar amino acid composition may alter the taste of the particular food that both bees and flies are able to detect (Gardener, Gillman, 2002).

The fact that the food resource on our study plots with different flower density was quite equal indicates that bees collect nectar according to the marginal value theory (Charnov, 1976). If the food resource is found in clumps or patches, the individual has to make decisions when to leave and how far to travel next. The marginal value theorem shows that a forager making optimum use of patches should leave each patch when the instantaneous intake rate falls to the average rate expected for the habitat. The main predictions from this theorem are that (1) patch residence time (PRT) and the total harvest per patch should increase with increasing patch quality; (2) PRT should be shorter in areas where average patch quality is higher; and (3) as average habitat quality increases more poor patches should be ignored by the forager. This model assumes that foragers have complete environmental information and can recognize patch quality instantaneously (Alonso et al., 1995). Neither the amount of food in flowers nor the number of flowers available is the most important cue for foraging bees. They judge the environment constantly and change their choice as the conditions alter.

Different fertilising affects the number of oilseed rape flowers but does not affect the amount of food resource left in flowers on average. Bees are the most important pollinators, foraging on flowers according to the marginal value theorem: they leave, when the instantaneous food (energy) intake rate per patch has fallen to the average intake rate for the habitat. Since nectar and pollen production rate in the case of different fertilisation was not studied, it should be the next step in understanding these effects.

Acknowledgements

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References

1. Alonso, J.C., Alonso, J.A., Bautista, L.M., Muñoz-pulido, R. 1995. Patch use in cranes: a field test of optimal foraging predictions. Animal Behaviour, 49, 1367—1379.
2. Australian Government. The biology and ecology of canola (*Brassica napus*). 2002. Office of the Gene Technology Regulator.
3. Becker, H.C., Damgaard, C., Karlsson, B. 1992. Environmental variation for outcrossing rate in rapeseed (*Brassica napus*). Theoretical and Applied Genetics, 84, 303—306.
4. Charnov, E.L. 1976. Optimal foraging, the marginal value theorem. Theoretical Population Biology, 9(2), 129—136.
5. Cook, S.M., Awmack, C.S., Murray, D.A., Williams, I.H. 2003. Are honey bees' foraging preferences affected by pollen amino acid composition? Ecolgical Entomology, 28, 622—627.
6. Corbet, S.A. 2000. Butterfly nectaring flowers: butterfly morphology and flower form. Entomologia Experimentalis et Applicata, 96, 289—298.
7. Cowgill, S.E., Wratten, S.D., Sotherton, N.W. 1993. The selective use of floral resources by the hoverfly *Episyrphus balteatus* (Diptera: Syrphidae) on farmland. Ann. Appl. Biol., 122, 223—231.
8. Delaplane, K.S., Mayer, D.F. 2000. Crop pollination by bees. Irrigated Agriculture Research and Extension Centre, Washington State University, USA, 185—189.
9. Faegri, K., van der Pijl, L. 1979. The Principles of pollination ecology. (3rd ed.) Pergamon, Oxford.
10. Free, J.B. 1970. The flower constancy of bumblebees. Journal of Animal Ecology, 39, 395—402.
11. Free, J.B. 1993. Insect Pollination of Crops. (2nd ed.) Academic Press, London, San Diego, New York, Boston, Sydney, Tokyo, 172—180.
12. Gardener, M.C., Gillman, M.P. 2001a. Analyzing variability in nectar amino acids: composition is less variable than concentration. Journal of chemical Ecology, 27, 12, 2545—2558.
13. Gardener, M.C., Gillman, M.P. 2001b. The effects of soil fertilizer on amino acids in the floral nectar of corncockle, *Agrostemma githago* (Caryophyllaceae). Oikos, 92, 101—106.
14. Gardener, M.C., Gillman, M.P. 2002. The taste of nectar — a neglected area of pollination ecology. Oikos, 98, 3, 552—557.
15. Heinrich, B. 1976. The foraging specialisations of individual bumblebees. Ecological Monographs, 46, 105—128.

16. Herbert, E.W. 1992. Honey bee nutrition. The Hive and the Honey Bee (ed. by J. M. Graham). Dadant & Sons, Hamilton, Illinois, 197—233.
17. Hrassnigg, N., Crailsheim, K. 1998. The influence of brood on the pollen consumption of worker bees (*Apis mellifera* L.). Journal of Insect Physiology, 44, 393—404.
18. Laloi, D., Sandoz, J.C., Picard-Nizou, A.L., Marchesi, A., Pouvreau, A., Taséi, J.N., Poppy, G., Pham-Delègue, M.-H. 1999. Olfactory conditioning of the proboscis extension in bumble bees. Entomologia Experimentalis et Applicata, 90, 123—129.
19. Laloi, D., Bailez, O., Blight, M.M., Roger, B., Pham-Delègue, M.-H., Wadhams, L.J. 2000. Recognition of complex odors by restrained and free-flying honeybees, *Apis mellifera*. Journal of Chemical Ecology, 26 (10), 2307—2319.
20. McGregor, S.E. 1976. Crop Plants and Exotic Plants. Rape. In: Insect Pollination of Cultivated Crop Plants. URL: http://gears.tucson.ars.ag.gov/book/.
21. Pernal, S.F., Currie, R.W. 2001. The influence of pollen quality on foraging behaviour in honeybees (*Apis mellifera* L.). Behav Ecol Sociobiol., 51, 53—68.
22. Pierre, J., Mesquida, J., Marilleau, R., Pham-Delégue, M.H., Renard, M. 1999. Nectar secretion in winter oilseed rape, *Brassica napus* — quantitative and qualitative variability among 71 genotypes. Plant Breeding, 118, 471–476.
23. Pierre, J., Marsault, D., Genecque, E., Renard, M., Champolivier, J., Pham-Delègue, M.H. 2003. Effects of herbicide-tolerant transgenic oilseed rape genotypes on honey bees and other pollinating insects under field conditions. Entomologia Experimentalis et Applicata, 108, 159—168.
24. Rasheed, S.A., Harder, L.D. 1997. Economic motivation for plant species preferences of pollen-collecting bumble bees. Ecological Entomology, 22, 209—219.
25. Steffan-Dewenter, I. 2003. Seed set of male-fertile oilseed rape (*Brassica napus*) in relation to pollinator density. Apidologie, 34, 227—235.
26. Teräs, I. 1985. Food plants and flower visits of bumblebees (Bombus: Hymenoptera, Apidae) in southern Finland. Acta Zoologica Fennica, 179, 1—120.
27. Teräs, I., Pohtio, I. 1995. Bumblebee visits to different colour morphs of the Washington lupine, *Lupinus polyphyllus*. Entomologica Fennica, 5, 139—151.
28. Treu, R., Emberlin, J. 2000. Pollen dispersal in crops Maize (*Zea mays*), Oil seed rape (*Brassica napus* ssp *oleifera*), Potatoes (*Solanum tuberosum*), Sugar beet (*Beta vularis* ssp. *vulgaris*) and Wheat (*Triticum aestivum*). Evidence from publications. A report for the Soil Association, Worchester, 18—24.

PESTS AND THEIR NATURAL ENEMIES IN OILSEED RAPE IN ESTONIA

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Abstract

The cultivation area of oil crops has expanded throughout the world. In Estonia, the main oil crop is oilseed rape (*Brassica napus* L.). Its sowing area has rapidly increased during the last few years. In 2003, rape was grown already on 44,300 hectares according to the data of the Statistical Office of Estonia. Spring oilseed rape is dominating on winter oilseed rape because the winter survival of the crop is always questionable. Big cultivation areas are providing good preconditions for the population growth of potential pests that is of the phytophagous insects specialised in cruciferous crops. In Europe, the most common pests of oilseed rape are pollen beetles (*Meligethes aeneus* Fabr., *M. viridescens* Fabr.), cabbage seed weevil (*Ceutorhyncus assimilis* Payk.), cabbage stem weevil (C. *pallidactylus* Panz), rape stem weevil (*C. napi*), brassica pod midge (*Dasineura brassicae* Winn.), cabbage stem flea beetle (*Psylliodes chrysocephala*), and flea beetles (*Phyllotreta nemorum, P. undulata, P. diademata*) (Free, Williams, 1978, 1979; Ekbom, Borg, 1993; Williams et al., 2002; Hansen, 2003). Pests populations are regulated by their natural enemies: hymenopterous parasitoids and predators, the influence is depending on the cropping system (Büchs, 2003).

In Estonian conditions the more careful study of the target rape entomofauna started two years ago with the EU-funded project MASTER: MAnagement STrategies for European Rape pests (Williams et al., 2002), which is aimed at developing economically viable and environmentally acceptable crop management strategies for winter oilseed rape which maximise the biocontrol of key pests (Williams et al., 2002). This requires much greater knowledge of pest, parasitoid and predator taxonomy and biology throughout Europe.

Key words: *Brassica napus*, pests, natural enemies.

Materials and Methods

In Southern Estonia in 2002 in an organic winter oilseed rape field and in 2003 in conventionally cultivated spring oilseed rape field, the studies were carried out for the establishment of rape pests, their hymenopterous parasitoids and carabids as predators. Pests and parasitoids were caught with yellow black water traps and carabids with pitfall traps from the beginning of the crop development in spring to the harvest time. Rape plants and pods were dissected for the establishment of weevils and pod midges damages.

Results and Discussion

Pests

Winter oilseed rape

The eleven species of crucifer-specialist insects were caught in the yellow water traps: *Meligethes aeneus, M. viridescens, Ceutoryncus assimilis, C. floralis, C. rapae, C. pleurostigma, Phyllotreta undulata, P. vittata, P. atra, P. armoraciae, P. nemorum. Psylliodes chrysocephala, Ceutorhyncus pallidactylus*, and *C. napi* were not found (Fig.1). There was no evidence of the plant damage by these stem borers. In the second week of May, *Ceutoryncus floralis* was abundant. The number of *Meligethes viridescens* increased in the first week of June but numbers still remained small. This species prefers to reproduce in wild cruciferous plants (Billqvist, Ekbom, 2001a, 2001b). From the second week of June until harvest, *Meligethes* spp. and *Ceutoryncus assimilis* were present but in low numbers. Their population peaks were at the beginning of June when 10—20% of rape pods had reached the final size. Neither *Meligethes* spp. larvae nor *Ceutoryncus assimilis* larvae or their damage in pods was detected. The absence of larvae indicates that these beetles used the plants for maturation feeding and not for reproduction.

Of the potential pests, *Meligethes aeneus* and *Ceutorhynchus assimilis* were the most numerous but did not cause any significant damage to the winter oilseed rape.

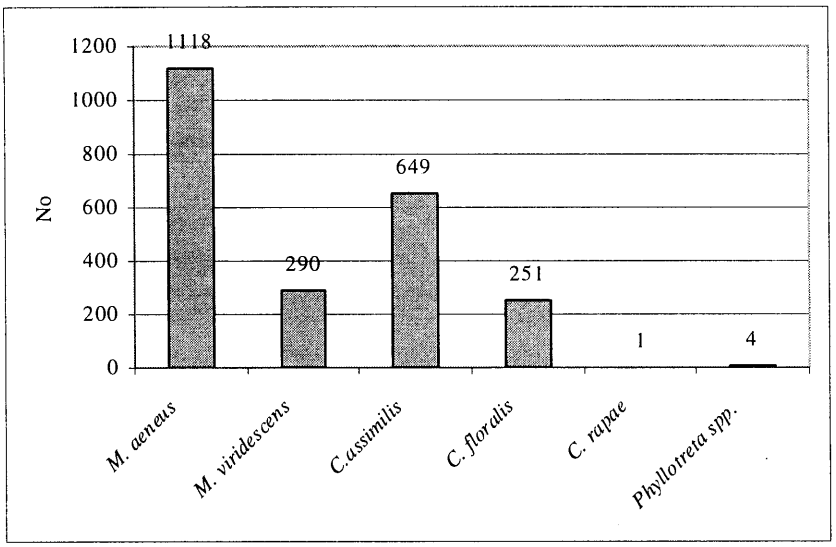

Fig. 1. The species' composition and the total number of crucifer specialist caught in the organic cropping system field of winter oilseed rape on the Puki Farm, the Tartu County in 2002

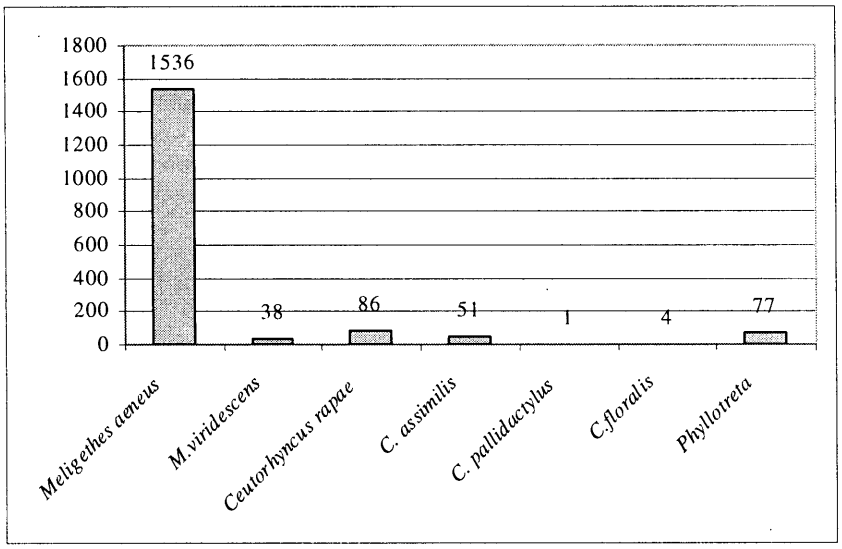

Fig. 2. The species' composition and the total number of crucifer specialist caught in the standard cropping system fields of spring oilseed rape on the Pilsu Farm, the Tartu County in 2003

Spring oilseed rape

Insects from seven different taxa were found: *Meligethes aeneus, M. viridescens, Ceutorhynchus assimilis, C. rapae, C. pallidactylus, C. floralis, C. pleurostigma,* and *Phyllotreta sp.* (Fig. 2).

Three of them are belonging to the key pests in Europe: *Meligethes aeneus, Ceutorhyncus assimilis,* and *C. pallidactylus.* Pollen beetles abundance and damage (per plant) were high. *C. assimilis* was not numerous — only 67 specimens were found (and their damage rate per plant was only 1%). *C. pallidactylus* — only single specimen was collected (Fig. 2). The pollen beetle population had two peaks. The first peak was when the first rape flowers opened and the second — in the stage of ripening of rape seed pods. The second peak was caused by emerged young beetles of new generation.

In 2003, only pollen beetles were real pests of spring oilseed rape in Estonian conditions. In general also flea beetles can cause damage but their number was very low, probably the very severe winter of 2002/2003 reduced their populations.

Parasitoids

In the yellow water traps in winter oilseed rape parasitic wasps, pollen beetle larval parasitoids: *Phradis morionellus, Mesopolobus morys, Stenomalina gracilis* and *Trichomalus perfectus*, larval parasitoids of *Ceutorhynchus assimilis* were caught. *T. perfectus* and *P. morionellus* were also present in spring oilseed rape. Three new pollen beetle parasitoids *Diospilus capito, P. interstitialis* and *Tersilochus heterocerus* were established in spring oilseed rape. But numbers of parasitoids were very low. The occurrence and abundance of different species depend on the factors such as the local climate, the crops grown during previous years and the cultivation techniques used (Nilsson, 2003). The spring

oilseed rape field was situated on the farm which has grown oilseed rape for 10 years already, this may be the reason why there were more pollen beetle parasitoids in spring oilseed rape field.

The presence of *P. morinellus* is very important because this species is considered to be an effective population regulator of *Meligethes* spp. (Hokkanen, 1989; Billqvist and Ekbom, 2001). *P. morinellus* was a most numerous pollen beetles' parasitoid species in the spring oilseed rape field and might have influenced the number of the new generation of pollen beetles.

Predators

In winter rape 41 and in spring rape 32 taxa of carabids were caught. The winter rape is offering good hibernation for carabids and therefore richer fauna. Dominant genera carabids were *Pterostichus, Amara, Agonum, and Harpalus* and *Carabus*.

In winter rape *Pterostichus* dominated, with *P. cupreus* and *P. melanarius* being the most numerous species.

In spring rape the most numerous species was *Harpalus pubescens* followed by *P. cupreus* and *P. melanarius*. In the rape field they were most abundant in the period when the larvae of pollen beetles finished feeding on flowers and dropped down into soil for pupation. At that time they were prey objects for carabids.

In the Estonian conditions, winter oilseed rape has still no serious pest problem. The phytophagous insects specialised in cruciferous crops *Meligethes aeneus, M. viridescens, Ceutoryncus assimilis, C. floralis, C. rapae, C. pleurostigma, Phyllotreta* spp. were found, but their abundance was low. Of the potential pests, *Meligethes aeneus* and *Ceutorhynchus assimilis* were the most numerous but did not cause any significant damage to winter oilseed rape.

In the spring oilseed rape, only pollen beetles were pests. In certain conditions flea beetles may cause serious damage or even destroy all the crop. In future, if the seed weevils' population is increasing, they might be serious pests in Estonia.

The occurrence of pests' natural enemies was established. From predators, carabids *Pterostichus cupreus* and *P. melanarius* were the dominating species. Their higher abundance in the period when the larvae of pollen beetles finished feeding on flowers is indicating on their role in the pollen beetle population regulation. Pollen beetle larval parasitoids: *Phradis morionellus, P. interstitialis, Diospilus capito, Tersilochus heterocerus, Mesopolobus morys, Stenomalina gracilis* and *Trichomalus perfectus*, larval ectoparasitoids of *Ceutorhynchus assimilis* were found. Their abundance was low, but could increase with the using of integrated cultivation techniques.

Acknowledgements

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References

1. Billqvist, A., Ekbom, B. 2001a. Effects of host plant species on the interaction between the parasitic wasp *Diospilus capito* and pollen beetles (*Meligethes* spp.). Agricultural and Forest Entomology, 3, 147—152.
2. Billqvist, A., Ekbom, B. 2001b. The influence of host plant species on the parasitism of pollen beetles (*Meligethes* spp.) by *Phradis morionellus*. Entomologia Experimentalis et Applicata 98, 41—47.
3. Büchs, W. 2003. Predators as Biocontrol Agents of Oilseed Rape pests. Biocontrol of Oilseed Rape Pests. Ed. Alford, D.V., Blackwell Science Ltd, 279—298.
4. Ekbom, B., Borg, A. 1993. Predators, *Meligethes* and *Phyllotreta* in unsprayed spring oilseed rape. IOBC/WPRS Bulletin, Vol. 16 (9), 175—179.
5. Free, J.B., Williams, I. H. 1978. The Insect Pest of Oil Seed Rape. Proceedings of the 5th International Rapeseed Conference, Vol. I. Malmö, Sweden, June 12—16.
6. Free, J.B., Williams, I. H. 1979. The distribution of insect pests on crops of oil-seed rape (*Brassica napus* L.) and the damage they cause. Journal of Agricultural Science, Cambridge, 92, 139—149.
7. Hansen, L.M. 2003. A model for determination of the numbers of pollen beetles (*Meligethes aeneus* F) (Col., Nitidulidae) per plant in oil-seed rape crops (*Brassica napus* L.) by estimating the percentage of plants attacked by pollen beetles. Journal of Applied Entomology, 127, 163—166.
8. Hokkanen, H. 1989. Biological and agrotehnical control of the rape blossom beetle *Meligethes aeneus* (Col., Nitid.). Acta Entomologica Fennica, 53, 25—29.
9. Nilsson, C. 2003. Parasitoids of Pollen Beetles. Biocontrol of Oilseed Rape Pests. Blackwell Science Ltd, 73—85.
10. Williams, I.H., Büchs, W., Hokkanen, H., Johnen, A., Klukowski, Z., Luik, A., Nilsson, C., Ulber, B. 2002. MASTER: Management Strategies for European Rape pests — a new EU Project. The BCPC Conference, 18—21 November 2002, Conference Proceedings Vol. 2, Brighton, UK.

FOOD PLANT PREFERENCE OF THE CABBAGE MOTH, *MAMESTRA BRASSICAE* (L.)

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Abstract

The aim of the present study was to elucidate plants preferred by cabbage moth, *Mamestra brassicae* larvae and the dynamics of their numbers on these plants. The experiment included white cabbage (*Brassica oleracea* var. *capitata* f. *alba*), rutabaga (swede) (*B. napus* var. *napobrassica*), collard (ornamental kale) (*B. oleracea* var. *acephala*), red cabbage (*B. oleracea* var. *capitata* f. *rubra*), and nasturtium (*Tropaeolum majus*). Our experiments revealed that cabbage moth larvae had feeding preferences within the same *Brassica* family. The *M. brassicae* preferred white cabbage; 51.2% of larvae counted during the observation period were gathered from this plant. The next choices were collard, by 32.1%, and red cabbage, by 16.5%. On rutabaga only few larvae of *M. brassicae* were found. No *M. brassicae* were discovered on nasturtium. White cabbage had the biggest number of larvae at the end of July; thereafter their numbers decreased, and in mid-August only single larvae were found there. Red cabbage had larvae only during the first two analyses; later samples did not show any larvae. Collard had a relatively small number of larvae during the first two observations, after that their number started to increase, reaching its peak in the fourth week of the observations. Thereafter the numbers decreased until by mid-August none remained.

Key words: *Mamestra brassicae*, foodplant preference, white cabbage, rutabaga, collard, red cabbage, nasturtium.

Introduction

The cabbage moth (CM), *Mamestra brassicae* (L) is a highly polyphagous species, particularly associated with cruciferous crops (Bretherton et al. 1979), but also feeding on a wide range of other plant species (Turnock & Carl, 1995). The occurrence of CM as a pest in Estonia is variable. During the last years, the population levels in general have been low, but with sporadic, local outbreaks. In different climatic zones, CM can produce a number of generations during a summer; in Estonia it is mainly an univoltine species, hibernating as diapausing pupae in the soil. The adults emerge at the end of June or at the beginning of July. Since the butterflies lay their eggs during a longer period of time, the larvae may be found in nature during a few months. Females oviposit at night and lay their eggs in a single-layered cluster mainly on the underside of host-plants leaves. Egg clustering may protect eggs from desiccation (Clark and Faeth, 1998) as well as from other detrimental environmental factors (Ulmer et al., 2003). In general there is a viewpoint that young larvae of CM remain clustered during the first instar, however, Johansen (1997) found that the larvae started to spread all over the host plant within a few hours after hatching, and continued to disperse radically from the original infested plant throughout the larval stage. In younger instars, the larvae feed mainly on the external leaves. From the fifth instar, they display a negative phototaxis (Omono et al., 1973) and they move into the central part of the plant in between young leaves where they complete their larval development.

It is generally known that various plant characteristics influence host plant selection in herbivorous insects, but plant chemistry can be especially important. For example, secondary plant metabolites are used by several insects for recognition of their host plants (Chew, 1988; Städler, 1992). The typical pattern of host location among adult *Lepidoptera* is the use of plant odours for longer-range detection and evaluation of potential host plants, followed by contact chemoreception for selection of oviposition sites (Schoonhoven et al., 1998). In the case of CM, it has been found that it mainly selects an oviposition site by odour cue, whereas the search process is, to some extent, influenced by visual cues (Rojas et al., 2000). The choice of egg-laying sites is also influenced by several other factors. The hypothesis that adult females prefer to oviposit on the plant species which had served as their larval food plant is known as the Hopkins host selection principle (Szentesi & Jermy, 1990). However, Rojas and Wyatt (1999[a]) discovered by their experiments that, there is no evidence that the adults of CM base their search of egg-laying sites on the needs of the larvae.

Several aspects of the *M. brassicae* biology have been studied in detail (Johansen, 1997; Rojas and Wyatt, 1999[b]; Rojas et al., 2000, 2001, etc.), but most experiments with the CM selection of host plant has been conducted with adults in wind tunnels in laboratories. Although larvae of CM can feed on many different host plant species, there is currently little knowledge on its feeding preferences and there are almost no data on relevant field observations. It is known that also larvae of highly polyphagous species are selective in their food choice and show preferences for some plants over others (Schoonhoven & Van Loon, 2002). The aim of this experiment was to establish whether CM larvae have feeding preferences for some more important garden culture in Estonia and whether there are any preferences within one plant genus. The criterion for choosing plants was that they all contained glucosinolates.

Materials and Methods

The experiments were carried out in the experimental garden of the Estonian Agricultural University in the summer of 2003. The experiment included white cabbage (*Brassica oleracea* (L) var. *capitata* f. *alba*), rutabaga (swede) (*B. napus* L. var. *napobrassica* DC. L. Reichenb.), collard (ornamental kale) (*B. oleracea* (L) var. *acephala*), red cabbage (*B. oleracea* (L) var. *capitata* f. *rubra*), and nasturtium (*Tropaeolum majus* L.). All plants were grown from

seed, kept in a glasshouse until they reached the 3 true leaf stage. In mid-May the plants were replanted in the experimental field. Each variant consisted of 9 plants per plot (three rows of three plants spaced at 70-cm intervals). All variants had three replications. To prevent larvae from leaving the experimental plots, a 20 cm wide strip of dill (*Anethum graveolens* L), which is not a food plant of CM larvae, was sown around each plot. Larvae of CM on all the experimental plots were sampled at 7-day intervals from 18 July to 05 September. Larvae were removed by hand picking them from plants to avoid repeated counting. Such repeated experiments enabled discovering, during the experimental period, also those larvae not found in earlier counts.

Data have been presented as mean ± standard deviation. Statistical comparisons were performed with paird Student's t-test or repeated-measures ANOVA by Tukey test. All means were considered significantly different at the $P < 0.05$ level.

Results and Discussion

The proportion of larvae of CM on different cultures

Analysing of material gathered during the experimental period showed that 52% of larvae counted during the observation period were gathered from white cabbage. The next choices were collard (ornamental kale), by 32%, and red cabbage, by 14%. On swede (rutabaga) only few larvae of CM were found (2%) (Fig.1). No CM were discovered on nasturtium.

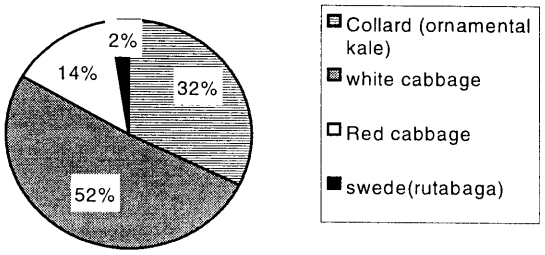
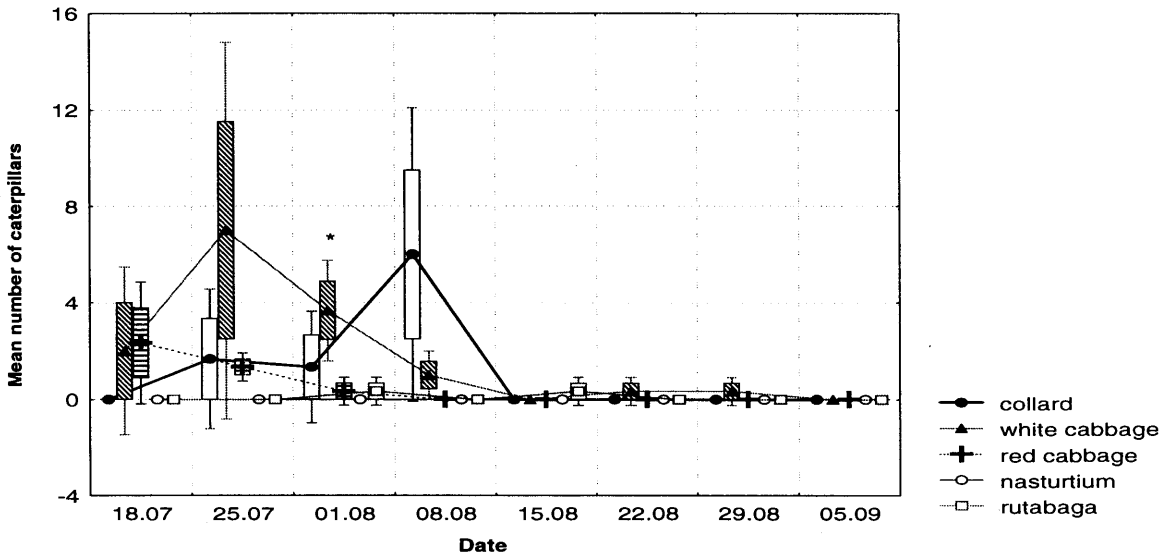


Fig. 1. The proportion of larvae of *Mamestra brassicae* on four different plant species

The dynamics of the number of CM larvae

During the first observation (18.07), there were few CM larvae and a nearly the same number of them were found both on white cabbage (M = 2.0) and red cabbage (M = 2.33). There was no statistically significant difference in the mean number of these variants (df = 4, F = 1.16, P = 0.38). There were no larvae on plants of the other variants. During the second observation (25.07), larvae were found again on white cabbage (M = 7.0) and, to a lesser extent, on red cabbage (M = 1.33), whereas only larvae of older instars were found on red cabbage. During this observation, larvae were first found on collard (ornamental kale), too (M = 1.66). On comparing the mean numbers in different variants, there was no statistically significant difference (df = 4, F = 1.8, P = 0.20), however, there were somewhat more larvae on white cabbage than in other variants. The third observation (01.08) revealed again larvae on white cabbage (M = 3.66) and ornamental kale (M = 1.33) but red cabbage had only few larvae of the last instar (M = 0.33). Larvae of the second and third instar were found on rutabaga (swede) (M = 0.33). A comparison of the variants showed that white cabbage had statistically reliably (df = 4, F = 3.27, P = 0.05) more larvae than the other variants. At the fourth observation (08.08) there were larvae of mainly the fourth instar on white cabbage. Their number was low (M = 1.0). This observation revealed a bigger number of larvae on ornamental kale (M = 6.0). As by the time of the observation larvae of the variant had mainly reached last instars, it may be concluded that young larvae had not earlier been found between the thick and wrinkled foliage. Later when larvae reached older instars and moved onto young leaves of ornamental kale, they were easily noticeable. However, the difference in the numbers of larvae on white cabbage and ornamental kale was not statistically reliable (df = 4, f = 2.68, P = 0.09). During the fifth observation (15.08), few larvae were found on rutabaga (M = 0.33). Other variants revealed no larvae. At the sixth (22.08) and seventh observation (29.08) only a few grown-up larvae were detected on white cabbage (M = 0.33). In September (05.09) no larvae were found on any plant, and the experiment was finished.

In brief, it can be concluded that white cabbage had the biggest number of larvae at the end of July; thereafter their numbers decreased, and in mid-August only single larvae were found there (Fig. 2). The occurrence of larvae on red cabbage showed nearly the same tendency. The red cabbage was more infested with larvae during the first three analyses; in later samples their number decreased constantly. On the basis of the results obtained with white and red cabbage, it can be concluded that there was repeated egg-laying of CM females on white cabbage as larvae of different instars were found within a long period of time. On red cabbage eggs were laid only during one period since each observation revealed larvae of older instars. Collard had a relatively small number of larvae during the first two observations, after that the number started to increase, reaching its peak in the fourth week of the observations. After that the numbers of larvae decreased until by mid-August none remained. Obviously, eggs were laid also on that plant only at the beginning of our observations as later counts revealed no younger instars. It must be added that the number of CM was low in all variants over the entire experimental period.



* statistical difference ($P < 0.05$).

Fig. 2. Dynamics of larvae of *Mamestra brassicae* on different cultures

Thus, in this experiment, CM larvae fed only on crucifers. According to Rojas and Wyatt (1999[a]), CM may have evolved the ability to recognise characteristic chemicals of this family group. Cruciferas contain a group of sulfur containing secondary metabolites known as the glucosinolates. Glucosinolates are found in all parts of the plant, and concentrations differ according to tissue type, physiological age, plant health, nutrition, etc. It is known that younger plants and plant parts have a higher glucosinolate content than the older ones. CM females always lay their eggs on outer, older cabbage leaves and younger larvae prefer those leaves as well. In an experiment by Boer (1999), young CM larvae chose to feed also on older leaves of the crucifer ragwort (*Senecio jacobaea* L) by constantly moving, when reaching older instars, onto younger parts of the plant. Our observations showed that larvae stayed on older cabbage leaves during their three first instars. Larvae already in the fourth instar started to move onto younger leaves of the plant and, at the end of the fourth and at the beginning of the fifth instar they penetrated inside cabbage head. As collard and rutabaga form no heads, we found older instars between leaves of the core. One reason for such relocation is considered be the fact that the larvae switch from carbohydrate (in older leaves) to protein content (in younger leaves) in their diet when they reach the last instar (Reavey, 1993). The products of hydrolysis may have important roles in the plant defence system against polyphagous insects, however, the defensive secondary metabolites of plants can be utilised by insects adapted to them. It is probably also the case with CM larvae who, by eating older leaves, adapt themselves to the chemistry of the food plant and the lesser amounts of defensive substances there. This enables them later to eat also younger plant parts.

Over the entire experimental period, no larvae were discovered on *T. majus*, despite the glucosinolate content of the plant. However, this species of the family Tropaeolaceae produces only a single glucosinolate — glucotropaeolin. This glucosinolate has long been known for its antimicrobial activity, in addition to which it also contains, for example, chlorogenic acid and isoquercitrin, known to be antifeedant for certain insect species (Duke, 1992; Huang and Renwick, 1995).

Our experiments also revealed that CM larvae had feeding preferences within the same *Brassica* family. Plant species have numerous chemical and physical differences (colour, plant surface waxes, trichome density, secondary chemicals, etc) that may influence preferences. As the largest number of larvae was found on white cabbage, there is a reason to believe that these were the chemical composition and physical properties of white cabbage that suited CM best in the experiment. It must be noted that certain colours are more or less attractive to different insect species. Radcliff and Chapman (1966) detected that a colour-related factor appeared to be important in determining host preferences for *Pieris brassicae* and *P. rapae*, and red cabbage varieties were less susceptible to oviposition than green varieties. Red colour of the plants is mainly caused by anthocyanins. These are members of a class of nearly universal, water-soluble, terrestrial plant pigments that can be classified chemically as both flavonoid and phenolic. There are 15 different anthocyanins in red cabbage (Saupe, 2002). Anthocyanins may inhibit larval growth in insects but also act as insect repellents (Thain et al., 2002). In our experiment red cabbage were less infested (14%) with CM larvae than white cabbage (52%). According to Hommes (1983), red cabbage was less infested with larvae of *P. rapae* than white cabbage. Probably a determinative factor here is the choice by adults who, on some reasons, lay less eggs on red-coloured plants. Our earlier feeding experiments in laboratory have shown that both larvae of *P. rapae* and *P. brassicae* grew and developed equally both on white and red cabbage. Besides, larvae of *P. brassicae* developed on red collard even more successfully than on white head cabbage (Metspalu et al., 2003).

However, to the lesser preference of red cabbage by larvae there were obviously some other reasons than the colour as on red collard there were noticeably more (32%) larvae than on red cabbage (14%). As well known, plant

surface may play an important role in the selection of both oviposition sites of adults and food plants of larvae. The plant cuticle acts as a first chemical and mechanical barrier against herbivorous insects. Characteristic cuticular compounds might act as deterrents against generalistic herbivores, while specialised insects could use them as clues for host-plant recognition. The plants contain a coating of wax on the surface of the cuticule. The wax restricts water loss to the atmosphere but its physiological role is not less important. Obviously both the chemistry of such leaf waxes and their physical attributes act together with some plant characteristics. So far it is not exactly known whether the active factors are the wax coating itself or secondary compounds of the plant, associated with the waxes (Eigenbrode, 1996). It has been found that on leaves with a thicker wax coating larvae waste more time in search of suitable feeding sites than with a thinner wax coating, due to which their feeding time shortens and the amount of food acquired decreases. The consequence here is their decelerated development (Eigenbrode, 1996). In addition, leaf surface may mechanically influence movement of the insect. For example, microscopic structures on the plant surface can reduce the adhesion of insect feet, thereby creating slippery grounds for the animals. In our experiment the wax coating of red cabbage was very thick compared with the wax coating of white cabbage or collard. Wax coating makes it difficult for females of CM to obtain information on the chemical composition of leaves since it prevents the discharge of specific odours from a leaf. The wax coating on rutabaga was noticeably thinner than that of red and white cabbage. There were fewer larvae found on rutabaga (2%). One reason here may have been that rutabaga contains an exceptionally high amount of glucosinolates, even more than other cabbage varieties. Fifteen glucosinolates have been found, whereas gluconasturtin, together with gluconapin, glucobrassicanapin and progoitrin have been reported to be the main glucosinolates from this plant (Kjaer, 1976). It may be that such an amount and composition of glucosinolates and their breakfown products are repellent already to adults of CM, on which reason lesser eggs are laid to rutabaga. Another possibility is that rutabaga leaves probably contain feeding deterrents for the larvae, due to which there were especially few larvae of younger instars on rutabaga leaves. According to our unpublished data, observations carried out in a field of rutabaga in Hiiumaa showed that, when eggs were laid on rutabaga, larvae hatched from egg clutches died in their second and third instar and only a few larvae reached older instars. Hairs, trichomes and thorns on the leaf surface also influence both the choices of oviposition sites of adults and feeding of larvae. Distribution and density of leaf trichomes and chemicals contained in the trichomes may be among the factors influencing the host plant and food plant selection by CM in rutabaga. Usually the youngest leaves have the highest density of trichomes, which certainly was one of the reasons why larvae appeared mainly on older, less hairy leaves of rutabaga. During the year of the experiment, the number of CM was low, and there was almost no competition for adult oviposition site. There could be no competition among larvae for feeding sites as during each observation larvae counted were removed from plants.

It can be concluded that glucosinolates are not the determinative factors in the choice of oviposition and feeding plants by CM, but other secondary compounds, exist in plants, might be responsible for variation in the acceptability of different plant species to cabbage moth. The balance between stimulatory compounds and deterrent ones determined the reaction of CM larvae to different food plants.

Acknowledgement

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Reference

1. Boer, de N. J. 1999. Pyrrolizidine alkaloid distribution in *Senecio jacobaea* rosettes minimalises losses to generalist feeding. *Entomol. Exp. Appl.* 91, 169—173.
2. Bretherton, R.F., Goater, B., Lorime, R.I. 1979. Noctuidae. In: J. Heath and A.M. Emmet (eds). The Moths and Butterflies of Great Britain and Ireland. Curwen Booka, London, 120—278.
3. Chew, F.S. 1988. Searching for defensive chemistry in the Cruciferae, or, do glucosinolates always control interactions of Cruciferae with their potential herbivores and symbionts? No! In: K.C. Spencer (ed), Chemical Mediation of Coevolution. Academic Press, San Diego, 81—112.
4. Clark, B.R., Faeth, S.H. 1998. The evaluation of egg clustering in butterflies: a test of the egg desiccation hypothesis. Evol. Ecol. 12, 543—552.
5. Duke, J.A. 1992. Chemicals in: *Tropaeolum majus* L. (Tropaeolaceae) Naturtium. Phytochemical Database, USDA-ARS-NGRL, Beltsville Agricultural Research Center, Beltsville, Maryland.
6. Huang, X.P., Renwick, J.A. 1995. Chemical and experimental basis for rejection of *Tropaeolum majus* by *Pieris rapae* larvae. J. Chem. Ecol., 21, 1601—1617.
7. Eigenbrode, S.D. 1996. Plant surface waxes and insect behaviour. In: G. Kerstiens (ed). Plant cuticule: An integrated functional approach. BIOS Scientific Publisher, Oxford, 201—222.
8. Hommes, M. 1983. Untersuchungen zur Populationsdynamik und integrierten Bekämpfung von Kohlschädlingen. Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem, 213, 3—210.
9. Johansen, N.S. 1997. Mortality of eggs, larvae and pupae and larval dispersal of the cabbage moth, *Mamestra brassicae*, in white cabbage in south-eastern Norway. Enomol. Exp. Appl., 83, 347—360.
10. Kjaer, A. 1976. Glucosinolates in the Cruciferae. In: J.G. Vaughan, A.J. MacLeod, and B.M. G. Jones (eds). The Biology and Chemistry of the Cruciferae. Academic Press, New York, 207—219.
11. Metspalu, L., Hiiesaar, K., Jõudu, J., Kuusik, A. 2003. Influence of food on the growth, development and hibernation of Large White Butterfly (Pieris brassicae). Agronomy Research, 1, 85—92.

12. Omono, T., Yokoi, S., Tsuji, H. 1973. Experimental studies on the daytime behaviour of Noctuid larvae, the cabbage armyworm, *Mamestra brassicae*, the tobacco cutworm, *Spodoptera litura* and the black cutworm, *Agrotis ipsilon*. Japanese J. Appl. Entomol. Zool., 17 (4), 215—220.
13. Radcliff, E.B., Chapman, R.K. 1966. Varieties resistance to insect attack in various cruciferous crops. J. Econ. Entomol., 59, 120—125.
14. Reavey, D. 1993. Why body size matters to larvae. In: N.E. Stamp & I.M. Casey (eds). Larvae. Chapman & Hall, New York, 29—91.
15. Rojas, J.C., Wyatt, T.D. 1999[a]. The role of pre- and post-imaginal experience in the host-finding and oviposition behaviour of the cabbage moth. Physiol. Entomol., 24, 83—89.
16. Rojas, J.C & Wyatt, T.D. 1999[b]. Role of visual cues and interaction with host odour during the host-finding behaviour of the cabbage moth. Entomol. Exp. Appl., 91, 59–65.
17. Rojas, J.C., Wyatt, T.D., Birch, M.C. 2000. Flight and oviposition behaviour toward different host plant species by the cabbage moth, *Mamestra brassicae* (L.) (Lepidoptera: Noctuidae). J. Insect Behavior, 13, 2, 247—254.
18. Rojas, J.C., Wyatt, T.D., Birch, M.C. 2001. Oviposition by *Mamestra brassicae* (L) (Lep., Noctuidae) in relation to age, time of day and host plant. J. Appl. Ent., 125, 161—163.
19. Saupe, S.G. 2002. Introduction to the Protoplast Lab. Plant Physiology, Col. St. Benedict, Biology Department, Collegeville. From web site: www.employees.csbsju.edu/ssaupe/index.html
20. Schoonhoven, L.M., Jermy, T., van Loon, J.J.A. 1998. Insect-plant biology from physiology to evolution. Chapman & Hall, London, UK.
21. Schoonhoven, L.M., van Loon, J.J.A. 2002. An inventory of taste in larvae: each species its own key. Acta Zoologica Academiae Scientiarum Hungaricae, 48, 215—263.
22. Szentesi, A., Jermy, T. 1990. The role of experience in host plant choice by phytophagous insects. In: A.A. Bernays (ed). Insect-Plant Interactions, Vol. II ed. CRC Press, Boca Raton, Florida, 3974 pp.
23. Städler, E. 1992. Behaviour responses of insects to plant secondary compounds. In: G.A. Rosenthal & M.R. Berenbaum (eds). Herbivores: Their Interactions with Secondary Plant Metabolites, Volume II: Evolutionary and Ecological Processes. Academic Press, San Dirgo, 45—88.
24. Thain, S.C., Murtas, G., Lynn, J.R., McGrath, R.B., Millar, A.J. 2002. The Circadian Clock That Controls Gene Expression in Arabidopsis Is Tissue Specific. Plant Physiology, 130 (1), 102—110.
25. Turnock, W.J., Carl, K.P. 1995. Evaluation of palearctic *Eurithia consobrina* (Diptera: Tachinidae) as a potential biocontrol agent for *Mamestra configurata* (Lepidoptera, Noctuidae) in Canada. Biocontr. Sci. Techn., 5, 55—67.
26. Ulmer, B., Gillott, C., Erlandson, M. 2003. Conspecific eggs and Bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae), oviposition site selection. Environ. Entomol., 32 (3), 529—534.

SPIDERS (*ARANEAE*) IN SUMMER OILSEED RAPE, WHEAT AND CLOVER FIELDS

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Abstract

The aim of the present work was to investigate the abundance and seasonal dynamics of spiders in summer oilseed rape, wheat and two-year-old clover fields. The experiments were carried out in the experimental field of the Estonian Agricultural University in the summer of 2003.

During the study period, 892 individuals of spiders were collected with pitfall traps. In all variants *Lycosidae, Linyphiidae* and *Opiliones* were dominating, but the total number of *Opiliones* was lower than the abundance of other dominating spider groups.

Compared with clover, the number of spiders was reliably lower on rape and wheat during the whole period of the experiments. Spiders preferred clover variant, where plant cover was greater, older and no fertilisers, herbicides or pesticides were used. Cutting had an immediate effect on the number of spiders in the clover variant. The prey conditions for spiders changed in this plot, and spiders left the clover variant for two weeks. The increase of ground cover density in plant communities could enhance the abundance of spider assemblages. On wheat variant the number of spiders was low during the whole period of the experiments. The number of spiders was the lowest on the intensive rape plot as in this variant fertilisers and herbicides were used, and it was treated with the pesticide Fastac twice.

The seasonal dynamic of spiders significantly depended on the intensity of field management practices, including pesticides spraying times and the periodically interrupted life cycles of spiders. After treatment with Fastac the number of spiders was lower but later it recovered again.

Keywords: spiders, oilseed rape, pest control, *Araneae, Lycosidae, Linyphiidae, Opiliones.*

Introduction

Spiders are well-known predators but, compared with insects such as ground beetles, they have received relatively little attention as natural enemies of crop pests. Spider abundance is correlated with the specific vegetation characteristics, suggesting that the availability of habitats is important for spider colonisation and establishment (Rypstra, Carter, 1995).

Of several factors influencing the development of the spider communities, more important could be the effect of pesticide treatments and weed cover. Increased weed coverage can result in higher numbers of spiders in the field (Frank, Nentwig, 1995), and can also lead to higher densities of foliage inhabiting spiders assemblages. This suggests that there are interactions between the communities in canopy and ground cover (Altieri, Scmidt, 1986; Wyss, 1995; Wyss et al., 1995). An additional factor could be the boundary effect, and in arable ecosystems these pesticide-free areas can conserve spider populations and thus represent an important source of immigration (Alderweireldt, 1989; Kromp, Steinberger, 1992; Toth et al., 1996). Field margins play an important agricultural role in providing a refuge for beneficial invertebrate predators facilitating movements of spiders (Duelli et al., 1990; Thomas et al., 1990) into the crop.

Spiders are often the first predators to enter a crop field after ploughing, establishing themselves within the field before most pests have an opportunity to colonise (Sunderland et al., 1999). Food is often limited in this initial period but spiders have adapted to withstand the starvation periods (Sunderland et al., 1999). When food becomes available, spiders are able to gorge themselves (Sunderland et al., 1999) and may even kill surplus prey.

Two families constitute the majority of individuals in agricultural fields in northern Europe: *Lycosidae*, particulary *Paradosa* species, and *Linyphidae*, notably *Oedothorax, Erigone, Bathyphantes, Meioneta,* and *Lepthyphantes* species (Nyffeler, Benz, 1988; Alderweireldt, 1994; Thomas, Jepson, 1997; Downie et al., 2000).

A wide range of species can occur in arable fields, of which money spiders (*Linyphiidae*) and wolf spiders (*Lycosidae*) are most abundant (Alford, 2003). Harenberg (1997) recorded the following as the most numerous species in oilseed rape fields in Germany: *Linyphiidae* (12 species), *Tetragnathidae* (1), *Theridiidae* (1), *Lycosidae* (1) and *Salticidae* (1).

The aim of the present work was to investigate the abundance and seasonal dynamics of spiders in summer oilseed rape, wheat, and clover fields.

Materials and Methods

The experiments were carried out in the experimental field of the Estonian Agricultural University in the summer of 2003. The experiment included four variants: intensive rape (fertiliser + Fastac), control rape (no chemicals), wheat (fertiliser + herbicide), and clover. In spring the plots of rape and wheat were mechanically cultivated two or three times and treated with the herbicide Trifluralin and fertilised with Opti Crop 21-8-12+S+Mg+B before seedling. For pest control, Fastac was used twice (28 May and 26 June) in the intensive rape variant, and the wheat variant plants were treated with an herbicide once (10 June). Clover had been growing in the same place also previous year. During the observation period, hay was made once (at the beginning of July) on the clover variant. Spiders were caught with pitfall traps. The size of each plot of rape and wheat was 1×10 m and each variant had three replications. In the clover

variant, the pitfall traps were situated 20 m from field margins, and there were three replications. Spiders in the traps were counted once every week during the observation period in all test variants.

Data are presented as mean ± standard error. The statistical comparison was performed by means of ANOVA and Student's *t*-test. All means were considered significantly different at the $p = 0.05$ level.

Results and Discussion

During the study period, 892 individuals were collected. In all experimental plots three spiders families dominated — *Lycosidae, Linyphiidae,* and *Opiliones*, but the total number of *Opiliones* was lower than the abundance of other dominating spider groups.

The results indicated that, compared with clover, the number of spiders was reliably lower on intensive rape ($t = 6.19$, $df = 16$ $p = 0.02$) and wheat ($t = 4.75$ $df = 16$ $p = 0.04$) (Fig. 1) during the whole period of the experiments. It appeared that spiders preferred habitats where plant cover was greater and older. Spiders can overwinter at field edge (Maelfait, Keer, 1990) and there is some potential for improving these habitats for spiders by vegetational diversification to include grass tussocks (Bayram, Luff, 1993). Our experimental conditions for the habitation and overwintering of spiders were probably better in the clover than in intensively cultivated variants. Clover had been growing in the same place also previous year and spiders had good opportunities for overwintering there. Intensive rape, control rape and wheat plots in our experiment were located inside of a big cereal field, which may have had an influence on the abundance of spiders. In addition, the field part where rape and wheat were sown this year had been before used for growing other intensively managed cultures (potato was in this place in previous year).

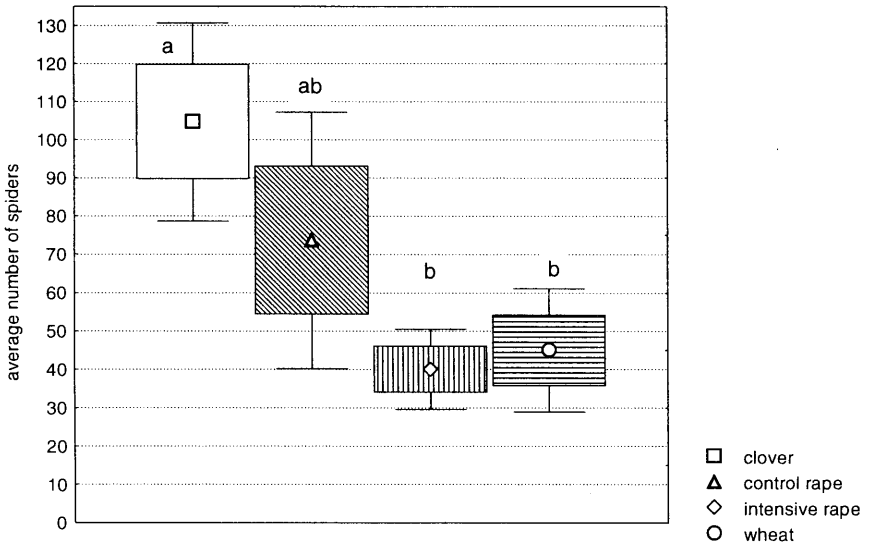

Fig. 1. Average number of spiders in different experimental plots in 2003. Means followed by same letter are not significantly different ($p < 0.05$) (means ± SD)

In the majority of such studies (including soybean, cereals and orchards), increases in spider densities at the edges were not translated into increases in the fields themselves, and especially in the centres of large fields (Altieri, Schmidt, 1986; Alderweireldt, 1989; Kemp, Barlett, 1989; Dennis, Fry, 1992; Kromp, Steinberger, 1992; Altieri, 1994; Vangsgaard, 1996; Toth, Kiss, 1997). This agrees with our work: plots of control rape, intensive rape and wheat were placed inside of a large wheat field. Spiders immigration to the field decreased from field margins to the centre, which is probably the reason why they did not prefer those experimental plots.

A comparison of intensive rape with control rape (Fig.1) revealed no reliable differences in the number of spiders, although there existed a slight tendency in favour of control rape. Both on intensive rape and wheat the number of spiders was lower during the whole period of the experiments, and a comparison of these variants showed no statistical reliability. In the clover and control rape variants, where no pesticide applications were used, significantly more spiders were found, probably because spiders like those areas. This agrees with laboratory studies of the effect of pesticide residues on the wolf spider *Paradosa agrestis* (Mansour et al., 1992). Most studies in different cultures compared spider densities in unsprayed and conventional field blocks. Specth and Dondale (1960) compared modified spray with unsprayed orchards. Spider densities were initially higher in the unsprayed programme, but the difference decreased through the season.

Studies have also shown that community composition changes with insecticide use. Chant (1956) identified only half as many species in sprayed than in unsprayed orchards. Specht and Dondale (1960) noted a lower proportion of hunting spiders in sprayed compared to unsprayed blocks (49% vs. 68%) and speculated that hunting forms might be more susceptible to insecticides. Probably the same fact had an effect on the intensive rape variant of our experiment, because spiders did not come from control rape and clover plots to the variant mentioned before, regardless that the plots were side by side. Another possible explanation for the highest number of spiders on clover is that there were many phytophagous pests and spiders who found there good prey and habitat conditions. The spider density can

actually be augmented by increasing the density of their fungifore and detritivore prey; this phenomen was studied in a forest floor system (Chen, Wise, 1999), and may also apply to crop systems. The sparse plant cover in the intensive rape and wheat variants caused there to be less phythophagous pests.

Intensively cultivated arable fields are not self-contained systems for invertebrate predators, because their life cycles are interrupted periodically by severe agricultural practices such as ploughing, sowing, spraying, etc. Intensively managed fields, where synthetic, broad-spectrum insecticide use is high year after year, have spider faunas of low density and diversity (Miliczky et al., 2000). This agrees with our work: in the intensive rape and wheat variants the life cycles of spiders were periodically interrupted with pest control spraying times. Spiders immigration to intensively cultivated areas may be limited, because most of the surrounding land is also insecticide-treated. The number of spiders was the lowest on the intensive rape plot as in this variant Fastac was used for pest control twice. Chemicals used and cultivation may be the reasons for the lower number of spiders in the intensive rape and wheat variants. Topping and Sunderland (1998) showed similar effects of ploughing and harvesting on numbers of spiders in cereal fields.

Weed density is a general phenomen connected with spiders and mentioned in many studies (Frank, Nentwig, 1995). However, some species entirely prefer microhabitats with low weed covers (Alderweireldt, 1989). In our work the abundance of spider assemblages could be enhanced by increasing the ground cover density in different plant communities. Greater numbers of spiders were observed within the clover, where the plant density was higher than on other experimental plots. The number of spiders was the lowest on the rape and wheat plots, probably because the plant cover was sparse in those variants. The number of spiders was to extent higher in control rape, because of the high weed density in this variant.

Field margins are one of the reasons that increase the number of source spider populations and thus the numbers of migrants entering the crop (Bishop, Riechert, 1990; Topping, Sunderland, 1994). Movement of spiders from field margins to the crop remains difficult to demonstrate in an experimental situation. Meadows are known to provide a more diverse immigration source than arable land (Tischler, 1965; Curry, 1994) and may account for significantly more species occurring in the control rape variant of our work. Between wheat and various grassy areas, the penetration of spiders into neighbouring habitats was also limited (Duelli et al., 1990; Kajak, Lukasiewicz, 1994). Studies conducted in the USA about weed strips of alfalfa fields sown at the edge had high density of spiders, however the spiders did not walk out into the crop. The authors suspected that the weed borders were so hospitable to spiders that they had no stimulus to disperse (Bugg et al., 1987). Probably the same fact had an effect on the intensive rape and wheat plots of our experiment, because spiders did not come from clover to the variants mentioned before, regardless that the variants were side by side.

The seasonal occurrence of spiders shows that spiders were very sensitive to different chemicals, used on our experimental plots in addition to other agricultural practices. The activity of spiders was low in the intensive rape (Fig. 2) and wheat (Fig. 3) variants in spring, on the basis of which it seems that the activity of spiders significantly depended on the pest spraying times in the experimental field and the intensive management of those plots. The activity of spiders was low in the control rape (Fig. 2) as well, probably because of the used different agricultural practices. In intensive rape and wheat the number of spiders started to increase, when the insecticide effect was ended, however, these were only small population peaks and decreased very quickly.

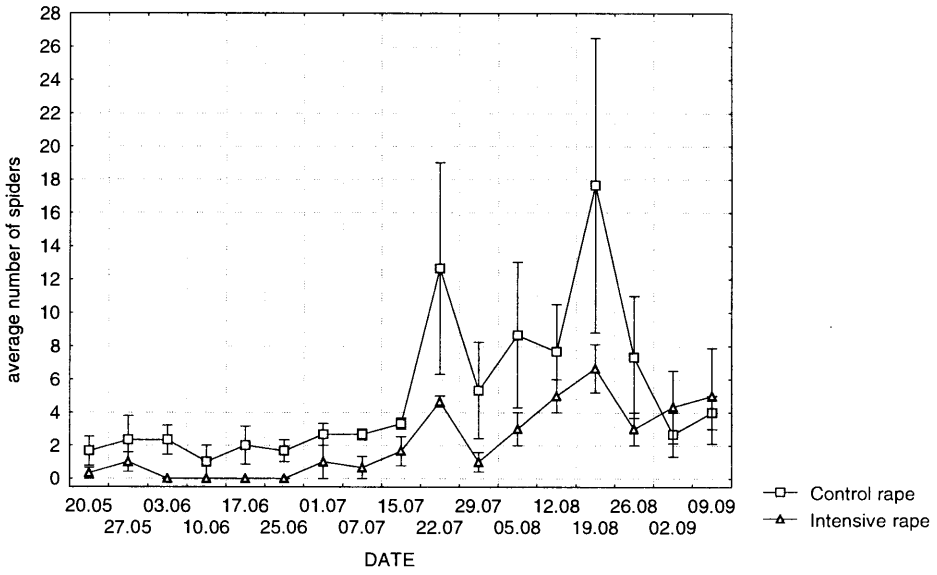

Fig. 2. Seasonal dynamics of spiders caught by pitfall traps in control rape and intensive rape variants during the observation period in 2003 (Means ± SE)

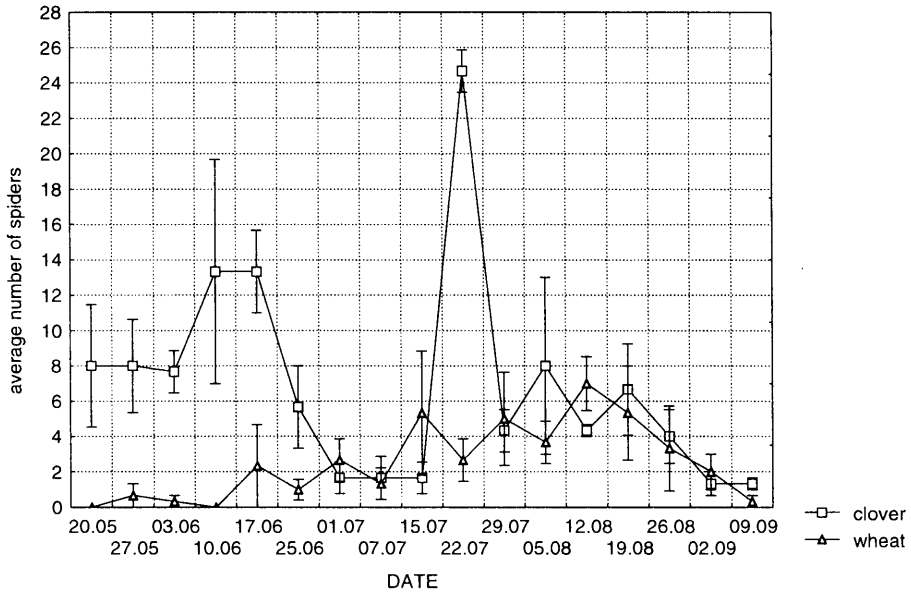

Fig. 3. Seasonal dynamics of spiders caught by pitfall traps in the clover and wheat variants during the observation period in 2003 (Means ± SE)

A probable reason for the small population peak in both variants was the appearance of a new generation — because a high number of juveniles were found in pitfall traps at this time of observation.

The seasonal occurrence of spiders in the clover variant (Fig. 3) was different in comparison with intensive rape, control rape, and wheat. In May, the number of spiders was low in the clover variant but started to increase quickly at the beginning of June as the plants had fully grown by this time. The plant cover affected favourably the dynamics of the spider population, particulary from late spring until summer. After the population peak, the number of spiders decreased again, because hay was made in the clover variant at the beginning of July. The cutting probably influenced the abundance of spiders on this plot negatively. Cutting had an immediate effect on the number of spiders in the clover variant. The prey conditions for spiders changed in our experimental plots, and they left this variant for two weeks. The same results showed a study conducted in UK grasslands, where cutting had an immediate effect on spiders numbers (Bell et al., 2002). Many authors agree that a summer cut reduces the number of spiders (Baines et al., 1998).

The activity of spiders significantly depended on the pesticides spraying times and plant cover (including weed cover) density in the experimental fields. Cutting had an immediate effect on the number of spiders in the clover variant. The increase of ground cover density in plant communities could enhance the abundance of spider assemblages. Field margins play an important role in spiders activity too. Spiders preferred clover variant, but they like areas, where fertiliser or chemical pest control were not used (control rape variant in our experimental field). In all variants there were dominating *Lycosidae, Linyphiidae* and *Opiliones*, but the total number of *Opiliones* was lower than the abundance of other dominating spider groups.

Acnowledgements

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References

1. Alderweireldt, M. 1989. An ecological analysis of the spider fauna (Araneae) occurring in maize fields, Italian ryegrass fields and their edge zones, by means of different multivariate techniques. Agric. Ecosys. Environ., 27(1—4), 293—305.
2. Alderweireldt, M. 1994. Prey selection and prey capture strategies of linyphiid spiders in high-input agricultural fields. Bull. Brit. Arachnol. So., 9, 300—308.
3. Alford, D.V. 2003. Biocontrol of Oilseed Rape Pests. Blackwell Sci. Ltd., 181—185.
4. Altieri, M. A., Schmidt, L.L. 1986. The dynamics of colonizing arthropod communities at the interface of abandoned, organic and commercial apple orchards and adjacent woodland habitats. Agric. Ecosys. Environ., 16, 29—43.
5. Altieri, M. A. 1994. Biodiversity and Pest Management in Agroecosystems. Food Products Press, New York, 185 pp.
6. Baines, M., Hambler, C., Johnson, P.J., Macdonald, D.W., Smith, H. 1998. The effects of arable field margin management on the abundance and species richness of the Araneae (spiders). Ecography, 21, 74—86.
7. Bayram, A., Luff, M.L. 1993. Winter abundance and diversity of lycosids (Lycosidae, Araneae) and other spiders in grass tussocks in a field margin. Pedobiol., 37, 357—364.
8. Bell, J.R, Johnson, P.J., Hambler, C., Haughton, A.J., Smith, H., Feber, R.E., Tattersall, F.H., Hart, B.H., Manley, W., Macdonald, D.W. 2002. Manipulating the abundance of *Lepthyphantes tenuis* (Araneae:Linyphiidae) by field margin management. Agric. Ecosys. Environ., 93, 295—304.
9. Bishop, L., Riechert, S.E. 1990. Spider coloniziation of agroecosystems: mode and source. Environ. Entomol., 19, 1738—1745.

10. Bugg, R.L., Ehler, L.E., Wilson, L.T. 1987. Effects of commonknotweed (*Polygonum aviculare*) on abundance and efficiency of insect predators of crop pest. Hilgardia, 55, 1—52.
11. Chant, D.A. 1956. Predacious spiders in orchards in south/eastern England. Journal of Horticultural Sience, 31, 35—46.
12. Chen, B.R., Wise, D.H. 1999. Bottom-up limitation of predaceous arthropods in a detritus-based terrestrial food web. Ecollogy, 80, 761—772.
13. Curry, J.P. 1994. Grassland invertebrates. Ecology, Influence on Soil fertility and effects on Plants Growth. Chapman & Hall, London, 437 pp.
14. Dennis, P., Fry, L.A. 1992. Field margins: can they enhance natural enemy population densities and general arthropod diversity on farmland? Agric. Ecosys. Environ., 40, 95—115.
15. Downie, I.S., Ribera, I., McCracken, D.I., Wilson, W.L., Foster, G.N., Waterhouse, A., Abernethy, V.J., Murphy, K.J. 2000. Modelling populations of *Erigone atra* and *E. dentipalpis* (Araneae: Linyphiidae) across an agricultural gradient in Scotland. Agric. Ecosyst. Environ., 80, 15—28.
16. Duelli, P., Studer, M., Marchand, I., Jakob, S. 1990. Population movements of arthropods between natural and cultivated areas. Biol. Conserv., 54, 193—207.
17. Frank, T., Nentwig, W. 1995. Ground-dwelling spiders (Araneae) in sown weed trips and adjacent fields. Acta Oecologica 16(2), 179–193.
18. Harenberg, A. 1997. Auswirkungen extensiv geführter Anbausysteme in verschiedenen Fruchtfolgen (Raps-, Zuckerrübenfructfolge) und einer selbstbegrünenden Dauerbache auf spinnen (Arachnida: Aranea). PhD. thesis, Technical University of Braunsweig, Germany.
19. Kajak, A., Lukasiewicz, J. 1994. Do semi-natural patches enrich crop fields with predatory epigean arthropods. Agric. Ecosys. Environ., 49, 149—161.
20. Kemp, J.C., Barrett, G.W. 1989. Spatial patterning: impact of uncultivated corridos on arthropod populations within soybean agroecosystems. Ecology, 70, 114—128.
21. Kromp, B., Steinberger, K.H. 1992. Grassy field margin and arthropod diversity: a case study on ground beetles and spiders in eastern Austria (Colepotera: Carabidae; Arachnida: Aranei, Opiliones). Agric. Ecosys. Environ., 40(1—4), 71—93.
22. Maelfait, J.P., De Keer, R. 1990. The border zone intensively gazed pasture as a corridor for spiders (Araneae). Biological Conservation, 54, 223—238.
23. Mansour, F., Heimbach, U., Wehling, A., 1992. Effects of pesticide residues on ground-dwelling lycosid and micryphantid spiders in laboratory tests. Phytoparasitica, 20(3), 195—202.
24. Miliczky, E.R., Calkins, C.O., Horton, D. 2000. Spider abundance and diversity in apple orchards under three insect pest management programmes in Washington State, U.S.A. Agricultural and Forest Entomology, 2, 203—215.
25. Nyffeler, M., Benz, G. 1988. Prey predatory importance of micryphantid spiders in winter wheat fields and hay meadows. J. Appl. Entomol., 105, 190—197.
26. Rypstra, A.L., Carter, P.E. 1995. The web-spider community of soybean agroecosystems in south-western Ohio. J. Arach. 23(3), 135—144.
27. Specht, H.B., Dondale, C.D. 1960. Spider populations in New Jersey apple orchards. Journal of Economic Entomology, 53, 810—814.
28. Sunderland, K.D., Greenstone, M.H., Symondson, B. 1999. Spiders for pest control. Pesticide Outlook, 10, 82—85.
29. Thomas, C.F.G., Hol, E.H.A., Everts, J.W. 1990. Modelling the diffusion component of dispersal during recovery of a population of Linyphiid spiders from exposure to an insecticide. Functional Ecology, 4, 357—368.
30. Thomas, C.F.G., Jepson, P.C. 1997. Field-scale effects of farming practices on linyphiid spider populations in grass and cereals. Entomol. Exp. Appl., 84, 59—69.
31. Tischler, W. 1965. Agrarökologie. Gustav-Fischer Verlag, Jena, 499 pp.
32. Topping, C.J., Sunderland, K.D. 1994. A spatial population dynamics model for *Lepthyphantes tenuis* (Areneae: Linyphiidae) with some stimulations of the spatial and temporal effects of farming operations and land-use. Agric. Ecosys. Environ., 48, 203—217.
33. Topping, C.J., Sunderland, K.D. 1998. Population dynamics and dispersal of *Lepthyphantes tenuis* in an ephemeral habitat. Entomol. Exp. Appl., 87, 29—41.
34. Toth, F., Kiss, J. 1997. Occurrence of *Paradosa* (Araneae, Lycosidae) species in winter wheat and in the field margin. Proc.16th Europ.Coll. Arachnol., Siedlce, 309—315.
35. Toth, F., Kiss, J., Samu, F., Toth, I., Kozma, E. 1996. Az öszibuza fontosabb pokfajainak (Araneae) jellemezese talajcsapdas gyüjtesre alapozva (Dominant spiders species (Araneae) in winter wheat in pitfall trap catches). Növenyvedelem 32(5), 235—239.
36. Vangsgaard, C. 1996. Spatial distribution and dispersal of spiders in a Danish barley field. Rev. Suisse Zool., hors serie: 671—682.
37. Wyss, E. 1995. The effects of weed strips on aphids and aphidophagous predators in an apple orchard. Entomol Exp. Appl., 75(1), 43—49.
38. Wyss, E., Niggli, U., Nentwig, W. 1995. The impact of spiders on aphid population in a strip-managed apple orchard. J.Appl. Entomol., 119(7), 473—478.

MORTALITY FACTORS OF *COTESIA GLOMERATA* (L.) (*HYMENOPTERA*: *BRACONIDAE*)

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Abstract

The parasitic wasp *Cotesia glomerata* (L.) (*Hymenoptera*: *Braconidae*) parasitises early instars of larvae of the family *Pieridae*. Our studies have shown that after successful completion of their endoparasitic development and egress from the host nearly 20% of wasp individuals perished. Most of them, 90%, had reached adult stage but died in the process of emerging. It is not often possible to determine reasons for each perishing. The effectiveness of a parasitoid may be influenced by superparasitism. In a clutch of first generation wasps, there were 29.8 ± 10.4 and in the second 34.7 ± 13.3 individuals on average, and with artificial manipulation 88.2 ± 12.2 individuals. 21,6% of *C. glomerata* clutches contained hyperparasitoids (*Hymenoptera*: *Chalcididae* and *Eulophidae*), but the number of hyperparasitised individuals per total cocoon masses was very low — only 1.2%. In the natural population of the parasitoid wasp, a disease caused by *Nosema (Vairimorpha) mesnili* (*Protozoa*) was widely spread, 73.5% of individuals perished in cocoon contained *N. mesnili* spores. Despite the high infectivity per clutch, many individuals within these clutches were uninfected. The most severe reason for the perishing was diapause failures in prepupae of the second generation, preparing for hibernation. In mid-September, diapause had been developed only by 43% of individuals of the second generation.

Key words: *Cotesia glomerata,* mortality, superparasitism, hyperparasitism, microsoridiosis, diapause disruption.

Introduction

Gotesia glomerata (*Hymenoptera*: *Braconidae*) is a gregarious endoparasitoid of *Pieridae* that deposits its eggs in first to third instar caterpillars, where its larvae develop (Laing & Levin, 1982). They regulate and control the population density of their herbivore hosts. Female adult wasps can find their hosts on *Crucifera*, *Rosaceae*, *Tropaeolaceae* or *Berberidaceae* plants (Feltwell, 1982). *C. glomerata* cannot be considered an ideal biocontrol agent as a parasitised caterpillar continues feeding until its last instar, by which time damage to the plant has already been caused (Wilkinson, 1966; Hamilton, 1979). Sometimes parasitisation of caterpillars entails increased loss as the energy consumption of the host increases. When wasps develop inside *Pieris rapae*, the feeding activity of caterpillars even increases (Rahman, 1970; Parker & Pinelli, 1973; Slansky, 1978). In the case of *P. brassicae,* there are contrary data, their feeding activity decreases when they are parasitised (Fuhrer & Keja, 1976). On the whole, wasps are important from the position of pest population abundance, as each parasitised host will be later eliminated. There is a large complex of factors in nature, having an impact on the effectiveness of *C. glomerata.*

The aim of this work was to determine the factors influencing the efficiency of *C. glometara*: the survival of individuals in clutch, the impact of hyperparasitoids, microsporidian disease, and the formation of diapause in second-generation larvae.

Materials and Methods

Clutches of *C. glomerata* were taken from three sources: 1. Last instar caterpillars of *Pieris brassicae* were collected in cabbage fields and reared on cabbage leaves until the egress of parasitic wasp larvae. 2. Wasp clutches of first and second generation, which had completed endoparasitic development, was collected directly in agricultural fields. 3. Wasp clutches were taken from *P. brassicae* caterpillars parasitised in laboratory at 20...+22 °C. For the purpose, second instar *P. brassicae* larvae were exposed to 2-day-old adult parasitoid *C. glomerata* in cage for 24 hours, with calculation 5 female parasitoid adults per one host cluster, ca 30—35 caterpillar of *P. brassicae*. Then parasitoids were removed and caterpillars were reared on cabbage leaves until the egress of parasitoid larvaes.

After completion of their development, wasp clutches were analysed: clutch sizes were determined, individuals perished in a clutch were counted, and their development stage was determined. It was tried to establish reasons for the perishing of the parasitoid. Part of the clutches was autopsied and phase contrast light microscopy was used to identify possible infections. Second-generation wasp clutches were kept, after their emergence from the host caterpillar, at outer temperature for 3 more weeks, to elucidate the formation of diapause. Thereafter cocoons were dissected and active specimens that completed their preimaginal development and diapausing ones were counted.

Results and Discussion

Mortality

An analysis of *C. glomerata* clutches revealed that after successful completion of endoparasitic development and egress from the host, part of wasp individuals perished, there were cocoons from which nothing emerged. There were no differences in mortality between parasitic wasps developed in nature or in laboratory conditions (Fig. 1). Most of dead individuals inside the cocoons had completed their development but adults did not emerge. The perished adults were externally perfect and the top part of their cocoons was open. Many adults had died in the process of emerging. It

seems that mortality among parasitic wasps is a common phenomenon, because among 43 of the clutches analysed there was only one from which all adults emerged successfully.

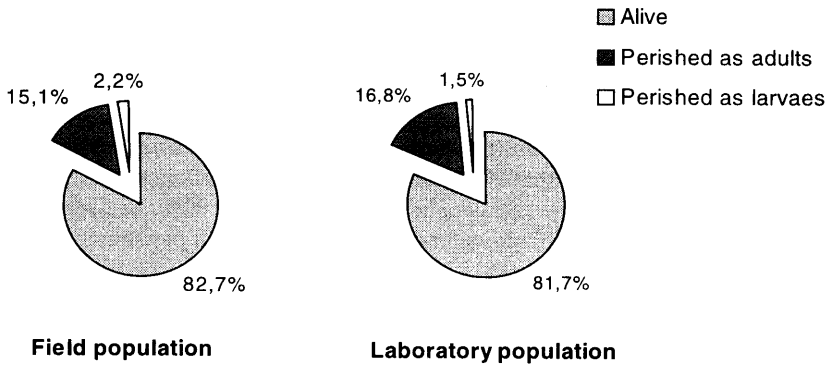

Fig. 1. Mortality of wasp *Cotesia glomerata* individuals after egression from host caterpillar by developing in field and laboratory conditions

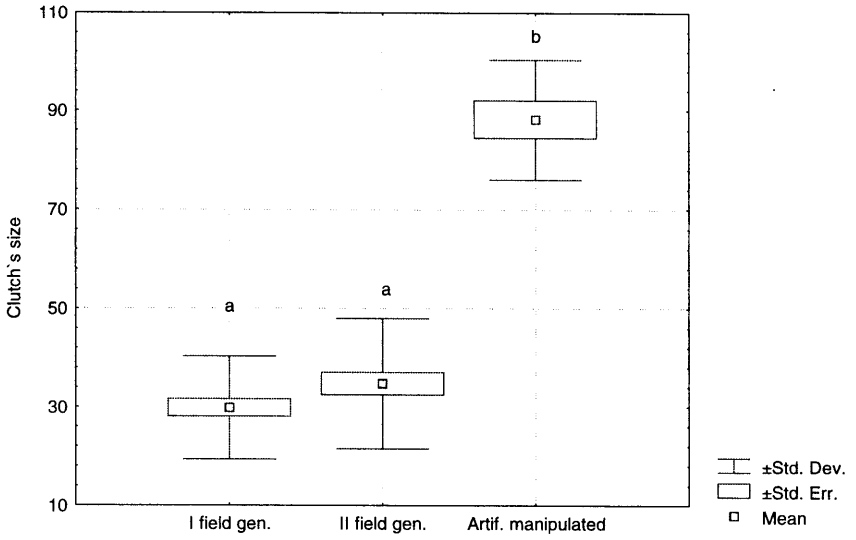

Fig. 2. Clutch sizes of first and second generation *Cotesia glomerata* in field conditions and artificially manipulated ones. Different letters above vertical bars indicate significant differences among columns

Sometimes it is difficult to identify real reasons of parasitic wasp mortality after the emergence from host caterpillar. In field conditions they may fail because of mechanical injures caused by predators or unfavourable weather conditions, diseases, hyperparasitoids, and because of disability to emerge from cocoons, or for unknown reasons.

Size of *C. glomerata* clutches. Superparasitism

Brood sizes of the gregarious wasp parasitoid *C. glomerata* may vary greatly. In nature it is influenced by many factors like the abundance of the host and the parasitoid, the synchronicity of development cycles and mutual contact possibilities, host species etc. According to our analysis, the clutch size of first generation wasps developed in natural conditions ranged from 17 to 36 individuals, with a mean of 29.8 ± 10.4, in the first generation, and from 14 to 58, with a mean of 34.7 ± 13.3, in the second generation (Fig. 2). No differences between brood sizes existed ($p = 0.22$), however, in the second generation there were broods with more individuals. In laboratory conditions where caterpillars of *P. brassicae* were manipulated artificially, remarkably larger brood sizes of *C. glomerata* were obtained, the number of individuals per clutch ranged from 69 to 110, with a mean of 88.2±12.2, statistically reliable difference between the both field generations exist, $p = 0.0001$ (Fig. 2).

Little is known about the dispersal of parasitoid wasps in nature (Nouhuys & Via, 1999). First generation female adults of *C. glomerata* had fewer chances to find a suitable host in field conditions because wasps hibernated far from fields. Search is time-consuming, but adults can survive with no additional food only for three days (Vos & Hemerik, 2002). For that reason, the egg reserve of first generation wasps may remain relatively small and its realisation may be difficult. It is easier for the second generation as their larvae pupate on plant leaves in a field and waste not much time in the search of a host.

In natural conditions, 3...158 parasitic wasp larvae have been found in one *P. brassicae* caterpillar (Gu et al., 2003). When a female parasitoid deposits in a host already parasitised by itself or conspecific females superparsitism results. Superparasitism may be suspected in case over 60 parasitoids develop within one host (Harvey, 2000). By

single piercing, a parasitic wasp inserts 10—20, maximum 40 eggs, inside a caterpillar (Johansson, 1951). Although *C. glomerata* is able to distinguish between already parasitised caterpillars and unparasitisised ones, its females deposit eggs in both (Tagawa, 1992), which was confirmed by our laboratory manipulation as well. In nature there rarely occur enormous parasitoid broods in *P. brassicae* since host caterpillars are together until their fourth instar, and when found by the parasitoid, the latter can choose between hosts and divide its egg reserve between different caterpillars (Le Masurier, 1994). In the case of *P. rapae*, superparasitism occurs more often as caterpillars lead solitary lives, and one single parasitoid may repeatedly infect a caterpillar, wasting no time on search of a new host. If there are sufficiently hosts, parasitoid broods have optimum sizes, which is 28 eggs for *P. rapae* (Le Masurier, 1991), there is no competition for food between larvae and they weigh more.

Superparasitism has both negative and positive sides for the parasitoid. If a first instar caterpillar is attacked repeatedly, it may perish even if no egg laying follows each piercing (Gu et al., 2003). In case there are more than 60 parasitoid larvae, 10% of these perish during the endoparasitic development already before emerging from the host (Johanssoni, 1951). Very small broods, under 10 individuals, may incapsulate in the host organism and they perish (Ikawa & Okabe, 1985). Successful incapsulation has, however, been noticed only with *P. rapae* as the host, with *P. brassicae* it has not been found (Brodeur & Vet, 1995). A small proportion of parasitised *P. brassicae* larvae have pupated in laboratory conditions (Gu et al., 2003). It is possible due to the peculiarities of the development of *C. glomerata* whose larvae feed only on the haemolymph of the host, without injuring its internal organs.

We assume superparasitism in the case of larger clutches. However, it cannot be eliminated with small broods. A third instar caterpillar attacked by a wasp defends itself actively, interrupting the egg laying prematurely, however, later the same host caterpillar will be attacked by the same or another female. Superparasitism has an adaptive value in habitats where resources available are scarce.

Impact of hyperparasitoids on *C. glomerata*

Many parasitoids have one or more hyperparasitoids. There exist scarce literature data on the spread and species of *C. glomerata*'s hyperparasitoids (Nealis, 1983; Gaines & Kok, 1999), Blunck (1954) counts 9 species. According to earlier investigations, the parasitisation by *C. glomerata* in Estonia is low (Merivee & Hiiesaar, 1970). The activity of hyperparasitoids has a negative impact on plant life as it eliminates parasitoids from the lives of plant pests.

Our aim was to determine the proportion of hyperparasitoids in the population of *C. glomerata* in field conditions. Although we did not succeed in identifying hyperparasitoids up to their species, we cannot ignore the impact of hyperparasitoids in investigating mortality factors of *C. glomerata*. Dissections and analyses of cocoons of second generation *C. glomerata* collected on cabbage leaves indicated that a relatively large proportion — 21.6% of the *C. glomerata* clutches — contained hyperparasitoids (*Hymenoptera*: *Chalcididae* and *Eulophidae*). But the number of hyperparasitised cocoons per total cocoon masses investigated was very low, only 1.2% (Fig. 3). Hyperparasitoids had a little effect on *C. glomerata* field population because of their low abundance at that time. This, however, does not serve as a ground for underestimating their role as the situation may change quickly. For instance, according to Gains and Kok (1999), in Southwestern Virginia only 9.7% of *C. glomerata* clutch were hyperparasitised in 1989, but in 1990 hyperparasitisation was sustained at levels ranging from 89 to 100%.

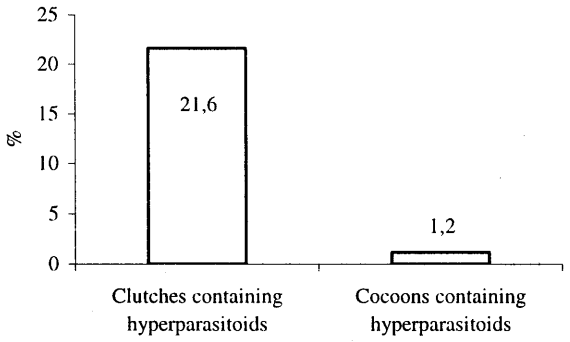

Fig. 3. Hyperparasitisation of the parasitic wasp *C. glomerata* per clutch and per total cocoon number (Number of clutches = 23; number of cocoons = 886)

Microsporidiosis

Microsporodiosis (*Protozoa*: *Microsporidia*) is widely spread in natural populations of *P. brassicae* (Blunck, 1956; Weiser, 1962; Issi, 1968; Hiiesaar, 1974) and hymenopterous parasitoids are also susceptible to disease that attack their host. During a microscopic analysis of *C. glomerata* cocoons, their infection with microsporodiosis, caused by *Nosema (Vairimorpha) mesnili* Paillot, was identified. The extensive spread of the disease in nature is confirmed by our analyses showing that 73.5% of perished individuals and 32.1% of living individuals contained *N. mesnili* spores (Fig. 4).

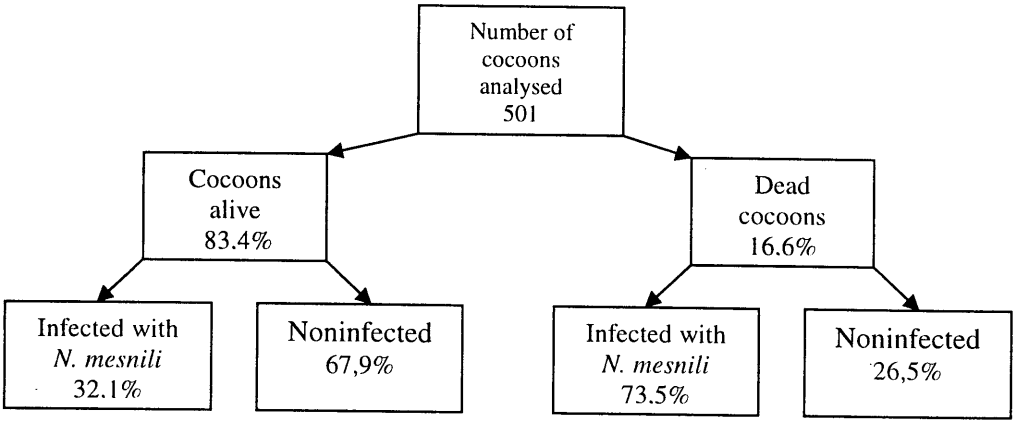

Fig. 4. A microscopic analysis of *Cotesia glomerata* cocoons indicating their infection with *Nosema mesnili*

It is difficult to determine the direct impact of microsporidiosis on the mortality of parasitoid wasps. In the case of a severe infection, the disease develops acute nature, after which the host and the parasitoid perish already at their larval stage prior to the egress of parasitoid wasps. In the case of a weak infection, the disease cannot either be considered harmless to the parasitoid. The disease destroys silk glands of wasps, their cocoons are sparse, covering the larva only partly. Our earlier studies showed that the weight of cocoons of uninfected wasps was ca 0.5 mg, with a medium infection the weight was 0.3 mg, and with a severe infection only 0.17 (Hiiesaar, 1979). Such cocoons do not protect larvae from the impact of the unfavourable environment conditions.

Parasitic wasps may become infected through the host, by feeding on haemolymph containing spores of microsporidia (Hostounsky, 1970). Spores are transferred to haemolymph from damaged organs after breaking the cell walls (Lipa & Steinhaus, 1962; Weiser, 1969). In our investigation not all individuals within one clutch were affected by the disease because simultaneously healthy and infected individuals occurred in the same *C. glomerata* clutch. In the case of weak infection of the host, the disease may be localised, few pathogens pass to haemolymph, and therefore there is no or partial infection of parasitoids. The another reason why part of the larvae were disease carriers and a part not may be superparasitism, where the offspring of different parasitoid adults form one clutch, whereas only some of them were infected.

Disruption of diapause

C. glomerata have facultative diapause, induced by phototermoperiod (Maslennikova, 1958; Zeleny, 1961). There are two full generations of *C. glomerata* in Estonia, and in the warm autumn of some year they have a third partly generation that is not able to develop fully in time. Prepupae of the second generation hibernate in the state of diapause. In our climatic conditions, prepupae of *C. glomerata,* emerged from mid-August to mid-September, must have developed diapause, only then they are able to hibernate successfully.

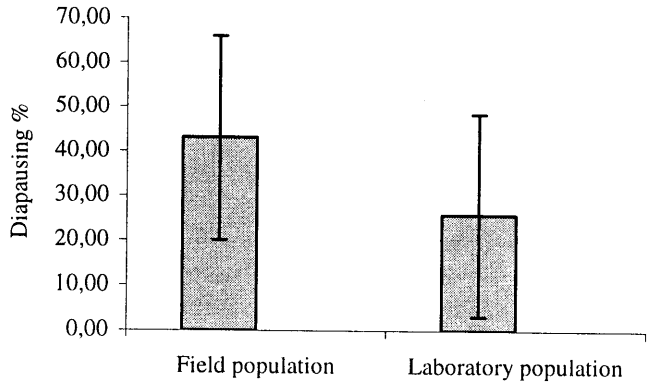

Fig. 5. Formation of diapause in 2nd generation of *Cotesia glomerata* who completed the endoparasitic development in field conditions and in laboratory at temperature 20 ± 2 °C (mean ± SD)

We analysed second generation clutches of *C. glomerata*: 1. emerged from *P. brassicae* collected in a field in mid-September 2. reared in a laboratory in natural light conditions at a temperature of 20 ± 2 °C. Diapausing individuals of *C. glomerata* cocoons collected from nature was 43%, while cocoons reared in the laboratory these were only 25% (Fig. 5). It means, that only such part of prepupae have chance to survive.

Although the diapause of parasitic wasps forms in the course of endoparasitic development, they are susceptible to the impact of the environment for some time after egress from the host (Lees, 1955). When day-lengths fall below the critical 15—16 hours, *C. glomerata* and *P. brassiae* the diapause was formed, however, the diapause of the parasitic wasp is largely corrected by temperature. If outer temperature is +25 °C during the endoparasitic development, they

have no diapause with whatever day-lengths (Maslennikova, 1958). Thus a dry and warm autumn may become fatal for parasitoids in nature. On the basis of our analyses, diapause disruptions may be considered the most important factor of second-generation parasitic wasp death in nature.

References

1. Blunck, H. 1954. Microsporidien bei *Pieris brassicae* L. ihren Parasiten und Hyperparasiten. Zeitschrift für angew. Entomology, B 36 H-3, Berlin, Hamburg, 316—333.
2. Blunck, H. 1956. Is there Possibility of Using Microsporidia for Biological Control of Pieridae? Proc. of the 10th Intern. Congress of Entomol., Montreal, 4, 703—710.
3. Brodeur, J., Vet, L.E.M. 1995. Relationships between parasitoid host range and host defence: a comparative study of egg encapsulation in two related parasitoid species. Physiological Entomology, 20, 7—12.
4. Feltwell, J. 1982. Large White Butterfly. The Biology, Biochemistry and Physiology of Pieris brassicae (Linnaeus). Dr. W. Junk Publishers, the Hague-Boston-London, p. 273.
5. Fuhrer, E., Keja, J. 1976. Physiologische wechselbeziehungen zwischen *Pieris brassicae* und dem endoparasiten *Apanteles glomeratus*. Der einfluss der parasitierung auf wachstum und korpergewicht des wirtes. Entomologia experimentalis et applicata, 19, 287—300.
6. Gaines, D.N., Kok, L.T. 1999. Impact of Hyperparasitoids on *Cotesia glomerata* in Southwestern Virginia. Biological Control, 14, 19—28.
7. Gu, H.N., Wang, Q., Dorn, S. 2003. Superparasitism in *Cotesia glomereta*: response of hosts and consequences for parasitoids. Ecological Entomology, 28, 422—431.
8. Hamilton, J.T. 1979. Seasonal abundance of *Pieris rapae*, *Plutella xylostella* and their diseases and parasites. Genera Applied Entomology, 11, 59—66.
9. Harvey, J.A. 2000. Dynamic effects of parasitism by an endoparasitoid wasp on the development of two host species: implication for host quality and parasitoid fitness. Ecological Entomology, 25, 267—278.
10. Hostounsky, Z. 1970. *Nosema mesnili* (Paillot) a Microsporidian of the cabbageworm, *Pieris brassicae* in the parasites *Apanteles glomeratus* (L)., *Hyposoter ebeninus* (Grav.) and *Pimpla instigator* (F.). Acta Ent. Bohemoslow., 67, 1—5.
11. Ikawa, T., Okabe, H. 1985. Regulation of egg number per host to maximize the reproductive success in the gregarious parasitoid, *Apanteles glomeratus* L. (Hymenoptera. Braconidae). Applied Entomology and Zoology, 19, 389—390.
12. Johansson, A.S. 1951. Studies on the relation between *Apanteles glomeratus* and *Pieris brassicae*. Norsk. Ent. Tidsckr., 6, 4—5: 145—186.
13. Laing, J.E., Levin, D.B. 1982. A review of the biology and a bibliography of *Apanteles glomeratus* (L.) (Hymenoptera: Braconidae). Biocontrol News and Information, 3, 7—23.
14. Lees, A.D. 1955. The physiology of diapause in arthropods. Cambridge Monogr. Exp. Biol., 4, 1—151.
15. Lipa, J., Steinhaus, E.A. 1962. Further report on identifications of Protozoa pathogenic for Insects. Acta Parasitol. Polonica, 10, 115—175.
16. Le Masurier, A.D. 1991. Effect of host size on clutch size in *Cotesia glomerata*. J. of Animal Ecology, 60, 107–118.
17. Le Masurier, A.D. 1994.Costs and benefits of egg clustering in *Pieris brassicae*. J. of Animal Ecology, 63, 677–685.
18. Merivee, E., Hiiesaar, K. 1970. Kapsaliblika juulukas on kapsaliblika looduslik vaenlane. Sots. Põllumajandus, 1, 38—39.
19. Nealis, V.G. 1983. *Tetrastichus galactopus* (Hymenoptera: Eulophidae), a hyperparasitoid of *Apanteles rubecula* and *Apanteles glomeratus* (Hymenoptera: Braconidae) in North America. J. Entomol. Soc. British Columbia, 80, 25—28.
20. Van Nouhuys, S., Via, S. 1999. Natural selection and genetic differentiation of behaviour between parasitoids from wild and cultivated habitats. Heridity, 83, 127—137.
21. Parker, F.D., Pinell, R.E. 1973. Effect on food consumption of the imported cabbageworm when parasitized by two species of *Apanteles*. Environmental Entomology, 2, 216—219.
22. Rahman, M. 1970. Effect of parasitism on food consumption of *Pieris rapae*. Journ. Econ. Entomol., 63, 820—821.
23. Slansky, F.Jr. 1978. Utilization of energy and nitrogen by larvae of imported cabbageworm, *Pieris rapae*, as infected by *Apanteles glomeratus*. Environmental Entomology, 7, 179—185.
24. Zeleny, J. 1961. Contribution to the knowledge of diapause in insects. Acta Soc. Zool. Bohemoslov., 25, 258—270.
25. Tagawa, J. 1992. Host discrimination by unmated individuals of the gragarious larval endoparasitoid wasp, Cotesia (=*Apanteles glomerata*) (Hymenoptera: Braconidae). Applied Entomology and Zoology, 27, 306—309.
26. Vos, M., Hemerik, L. 2002. Linking foraging behaviour in lifetime reproductive success for an insect parasitoid: adaptation to host distributions. Behavioural Ecology, 14, 236—245.
27. Weiser, J. 1962. Biological Insect Control. Advances in Biological Sciences, 123—138.
28. Weiser, J. 1969. Immunity of insects to Protozoa. Immunity to parasitic animals, 1: 129—147.
29. Wilkinson, A.T.S. 1966. *Apanteles rubecula* and other parasits of *Pieris rapae* in British Columbia. Journal of Economical Entomology, 59, 1012—1013.
30. Исси, И.В. 1968. Микроспоридии регулирующие численность вредных насекомых. Труды ВИЗР, 31, "Kolos", 300—330.
31. Масленникова, В.А. 1958. Об условиях определяющих диапаузу перепончатокрылых *Apanteles glomeratus* (*Hymenoptera: Braconidae*) и *Pteromalus puparum*. Энтомол. обозрение, 37, 538—545.
32. Хиесаар, К. 1979. Влияние микроспоридиоза на состояние покоя *Apanteles glomeratus*. Тезисы докладов научно-производственной конференции. Минск, 102—104.

THE EFFECT OF MICROFERTILISERS ON THE NUMBER OF POLLEN BEETLES ON SPRING OILSEED RAPE

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Abstract

The pollen beetle (*Meligethes aenus* F.) is an important pest of spring oilseed rape (*Brassiaca napus* var. *oleifera*) crop. Both *Meligethes* adults and larvae feed on pollen of oilseed rape. The beetles use olfactory and pollen odour cues to locate food resource. Here we report on pollen beetle responses to the pollen of oilseed rape fertilised with different microelements.

The study was carried out in an experimental field near Tartu in July 2003. The field consisted of 36 plots (à 10 m^2), which were treated with eight different microfertilisers (Micro Mo, NewRaps, Micro Mn, MicroRaps, HydroMag 300, Sulphur F3000, Micro B, Micro Cu). Each treatment was conducted in four replications. To quantify pollen availability, twelve flowers were collected randomly from each plot in the peak flowering of oilseed rape in mid-July. Pollen beetles were gathered by the shaking method.

The mean number of pollen grains per flower depended significantly on the fertilising system and the pollen available in the flowers. The plots fertilised with sulphur, magnesium, copper and boron distinguished from all other variants by a higher number of pollen grains per flower. At the same time, the number of pollen beetles was the lowest on the plots fertilised with sulphur, magnesium, copper and boron, and the highest on the untreated plots and on the plots fertilised with molybdenum. Our investigations suggest that adult pollen beetles seemed to be able to discriminate flowers fertilised with different nutrients.

Key words: *Brassica napus,* spring oilseed rape, microfertilisers, pollen, pollen beetle.

Introduction

The oilseed rape (*Brassiaca napus* L var. *oleifera*) crop is one of the most important sources of vegetable oil in the world. Winter oilseed rape (*Brassiaca napus* var. *oleifera* subvar. *biennis*) is the dominating rape crop grown in the world, compared with the spring form (*Brassiaca napus* var. *oleifera* subvar. *annua*). However, spring oilseed rape is the most adapted to the North European climatic conditions. In Estonia, the growing area of spring rape has increased from one thousand hectares to forty thousand hectares during the last ten years. However, the level of yields is low and fluctuating due to agrotechnologies, fertilising, and plant protection activities (data of the Statistical Office of Estonia from 2002). Spring oilseed rape is infested by numerous pests, which cause more losses in yields or higher cost of chemical control, compared with the winter rape form (Ekbom, 1995).

The most important key pest of spring oilseed rape crop all over Europe is the pollen beetle (*Meligethes aenus* F.) (Lamb, 1989; Winfield, 1992; Ekbom, 1995). The hibernating adults appear in spring, when the temperature reaches 10°C. After a short period of feeding, the adults migrate to oilseed rape crops where the females begin laying eggs. They bite a small hole at the base of the flower bud and deposit eggs on the stamens or pistil. Females prefer buds with sizes between 2—3 mm and usually lay one to six eggs per bud (Ekbom and Borg, 1996). One female can lay up to 150 eggs in Swedish conditions (Nilsson, 1998). The eggs hatch within 4 to 9 days, and the larvae remain in the flower bud. The larvae have two instars over a period of 30 days. The second instars migrate to other buds, then drop to the ground to pupate in a shallow earthen cell. Pollen beetles are of particular importance in northern countries, such as Finland and Sweden, where spring rape dominates (Hokkanen, 1993).

Both adults and larvae feed on the pollen of oilseed rape (Kirk-Springs, 1996), which provides a variety of essential nutrients, including protein, starch, steroids, lipids, vitamins, and minerals (Roulson and Cane, 2000). The oviposition and feeding damage by both adults and larvae may cause bud abscission and loss of seed yield, particularly in spring oilseed rape (Williams and Free, 1978; Lamb, 1989). These crops are particularly susceptible at the early bud stages and become less and less sensitive as the plants develop (Nilsson, 1988). Winter rape is often past the susceptible bud stages before the main invasion of beetles to the crop occurs.

Phytophagous insects like pollen beetles orient to plants for both feeding and oviposition under the influence of visual and odour stimuli (Blight and Smart, 1999). It is well known that the yellow colour of oilseed rape flowers attracts pollen beetles (Laska and Kocourek, 1991; Giamoustaris and Mithen, 1996; Blight and Smart, 1999; Cook et al., 2002). Both male and female beetles are attracted to the odours of a whole oilseed rape plant (Evans and Allen-Williams, 1994; Ruther and Thiemann, 1997). Some components are released only from the flowers (Jakobsen et al., 1994) and at least part of the attractive odour of oilseed rape emanates from pollen which is quantitatively and/or qualitatively different from the odour of the rest of the flower (Cook et al., 2002). Recent investigation shows that, for the pollen beetle, pollen availability influences adult incidence and oviposition site selection during the flowering (Cook et al., 2004). Laboratory experiments confirmed that a diet with pollen led to increased weight of larvae and adult herbivorous insects (Carisey and Bause, 1997; Cook et al., 2004). Adult body weight in autumn was found to be the most important factor in the survival of overwintering pollen beetles; heavier beetles survive better than lighter ones (Hokkanen, 1993). However, pollen is not obligatory for larval survival and development of pollen beetles. The larvae

reared on male-sterile flowers (without pollen) successfully developed to adulthood (Cook et al., 2004). So, factors other than pollen enabled larval development in oilseed flowers.

Once pollen beetles have located pollen, olfactory, tactile and gustatory phagostimulats induce their feeding. The availability of dehisced pollen has the effect of retaining beetles on a plant more than its absence (Cook et al, 2002). Since beetles remain on a plant for some time to feed, this has the effect causing aggregations of beetles on flowers. This effect could be due to aggregation feromones, released in the presence of a host plant suitable for reproduction. The criteria used by pollen beetles in host-quality assessment have been little studied. Many studies focus on the effects of nutrients on the oilseed rape growth, seed set, and oil content in seeds (Zhao et al., 1995; Haneklaus et al., 1999; Fismes et al., 2000). Still, no studies have considered the effects of different fertilisers on the host-plant selection by adult pollen beetles. The aim of this study was to investigate pollen beetle responses to the pollen of oilseed rape fertilised with different microfertilisers.

Materials and Methods

The study was carried out in an experimental field of the Estonian Agricultural University near Tartu, in Estonia, in July 2003. The spring oilseed rape variety Mascot, bred and produced by the Swedish company Weibull was used. Technical data of the variety: raw fat content 40—43%, mass of grains 3.5—4.5 g, glucosinolates 20 μmol/g, lodging resistance 6—8 points, height of plant 98—108 cm, growth period 90—108 days (Velička, 2003).

Spring oilseed rape seeds were sown on 15 May by calculating 200 germinating seeds per m^2, sowing depth 2—3 (4) cm, pre-crop being potato. The field consisted of 36 plots (à 10 m^2) that were treated with eight different microfertilisers. Each treatment was conducted in four replications. The trial variants were:

1. 0 (no pesticides or mineral fertilisers were used);
2. HydroPlus™ Micro Molybdenum (Mo) (active substance agent 0.25 l ha^{-1});
3. HydroPlus™ New Rape (New) (active substance agent 2 kg ha^{-1});
4. HydroPlus™ Micro Manganese (Mn) (active substance agent 1 l ha^{-1});
5. HydroPlus™ Micro Rape (Micro) (active substance agent 2 kg ha^{-1});
6. Hydromag 300 (Mg) (active substance agent 7 l ha^{-1});
7. Sulphur F3000 (S) (active substance agent 7 l ha^{-1});
8. HydroPlus™ Boron (B) (active substance agent 2 l ha^{-1});
9. HydroPlus™ Micro Copper (Cu) (active substance agent 0.5 l ha^{-1}).

Prior to the sowing, the field was sprayed with the intra soil herbicide EK Trifluralin (0.15 l ha^{-1}) and the mineral complex granular combined fertiliser OptiCropNPK 21-08-12+S+Mg+B+Ca, calculating 120 kg of the active substance agent of nitrogen per hectare (exclusion variant 0). Liquid microfertilisers (water amount 400 l ha^{-1}) were sprayed on oilseed rape leaves on 26 June, when the plants had reached the growth stage 27—31, according to the BBCH scale (Lancashire et al., 1991).

For pest control, Fastac (alphacypermethrin) was used, calculating 0.15 l ha^{-1} (active substance agent). The plants were sprayed two times: the first time on 28 May and the second time on 3 July (exclusion variant 0).

Adult pollen beetles were gathered in the peak flowering of oilseed rape on 17 July, when 80% of buds on the raceme were flowering (crop growth stage 4.8). Beetles were collected randomly from three plants on each plot using the shaking method.

To estimate the amount of forage resource available, the number of flowers in random 1 m^2 quadrants on each plot were counted. To quantify pollen availability, twelve flowers were collected randomly from the plant main raceme from each plot in the morning and stored separately in microcentrifuge tubes. The flowers with pollen were later acetolysed (Faegri and Iverson, 1989) to digest both floral tissue and pollen contents, leaving pollen exines intact. Separated pollen was dispersed in distilled water (1 ml). Number of pollen grains was counted with light microscope using the Fuchs-Rosenthal chamber (3.2 mm^3). These data were used to calculate the number of pollen grains per flower.

Statistical analyses were performed using the Statistica 6 version. The number of pollen beetles was normalised by transformation [$\log_{10}$ (no. beetles)] for all surveys and analysed by mixed ANOVA. Differences between mean number of oilseed rape pollen and pollen beetles were inspected using Fisher's protected significant difference post hoc analysis.

Results and Discussion

The mean number of pollen beetles per oilseed rape plant depended significantly both on the fertilising system and pollen available in the flowers (Table 1).

Table 1

Mixed ANOVA table of *F*-values on the effect of different microfertilisers and pollen available in the flowers on the number of pollen beetles per plant

Effect	*df*	SS	*F*	*P*
Microfertiliser (M)	1	47.0	165.5	<0.001
Pollen grains per flower (P)	1	24.8	87.4	<0.001
M * P	1	15.8	55.5	<0.001
Residual	57	16.2		

The folian application of microfertilisers had a positive effect on the number of flowers ($F_{1,38}$ = 12.3; P = 0.001) and on the amount of pollen per flower ($F_{1,58}$ = 7.4; P = 0.009). The average number of flowers per 1m^2 on treated plot was significantly higher (mean 362.7 ± S.E. 26.0) compared with control (accordingly 176.0 ± 1.5). The mean number of pollen grains available averaged 26700 ± 1000 (S.E.) per flower. The plots fertilised with sulphur, magnesium, copper and boron distinguished from all other variants by the higher number of pollen grains per flower (Fig. 1). At the same time, the number of pollen beetles was the lowest on the plots fertilised with sulphur, magnesium, copper and boron, and the highest on the untreated plots and on the plots fertilised with molybdenum (Fig. 2).

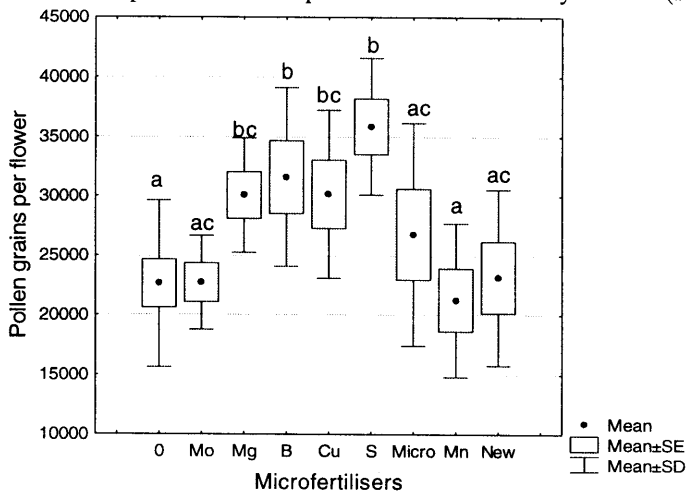

Fig. 1. The mean number of pollen grains per oilseed rape flower on differently fertilised plots during the peak of the flowering period. See Material and Methods for abbreviations of microfertilisers. Treatments with different letters are significantly different with α = 0.05 (Fisher's test)

Although interest in interactions between pollen beetles and oilseed rape plants has increased in recent times, no studies have considered the effects of microfertilisers with folian fertilisation on the flower selection of adult pollen beetles. The results of this paper show that adult pollen beetles seemed to be able to discriminate the flowers fertilised with different nutrients. Pollen beetles were more abundant on the plots fertilised with molybdenum but avoided plants fertilised with sulphur, magnesium, and boron. The pollen content of flowers was the highest on the latest plots. This result may be due to attraction of the beetles to olfactory cues from exposed flowers.

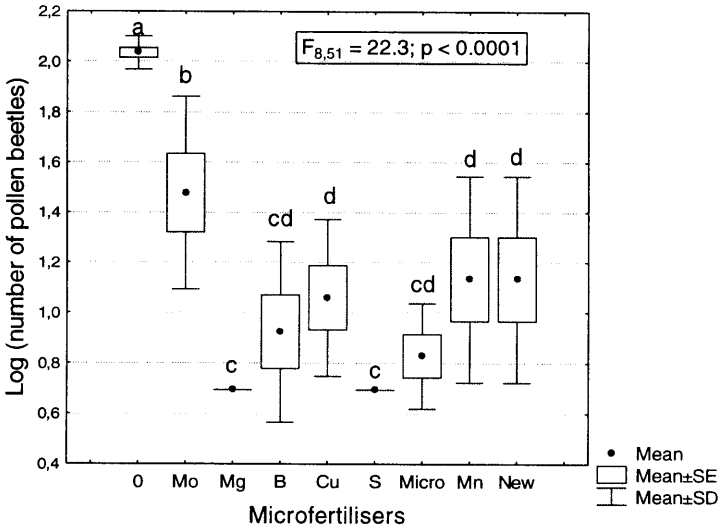

Fig. 2. The effect of different microfertilisers on the number of pollen beetles (*Meligethes aenus*) during the peak of oilseed rape flowering period. See Material and Methods for abbreviations of microfertilisers. Treatments with different letters are significantly different with α = 0.05 (Fisher's test)

Several components of plant quality may influence the host plant selection of pollen beetles on oilseed rape. The crop has a high demand for sulphur, requiring approximately 16 kg S to produce 1 t of seeds (Grant and Bailey, 1990). This has been attributed to the biosynthesis and accumulation of glucosinolates, which can account for up to 50% of the total S in seeds. The foliar fertilisation of oilseed rape with ionic sulphur and soil fertilisation with elemental sulphur increased not only the content of oil in seeds but also the content of glycosinolates proportionally to the dose applied (Szulc et al., 2003). Glucosinolates constitute a potent defensive system in Crucifers, active against a variety of pests and pathogens. Pollen beetles, like many brassica-feeding insects, are attracted to isothiocyanates, which are volatile

catabolites of glucosinolates (Pivnick et al., 1992). These compounds are produced constitutively, and are present in all tissues.

In our experiment, pollen beetles preferred unfertilised flowers compared with flowers treated with sulphur and other microfertilisers. The number of pollen beetles on the treated plots (insecticide + microfertilisers) was continuously lower compared with control. In addition, pollen beetles were quite settled in one place and moved mostly within the range of the raceme. Colonisation of the treated plots by pollen beetles from the untreated plots did not occur. This suggests that the quality of a host plant influenced the host plant selection of pollen beetles. The actual role of glucosinolates and micronutrients in pest resistance is still uncertain and more detailed observations about the effect of microfertilisers on the host plant selection behaviour are planned for the future.

Acknowledgements

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References

1. Blight, M. M., Smart, L. E. 1999. Influence of visual cues and isothiocyanate lures on capture of the pollen beetle, *Meligethes aenus* in field traps. Journal of Chemical Ecology, 25, 1501—1516.
2. Carisey, N., Bause, E. 1997. Impact of balsam fir flowering on pollen and folige biochemistry in relation to spruce budworm growth, development, and food utilization. Entomologia Experimentalis et Applicata, 85, 17—31.
3. Cook, S.M., Barlet, E., Murray, D.A., Williams, I. H. 2002. The role of pollen odour in the attraction of pollen beetles to oilseed rape flowers. Entomologia Experimentalis et Applicata, 104, 43—50.
4. Cook, S.M., Murray, D.A., Williams, I. H. 2004. Do pollen beetles need pollen? The effect of pollen oviposition, survival, and development of a flower-feeding herbivore. Ecological Entomogia, 29, 164—173.
5. Ekbom, B. 1995. Insect pests. In: D. Kimber, D., McGregor, D.I. (eds) Brassica Oilseeds: Production and Utilization. CAP International, Wallingford, U.K., 141—152.
6. Ekbom, B., Borg, A. 1996. Pollen beetle (*Meligethes aenus*) oviposition and feeding preference on different host plant species. Entomologia Experimentalis et Applicata, 78, 291—299.
7. Evans, K.A., Allen-Williams, L.J. 1994. Laboratory and field response of the pollen beetle, Meligethes aenus, to the odour of oliseed rape. Physiological Entomology, 19, 285—290.
8. Faegri, K., Iverson, J. 1989. Textbook of Pollen Analysis, 4th edn. John Wiley and Sons Ltd, Chichester, U.K.
9. Fismes, J., Vong, P.C., Guckert, A., Frossard, E. 2000. Influence of sulphur on apparent N-use efficiency, yield and quality of oilseed rape (*Brassica napus* L.) grown on a calcareous soil. Europ. J. Agron., 12, 127—141.
10. Giamoustairis, A., Mithen, R. 1996. The effect of flower colour and glucosinolates on the interaction between oilseed rape and pollen beetles. Entomologia Experimentalis et Applicata, 80, 206—208.
11. Grant, C.A., Bailey, L.D. 1990. Fertility management in canola production. Proceedings of International Canola Conference, April 1990, Atlanta, GA, USA. Potash and Phosphate Institute, Atlanta, GA, USA.
12. Haneklaus, S., Paulsen, H.M., Gupta, A.K., Bloem, E., Schnug, E. 1999. Influence of sulphur fertilisation on yield and quality of oilseed rape and mustard. 10th International Rapeseed Congress, Canberra, Australia, CD ROM.
13. Hokkanen, H.M.T. 1993. Overwintering, survival and spring emergence in *Meligethes aenus*: effects of body weight, crowding, and soil treatment with *Beauveria bassiana*. Entomologia Experimentalis et Applicata, 67, 241—246.
14. Jakobsen, H.B., Friis, P., Nielsen, J.K., Olsen, C.E. 1994. Emission of volatiles from flowers and leaves of *Bassica napus in situ*. Phytochemistry, 37, 695—699.
15. Kirk-Springs, A.H. 1996. Pollen Beetles. Coleoptera: Kteretidae and Nitidulidae: Meligethinae. Handbooks for the identification of British Insects, Vol. 5 (6a). Royal Entomological Society of London, London.
16. Lamb, R.J. 1989. Entomology of oilseed Brassica crops. Annual Review of Entomology, 34, 211—129.
17. Lancashire, P.D., Bleiholder, H., Boom, T. van den, Langelüddeke, P., Strauss, R., Weber, E., Witzenberger, A. 1991. A uniform decimal code for growth stages of crops and weeds. Annals of Applied Biology, 119, 561—601.
18. Laska, P., Kocourek, F. 1991. Monitoring of flight activity in some crucifer-feeding pests by means of yellow water-traps. Acta Entomologica Bohemoslovaca, 88, 25—32.
19. Nilsson, C. 1988. Pollen beetle (*Meligethes aenus* F.) and flowering in rape. Swedish Journal of Agricultural Research, 18, 113—118.
20. Pivnick, K.A., Lamb, R.J., Reed, D. 1992. Response of flea beetles, *Phyllotrata* spp., to mustard oils and nitriles in field trapping experiments. Journal of Chemical Ecology, 18, 863—873.
21. Roulson, T.H., Cane, J.H. 2000. Pollen nutritional content and digestibility for animals. Plant Systematics and Evolution, 222, 187—209.
22. Ruther, J., Thiemann, K. 1997. Response of the pollen beetle Meligethes aenus to volatiles emitted by intact plants and conspecifies. Entomologia Experimentalis et Applicata, 84, 183—188.
23. Szulc, P.M., Drozdowska, L., Kachlicki, P. 2003. Effect of sulphur on the yield and content of glucosinolates in spring oilseed rape seeds. Electronic Journal of Polish Agricultural Universities, Agronomy, 6(2): URL: http/www.ejpau.media.pl/series/volume6/issue2/agronomy/art-01.html
24. Zhao, F., Evans, E.J., Bilsborrow, P.E. 1995. Varietal differences in sulphur uptake and utilization in relation to glucosinolate accumulation in oilseed rape. 9th International Rapeseed Congress, Cambridge, 271—273.
25. Velička, R. 2003. Rape. Summary of monograph, presented for hibernation conference. Lithuanian University of Agriculture, Biomedical Sci., Agronomy, Kaunas, 78 pp. .
26. Williams, I.H., Free, J.B. 1978. The feeding and mating behaviour of pollen beetles (*Meligethes aenus* Fab.) and seed weevils (*Ceutorhynchus assimilis* Payk.) on oilseed rape (*Bassica napus* L.). Journal of Agricultural Science, Cambridge, 91, 453—459.
27. Winfield, A.L. 1992. Management of oilseed rape pests in Europe. Agric. Zool. Rev., 5, 512—95.

PREDATORS IN APHID COLONIES INHABITING DECORATIVE SHRUBS AND TREES IN LUBLIN

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Abstract

The purpose of the studies conducted during the years 1999—2001 was to find out the presence and establish the number of predatory arthropods occurring in aphid colonies inhabiting some ornamental shrubs and trees in the urban green areas of Lublin. The observations were performed in two sites: a street (A) and a park (B). The studies comprised eight species of deciduous and coniferous ornamental shrubs and trees: *Cornus alba* L., *Crataegus* x *media* Bechst., *Cotoneaster divaricatus* Rehder et E. H. Wilson, *Rosa* sp., *Spiraea japonica* L. f., *Juniperus communis* L., *Juniperus* x *pfitzeriana* (L. Späth) P. A. Schmidt, and *Pinus mugo* Turra. No predators were found on the shrubs of *J.x pfitzeriana*, while on other plants the studies observed occurrence of young and adult specimens of *Araneida*, eggs, larvae and adults *Coccinellidae*; larvae and adults *Dermaptera*; larvae *Cecidomyiidae*; eggs and larvae *Syrphidae*; larvae *Chamaemyiidae*; eggs, larvae and adults *Chrysopidae* and larvae and adults *Anthocoridae.* In site A, the greatest number of predatory arthropods from various systematic groups were found on trees *C.* x *media*, but in site B — on shrubs *C. alba.* Comparing the number of predators in both sites, more predators were found in site A, probably due to a considerably higher number of aphids in that site.

Key words: aphids, predators, ornamental shrubs, and city green areas.

Introduction

Deciduous and coniferous ornamental trees and shrubs growing in the urban green areas are the nutrition base for the phytophagous species, among which aphids are the most numerous. Because of a limited possibility of using insecticides in cities, the role of natural enemies limiting their populations is increasing. The most important include specialized and polyphagous predatory arthropods. The specialized arthropods include the dipterous from the family *Chamaemyiidae*, *Cecidomyiidae* and *Syrphidae*, while polyphagous predators include ladybirds, golden-eyed flies, spiders, heteroptera, and earwigs.

The purpose of the present paper was to find out the presence and establish the number of predatory arthropods occurring in aphid colonies inhabiting selected ornamental shrubs and trees in the urban green areas of Lublin.

Materials and Methods

Observations concerning the occurrence and number of predators in aphid colonies inhabiting some decorative shrubs and trees in the urban green areas of Lublin were conducted in two sites: a street (A) and a park (B) during the years 1999—2000. The studies comprised eight plant species: *Cornus alba* L., *Crataegus* x *media* Bechst., *Cotoneaster divaricatus* Rehder et E.H. Wilson, *Rosa* sp., *Spiraea japonica* L.f., *Juniperus communis* L., *Juniperus* x *pfitzeriana* (L. Späth) P.A. Schmidt, and *Pinus mugo* Turra. Five plants of the same species growing close to each other were chosen in each site. Aphids and accompanying predatory arthropods were counted on each plant on five randomly selected shoots of similar length. The monitoring of shrubs and trees began early in spring and finished late in autumn, with about 10-days intervals.

Results and Discussion

Predatory arthropods were observed in aphid colonies only on seven out of eight species of shrubs and trees (*Cornus alba* L., *Crataegus* x *media* Bechst., *Cotoneaster divaricatus* Rehder et E.H. Wilson, *Rosa* sp., *Spiraea japonica* L.f., *Juniperus communis* L., and *Pinus mugo* Turra). The occurrence of young and adult specimens of *Araneida*, eggs, larvae and adults *Coccinellidae*; larvae and adults *Dermaptera*; larvae *Cecidomyiidae*; eggs and larvae *Syrphidae*; larvae *Chamaemyiidae*; eggs, larvae and adults *Chrysopidae* and larvae and adults *Anthocoridae* was found out.

In the course of three years of studies, in site A the eggs and larvae of *Syrphidae* occurred in the greatest number (Table 1). Their biggest number was found on the shrubs of *C. alba* and the trees of *C.* x *media*, they were also observed in fairly big numbers on *Rosa* sp. No *Syrphidae* eggs were observed on the shrubs *P. mugo* while the larvae were found only in one year of studies (2000). The eggs of *Chrysopidae*, which were most numerous on *C.* x *media* and *J. communis*, also occurred in big number near the aphid colonies. Larvae and adult specimens of *Coccinellidae* had a distinct effect on the reduction of populations of different aphid species. Ladybird larvae were observed in the greatest number on the shrubs of *J. communis* between May and September. Among those, the larvae of *Chilocorus bipostulatus* (L.) (subfamily *Coccinellinae*, genus *Chilocorus* (Leach)) were numerous. No ladybirds larvae were found on the shrubs of *C. alba*, *C. divaricatus*, *S. japonica* and *P. mugo.* On the other hand, adult forms of *Coccinelidae* were observed in the greatest numbers on the shrubs of *P. mugo* (6.4 specimens/shrub) and *J. communis* (4.4 specimens/shrub). Their number on the other plants was smaller and it did not usually exceed 2 specimens/shrub.

Table 1

The number of predators occurring in aphid colonies inhabiting ornamental shrubs and trees in site A

Predators		Number/shrub																											
		Cornus alba L.				*Crataegus* x *media* Bechst.				*Cotoneaster divaricatus* Rehder et E.H. Wilson				*Rosa* sp.				*Spiraea japonica* L.f.				*Juniperus communis* L.				*Pinus mugo* Turra			
		1999	2000	2001	Σ	1999	2000	2001	Σ	1999	2000	2001	Σ	1999	2000	2001	Σ	1999	2000	2001	Σ	1999	2000	2001	Σ	1999	2000	2001	Σ
Araneida	young and adults	2	-	0,4	2,4	1,2	2,8	-	4	2	2	-	4	1,6	0,8	-	2,4	0,8	0,4	-	1,2	0,4	0,4	-	0,8	2,8	0,8	1,6	5,2
Coccine-llidae	eggs	-	-	-	-	0,4	4,8	-	5,2	-	0,4	-	0,4	-	0,4	0,4	0,8	-	-	-	-	-	-	-	-	-	-	-	-
	larvae	-	-	-	-	0,4	7,2	1,6	9,2	-	-	-	-	0,4	0,8	-	1,2	-	-	-	-	5,4	8,6	10,4	24,4	-	-	-	-
	adults	-	0,8	1,2	2	-	2,4	-	2,4	0,4	1,2	-	1,6	0,8	0,8	-	1,6	1,2	-	0,4	1,6	-	0,8	3,6	4,4	2,8	3,6	-	6,4
Dermaptera	larvae and adults	-	-	-	-	-	0,4	-	0,4	-	-	-	-	-	0,4	-	0,4	1,2	-	-	1,2	-	0,4	-	0,4	-	0,4	0,4	0,8
Cecido-myiidae	larvae	0,4	4	0,4	4,8	-	8,8	-	8,8	-	-	-	-	-	1,2	-	1,2	-	-	-	-	-	-	-	-	-	-	-	-
Syrphidae	eggs	2,8	25,6	0,8	29,2	1,6	20,4	6,8	28,8	-	2,4	2	4,4	-	9,2	9,6	18,8	-	-	2,4	2,4	0,8	-	0,4	1,2	-	-	-	-
	larvae	4,4	14,4	2	20,8	-	38,8	1,6	40,4	1,8	-	-	1,8	-	7,6	0,8	8,4	-	-	0,8	0,8	-	0,4	-	0,4	-	1,6	-	1,6
Chamae-myiidae	larvae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chrysopidae	eggs	0,4	0,8	-	1,2	16,4	19,6	-	36	-	14	-	14	0,4	18	-	18,4	1,6	2,8	-	4,4	5,2	23,6	-	28,8	2,4	1,6	-	4
	larvae	-	-	0,4	0,4	0,4	1,2	-	1,6	-	-	-	-	-	1,2	-	1,2	-	-	-	-	-	0,4	-	0,4	-	-	-	-
	adults	-	-	-	-	-	0,4	-	0,4	-	0,8	-	0,8	0,8	1,2	-	2	-	-	-	-	-	-	-	-	-	0,8	-	0,8
Anthoco-ridae	larvae and adults	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0,4	-	0,4	-	-	-	-

Table 2

The number of predators occurring in aphid colonies inhabiting ornamental shrubs and trees in site B

Predators		Number/shrub																											
		Cornus alba L.				*Crataegus* x *media* Bechst.				*Cotoneaster divaricatus* Rehder et E.H. Wilson				*Rosa* sp.				*Spiraea japonica* L.f.				*Juniperus communis* L.				*Pinus mugo* Turra			
		1999	2000	2001	Σ	1999	2000	2001	Σ	1999	2000	2001	Σ	1999	2000	2001	Σ	1999	2000	2001	Σ	1999	2000	2001	Σ	1999	2000	2001	Σ
Araneida	young and adults	-	0,8	-	0,8	-	1,2	0,8	2	0,8	-	-	0,8	0,8	-	2	2,8	0,4	0,4	-	0,8	2	2,4	-	4,4	0,8	3,6	-	4,4
Coccine-llidae	eggs	-	-	-	-	-	-	0,4	0,4	-	0,8	-	0,8	-	0,4	-	0,4	-	-	-	-	-	-	-	-	-	-	-	-
	larvae	-	-	-	-	-	0,4	1,6	2	-	0,8	-	0,8	-	-	-	-	-	-	-	-	2,4	2,8	3,2	8,4	-	-	-	-
	adults	0,4	-	-	0,4	0,4	0,4	2,8	3,6	0,4	1,6	0,4	2,4	-	0,4	0,4	0,8	0,8	0,4	-	1,2	-	0,8	2,4	3,2	2,4	4,8	0,4	7,6
Derma-ptera	larvae and adults	-	0,4	0,4	0,8	-	-	-	-	-	0,4	-	0,4	-	-	-	-	-	0,4	-	0,4	-	-	-	-	-	-	-	-
Cecido-myiidae	larvae	-	8	4,8	12,8	-	-	-	-	-	2,4	-	2,4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Syrphidae	eggs	3,6	66,8	0,4	70,8	-	1,6	-	1,6	-	2,4	0,4	2,8	-	2	1,2	3,2	-	-	-	-	-	-	-	-	-	-	-	-
	larvae	4	8	1,2	13,2	-	2	-	2	0,2	0,4	0,4	1	-	1,6	0,8	2,4	-	-	-	-	0,4	-	0,4	0,8	-	-	2,8	2,8
Chamae-myiidae	larvae	-	1,2	-	1,2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chrysopidae	eggs	-	-	-	-	3,6	26	0,4	30	-	7,6	-	7,6	1,6	21,6	-	23,2	0,4	12,8	-	13,2	-	10,8	2	12,8	1,6	2,8	0,4	4,8
	larvae	-	0,4	-	0,4	0,4	-	-	0,4	-	-	-	-	-	0,4	-	0,4	-	-	-	-	-	-	-	-	-	-	-	-
	adults	-	-	-	-	-	0,8	-	0,8	-	-	-	-	-	-	-	-	-	-	-	-	-	0,4	-	0,4	-	-	-	-
Anthoco-ridae	larvae and adults	-	-	-	-	-	-	-	-	-	0,8	-	0,8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

A considerable role in limiting the aphid population was also played by young and adult network and no-network spiders. Totally, their number ranged from 0.8 specimens/shrub (*J. communis*) to 5.2 specimens/shrub (*P. mugo*) on all decorative shrubs and trees during the three years of studies.

The larvae of *Cecidomyiidae* occurred in fairly big numbers but only on some plants. They were found to be the most numerous on *C.* x *media* (8.8 specimens/shrub) but only in 2000. Those larvae occurred on the shrubs of *Rosa* sp. but only in 2000 and in much smaller numbers (1.2 specimens/shrub). On the other hand, the presence of *Cecidomyiidae* larvae on *C. alba* was observed in each year of the studies, their number ranging from 0.4 to 4.0 specimens/shrub. They were not found on the shrubs of *C. divaricatus*, *S. japonica*, *P. mugo*, and *J. communis.*

Larvae and adult *Dermaptera* were observed on a few shrubs and trees (*C.* x *media*, *Rosa* sp., *S. japonica*, *J. communis*, *P. mugo*). Their numbers were very small and did not usually exceed 1 specimen/shrub. Larvae and adult *Anthocoridae* were observed only on *J. communis* in 2000, and their number was 0.4 specimens/shrub. Because of the low numbers of larvae and adult specimens of *Dermaptera* and *Anthocoridae*, they did not have any significant influence on the reduction of the aphid population.

No larvae of *Chamaemyiidae* were observed on any of the examined decorative shrubs and trees.

In site B, the eggs of *Chrysopidae* as well as the eggs and larvae of *Syrphidae* were most numerous near aphid colonies (Table 2). The greatest number of *Chrysopidae* eggs were found on the trees of *C.* x *media* and on the shrubs of *Rosa* sp., while the eggs and larvae of *Syrphidae* were most numerous on *C. alba.*

A significant role in limiting the population of aphids was played by the larvae and adults of *Coccinellidae.* The larvae were observed only in certain years of studies on *J. communis*, *C.* x *media* and *C. divaricatus*, and their total number ranged from 0.8 to 8.4 specimens/shrub, depending on the studied plant. The greatest number of adult specimens was observed on the shrubs of *P. mugo* (7.6 specimens/shrub), while the smallest on *C. alba* and *Rosa* sp., where their number did not exceed 1 specimen/shrub.

A big influence on the reduction of the aphid population, especially on *C. alba*, was exerted by the larvae *Cecidomyiidae*, whose total number on that plant was 12.8 specimens/shrub. The occurrence of those larvae was also found out on the shrubs of *C. divaricatus* (2.4 specimens/shrub), but only in the year 2000. *Cecidomyiidae* larvae did not occur on the other ornamental shrubs.

Young and adult specimens of *Araneida* were observed on all the studied plants, however, not in all the studied years. Their total numbers ranged from 0.8 to 4.4 specimens/shrub, depending on the plant species.

Larvae and adult specimens of *Dermaptera* and *Anthocoridae* as well as the larvae of *Chamaemyiidae* were observed only scarcely and only on certain shrubs. Larvae and adult specimens of *Dermaptera* were observed on *C. alba*, *C. divaricatus* and *S. japonica*, their numbers on each shrub ranging from 0.4 to 0.8 specimen/shrub in the course of three years of studies. Larvae and adult specimens of *Anthocoridae* were found only on *C. divaricatus* in 2000 (0.8 specimen/shrub). On the other hand, the larvae of *Chamaemyiidae* were observed on *C. alba* shrubs only in the year 2000 (1.2 specimens/shrub). Their scarce presence did not have any significant effect on the population of aphids colonizing the examined shrubs.

A remarkably higher number of predators was stated in site A as compared to site B. In site A, the greatest number of predatory arthropods from various systematic groups was found on the trees of *C.* x *media*, while in site B — on the shrubs of *C. alba.* This was probably due to the big number of aphids preying on those plants.

The studies conducted on ornamental shrubs and trees near the colonies of aphids observed the presence of predatory arthropods: young and adult specimens of *Araneida*, eggs, larvae and adults *Coccinellidae*; larvae and adults *Dermaptera*; larvae *Cecidomyiidae*; eggs and larvae *Syrphidae*; larvae *Chamaemyiidae*; eggs, larvae and adults *Chrysopidae* and larvae and adults *Anthocoridae.*

Totally in the three years of studies the eggs and larvae of *Syrphidae* were the most numerous in site A, while in site B — the eggs of *Chrysopidae* as well as the eggs and larvae of *Syrphidae. Syrphidae* larvae are most often polyphagous, feeding above all on aphids, while the adults feed on the plant pollen and nectar (Barczak, 1994). Remarkable voracity of larvae in orchards is pointed out by Olszak (1991) and Wnuk (1972), while in agricultural cultivations — by Malinowska (1979) and Ciepielewska (1993). Differences in the communities of *Syrphidae* in various environments are connected with the development of aphids and the life cycle of those predators and the species variability of plants (Grabarkiewicz, Jaśkiewicz, 2001). According to the model of Lotka-Volter, the maximum occurrence of the predator is always preceded by the maximum number of victims (Hurej, Ignatowicz, 1981). In the authors' own studies, the period of the most numerous occurrences of *Syrphidae* larvae was correlated in time with the highest number of aphids on the examined plants, which is consistent with the results obtained by Ziarkiewicz and Kozłowska (1973). The amount and the quality of the food taken by the larvae of *Syrphidae* is related to the density of the aphid population, the size of predators and their victims, the species of the predator and the aphid as well as their development stage (Ciepielewska, Żurańska, 1985).

Larvae and adults of *Chrysopidae* are polyphagous. They require a variety of feeds for their development but they prefer the aphids (Barczak, 1994). The presence of particular species and their effect is related to the site (Werstak, 1994). They are more numerous in aphid colonies in field cultivations rather than in trees (Cichocka, 1980). They show greater resistance to pesticides than other predators (Kowalska, 1978).

A lot of authors point out that the most important role in limiting the population of aphids is played by ladybirds, especially those from the family *Coccinellinae* (Cichocka, 1980; Olszak, 1982; Wiąckowski, Wiąckowska, 1968; Wójtowska, 1990). These are usually polyphagous species, the larvae and adults of which are predators (Barczak, 1994). Ladybirds can be found in all environments where aphids occur. The number of particular ladybird species on

decorative shrubs is probably related to the site where those shrubs grow (Barczak et al., 1996). Pruszyński and Lipa (1970) state that *Adalia bipunctata* (L.) most readily eats the aphids colonizing shrubs and trees. In the authors' own studies, the greatest number of ladybird larvae was observed on *C.* x *media* and *J. communis*, while adult specimens most frequently appeared on coniferous shrubs — *J. communis* and *P. mugo.*

The larvae of *Cecidomyiidae*, which were most numerous in aphid colonies inhabiting the shrubs of *C. alba*, played a slight role in limiting the aphid population. They are not able to reduce the population of those insects in a considerable way because of their small size (2—3 mm).

Among the predatory Diptera, the family *Chamaemyiidae* is the least studied. Their larvae, due to considerable similarity to *Syrphidae* larvae, are probably mistaken for them (Wnuk, 1978). The authors' own studies found their presence on the shrubs on *C. alba* only in one year of the studies.

The other predatory insects (*Anthocoridae* and *Dermaptera*) occurred scarcely and no significant effect on the aphid population was found out.

A big role in limiting the aphid number was played by network and no-network spiders, the presence of which was observed on all species of ornamental plants colonized by predators. This is consistent with the results of studies conducted on maples by Cichocka et al. (1998).

A considerable higher number of predators in the street site (A) was connected with bigger number of aphids in this site. Similar dates are given by Cichocka et al. (1998).

1. The occurrence of predators: young and adult specimens of *Araneida*, eggs, larvae and adults *Coccinellidae*; larvae and adults *Dermaptera*; larvae *Cecidomyiidae*; eggs and larvae *Syrphidae*; larvae *Chamaemyiidae*; eggs, larvae and adults *Chrysopidae* and larvae and adults *Anthocoridae*, was observed near the aphid colonies inhabiting decorative shrubs and trees.
2. The most important role in limiting the aphid population was played by *Syrphidae*, *Chrysopidae*, and *Coccinaellidae.*
3. A considerably greater number of predatory arthropods were observed in site A as compared to site B. The increase in the aphid number caused increase in the number of predators.
4. The greatest number of predators from various systematic groups in site A was found on the trees of *Crataegus* x *media* Bechst., while in site B — on shrubs *Cornus alba* L.

References

1. Barczak, T. 1994. Naturalni wrogowie mszyc i ich znaczenie w ochronie roślin. Wiad. Entomol., 13, 3, 141—152.
2. Barczak, T., Kaczorowski, G., Burmistrzak, M. 1996. Coccinellid beetles associated with population of *A. fabae* Scop. — complex (*Aphididae*, *Hom.*) on spindle bush. Preliminary results. Aphids and Other Homopterous Insects, 5, PAS, Skierniewice, 15—22.
3. Cichocka, E. 1980. Mszyce roślin sadowniczych Polski. PWN, Warszawa, 119 pp.
4. Cichocka, E., Goszczyński, W., Szybczyński, K. 1998. Mszyce i ich naturalni wrogowie na klonach w Warszawie. W: Fauna miast — Urban fauna [red. Barczak T., Indykiewicz P.], Wyd. ATR, Bydgoszcz, 83—88.
5. Ciepielewska, D. 1993. Drapieżne *Syrphidae* (*Diptera*) występujące na uprawach roślin motylkowatych w rejonie Olsztyna. Pol. Pismo Entomol., 62, 231—241.
6. Ciepielewska, D., Żurańska, I. 1985. Występowanie bzygowatych na roślinach motylkowatych i ich rola w redukcji populacji mszyc. Ochrona Roślin, 9, 7—8.
7. Grabarkiewicz, A., Jaśkiewicz, B. 2001. Wpływ ochrony chemicznej roślin motylkowatych na występowanie drapieżnych muchówek bzygowatych (*Diptera, Syrphidae*). Wiadomości Entomologiczne, Poznań, XIX, 3—4, 179—186.
8. Hurej, M., Ignatowicz, S. 1981. Przykłady skutecznego ograniczania liczebności mszycy trzmielinowo-burakowej przez larwy bzygowatych w uprawie buraka cukrowego. Ochrona Roślin, 5, 5—7.
9. Kowalska, T. 1978. Drapieżne siatkoskrzydłe. Biologiczne metody walki ze szkodnikami roślin [red. Boczek J., Lipa J. J.] PWN, Warszawa, 211—224.
10. Malinowska, D. 1979. Communites of Aphidophagous Syrphids (*Diptera, Syrphidae*) in Lublin Region. Memorabilia Zoologica, 30, 37—62.
11. Olszak, R. W. 1982. Perspektywy wykorzystania biedronek w integrowanych metodach zwalczania mszyc w sadach. Zesz. Probl. Post. Nauk Rol., 251, 63—68.
12. Olszak, R. W. 1991. Ocena skuteczności zespołu afidofagów występujących w sadach jabłoniowych. W: Mszyce — ich bionomia, szkodliwość i wrogowie naturalni. (red. Cichocka, Goszczyński), PAN, Warszawa, 107—113.
13. Pruszyński, S., Lipa, J. J. 1970. Obserwacje nad cyklem rozwojowym i specjalizacją pokarmową biedronki dwukropki *Adalia bipunctata* L. (*Coleoptera, Coccinellidae*). Prace Nauk. Inst. Ochr. Rośl., 12, 2, 99—116.
14. Werstak, K. 1994. Green Lacewings (*Neuroptera, Chrysopidae*) on the forest and field biocenosis. Roczniki Nauk Rolniczych, E, T. 24, 1/2, 27—32.
15. Wiąckowski, S., Wiąckowska, I. 1968. Badania nad entomofauną towarzyszącą mszycom drzew i krzewów owocowych. Pol. Pismo Ent., 38, 2, 32—36.
16. Wnuk, A. 1972. Badania nad składem gatunkowym drapieżnych bzygowatych (*Syrphidae, Diptera*) występujących w koloniach mszyc na drzewach i krzewach owocowych. Pol. Pismo Entomol., 42, 235—247.
17. Wnuk, A. 1978. Drapieżne muchówki. W: Biologiczne metody walki ze szkodnikami roślin. (red. Boczek J., Lipa J. J.), PWN, Warszawa, 267—286.
18. Wójtowska, M. 1990. Wpływ różnych czynników na liczebność mszycy grochowianki na różnych roślinach motylkowych. Zesz. Probl. Post. Nauk Rol., 392, 161—169.
19. Ziarkiewicz, T., Kozłowska, A. 1973. Materiały do poznania składu gatunkowego drapieżnych bzygowatych (*Syrphidae, Diptera*) występujących w koloniach mszyc na krzewach ozdobnych. Pol. Pismo Entomol., 43, 621—626.

THE SCALE INSECTS OF SOME TROPICAL FRUIT PLANTS IN GREENHOUSES OF THE BOTANICAL GARDEN IN LUBLIN (POLAND)

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Abstract

The aim of presented investigations was to determine the composition of scale insects species and intensity of their occurrence on some tropical plants of greenhouses. The investigations were carried out in the greenhouses of the Maria Curie Skłodowska Botanical Garden in Lublin during the years 2002—2004. Ten species belonging to seven genera of tropical fruit plants were observed: *Ficus*, *Musa*, *Eugenia*, *Ananas*, *Feijoa*, *Citrus*, and *Eriobotrya*. The quantitative analysis of the studied material was performed making use of the following ecological indicators: number, frequency, and density. Identification of scale insect species was performed on the basis of microscope sliders.

Four species of scale insects belonging to two families were observed on tropical fruit plants: *Pseudococcidae* (*Pseudococcus maritimus, Planococcus citri*) and *Coccidae* (*Coccus hesperidum*, *Saissetia coffeae*). The scale insects were noted on nine species of tropical plants, they were not observed on *Ananas comosus*. The observed scale insects are typical polyphagous and all of them are considered as harmful pests in greenhouses. However, two species — *P. citri* and *S. coffeae* — seem to be of major importance as pests of tropical fruit plants in observed greenhouses.

Key words: scale insects, tropical fruit plants, greenhouses, botanical garden, number, frequency, density.

Introduction

The scale insects are a group of insects, which occur in all climatic zones; however, in tropical and subtropical zones they are the most serious pests. Scale insects occupy citrus, pineapple, coffee and tea plantations (Kosztarab, 1996; Ben-Dov, Hodgson, 1997).

Some of them have been noted for years in Polish greenhouses. Currently, over 40 species of scale insects have been observed on various plant species cultivated in greenhouses in Poland (Koteja, 1996). These are cosmopolitic and polyphagous species, spread through trade and exchange of plant material. Polyphagous species occupying new areas often become a dangerous pest, mainly due to the lack of natural enemies, great reproductive potential (parthenogenesis) and specific morphological structure (protective shields, waxy powder and threads, sclerotized dorsal side) making chemical control difficult (Dziedzicka, 1988b; Łagowska, 1995a; Ben-Dov, Hodgson, 1997).

The main injury caused by scale insects is the ingestion of plant sap, resulting in the loss of plant vigour, poor growth, leaf drop, dieback of twigs, or sometimes the death of the plant. Moreover, while piercing plant tissue, the saliva secreted by the insect may be toxic for the plant and produce chlorotic yellowing of leaves, discoloration of leaves and fruits, and deformation of plants. Some species of scale insects can transmit plant virus diseases, and by injuring the plant surface enable the entry of other pathogens (Dziedzicka, 1988b; Kosztarab, 1996). Through excreting honeydew coating plant surfaces, assimilation and photosynthesis are inhibited which results in poor growth, poor development of plants, leaf drop, less sugar in fruits, and also spoil the appearance of ornamental plants. In addition, dust and other contaminants settle on honeydew, but primarily sooty moulds grow which coat the plant surface with a black layer. Honeydew also attracts other insects (ants), the presence of which decreases the aesthetic value of plants.

Materials and Methods

The studies were conducted in the greenhouses of the Maria Skłodowska University Botanical Garden in Lublin during during the years 2002—2004. During this period, chemical, biological and mechanical plant protection was carried out. The plants observed were cultivated in greenhouses of the total area of 270 m^2. The studies covered 10 tropical plant species of edible fruits, belonging to 7 genera: *Ananas, Citrus, Eriobotrya, Eugenia, Feijoa, Ficus,* and *Musa*. In the study, 30 centimetre long fragments on each plant were selected at random. Observations of the selected fragments (twigs, leaves) were made every 14 days. In order to identify species, several individual scale insects were collected from each examined plant, and fixed microscopic specimens were prepared by Williams and Kosztarab method (1972).

Quantitative analysis of the collected material was conducted with the use of ecological indicators: numbers, frequency (percentage of samples in which an individual species occurred — Szujecki, 1980), density (number of species present in an individual environment on a defined surface unit — Górny, Grüm, 1981). The density of scale insects on examined plant fragments was carried out based on a 5-degree scale: 0 — lack of scale insects; I — single scale insects; II — up to 25% of surface affected by scale insects; III — up to 50% of surface affected by scales; IV — up to 100% of surface affected by scales (mass density).

Results and Discussion

Based on the results of studies conducted on 10 tropical plant species, 4 scale insect species, belonging to 2 families, were observed: *Pseudococcidae — Pseudococcus maritimus* (Ehrh.) (grape mealybug), *Planococcus citri* (Risso) (citrus mealybug), *Coccidae — Coccus hesperidum* L. (brown soft scale), and *Saissetia coffeae* (Walker) (hemispherical scale).

The presence of scale insects was noted on 9 plant species belonging to the families: *Moraceae, Musaceae, Myrtaceae, Rosaceae, Rutaceae*. Scales insect did not occupy *Ananas cosmosus*. *P. citri*, which was observed on 6 plant species, was characterised by the largest number of host plants. *P. maritimus* and *S. coffeae* were noted on 3 species of hosts (Table 1). *C. hesperidum* was observed on the smallest number of host species (2); however, its total number on these plants was the highest (1036 individuals), compared to the remaining 3 species of scale insects. *P. maritimus* was characterised by the smallest total number of individuals (692) (Fig. 1).

Table 1

Occurrence of scale insects on 10 species of tropical fruit plants, their number and density

Species of scale insects	Species of tropical plants	Number of individuals of scale insects	Density
Pseudococcus maritimus (Ehrh.)	*Musa* sp. L.	359	III
	Eugenia uniflora L.	290	III
	Eribotrya japonica (Thunb)	43	I
Planococcus citri (Risso)	*Ficus carica* L.	147	I
	Feijoa sellowiana O. Berg	99	I
	Citrus grandis 'Pompela' (L.)	276	II
	Citrus limon (L.) Burm. F.	329	III
	Citrus reticulata Blanco	12	I
	Citrus paradis 'Grapefruit' (Swingle)	90	II
Coccus hesperidum L.	*Citrus reticulata* Blanco	387	III
	Citrus paradis 'Grapefruit' (Swingle)	649	III
Saissetia coffeae (Walker)	*Eugenia uniflora* L.	2	I
	Feijoa sellowiana O. Berg	875	III
	Citrus grandis 'Pompela' (L.)	100	II

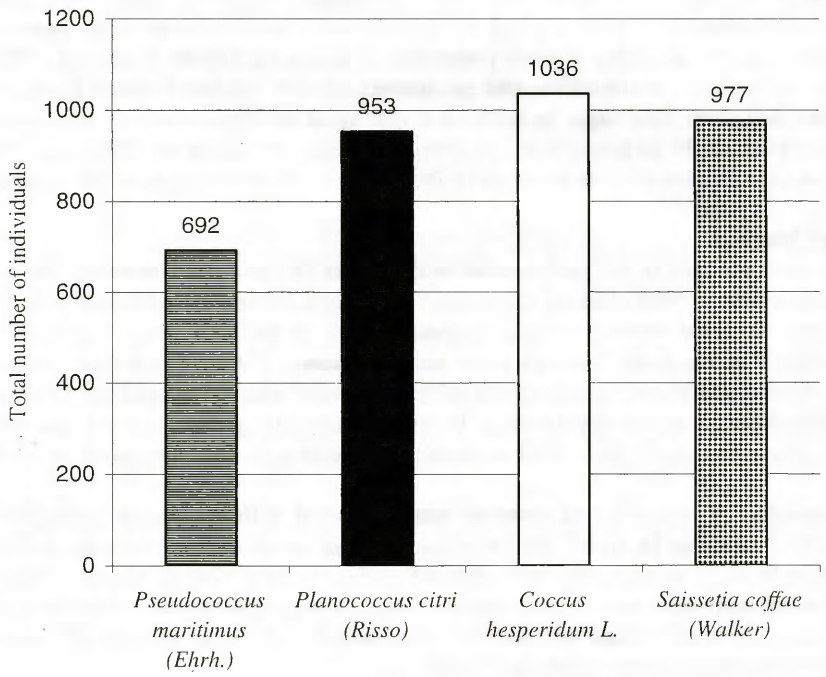

Fig. 1. The total number of scale insects on tropical fruit trees in the greenhouse of the Botanical Garden Lublin

Among scale insects observed on this group of plants, the following species of *Coccidae* family appeared in abundance on individual hosts: *S. coffeae* — the greatest number occurred on *Feijoa sellowiana* (875 individual scales)

and *C. hesperidum* — on *Citrus paradis 'Grapefruit'* (649 individual scales). The species *S. coffeae* on *Eugenia uniflora* was found in the smallest number — 2 individual scales (Table 1).

Frequency of individual species varied in the greenhouses in the study. In the group of scale insects found on tropical plants it was the highest for the species *P. maritimus* on *Musa banan* (F = 100%) and *C. hesperidum* on *Citrus reticulata* (F = 90%), whereas the lowest frequency was noted for *S. coffeae* species on *Eugenia uniflora* (F = 10%). The frequency of scale insects on the remaining plant species ranged from 30 to 85% (Fig. 2).

On the examined plants, the colonies of scale insects occurred in classes I, II and III of density, while their appearance in mass (class IV) was not observed. On the majority of plants, class III of scale insects density was noted (Table 1).

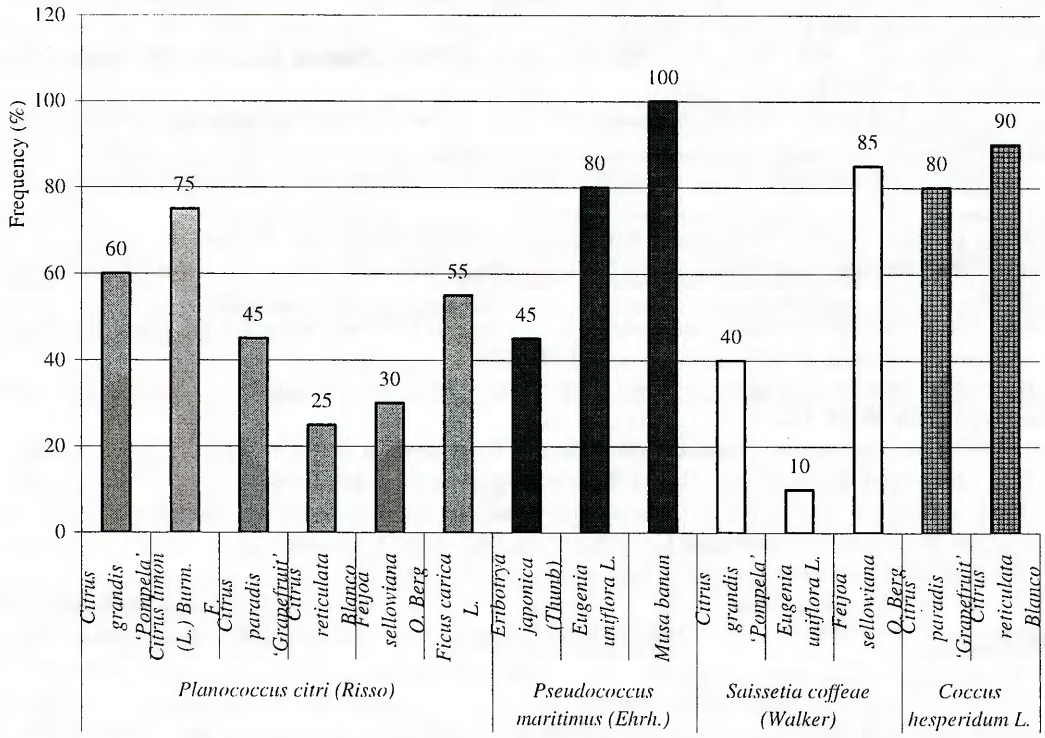

Fig. 2. The frequency (%) of scale insects occuring on some tropical fruit plants

Scale insects were found in the greenhouses despite intensive plant protection being carried out. According to literature (Dziedzicka, 1988b; Łagowska, 1995b), scale insects are present even in the best maintained greenhouses due to their peculiar morphology and biology.

The found species belong to most abundant and dangerous scale insects observed in Poland's greenhouses (Łagowska, 1995a, b; Dziedzicka, 1988a, b). According to literature reports, the *Pseudococcidae* family in Poland is represented by 5 greenhouse species, whereas *Coccidae* — by 9 scale insect species (Dziedzicka, Madro, 1999; Koteja, 1996). Studies conducted in the greenhouses of the Botanical Garden in Lublin on 10 tropical plant species showed the presence of 2 species from *Pseudococcidae* family and 2 species from *Coccidae* family. The presence of scale insects from the *Diaspididae* family, which is represented in Poland by 27 greenhouse species (Koteja, 1996), during the studies was not observed.

Among scale insects noted on tropical plants in the greenhouses of the Maria Skłodowska Curie Botanical Garden, *P. citri* occurred on the greatest number of plant species. This species was noted on plants of *Citrus* and *Ficus* genera, which was confirmed by literature reports (Ben-Dov, 1993; Ben-Dov, Hodgson, 1997; Dziedzicka, 1988a; Łagowska, 1995b).

All the scale insects observed were cosmopolitic and polyphagous species and occurred in almost every greenhouse (Dziedzicka, 1988b). Although all plants in the study grew in the same environmental conditions, making a compact composition, they were occupied by scale insects to a various degree. These insects appeared on 9 host plant species. The only species on which no scale insects were found was *Ananas comosus*. During the two years of the study, the presence of scale insects on this plant was not noted, although literature reports mention *Ananas* genus as a host for more than 10 species, including *Coccus hesperidum* and *Planococcus citri.*

While investigating the occurrence of scale insects on several plant species it may be presumed that the host species exerts a great effect on their occupancy. An example may be *S. coffeae*, the numbers of which on *E. uniflora* were the smallest (2 individual scale insects), while it was many times higher on *F. sellowiana* (875 individual scale insects). According to Piechota (1981), certain properties of plants may condition their defensive reaction. These are the features of morphologic structure (height and shape of plants, size of leaves, occurrence of hairs and furrows on leaf surface), anatomical structure (number and size of stomatal apparatuses, thickness and degree of lignification of cellular

walls, as well as thickness of sclerenchyma's conducting tissue), or physiological reaction (synchronisation of the development phase of a plant with the requirements defined by life cycle of an insect). Tingle and Copland (1988) investigated the effect of host plant on *P. citri* population.

The present study showed that the condition of the host plant is one of major factors affecting the size of this species population. Occupancy on individual plant species may also be affected by specific odorous substances produced by them, which by attracting scale insects parasites result in the plants being less affected by these insects (Van Alphen, Ren, 1990).

References

1. Ben-Dov, Y. 1993. A systematic catalogue of the soft scale insects of the world. Sandhill Crane Press, inc., Gainesville (Florida) and Leiden (The Netherlands), 536 pp.
2. Ben-Dov, Y., Hodgson, C.J. 1997. Soft Scale Insects – Their Biology, Natural Enemies and Control. Elsevier, Amsterdam & New York, 452 pp.
3. Dziedzicka, A. 1988a. Wełnowce szklarniowe (*Homoptera, Coccinea, Pseudococcidae*). Zesz. Probl. Post. Nauk Roln., 333, 87—91.
4. Dziedzicka, A. 1988b. Czerwce szklarniowe (*Coccinea*) Polski. Rocz. Nauk. — Dydakt. WSP Kraków, 123, 79—91.
5. Górny, M., Grüm, L. 1981. Metody stosowane w zoologii gleby. PWN, Warszawa, 483 pp.
6. Kosztarab, M. 1996. Scale Insects of Northeastern North America. Virginia Museum of Natural History, Sp. Publ. Nb 3, Martinsville.
7. Koteja, J. 1996. Jak rozpoznać czerwce (*Homoptera, Coccinea*). W: Boczek J. (red.), Diagnostyka szkodników roślin i ich wrogów naturalnych. SGGW Warszawa, 2, 139—231.
8. Łagowska, B. 1995a. Możliwości biologicznego zwalczania czerwców (*Homoptera, Coccinea*) na roślinach ozdobnych w szklarniach. Wiad. Entomol., T.14 (1), 5—10.
9. Łagowska, B. 1995b. Występowanie czerwców (Homoptera, Coccinea) na doniczkowych roślinach ozdobnych w szklarniach. Mat. Ogólnopol. Konf. Nauk. "Nauka Praktyce Ogrodniczej", AR–Lublin.
10. Piechota, J. 1981. Podatność wybranych odmian pszenicy jarej na mszycę zbożową (*Sitobion avenae*) i mszycę czeremchowo-zbożową (*Rhopalosiphum padi* L.). Praca doktorska, SGGW Warszawa.
11. Szujecki, A. 1980. Ekologia owadów leśnych. PWN, Warszawa, 603 pp.
12. Wiliams, M., Kosztarab, M. 1972. Morphology and systematics of the *Coccidae* of Virginia with notes on their biology (*Homoptera*: *Coccoidea*). Res. Div. Bull. Virginia Polytech., Inst. and State Univ., Blacksburg, 74, 1—215.

STUDIES ON PEST CONTROL IN GREENHOUSES OF THE BOTANICAL GARDEN IN LUBLIN (POLAND)

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Abstract

The sucking insects are most frequent and dangerous pests in greenhouses. They belong to *Hemiptera* (*Coccoidea, Aphidoidea, Aleurodidae*) and *Thysanoptera.* Studies were carried out in a greenhouse of the Maria Curie-Skłodowska University (UMCS) Botanical Garden in Lublin during the years 2002—2004. The purpose of the studies was to determine the influence of the applied methods, especially chemical control, on the number of pests and the intensity of plant infestation. The main method was chemical control applied throughout the growing season. The most frequently applied insecticide was Actellic 50 EC. The biological method was started in 1999 and continued till 2002. Two kinds of natural enemies were used against the pests in the greenhouse: parasitoids and predators. The aim of the mechanical method was to eliminate the most invaded parts of plants and to remove the pests using a mixture of water and methylated spirit in the proportion 1:1 once a month. Conventional methods of pest control turned out to be ineffective and did not produce the expected results. The biological method was found to be more effective and safe. It reduced the number of pests considerably. The mechanical method was very short-lasting and less effective. However, the best effect in controlling the pests of greenhouse cultivations is achieved combining a few methods (biological, chemical and mechanical ones).

Key words: botanical garden, greenhouse, pests, predators, parasitoids, biological control, chemical control.

Introduction

The most frequent and troublesome pests that are of importance in the cultivations under cover include sucking-plant insects belonging to *Hemiptera* (*Coccoidea, Aphidoidea, Aleurodidae*) and *Thysanoptera.* Convenient conditions in greenhouses (raised temperature and humidity), vegetative reproduction of plants, polyphagism and frequent parthenogenesis of the pests are conducive to fast reproduction and spread.

Considering the biological and morphological features of greenhouse pests, the choice of proper control is very important. The chemical method is one of the most common ones. The effectiveness of chemical control depends not only on the kind of the applied preparation but to a large extent, also to the date of the treatment. Small dimensions of those insects often hiding in the leaf corner and the cracks of bark, which makes them hardly visible, are an obstacle in fixing of the proper date and method of control. The all-year development of insects in greenhouses determines the necessity of frequent application of insecticides. As early as in the 1980s, the range of the application of parasitic and predatory species in glasshouses increased. Until 1990, in Poland the areas subjected to the biological method included totally about 500 ha (Pruszyński et al., 1991).

Materials and Methods

The studies were conducted in the greenhouses of the Botanical Garden of UMCS in Lublin during the years 2002—2004. 29 exotic plants growing on the area of 270 m^2 were chosen for observation. Three methods of controlling the pests were applied in the examined greenhouses: chemical, biological and mechanical ones. Chemical control was the leading method applied throughout all the season with the use of Actelic 50EC (0.1%) and Confidor 200 SL (0.15%). The chemical treatments were applied in three cycles (I cycle: 29.11.2002—28.02.2003, II cycle: 09.09.2003—28.11.2003, and III cycle: 27.02.2004—20.04.2004), while spraying was performed once a week. In 2002 the biological control was continued which began in 1999 with the use of parasites (*Encarsia formsa, Aphidius ervi, Aphidius colemani*) and predators (*Amblyseius cucumeris, Orius laevigatus, Orius insidiosus, Cryptolaemus montrouzieri*). The mechanical method consisted of removing the most infected fragments of plants and washing off the pests using water and methylated spirit in the proportion 1:1 once a month.

Three shoot fragments 30 cm in length were chosen at random on each of the studied plants. The monitoring of the marked fragments (shoots and leaves) was performed every 14 days. The quantitative analysis of the examined material was made on the basis of the number of specimens.

The purpose of the studies was to determine the effect of the applied methods of control, especially chemical ones, on the population of pests.

Results and Discussion

The occurrence of pests belonging to a few families of *Hemiptera* and *Thysanoptera* was found out during the years 2002—2004 in the greenhouses of the Botanical Garden of the UMCS. The studies observed the appearance of *Myzus persicae* Sulz. on *Citrus grandis* "Pompela" and on four plant species from the genus *Piper:* (*P.nigrum, P.celtidifolium, P.longum, P. microphyllum*), while *Trialeurodes vaporariorum* Westw. was found on *Abutilion striatum* cv. 'Thomsoni'.

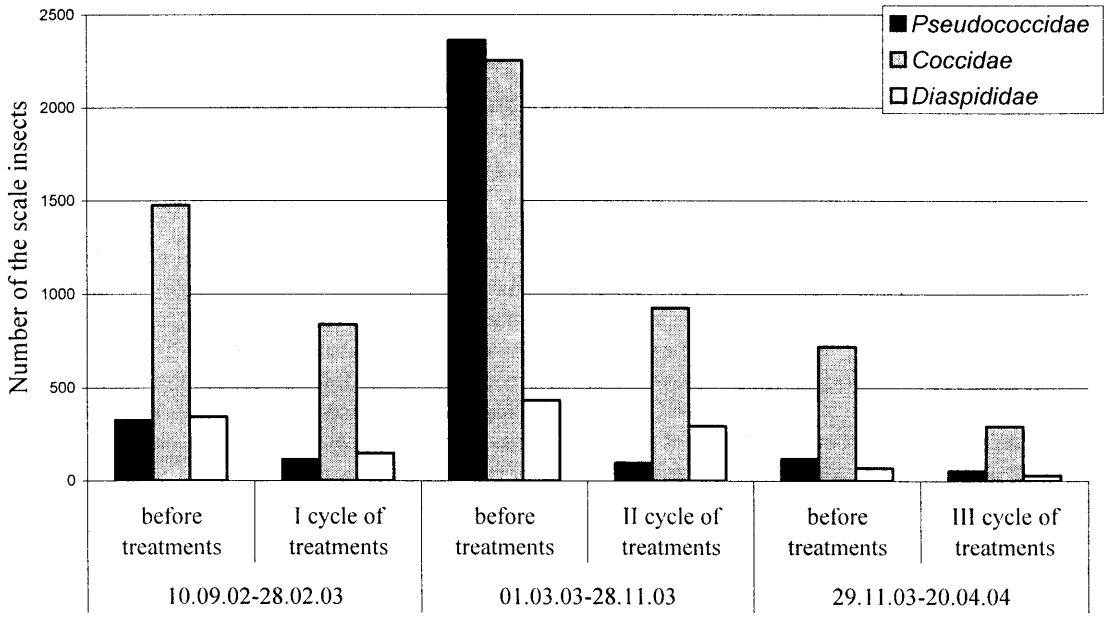

Fig. 1. Differences in number of scale insects specimens during three cycles of applied control methods

The most numerous and most frequent were pests representing three scale insect families: *Pseudococcidea* (mealybugs), *Coccidae* (soft scales), and *Diaspididae* (armoured scales). In the greatest number of host plants occurred mealybugs (22 plant species) and soft scales (18 plant species) (Tables 1, 2). Scale insects from the family *Diaspididae* inhabited the smallest number of hosts, namely 4 plant species (Table 3).

Totally, 8243 specimens of scale insects were found on the studied plants. The soft scales and mealybugs (2805 specimens) were most numerous (4594 specimens), while the least numerous included armoured scale insects (844 specimens) (Tables 1, 2, 3, Fig. 1).

Eugenia uniflora was the species of the host plant that was inhabited by scale insects in the greatest numbers. There were 655 soft scales and 284 specimens of mealybugs on it (Tables 1, 2). Out of 29 examined plants, the only species that was not inhabited by pests was *Vanilla planifolia* (Tables 1, 2, 3).

The greatest — 10-fold — decrease of the population of pests was observed in the case of mealybugs. In the second cycle of applied methods of control, the reduction of the number of specimens was the greatest and it was from 2362 to 96 specimens (Table 1, Fig. 1). The number of scale insects from the family *Coccidae* and *Diaspididae* after the treatments decreased by 2.4 and 1.8 times, respectively (Tables 2, 3, Fig. 1).

Despite the fact that during the studies no useful organisms were introduced to the greenhouses, the occurrence of the larvae of a predatory ladybird *Cryptolaemus montrouzieri* was observed in the colonies of mealybugs. That predator occurred in the greatest population in the spring of 2003 on the plants from the genera *Citrus, Coffe, Dizygoteca, Eriobotrya, Feijoa, Ficus, Musa,* and *Ruscus.*

Table 1

The number of specimens of *Pseudococcidae* in three cycles of applied control methods

Species of host plant	before treatments	I cycle of treatments	before treatments	II cycle of treatments	before treatments	III cycle of treatments	Total	
	10.09.02—29.11.02	29.11.02—28.02.03	01.03.03—08.09.03	09.09.03—28.11.03	29.11.03—26.02.04	27.02.04—20.04.04	before treatments	Treatments
Ficus carica	18	10	126	0	2	0	146	10
Musa sp.	24	23	271	24	20	17	315	64
Eugenia uniflora	12	6	264	1	8	7	284	14
Feijoa sellowiana	0	0	95	3	1	0	96	3
Citrus grandis 'Pompela'	36	4	230	0	1	1	267	5
Eriobotrya japonica	14	7	29	0	0	0	43	7
Citrus limon	8	7	284	17	2	2	294	26
Citrus reticulata	4	0	8	0	0	0	12	0
Citrus paradis 'Grapefruit'	5	0	78	7	0	0	83	7
Punica granatum	24	4	9	0	0	0	33	4
Rozmarinus officinalis								
Myrtus communis								
Laurus nobilis								
Vanilla planifolia								
Piper microphyllum	0	0	3	0	3	0	6	0
Piper celtidifolium	0	0	20	0	0	0	20	0
Piper longum	0	0	80	0	2	0	82	0
Piper nigrum								
Coffe arabica	20	11	134	3	14	9	168	23
Citrus medica var.'Buddhas Hand'	0	0	27	18	25	7	52	25
Abutilion striatum cv. 'Thomsoni'	14	2	25	0	0	0	39	2
Dezygotheca elegantissima	13	3	3	1	17	1	33	5
Hypoestes hyllostachya	2	0	5	0	0	0	7	0
Hedera helix	0	0	1	0	0	0	1	0
Ruscus aculeatus	95	31	370	19	20	9	485	59
Nerium oleander	0	0	3	0	0	0	3	0
Passiflora guadrangularia	36	7	297	3	3	0	336	10
Cyrtomium falcatum								
Total number of scale insects	325	115	2362	96	118	53	2805	264

Table 2

The number of specimens of *Coccidae* in three cycles of applied control methods

Species of host plant	*Coccidae*							
	before treatments	I cycle of treatments	before treatments	II cycle of treatments	before treatments	III cycle of treatments	Total	
	10.09.02—29.11.02	29.11.02—28.02.03	01.03.03—08.09.03	09.09.03—28.11.03	29.11.03—26.02.04	27.02.04-20.04.04	before treatments	treatments
Ficus carica								
Musa sp.								
Eugenia uniflora	0	0	1	1	0	0	1	1
Feijoa sellowiana	278	97	275	77	102	46	655	220
Citrus grandis 'Pompela'	5	0	21	0	76	3	102	3
Eriobotrya japonica								
Citrus limon								
Citrus reticulata	119	63	106	47	49	10	274	120
Citrus paradis 'Grapefruit'	128	65	290	88	76	73	494	226
Punica granatum	5	0	87	53	0	0	92	53
Rozmarinus officinalis	28	1	0	0	0	0	28	1
Myrtus communis	21	21	62	55	35	0	118	76
Vanilla planifolia								0
Laurus nobilis	3	0	0	0	0	0	3	0
Piper microphyllum								
Piper celtidifolium								
Piper longum								
Piper nigrum	106	18	28	0	0	0	134	18
Coffe arabica	61	56	121	73	93	62	275	191
Tea sinesis	18	7	25	15	6	2	49	24
Citrus medica var.'Buddhas Hand'	82	60	35	32	5	0	122	92
Abutilion striatum cv. 'Thomsoni'	123	1	181	161	0	0	304	162
Dezygotheca elegantissima	133	159	452	224	263	96	848	479
Hypoestes hyllostachya	109	87	153	73	16	0	278	160
Hedera helix								
Ruscus aculeatus								
Nerium oleander	72	12	44	7	0	0	116	19
Passiflora guadrangularia								
Cyrtomium falcatum	307	71	394	0	0	0	701	71
Total number of scale insects	1598	718	2275	906	721	292	4594	1916

Table 3

The number of specimens of *Diaspididae* in three cycles of applied control methods

Species of host plant	*Diaspididae*							
	before treatments	I cycle of treatments	before treatments	II cycle of treatments	before treatments	III cycle of treatments	Total	
	10.09.02—29.11.02	29.11.02—28.02.03	01.03.03—08.09.03	09.09.03—28.11.03	29.11.03—26.02.04	27.02.04—20.04.04	before treatments	treatments
Ficus carica								
Musa sp.								
Eugenia uniflora								
Feijoa sellowiana								
Citrus grandis 'Pompela'								
Eriobotrya japonica								
Citrus limon								
Citrus reticulata								
Citrus paradis 'Grapefruit'								
Punica granatum								
Rozmarinus officinalis	0	0	14	0	0	0	14	0
Myrtus communis								
Laurus nobilis	136	69	175	92	47	29	358	190
Vanilla planifolia								
Piper microphyllum	0	0	4	0	0	0	4	0
Piper celtidifolium								
Piper longum								
Piper nigrum								
Coffe arabica								
Tea sinesis								
Citrus medica var.'Buddhas Hand'								
Abutilion striatum cv. 'Thomsoni'								
Dezygotheca elegantissima								
Hypoestes hyllostachya								
Hedera helix	208	79	239	202	21	0	468	281
Ruscus aculeatus								
Nerium oleander								
Passiflora guadrangularia								
Cyrtomium falcatum								
Total number of scale insects	344	148	432	294	68	29	844	471

Pests occurring in greenhouses belong to insects that are very hard to control, which is due to their characteristic biology and morphology (polyphagism, considerable reproductiveness, parthogenesis and specific covers of the body).

The all year development of insects in greenhouses determines the necessity to apply chemical treatment repeatedly. However, when they are used, it is important to monitor the development of pests in order to establish the date of the treatment in time (Ronse, 1990). The moment when the larvae leave the eggs and disperse over the plants is the optimum date of controlling scale insects. At this time insects do not have well developed covers, already possessed by the specimens of older larval stages, protecting them from the effect of chemical preparations. Females with chitinized body covers also protect the eggs from the effect of insecticides (Łagowska, 1995a).

While analyzing the effect of the applied forms of control, it turned out that the highest reduction of individuals was observed in the case of mealybugs. The population of those insects on the observed plants underwent a 10-fold decrease. The greatest effect was found in the second cycle of treatments. The population of scale insects from this family in the discussed period was reduced by 4%, as compared to the number of individuals before the cycle of spraying. Most probably this reduction of individuals was influenced by chemical treatments and the predatory ladybird *C.montrouzieri.* The greatest number of its predatory larvae was observed in the discussed period. The temperature conducive to the activity of this species is above 16 °C, which additionally accounts for the appearance of the ladybird in that period. The species *C.montrouzieri* is used to control all developmental stages of mealybugs. It has a tendency to migration and it does not reproduce in the greenhouse, that is why the number of individuals of the predator in greenhouses is decreasing fast (Ronse, 1990; Łagowska, 1995a).

Dziedzicka (1988b) states that mechanical control gives good effects in controlling scale insects. It consists of washing off the pests (by means of a brush or a cloth) or a few hours' bath of the whole plant in water. Monthly washing off and cutting of the most infected shoots, which was performed in UMCS greenhouse, additionally affected the reduction of the population of scale insects.

Among *Pseudococcidae, Coccidae* as well as *Diaspididae,* it was just mealybugs that seem the best noticeable on plants because of the characteristic white waxy covers of the body. It results from the observations that the plant shoots where mealybugs fed were most frequently removed by the workers of the greenhouse.

Scale insects occur in greenhouses despite the intensive chemical control performed there. Conventional control of pests turns out to be of little effectiveness; it is short lasting and does not bring the anticipated results. The applied insecticides considerably reduce the population of scale insects but they do not eliminate them completely (Łagowska, 1995b). According to literature (Dziedzicka, 1988b; Łagowska, 1995b), the presence of one female on the plant makes it impossible to reconstruct the population consisting of 500—2000 specimens within two months. This is confirmed by the authors' own studies. After a cycle of spraying was used in greenhouses, the population of pests got reduced for a short time, after which it was quickly reconstructed.

As stated by a number of authors (Summy et al., 1986; Tingle, Copland, 1988; Ronse, 1990), biological control brings better results in reducing the population of pests as compared to the conventional method. However, the best effect in controlling the pests of greenhouse cultivations is achieved combining a few methods (biological, chemical and mechanical ones).

References

1. Dziedzicka, A. 1988a. Wełnowce szklarniowe (*Homoptera, Coccinea, Pseudococcidae*). Zesz. Probl. Post. Nauk Roln., 333, 87—91.
2. Dziedzicka, A. 1988b. Czerwce szklarniowe (*Coccinea*) Polski. Rocz. Nauk.-Dydakt. WSP Kraków, 123, 79—91.
3. Łagowska, B. 1995a. Możliwości biologicznego zwalczania czerwców (*Homoptera, Coccinea*) na roślinach ozdobnych w szklarniach. Wiad. Entomol., T.14 (1), 5—10.
4. Łagowska, B. 1995b. Występowanie czerwców (*Homoptera, Coccinea*) na doniczkowych roślinach ozdobnych w szklarniach. Mat. Ogólnopol. Konf. Nauk. "Nauka Praktyce Ogrodniczej", AR–Lublin, 504, 375—377.
5. Pruszyński, S., Piątkowski, J., Domagała, T. 1991. Stan badań i zakres zastosowania biologicznej metody zwalczania szkodników upraw szklarniowych. Mat. XXXI Sesji Nauk. Inst. Ochr. Roślin, 399, 58—71.
6. Ronse, A. 1990. Integrated pest management in the greenhouses of the national botanic garden of Belgium. Landbouwtndschnit, Revue de l'Agriculture, Vol. 43(3), 429—436.
7. Summy, K.R., French, J.V., Hart, W.G. 1986. Citrus mealybug (*Homoptera: Pseudococcidae*) on greenhouse citrus: density-dependent regulation by an encyrtid parasite complex. J. Econ. Ent., 79, 891—895.
8. Tingle, C.C.D., Copland, M. J. W. 1988. Effects of temperature and host-plant on regulation of glasshouse mealybug (*Hemiptera: Pseudococcidae*) populations by introduced parasitoids (*Hymenoptera: Encyrtidae*). Bull. ent. Res., 78, 135—142.

IDENTIFICATION OF TOBACCO NECROSIS VIRUS FROM STRAWBERRY IN LITHUANIA

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Abstract

Garden strawberry (*Fragaria ananassa* Duch.) plants with virus-like slight leaf mottle and deformation symptoms were detected in orchards from several locations of Lithuania. The objective of this study was to identify causal agents of the disease. A preliminary electron microscopic investigation revealed presence of spherical virus-like particles. For mechanical virus transmission to plant indicator species, buffered solutions containing nicotine and other antioxidant substances were used. For the primary isolation of the causal agent, most sensitive and suitable plants appeared to be *Atriplex hortensis* L. and *Tetragonia expansa* Murr. From these plants virus infection subsequently was transmitted to nearly 20 species causing only local reaction. Among locally infected were many *Chenopodium* and *Nicotiana* species.

In electron microscopic preparations from such infected plants only polyhedral virus particles about 28 nm in diameter were detected. According to host range studies, symptom expression and morphological properties causal virus was identified as *Tobacco necrosis necrovirus* (TNV). This assumption was supported by DAS-ELISA tests using commercially available kit for detection of TNV, and DAS-ELISA tests using antibodies and horse radish peroxidase conjugate prepared during this investigation. Polyclonal antiserum prepared against TNV isolate from plum tree (TNV-2) greatly increased the number of virus particles trapped on electron microscopic grid from virus infected plant sap. RT-PCR amplification reactions using several primer pairs confirmed that TNV isolate from strawberry is closely related to A strain of TNV.

Key words: strawberry, *tobacco necrosis virus,* identification, ELISA, RT-PCR.

Introduction

Garden strawberry (*Fragaria ananassa* Duch.) is one of major berryfruit crops cultivated in Lithuania and comprise more than 1000 ha. Strawberry yield and fruit quality are greatly influenced by pest and diseases. Currently more than thirty viruses and phytoplasmas have been reported in *Fragaria* (Martin et al., 2001). Due to mainly vegetative propagation of strawberry virus disease agents have tendency for gradual accumulation and rather often they occur in complexes. Detection and identification of strawberry viruses is very problematic due to their low concentration in plant tissues and in many cases for symptomless infection. Majority of viruses can be detected only by graft–inoculation to more sensitive hosts (Frazier, 1974).

A survey of strawberry virus diseases conducted in former Yugoslavia showed that strawberry were most frequently infected by *Strawberry mottle virus* (SMV), *Strawberry mild yellow edge virus* (SMYEV) and occasionally by *Strawberry crinkle virus* (SCV) (Dulic-Markovic et al., 1998). Investigation of virus diseases according to strawberry certification scheme in Israel revealed that SMYEV was commonly present. SMV and *Tobacco necrosis virus* (TNV) were found occasionally (Spiegel, 1998). Thorough investigation of viruses and virus-like diseases of strawberries in the Czech Republic revealed presence of SCV, SMV, *Strawberry latent ringspot virus* (SLRV), *Strawberry vein banding virus* (SVBV), *Arabis mosaic virus* (AMV), and *Tobacco necrosis virus* (TNV) (Franova, 2001; Franova et al., 2001). Major aphid-borne viruses in Italy recently were considered to be SMV, SCV, SMYEV, and SVBV (Cardoni et al., 2002). Survey of strawberry viruses occurring in commercial plantings in eastern North America revealed presence of SMYEV, occasionally *Tobacco streak virus* and *Tomato ringspot virus*. However, this survey also revealed that strawberry palidosis is the major virus or virus-like largely uncharacterized disease (Martin et al., 2001).

Recent investigation of strawberry viruses in Lithuania has revealed occurrence of *Strawberry mottle virus* (SMV), detected by graft-inoculation to standard indicator clones. According to DAS-ELISA test results, SMYEV and strawberry infecting *Nepoviruses* were not present in investigated commercial cultivars (Stankiene, Cieslinska, 2003).

TNV, member of genus *Necrovirus*, recently was isolated from plum trees in Lithuania (Staniulis, Rabenstein, 2002; Staniulis, 2003). TNV first discovered in tobacco, in Europe often causes cucumber necrosis (Sutic et al., 1999), is preserved in infected plant parts in the soil, wherefrom it is transmitted by zoospores of chytrid soil fungus *Olpidium brassicae* (Kassanis, MacFarlane, 1964). Soil water movement contributes to zoospore and virus spread. A strain of TNV was isolated from a lake and rivers in England (Tomlinson et al., 1983) and from a nutrient feeding solution (Adam et al., 1990). The virus occurs in the roots of a wide variety of plants and can be transmitted mechanically to leaves where virus infection is restricted to inoculated parts of plant and cause necrotic local lesions. Among 25 species of natural hosts, several are important cultivated herbaceous (*Nicotiana tabacum, Cucumis sativus, C. melo, Fragaria vesca, Lactuca sativa, Phaseolus vulgaris, Pisum sativum,* and *Solanum tuberosum*) and woody (*Malus sylvestris, Prunus domestica, Pyrus domestica*) plants (Sutic et al., 1999). TNV has a comparatively broad host range, has been transmitted experimentally to many species of plants (Price, 1940). In recent review it has been indicated TNV to infect

298 species in 167 genera of 54 families (Edwardson, Christie, 1997). Detailed descriptions of TNV are presented by Kassanis (Kassanis, 1970) and Uyemoto (Uyemoto, 1981). TNV has been described in fruit trees in Germany (Kegler et al., 1969) and in plums in former Czechoslovakia (Paulechova, 1980; Paulechova, Baumgartnerova, 1980).

Tobacco necrosis virus (TNV) was first reported in *Fragaria* in Arkansas (Fulton, 1952) and California (Frazier, 1955). Later on this virus has been reported in cultivated strawberries in Italy (Faccioli, 1970), Bulgaria, Japan (cited after Franova-Honetšlegrova et al., 1998), and the Czech Republic (Franova-Honetšlegrova et al., 1998).

In this article evidence based on biological, immunological properties and procedure based on RT-PCR technology of the occurrence of TNV in strawberry plants in Lithuania are presented.

Materials and Methods

Plant material

During survey of *Fragaria* virus diseases in 2001—2003 *Fragaria ananassa* Duch. plants with vein clearing and slight leaf deformation symptoms were observed and collected in Panevezys and Vilnius region. After preliminary detection of polyhedral virus-like particles, attempts were made to transmit possible virus diseases agents to herbaceous plants. Virus mechanical transmission experiments to test plants were conducted in greenhouse conditions. The inoculations were performed with an aid of carborundum powder as abrasive. Phosphate buffer solutions containing antioxidant substances including nicotine were used in mechanical inoculation procedures under greenhouse conditions.

ELISA

For immunological identification of isolated virus, two different ELISA procedure sets were used: commercially available, containing alkaline phosphatase (AP)-antibody conjugate (1) and prepared during this investigation, with horse radish peroxidase (HRP) as IgG coupling enzyme (2). The kit from Deutsche Samlung von Mikroorganismen und Zellkulturen GmbH (Germany) consisted of antibodies to Tobacco necrosis virus (strain D) and the antibodies conjugated with alkaline phosphatase. DAS-ELISA was conducted according to standard procedure using p-nitrophenyl phosphate substrate and the results were evaluated photometrically at 405 nm (Clark, Adams, 1977). For another variant of DAS-ELISA tests with components prepared during this investigation, antibodies in rabbit were raised against TNV isolate from plum tree purified as indicated in previous publication (Staniulis, 2003). The IgG fraction of the antiserum was obtained by ammonium sulfate precipitation, dialyzed against 10 mM Na carbonate buffer pH 9,5 and concentration adjusted to 5 mg/ml. Conjugation procedure of the antibodies with horse radish peroxidase (MERCK) was followed essentially as described by Nakane and Kawaoi (1974) and Clark et al. (1986). 5 mg peroxidase (HRP) was dissolved in 1 ml H_2O and 250 µl of 0,2 M freshly prepared water solution of $NaIO_4$ was added. After shaking for 30 min at room temperature in the dark, solution was dialyzed against 0,01 M Na acetate buffer pH 4,2 (or filtered through column of Sephadex G-25). pH of the solution was adjusted to 9,5 with 1 M Na_2CO_3. Peroxidase solution was mixed with antibodies from IgG fraction adjusted to pH 9,5. After incubation for 2 h at room temperature, 0,1 ml of 4 mg/ml $NaBH_4$ was added and incubated for 2 h at +4 °C. The conjugate was dialyzed against PBS, BSA (0,1%) was added and after mixing with 50% glycerol was stored at –20 °C. As a substrate for HRP 3,3', 5.5'-tetramethylbenzidine (TMB) TMB One ready-to-use (MBI Fermentas, Lithuania) was used. After addition of the substrate and incubation at room temperature for 30—60 min the enzyme reaction was stopped with 4% of H_2SO_4. The absorbance was read at 450 nm in microplate reader (Multiskan RC Labsystems, Finland).

RT-PCR

For molecular identification of polyhedral virus isolated from *Fragaria* and indicating similarity with TNV, polymerase chain reaction method was used (Saiki et al., 1988). Sequence-specific reverse transcription-polymerase chain reaction (RT-PCR) was adopted for identification of TNV isolate from strawberry (Robinson, 1992). For the detection of TNV by RT-PCR, frozen or fresh tissues of infected *Nicotiana rustica* or *Phaseolus vulgaris* plants were used. RNA extraction was carried out according to the instruction of "QuickPrep total RNA extraction kit for the direct isolation of total RNA from most eukaryotic tissues or cells". Tissue samples were grounded in liquid nitrogen and transferred to microfuge tubes. 150 µl volume of the extraction buffer was poured in the tube and 3 µl of 14.3 M 2-mercaptoethanol was added. 350 µl of lithium chloride (LiCl) and 500 µl of caesium trifluoracetate (CsTFA) solutions were added to the homogenized samples and mixed well. The tubes were spun for 15 min at 14000g. The RNA formed a pellet at the bottom of the microtubes. The proteins form a coat at the top of the tubes and DNA remains in the liquid phase. The protein coat and the liquid phase were carefully removed, and proceeded to wash the total RNA pellets with three "kit" components. The samples were spun in a microcentrifuge at 14000 g. The supernatant was carefuly discarded without disturbing the pellets. 1 ml of 70% ethanol to the samples was added. DEPC-treated (Diethyl Pyrocarbonate) water containing 1 µl of RNAse inhibitor was added to the RNA pellets. Primers used in RT-PCR were designed for TNV according to the sequences published in NCBI data bank. The primer pairs used in this investigation were synthesized according to the sequences of few TNV strains. Primers synthesized according to TNV „Nebraska" isolate RNA coat protein gene appeared most effective. Primer F included nucleotides from 910 to 930 (5' – ACA ATA GTC TCC AAC TCG GAG – 3'). Primer R was complimentary to nucleotides from 1192 to 1209 (5' – ATC ATA ACC TGC GTA AGG – 3'). Other two pairs used for another strains of TNV proved not efficient and are not indicated.

The dissolved RNAs were used in experiments for detection of TNV by RT-PCR. Pellets of RNA were resuspended in the solution containing RNAse inhibitor, primer R (reverse) and PCR water and incubated at 70 °C for

10 min. For the first strand cDNA synthesis the RNA pellet solutions to the mixture containing reaction buffer, RNAse inhibitor, dNTP mix and M-MLV reverse transcriptase (MBI Fermentas, Vilnius, Lithuania) were added. The first strand cDNA synthesis was carried out at 37 °C for 60 min and 70 °C for 10 min. DNA amplification was performed in reaction mixtures containing dNTP mix, both primers, PCR buffer with $MgCl_2$ and recombinant *Taq* polymerase (MBI Fermentas) using Eppendorf Mastercycler Personal. PCRs were carried out for 40 cycles using the following parameters: 1 min at 94 °C (4 min for the first cycle), 2 min at 55 °C and primers extension for 2 min (10 min in the final cycle) at 72 °C. PCRs products were analysed by electrophoresis in 5% polyacrylamide gel, stained with ethidium bromide, and DNA bands were visualized using a UV transilluminator. DNA fragment size (bp) standard was Phix174 RFI DNA (*Hae* III) digest (MBI Fermentas) (from top to bottom: 1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, 72).

Electron microscopy

Morphological properties of the isolated virus particles were observed in negatively stained with 2% uranyl acetate preparations for electronmicroscopic examination using JEOL JEM-100S transmission electron microscope. For immunosorbent electron microscopy the grids were sensibilized with antibodies to TNV-2 diluted 1:50.

Results and Discussion

During survey of virus diseases of horticultural plants in 2001, strawberry plants with slight mottling and rugosity symptoms were detected in strawberry plantation from Panevezys region. Virus-like symptoms exhibiting plant *Fragaria ananassa* contained polyhedral virus particles and therefore attempts were undertaken to isolate the virus. After few unsuccessful trials to transmit infection to herbaceous plants, infection succeeded using buffer containing antioxidants, 2% nicotine including. First symptoms appeared on inoculated leaves of *Nicotiana megalosiphon*, as a few local lesions. From this plant infection was transmitted to *Atriplex hortensis* (LL) (Fig. 1), *Tetragonia expansa* (LL), *Zinnia elegans* (LL), *Chenopodium amaranticolor* (LL) (Fig. 2), *Phaseolus vulgaris* 'Bataaf' (LL) (Fig. 3), *Nicotiana rustica* (LL), *Chenopodium foetidum* (LL). In all of inoculated and locally infected plants polyhedral virus particles were detected. However, experimentally infected *Fragaria* plants in greenhouse conditions revealed definit virus-like symptoms of slight rugosity and stunting (Fig. 4). Experimental host range and symptoms caused by this isolate (TNV-S) are presented in Table 1 and are characteristic of *Tobacco necrosis necrovirus* described by many previous investigations.

Fig. 1. Local lesions on *Atriplex hortensis* inoculated with TNV isolate from strawberry

Fig. 2. Local lesions on *Chenopodium amaranticolor* inoculated with TNV isolate from strawberry

Another successful isolation of polyhedral virus was from garden strawberry with leaf rugosity symptoms from Vilnius region (Ozkiniai). In this case, infection at first was transmitted to *Atriplex hortensis* (LL), later on to *Chenopodium quinoa, N. rustica* and other species causing local lesions only. According to preliminary data, this isolate revealed no differences from TNV-S and was not yet further investigated.

Table 1

Reaction of test plants to infection by virus isolate from strawberry

Plant species	Symptoms	Back inoculation
Amaranthus caudatus	LL/0	+
Atriplex hortensis	LL/0 (Fig. 1)	+
Capsicum annuum	LL/0	-
Celosia cristata	LL/0	+
Chenopodium amaranticolor	LL/0 (Fig. 2)	+
Chenopodium foetidum	LL/0	-
Ch. murale	LL/0	-
Chenopodium quinoa	LL/0	+
Ch. urbicum	LL/0	-
Cucumis sativus, cv. 'Kauniai'	LL/0	+
Datura stramonium	0/0 (LL/0)	-
Nicotiana clevelandii	LL/0	-
Nicotiana debneyi	LL/0	-
Nicotiana megalosiphon	LL/0	+
N. occidentalis	LL/0	+
N. rustica	LL/0	+
N. tabacum, cv. 'Xanthi NC'	LL/0	+
N. tabacum, cv. 'Samsun'	LL/0	-
Phaseolus vulgaris, cv. 'Bataaf'	LL/0 (Fig. 3)	+
Tetragonia expansa	LL/0	+
Zinnia elegans	LL/0	+

Note: LL local lesions, 0 – no infection, – not inoculated, + back inoculation.

Fig. 3. Local lesions on leaf of *Phaseolus vulgaris* cv. 'Bataaf' inoculated by TNV isolate from strawberry

Fig. 4. Symtoms on *Fragaria vesca* plants experimentally infected by TNV isolate from strawberry

For identification of TNV isolate from strawberry (TNV-S) in DAS-ELISA procedure 1 with AP as conjugate enzyme, the micro-plate (NUNC) wells were coated with antibodies to TNV (strain D) diluted to 1:1000. After washing antigen consisting of 1:20 diluted clarified by centrifugation at 10000g sap of TNV-S infected *N. rustica* with LL fresh or held refrigerated at –20 °C for several months was added. After appropriate washing, AP enzyme conjugate diluted to 1:1000 was used according to the manufacturer's instructions. A sample was scored positive if the A_{405} was twice that of the healthy control. It was found that in TNV-S infected plant extracts TNV was detectable up to dilution of 1:5000.

In DAS-ELISA procedure 2 using HRP as conjugation enzyme and TMB as substrate, the micro-plate wells were coated with IgG to TNV from plum (TNV-2) diluted to 1:500. HRP conjugate was used of dilution 1:400. Reading of absorption results at 450 nm indicated that it was possible to detect antigen to 5 ng/ml concentration and prepared HRP conjugate is suitable for diagnosis of TNV in infected plants.

Specific sequences of TNV RNA present in total RNA were detected following amplification by PCR. In RT-PCR using primer pair F (5' – ACA ATA GTC TCC AAC TCG GAG – 3') and R (5' – ATC ATA ACC TGC GTA

AGG – 3'), a product of expected size, approximately 300 bp, from extracts of TNV-2 and TNV-S infected *Tetrgonia expansa* and *Nicotiana rustica* plants was amplified (Fig. 5). It indicates that the primers used efficiently amplified TNV cDNA templates in RT-PCR irrespective of different plant source or TNV isolates used. The results suggest that both isolates investigated have close relationship with TNV 'Nebraska' isolate from group of A strains.

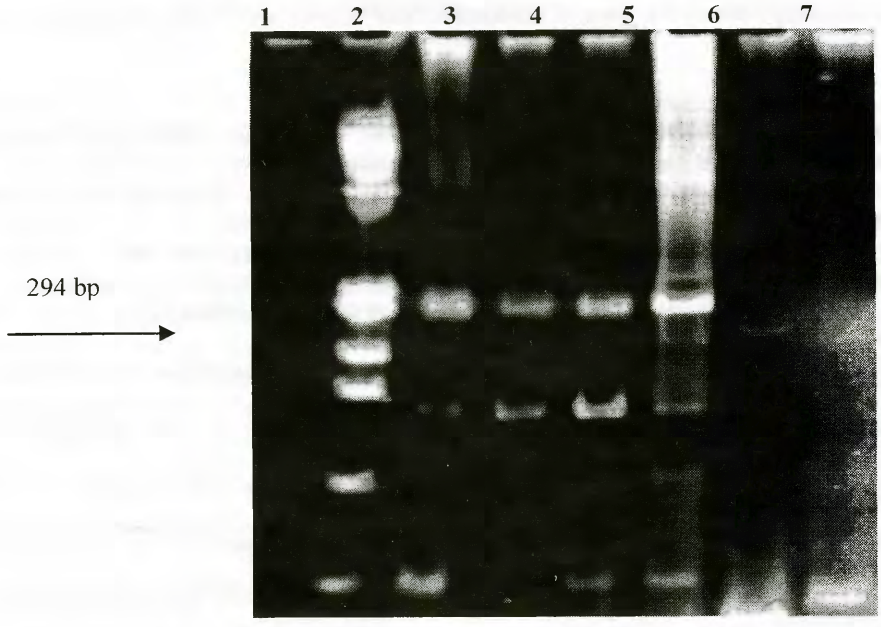

Fig. 5. Ethidium bromide stained 5% polyacrylamide gel electrophoresis of RT-PCR products of amplified TNV samples using primer pair specific for TNV strain A 'Nebraska' isolate. Lane 1 — DNA ladder — PhiX174 RFI DNAvHaeIII digest, fragment sizes (bp) from top to bottom: 1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, 72; Lane 2 and 3 — TNV isolate from plum (2 — from *T. expansa*, 3 — from *N. rustica*); Lane 4 and 5 — TNV isolate from strawberry (4 — from *T. expansa*, 5 — from *N. rustica*); Lane 6 — uninfected control; Lane 7 — water control

Electron microscopic observation of virus particles in preparations from plants infected with TNV-S revealed presence of polyhedral virus particles of 26—28 nm in diameter. Treatment of electron microscopic grids with antibodies to TNV-2 greatly increased the number of trapped virus particles from extracts of plants infected with TNV-S in comparison with untreated grids, indicating close affinity of the isolates. Instead of single particles, in case of untreated grids, they usually were spread in clusters (Fig. 6).

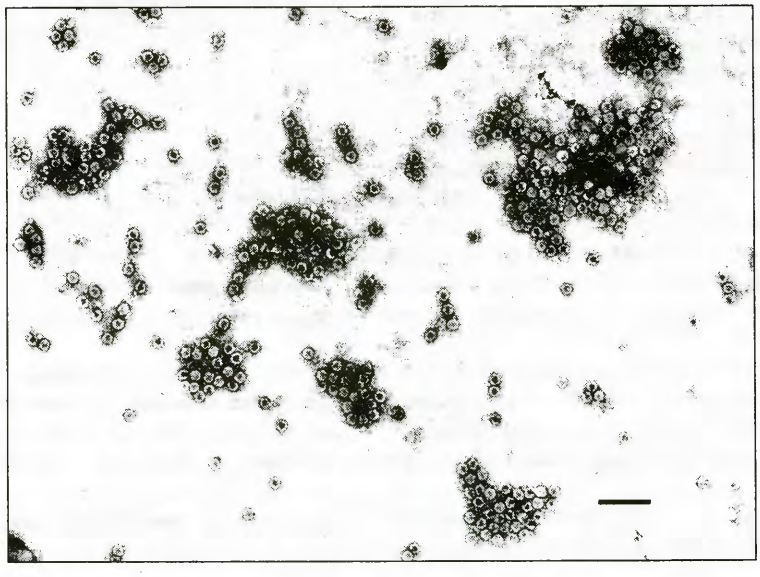

Fig. 6. Clusters of polyhedral virus particles TNV isolate from strawberry (TNV-S) in electron microscopic preparation from crude sap of *Nicotiana rustica* trapped on the grids treated with antibodies to TNV-2 isolate from plum. Scale bar represents 100 nm

In summary it can by concluded that from strawberry in Lithuania isolated polyhedral virus according to its biological, morphological properties, data of ELISA tests using two different sources of antibodies and conjugate, and RT-PCR results confirm virus identity with *tobacco necrosis virus*.

Acknowledgement

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References

1. Agrios, G. N. 1988. Plant Pathology. Academic Press, New York, 883 pp.
2. Adam, G., Winter, S., Lesemann, D.-E. 1990. Characterization of a new strain of tobacco necrosis virus isolated from nutrient feeding solution. Annals Applied Biology, V. 116, 523—526.
3. Brunt, A. A., Crabtree, K., Dallwitz, M. J., Gibbs, A. J., Watson, L. 1996. Viruses of Plants. Descriptions and Lists from the VIDE Database. CAB International. Cambridge University Press, 1256—1259.
4. Cardoni, M., Babini, A. R., Bissani, R. 2002. Occurrence and detection of strawberry viruses and virus-like diseases in Italy. J. Plant Pathology, V. 84(3), 171—178.
5. Clark, M.F., Adams, A.N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. General Virology, V. 34, 475—483.
6. Clark, M.F., Lister, R.M., Bar-Joseph, M. 1986. ELISA techniques. In: Methods in Ezymology, V. 118: Plant Molecular Biology. Acad. Press, 742—766.
7. Dulic-Markovic, I., Rankovic, M., Converse, R.H. 1998. Occurrence of strawberry viruses in Yugoslavia. Acta Horticulturae, N. 471, 35—37.
8. Edwardson, J.R., Christie, R.G. 1997. Viruses infecting peppers and other solanaceous crops. Vol. 1, University of Florida, Monograph 18—1, Gainesville, 320—336.
9. Franova, J. 2001. Occurrence of graft-transmissible virus diseases of the strawberry in the Czech Republic. Acta Virologica, V. 45, 151—157.
10. Franova-Honetšlegrova, J., Špak, J., Erbenova, M., Nebesarova, J., Martin, R.R. 1998. Detection and identification of tobacco necrosis virus in strawberry leaves in the Czech Republic. Acta Horticulturae, N. 471, 39—43.
11. Franova, J., Mraz, I., Petrzik, K., Špak, J., Šip, M., Bertacini, A., Erbenova, M., Karešova, R. 2001. The occurrence of strawberry viruses and phytoplasmas in the Czech Republic. Acta Horticulturae, N. 551, 81—86.
12. Frazier, N.W. 1955. Tobacco necrosis virus on strawberries. Plant Disease Reporter, V. 39, 143—147.
13. Frazier, N.W. 1974. Detection of graft-transmissible diseases in strawberry by a modified leaf grafting technique. Plant Disease Reporter, V. 58, 203—207.
14. Fulton, J. P. 1952. A tobacco necrosis virus associated with strawberry plants. Plant Disease Reporter, V. 36, 313—314.
15. Kassanis, B. 1970. Tobacco necrosis virus. CMI/AAB Descriptions of Plant Viruses, No. 14, p. 4.
16. Kassanis, B., MacFarlane, I. 1964. Transmission of tobacco necrosis virus by zoosspores of Olpidium brassicae. Journal of General Microbiology. Vol. 36, 79—93.
17. Kegler, H., Proll, E., Schmidt, H.B., Opel, H. 1969. Nachweis des Tabaknekrosevirus (tobacco necrosis virus) in Obstgeholzen. Phytopathologische Zeitschrift, B. 65(1), 21—42.
18. Martin, R. R., Heflebower, R. F., Hokanson, S. C, Maas, J. L., Rouse, R. 2001. Survey of strawberry viruses occurring in commercial plantings in the State of Maryland, USA. Acta Horticulturae, N. 551, 71—74.
19. Nakane, P.K., Kawaoi, A. 1974. Peroxidase labeled antibody a new method of conjugation. J. Hystochemistry and Cytochemistry, V. 22, N. 12, 1084—1091.
20. Paulechova, K. 1976. Evidence of the occurrence of tobacco necrosis virus in plum trees. Plant Virology. Proceedings of the 8th conference of Czechoslovak plant virologists, Bratislava, 387—394.
21. Paulechova, K., Baumgartnerova, H. 1980. Some properties of tobacco necrosis virus isolated from plums. Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15(1—4), 119—122.
22. Robinson, D.J. 1992. Detection of tobacco rattle virus by reverse transcription and polymerase chain reaction. J. Virological Methods, V. 40, 57—66.
23. Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B., Erlich, H.A. 1988. Primer-directed enzymatic amplification of DNA with a thermostabile DNA polymerase. Science, V. 239, 487—491.
24. Spiegel, S. 1998. The strawberry virus program in Israel — recent progress and future prospects. Acta Horticulturae, N. 471, 57—60.
25. Staniulis, J. 2003. Detection of Tobacco necrosis virus in horticultural plants. Scientific Works of the Lithuanian Institute of Horticulture and Lithuanian University of Agriculture. Horticulture and Vegetable Growing, V. 22(3), 72—82.
26. Staniulis, J., Rabenstein, F. 2002. Isolation of Tobacco necrosis virus from plum trees infected by Plum pox potyvirus. Abstracts of VIIIth International Plant Virus Epidemiology Symposium. Aschersleben, Germany, 12—17 May, 2002, p. 120.
27. Stankiene, J., Cieslinska, M. 2003. Ocurrence of strawberry viruses and phytoplasmas in Lithuania. Scientific Works of the Lithuanian Institute of Horticulture and Lithuanian University of Agriculture. Horticulture and Vegetable Growing, V. 22(1), 78—85.
28. Šutič, D.D., Ford, R.E., Tošič, M.T. 1999. Handbook of Plant Virus Diseases. CRC Press, New York, 321—432.
29. Tomlinson, J.A., Faithfull, E.M., Webb, M.J.W., Fraser, R.S.S., Seeley, N.D. 1983. Chenopodium necrosis: a distinctive strain of tobacco necrosis virus isolated from river water. Annals Applied Biology, Vol. 102, 135—147.
30. Uyemoto, J.K. 1981. Tobacco necrosis and satellite viruses. In: Handbook of Plant Virus Infections and Comparative Diagnosis (E.Kurstak ed.). Elsevier/North Holland Biomedical Press, New York, 123—146.

DETECTION OF INJURIOUS VIRUSES IN TOMATOES

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Abstract

Samples of tomatoes exhibiting symptoms characteristic of viral diseases were collected in various growing places and at a supermarket. The investigation of virus diseases of tomato crops in Lithuania revealed that some agents of tomato virus diseases have been brought to this country with seed material and commercial production. *Tomato ringspot nepovirus* (ToRSV) and *Pepino mosaic potexvirus* (PepMV) as harmful for tomatoes viruses were isolated and identified. PepMV was inscribed in EPPO Alert List as a phytosanitary risk for Europe and as an economically important for tomato and other solanaceous crops. ToRSV was detected in tomato cultivars 'Olan', 'Dombello', 'Raissa' and 'Sonata', which showed specific ringspotting symptoms on leaves. This virus infected a wide range of experimental host-plants and induced on them typical severe symptoms. Lithuanian State Plant Protection Service has carried out a survey for the presence of PepMV since 2001. In 2002, PepMV was detected on tomato fruits imported from Spain and the Netherlands at a supermarket in Vilnius.

The identification of ToRSV and PepMV was based on the data of symptoms on host- and test plants, morphology of virus particles by transmission electron microscopy, positive DAS-ELISA tests and by using of specific nucleotide primers in RT-PCR. Antigens have been purified, the specific immunoantisera were prepared in rabbits and the immunodiagnostical assays were carried out.

Key words: tomato, *Tomato ringspot virus*, *Pepino mosaic virus*.

Introduction

Among others, widespread virus disease agents are *Tomato ringspot nepovirus* (ToRSV) and *Pepino mosaic potexvirus* (PepMV) reported as economically important pathogens for tomato (*Lycopersicon esculentum* Mill.) crops. ToRSV is a member of the *Nepovirus* group of plant viruses and causes economically important diseases in a range of crops. ToRSV is found in many perennial crops in North America, in Europe, Asia, Australia and South America (Brunt et al., 1996). It affects 285 plant species in 159 genus of 55 botanical families (Edwardson, Christie, 1997). ToRSV is readily transmissible by sap inoculation. It is transmitted by nematode. Seed transmission of ToRSV has been reported in several crops. The virus is also transmitted by vegetative propagation and pollen. The virions are icosahedral, about 28 nm in diameter, sedimenting as three components. The most characteristic type of foliar symptom induced by ToRSV is ring spotting.of leaves. This virus was isolated from many ornamental (Navalinskienė et al., 1997) and cucurbitaceous species (Zitikaitė, 1999) in Lithuania.

PepMV is an important new pathogen infecting tomato and has become a serious problem for tomato production in Europe (Van der Vlugt et al., 2002). Originally PepMV was isolated from infected pepino (*Solanum muricatum* Ait.) plants in the regions of Peru (Jones et al., 1980). Its experimental host range is narrow, infecting 30 of 32 solanaceous species including potato and tomato. PepMV occurrence has been confirmed in many countries of EC (Sweden, Finland, Germany, Austria, Belgium, France, Italy, Portugal, and UK). The virus is highly contagious and rapidly spreads by mechanical means. It is unlikely that PepMV has true seed transmission. This virus was naturally present in wild *Lycopersicon* sp. as well as in cultured tomato (Soler et al., 2002). PepMV is not yet considered as a quarantine pathogen in Europe but is inscribed in EPPO Alert List (Anonymous, 2000).

The aim of this study was to present data on occurence and identification of ToRSV and PepMV isolates in Lithuania based on the results of host range and morphology of virus particles, immunological properties of antigens, and to adapt a sensitive and accurate molecular method for specific detection of ToRSV and PepMV in tomato plants and test plants by RT-PCR technique.

Materials and Methods

The material for investigation was collected in different private gardens of vegetable crops, in vegetable collections and experimental fields. The samples were collected from tomato plants expressing visual virus-like symptoms on leaves and fruits. Viruses have been identified by test-plant reaction and of virus particle morphology, double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA). DAS-ELISA was carried out according to standard protocol (Clark, Adams, 1977). The inoculum for mechanical inoculation was prepared by homogenizing infected plant material with 0.1 M phosphate buffer, pH 7.0, containing stabilizing agents 0.02% 2-mercapthoethanol or 0.01 M Na DIECA. Virus particles were visualized in negatively stained dip preparations using JEOL JEM-100S transmission electron microscope (EM) (Dijkstra, de Jager, 1998). The following ToRSV isolates were used: isolates from tomato detected in Kėdainiai (9801) and Vilnius (0011) regions. For antigen preparation, ToRSV isolate was purified by a modified method (Stace-Smith, 1984). Frozen leaves of *Nicotiana rustica* L. showing systemic symptoms of ToRSV infection were grinded in 0.2 M phosphate buffer (pH 7.2) containing 0.1% 2-mercaptoethanol. The extract was clarified by 10% (volume) chloroform. Virus particles were precipitated with w/v 10% polyethylene glycol (PEG)

(M 6000) and 0.2 M Na chloride. Final purification was accomplished by two cycles of differential centrifugation (10 min at 8000 g in K-24 centrifuge, and 3 h at 27000 g in VAC-601) by sedimentation through 20% sucrose cushion.

The PepMV isolate was maintained in test plants and purified according to Jones et al. (1980) with slight modification by homogenising of frozen systemically-affected *L. esculentum* leaves in buffer solution containing 0.05 M disodium tetraborate, 0.5 M boric acid, 0.2% ascorbic acid, and 0.2% sodium sulphite, at pH 7.8. The homogenate was centrifuged for 20 min at 6000 g. 4% chlorophorm was added to the supernatant fluid. The mixture was stirred for 3 h at room temperature. This solution was centrifuged for 20 min at 6000 g. Then 4% PEG was added to the supernatant fluid and stored at 4 °C overnight. After centrifugation the precipitate was collected and resuspended in a solution containing 0.05 M disodium tetraborate, 0.5 M boric acid, 0.5 M urea and 0.1 % 2-mercaptoethanol at pH 7.8. After three cycles of differential centrifugation the final pellet was resuspended in 0.01 M Tris-HCl buffer, pH 7.8. The concentration and purity of ToRSV and PepMV preparations were estimated by EM and spectrophotometrically.

For preparation of specific antisera against ToRSV and PepMV rabbits were immunized using schemes (Vaitukaitis et al., 1971; Jones et al., 1980; Gnutova, 1985).

For detection of ToRSV isolate by RT-PCR, the infected *N. rustica* plant was used. Nucleic acid from test-plants infected by ToRSV was extracted using the small-scale procedure as proposed for extraction of nucleic acids from woody plants (Zhang et al., 1998) with slight modifications. Tissue samples of infected test-plants were ground in liquid nitrogen and transferred to microtubes. 600 µl 1xSTE buffer (0.1 M Na Cl, 0.001 M Tris, 0.001 EDTA, pH 6.9), 80 µl of 10% SDS and and 800 µl of 2xSTE-saturated phenol was added to the powdered tissues. The mixture was centrifuged 5 min at 16000 g. Aqueous phase was removed and transferred to a clean microfuge tube. Ethanol to the final concentration of 30% was added, then ~10 mg cellulose (whatman CF-11). Cellulose CF-11 was washed by vortexing 3 times with 1 ml of 1xSTE/30% ethanol, collecting cellulose by centrifugation between washes and discarding supernatants. RNA from cellulose CF-11 was eluted by adding 200 µl of 1xSTE buffer, and centrifugation for 5 min. Supernatant was transferred to a clean tube. For precipitation of RNA, 40 µl of 3 M sodium acetate and 1 ml of ethanol were added. The tube was incubated at –20 °C for 2 h., centrifuged for 10 min. at 16000 g, and the pellet was incubated with 80% of ethanol at –20 °C, and air-dried. Pellets of RNA were resuspended in the mixture containing 1% of RNAse inhibitor, 0.4 µM of primer D1 and PCR water. Primers used in RT-PCR were designed from ToRSV viral sequence information (Griesbach, 1995). Primers included U1, (5' to 3') GACGAAGTTATCAATGGCAGC nt 1.078 to 1.098) and D1, TCCGTCCAATCACGCGAAT (nt 1.506 to 1.527) of the putative viral polymerase gene.

For detection of PepMV by RT-PCR from imported tomato fruits, frozen tissues of infected tomato fruits and experimentally inoculated *D. stramonium L.* and *N. debneyi* Domin. plants were used. RNA extraction was carried out according to the instruction of "QuickPrep total RNA extraction kit for the direct isolation of total RNA from most eukaryotic tissues or cells". Frozen tissue samples were grounded in liquid nitrogen and transferred to microfuge tubes. 150 µl volume of the extraction buffer was poured in the tube and 3 µl of 14.3 M 2-mercaptoethanol was added. 350 µl of lithium chloride (LiCl) and 500 µl of caesium trifluoracetate (CsTFA) solutions were added to the homogenized samples and mixed well. The tubes were spun for 15 min. The RNA formed a pellet at the bottom of the microtubes. The proteins form a coat at the top of the tubes and DNA remains in the liquid phase. The protein coat and the liquid phase were carefully removed, and proceeded to wash the total RNA pellets with three "kit" components. The samples were spun in a microcentrifuge at full speed. The supernatants were discarded without disturbing the pellets. 1 ml of 70% ethanol was added to the samples. DEPC-treated (Diethyl Pyrocarbonate) water containing 1 µl of RNAse inhibitor was added to the RNA pellets. Primers used in RT-PCR were designed for PepMV isolate from the UK according to sequence information (Mumford, Metcalfe, 2001). They included TGB F (Forward) (5' to 3') CACACCAGAAGTGCTTAAAGCA and UTR R (5' to 3') CTCTGATTAAAGTTTCGAGTG.

The dissolved RNAs were used in experiments for detection of ToRSV and PepMV by RT-PCR. Pellets of RNA were resuspended in the solution containing RNAse inhibitor, primer R (reverse) and PCR water and incubated at 70 °C for 10 min. For the first strand cDNA synthesis, the RNA pellet solutions to the mixture containing reaction buffer, RNAse inhibitor, dNTP mix and M-MLV reverse transcriptase (MBI Fermentas, Vilnius, Lithuania) were added. The first strand cDNA synthesis was carried out at 37 °C for 60 min and 70 °C for 10 min. DNA amplifications were performed in reaction mixtures containing dNTP mix, both primers, PCR buffer with $MgCl_2$ and recombinant *Taq* polymerase (MBI Fermentas) using Eppendorf Mastercycler Personal. PCRs were carried out for 40 cycles using the following parameters: 1 min at 94 °C (4 min for the first cycle), 2 min at 55 °C and primers extension for 2 min (10 min in the final cycle) at 72 °C. PCRs products were analysed by electrophoresis in 5% polyacrylamide gel, stained with ethidium bromide, and DNA bands were visualized using a UV transilluminator. DNA fragment size (bp) standard was Phix174 RFI DNA (*Hae* III) digest (MBI Fermentas) (from top to bottom: 1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, 72).

Results and Discussion

ToRSV was isolated and identified from two naturally infected tomato plants expressing specific for this virus symptoms. Naturally infected tomato plants showed mottling, chlorotic or necrotic spots on leaves. This virus induced typical severe symptoms on the main diagnostic plant species: *Chenopodium ambrosioides* L., and *Celosia argentea* f. *cristata* (L.) Kuntze (necrotic local lesions and systemic apical deformation); *Gomphrena globosa* L. (local white ring spots); *N. rustica* (local ring flecks and systemic mottling). Our assays determined that ToRSV infected a wide experimental host range from *Aizoaceae* Rudolphi, *Amaranthaceae* Juss., *Cucurbitaceae* Juss., *Chenopodiaceae* Vent.,

Fabaceae Lindl., *Solanaceae* Juss. and other families. EM investigation revealed isometric virus particles about 28 nm in diameter in preparations made from naturally infected tomato plants, and from various infected test-plants.

ToRSV tomato isolate (N 9801) was purified. For purification, ToRSV isolate was propagated in *N. rustica.* The purified virus preparations had A max at 260 nm and A min at 240 nm, A 260/280 ratio being 1.07. The yield of purified virus, taking into account that ToRSV specific absorbance A 0,1% / 260 nm being 10.0 (Stace-Smith, 1984), was calculated to be 26.7 mg from 1 kg of infected plant tissue. Partially purified virus preparation contained isometric particles about 28 nm in diameter (Fig. 1).

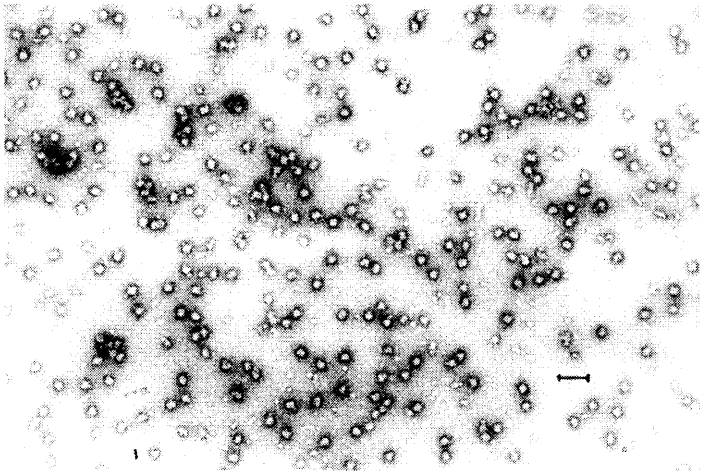

Fig. 1. Purified ToRSV particles from infected *Nicotiana rustica* plants. Bar represents 100 nm

In commercial lots of tomato fruits imported to Lithuania from Spain, some tomato fruits have been found showing irregular discoloration pattern and surface roughness: small spots, yellow blotches and areas, bright mottling, striation and yellow ring-like pattern. Analysis of samples from the tomato fruits by test plant and EM revealed presence of a new agent of virus disease of tomato recently detected in tomato crops of many European countries — PepMV. The virus isolates from tomato fruits systemically infected plants of all investigated tomato cultivars. The reaction of inoculated test plants to the virus was characteristic to PepMV recently described by other workers (Jorda et al., 2001; Soler et al., 2002). According them, symptoms consist of distorted leaf and discolored fruit development. Other plants show a dark green mosaic and bubbling of the leaf surface. EM revealed filamentous virus particles with normal length of about 500 nm in preparations made from test-plants: *L. esculentum, D. stramonium* L., *N. debneyi* Domin. The virus had filamentous particles typical of the *Potexvirus* group (Jones et al., 1980; Purcifull, Edwardson, 1981).

PepMV isolate propagated in *L. esculentum* plants was chosen for the purification of antigen, because of higher virus concentration. The purified virus preparations had A max at 260 nm and A min at 240 nm, A 260/280 ratio being 1.04-1.08. The yield of purified virus, taking into account PepMV specific absorbance A 0.1% / 260 nm for *Potexviruses* being 3 (Purcifull, Edwardson, 1981), was calculated to be 46.2—75.6 mg from 1 kg of infected *L. esculentum* plant tissue. Morphology of detected virus particles (filamentous about 500 nm in length) was characteristic of PepMV (Fig. 2) and in accordance with data of other workers (Jones et al., 1980; Brunt et al., 1996).

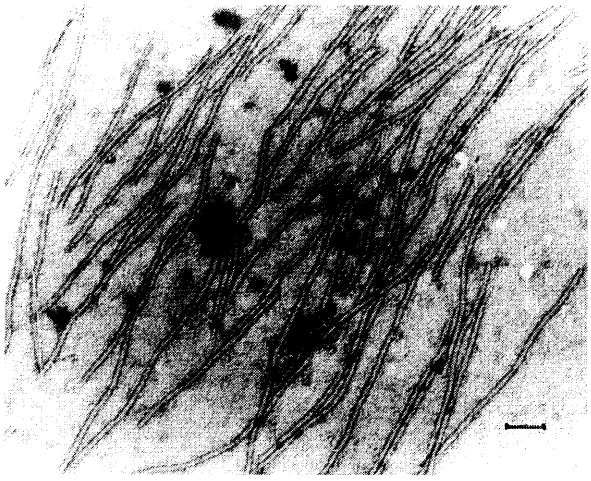

Fig. 2. Purified PepMV particles from infected tomato plants. Bar represents 100 nm

Identification of ToRSV and PepMV isolated from tomato plants was confirmed by a positive reaction in DAS-ELISA tests. The positive results in these tests for detection of ToRSV were obtained also with inoculated test-plants *N. rustica* (isolate N 9801) and *G. globosa* (isolate N 0011). Analysis of samples from the tomato fruits and inoculated test plants *D. stramonium* (isolate N 0236) and *N. debneyi* (isolate N 0232) by DAS-ELISA confirmed the presence PepMV in Lithuanian isolates from tomato.

The purified ToRSV and PepMV preparations were used as antigens for diagnostic antisera preparations in rabbits. The antiserum to ToRSV had a very low titre of 1:16, as determined by gel diffusion test and gave a precipitation line with the homologous antigen, but did not react visibly with healthy plant extracts. The titre of the PepMV specific IgG (immunoglobulin) in serological reactions of the microprecipitation test was found to be 1:1024. Our prepared antiserum against PepMV was applied successfully for diagnostic of PepMV in commercial tomato fruits.

For molecular confirmation of detection of ToRSV and PepMV in tomato by RT-PCR, Lithuanian isolates of ToRSV and PepMV were used. The primer pairs designed on basis of published sequences successfully amplified ToRSV and PepMV cDNA templates in RT-PCR using ToRSV and PepMV isolates from tomato crops. The specific PCR product was obtained in all investigated isolates but not with negative control. Specific bands were observed in gel electrophoretic analysis at the position corresponding to the expected size of the amplification products of 499 bp for ToRSV (Fig. 3) and of about 840 bp for PepMV (Fig. 4). RT-PCR products of ToRSV and PepMV isolates from tomato crops showed identity with different isolates of ToRSV from perennial ornamental and cucurbitaceous species in Lithuania (Samuitienė et al., 2003) and with European PepMV isolates (Mumford, Metcalfe, 2001).

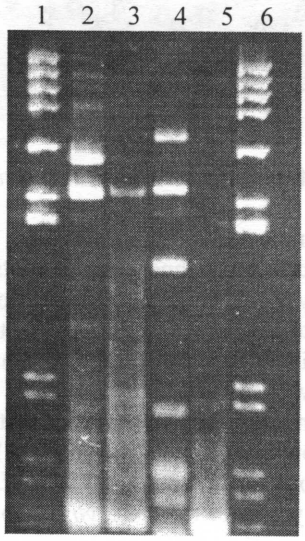

Fig. 3. Gel electrophoresis of PCR product (499 bp) of an amplified ToRSV tomato sample. Lane 1 and Lane 6 — DNA ladder; Lane 2 — ToRSV isolated from cucumber; Lane 3 — ToRSV isolated from tomato and infected *N. rustica* tissue; Lane 4 — ToRSV isolated from iris; Lane 5 — control

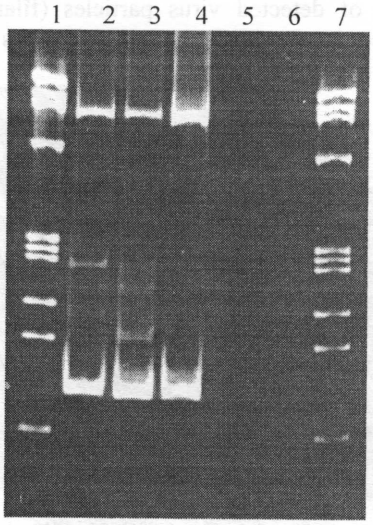

Fig. 4. DNA products (840 bp) amplified in PCR from plant samples infected by PepMV. Lane 1 and Lane 7 — DNA ladder; Lane 2 — PepMV from naturally infected tomato fruits; Lane 3 — PepMV infected *D. stramonium* tissue; Lane 4 — PepMV infected *N. debneyi* tissue; Lane 5 and Lane 6 — controls

The investigation of virus diseases of tomato crops in Lithuania revealed that some agents of tomato virus diseases have been brought to this country with seed material and commercial production. ToRSV and PepMV affected tomato crops are important and risk pathogens. The RT-PCR data confirmed results of ToRSV and PepMV identification obtained by investigating host range, virus morphological properties and results of DAS-ELISA assays. One of the important tasks of plant quarantine service is to prevent the introduction of harmful viruses into the country.

References

1. Anonymous. 2000. Addition of *Pepino mosaic potexvirus* to the EPPO Alert List. EPPO Report, 1.
2. Brunt, A.A., Crabtree, K., Dallwitz, M.J., Gibbs, A.J., Watson, L. 1996: Pepino mosaic virus; Tomato ringspot virus. Viruses of Plants. Descriptions and Lists from the VIDE Database, Cambridge, 942—943; 1309—131,.
3. Dijkstra, J., de Jager, C.P. 1998. Practical Plant Virology. Protocols and Exercises. Berlin-Heidelberg.
4. Clark, M.F., Adams, A.N. 1977. Characteristic of the microplate method of enzyme linked immunosorbent assay for the detection of plant viruses. J. Gen. Virology, 34, 475—483.
5. Edwardson, J.R., Christie, R.G. 1997. Viruses infecting peppers and other solanaceous crops. University of Florida.
6. Griesbach, J.A. 1995. Detection of Tomato ringspot virus by Polymerase Chain Reaction. Plant Disease, 79(10), 1054—1056.
7. Jones, R.A.C., Koenig, R., Lesemann, D.E. 1980. Pepino mosaic virus, a new potexvirus from pepino (*Solanum muricatum*). Ann. Appl. Biol., 94, 61—68.
8. Jorda, C., Lazaro, P.A., Martinez, P.V., Lacasa, A. 2001. First report of *Pepino mosaic virus* on natural hosts. Plant Disease, 85(12), 1292.
9. Mumford, R.A., Metcalfe, E.J. 2001. The partial sequencing of the genomic RNA of a UK isolate of *Pepino mosaic virus* and the comparison of the coat protein sequence with other isolates from Europe and Peru. Arch. Virol., 146, 2455—2460.
10. Navalinskienė, M., Samuitienė, M., Jackevičienė, E. 1997. Pomidorų žiediškosios dėmėtligės viruso identifikavimas iš daugiamečių gėlių. Ecological effects of microorganism action. Vilnius, 276—279.
11. Purcifull, D.E., Edwardson, J.R. 1981. Potexviruses. In: Kurstak (ed): Handbook of Plant virus infections. Comparative diagnosis. Amsterdam, 627—694.
12. Samuitienė, M., Zitikaitė, I., Navalinskienė, M., Valiūnas, D. 2003. Identification of tomato ringspot nepovirus by RT-PCR. Biologija, 4, 35—38.
13. Soler, S., Prohens, J., Diez, M.J., Nuez, F. 2002. Natural occurrence of *Pepino mosaic virus* in *Lycopersicon* species in central and southern Peru. J. Phytopathology, 150(2), 49—53.
14. Stace-Smith, R. 1984. Tomato ringspot virus. C.M.I./A.A.B. Descriptions of Plant viruses, p. 290. (No. 18 revised.)
15. Vaitukaitis, J., Robbins, J.B., Niesclag, E., Ross, G.T. 1971. A method for producing specific antisera with small doses of immunogen. J. Clin. Endocriol., 33(6), 988—991.
16. Van der Vlugt, R.A.A., Cuperus, C., Vink, J., Stijger, I.C.M.M., Lesemann, D.-E., Verhoeven, J.Th.J., Roenhorst, J.W. 2002. Identification and characterizaton of Pepino mosaic potexvirus in tomato. OEPP/EPPO Bulletin, 32, 503—508.
17. Zhang, Y.-P., Uyemoto, J.K., Kirkpatrick, B.C. 1998. A small-scale procedure for extracting nucleic acids from woody plants infected with various phytopathogens for PCR assay. Journal of Virological Methods, 71, 45—50.
18. Zitikaitė, I. 1999. Pomidorų žiediškosios dėmėtligės virusas daržovėse. Botanica Lithuanica, suppl. 3, 73—78.
19. Гнутова, Р.В. 1985. Иммунологические исследования в фитовиросологии. Москва, 183 с.

IDENTIFICATION OF VIRUSES AND PHYTOPLASMA INFECTING DELPHINIUM (*DELPHINIUM* L.) PLANTS IN LITHUANIA

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Abstract

Plants of delphinium (*Delphinium* L.) exhibiting symptoms characteristic of viral and phytoplasmal diseases were collected at Botanical gardens and different floriculture farms in Lithuania. Viral diseases were expressed by symptoms of leaf distortion, mottling, light green or yellow ringspotting. The causal agents of viral diseases, *Cucumber mosaic cucumovirus* (CMV) and *Tomato ringspot nepovirus* (ToRSV), were isolated and identified by the methods of test-plants and electron microscopy. Phytoplasmal symptoms of delphinium included general yellowing and stunting of plants, and virescence of flowers. Amplification of phytoplasmal 16S rRNA gene sequence, in polymerase chain reactions (PCRs) containing phytoplasma universal primer pair R16F2n/R2 and template DNA extracted from diseased plants, confirmed that the plants were infected by phytoplasma. The 1.2 kbp 16S rDNA product was subjected to single enzyme digestions with 10 different restriction endonucleases. RFLP analysis revealed that the delphinium plants were infected by a phytoplasma belonging to group 16SrI (aster yellows phytoplasma group), subgroup B (I-B, aster yellows subgroup).

Key words: delphinium, identification, virus, phytoplasma.

Introduction

The genus of delphinium (*Delphinium* L.) is a member of *Ranunculaceae* Juss. family and includes about 250 species which as annual and perennial herbaceous plants spontaneously grow in Europe, North and South America, Asia, Africa and Australia. The most decorative species of delphinium are grown as garden flowers. This flower has been grown since ancient times and is a popular garden flower in Lithuania. Today there are several new cultivars of delphinium suitable to grow as cut flowers and for garden planting.

Several viruses affecting delphinium were isolated and described in Great Britain: *Arabis mosaic nepovirus, Broad bean wilt fabavirus, Cherry leaf roll nepovirus, Cucumber mosaic cucumovirus, Potato virus X potexvirus, Raspberry ringspot nepovirus,* and *Strawberry latent ringspot nepovirus* (Ahmed, Bailiss, 1975). Disease of phytoplasma etiology on delphinium expressed by symptoms of flower virescence and phyllody was described in Germany (Marwitz, Petzold, 1976). In Lithuania, delphinium plants showing characteristic of phytoplasmal diseases symptoms were observed for the first time in 1969, later diseased plants have been found frequently (Макутенайте-Навалинскене, 1981). Phytoplasmas were first studied by the use of electron microscopy (EM) and were detected in more than 40 herbaceous plant species (Staniulis, 1988). EM enabled to establish the etiology of disease, but not to identify and estimate biodiversity of phytoplasmas. Recently molecular methods have been used to detect and identify diverse phytoplasmas associated with diseases of plants in Lithuania. Phytoplasmas associated with diseases of vegetables, legumes, forest trees, weeds, and cereals have been identified and classified on basis of 16 S rRNA gene sequence analyses (Jomantiene et al., 2000, 2002; Valiunas et al., 2000, 2001a,b; Staniulis et al., 2000; Valiūnas, 2003). Phytoplasmas were similarly identified in ornamental plants including primula, phlox, and hyacinth (Valiunas et al., 2000a; Alminaite et al., 2001), daisy (Samuitiene et al., 2002), and hellebore (Navalinskiene et al., 2003).

The objective of this study was to determine possible association of viruses and phytoplasma with diseases in delphinium and to identify the causal agents.

Materials and Methods

The plant material for investigation was collected at Botanical Gardens of Vilnius and Kaunas Vytautas Magnus Universities, Experimental Station of Field Floriculture and other floriculture farms in Lithuania. The experimental work was carried out at the Plant virus laboratory of the Institute of Botany. Viruses have been identified by electron microscopy (EM) negative staining technique (Robinson et al., 1987; Dijkstra, de Jager, 1998) and test-plant method (Bos, 1983; Stace-Smith, 1984; Francki et al., 1979; Brunt et al., 1996). The inocula for mechanical inoculation were prepared by homogenizing infected leaves with 0.1 M phosphate buffer (pH 7.0), containing as virus-stabilizing additives 0.2% 2-mercaptoethanol or 0.01 M sodium diethyldithiocarbamate. The following test-plants were used: *Amaranthus caudatus* L., *A. paniculatus* L., *Atriplex hortensis* L., *Celosia argentea f. cristata* (L.) Kuntze, *Chenopodium amaranticolor* Coste et Reyn, *C. ambrosioides* L., *C. hybridum* L., *C. quinoa* Willd., *C. urbicum* L., *Cucumis sativus* L.'Rodničok' 'Delikates', *Datura stramonium* L., *Gomphrena globosa* L., *Lycopersicon esculentum* Mill., *Nicandra physalodes* (L.) Gaertn., *Nicotiana alata* Link et Otto, *N. glutinosa* L. , *N. rustica* L., *N. tabacum* L. 'Samsun', 'Xanthi', *Petunia hybrida* Vilm., *Tetragonia expansa* Murr., *Zinnia elegans* Jack.

Phytoplasma was detected in polymerase chain reactions (PCRs). Nucleic acid for use as a template in PCR was extracted from the frozen tissue using the Fermentas Genomic DNA Purification Kit (Fermentas AB, Vilnius, Lithuania). Ribosomal (r) DNA was amplified in a nested PCR (Jomantiene et al., 1998 a). In the nested PCR, phytoplasmal rDNA was initially primed by primer pair P1/P7 (Deng, Hiruki, 1991). The amplified DNA product was diluted 1 : 50 with sterile water and used as template in the second (nested) PCR primed by primer pair R16F2n/R2

(Gundersen, Lee, 1996). All PCRs were carried out for 35 cycles using the following parameters: 1 min (3 min for the first cycle) denaturation at 94 °C, annealing for 2 min at 55 °C, and primer extension for 3 min (10 min in final cycle) at 72 °C in Perkin Elmer PCR buffer, 0.25 mM dNTP, 0.4μM of each primer, and 1 unit of recombinant *Taq* polymerase per 50 μl of reaction mixture. Resulting PCR products were analysed employing electrophoresis through 1% agarose gel stained with ethidium bromide, and DNA bands were visualized using an UV transilluminator. DNA fragment size standard was PhiX174 RFI DNA *Hae*III digest (Fermentas AB).

Products, from nested PCR primed by R16F2n/R2, were analysed by single enzyme digestion, according to manufacturer's instructions with 10 different restriction endonucleases: *Alu*I, Mse*I, Rsa*I, *Hpa*II, *Hae*III, *Hinf*I, *Sau3A*I, *Hha*I, *Kpn*I, and *Taq*I (Fermentas AB). The RFLP profiles of digested DNA were analysed by electrophoresis through 5% polyacrilamide gel, stained with ethidium bromide, and visualised using UV transilluminator. RFLP patterns were compared with previously published (Jomantiene et al., 1998a, b; Lee et al., 1998; Marcone et al., 2000).

Results and Discussion

Cucumber mosaic cucumovirus (CMV)

CMV was isolated from delphinium plants bearing conspiquous viral symptoms on leaves. Pattern of chlorotic concentric commonly irregular rings, 1—5 mm in diameter, with yellow or green areas in center appeared on young leaves of delphinium. There were chlorotic areas between rings, which were mostly arranged at the margins of leaf lamina, but sometimes covered all leaf. Symptoms made progress later in season, rings became larger extending till 10 mm in diameter, chlorotic patches became necrotic, necrosis extended until the whole leaf was killed. Considerable variation on symptom expression could be noticed depending on delphinium cultivar. Symptoms could be milder, expressed by light green to yellowish sinuous line pattern on the leaves (Fig. 1).

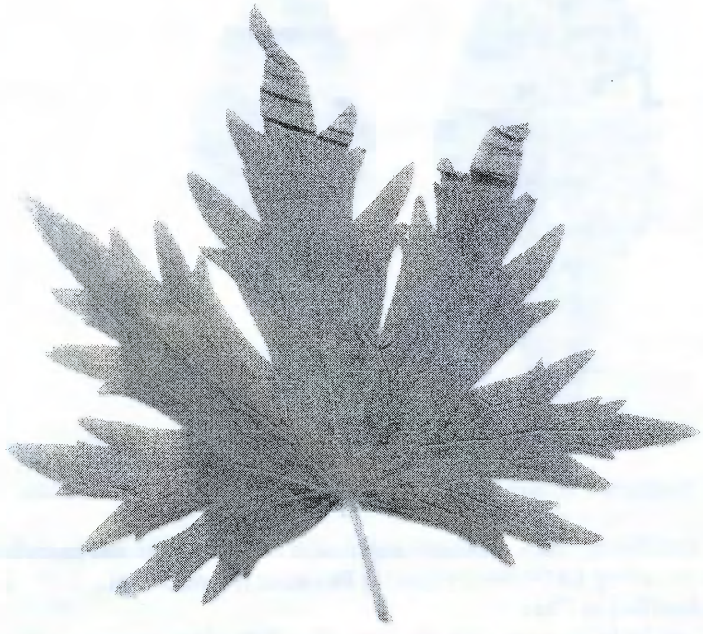

Fig. 1. Symptoms of CMV in delphinium

CMV was identified on the basis of symptom expression in mechanically inoculated test-plants. The results are presented in Table 1. The most characteristic for CMV symptoms were induced on following test-plants: *Atriplex hortensis* (local reaction, L) (Fig. 2), *Cucumis sativus* (L) and systemic (S), *Nicandra physalodes* (L and S), *Nicotiana alata* (L and S) (Fig. 3), *N. glutinosa* (S). EM revealed isometric virus particles about 30 nm in diameter in the preparations made from naturally infected delphinium plants and from inoculated test-plants. Identification of CMV was confirmed by a positive reaction in serological test. Mechanical inoculation with sap from delphinium was accomplished with difficulty due to presence of inhibitory substances in sap. In order to avoid virus inactivation, inocula were prepared by homogenizing infected leaves with 0.1 M phosphate buffer (pH 7.0), containing virus-stabilizing additives.

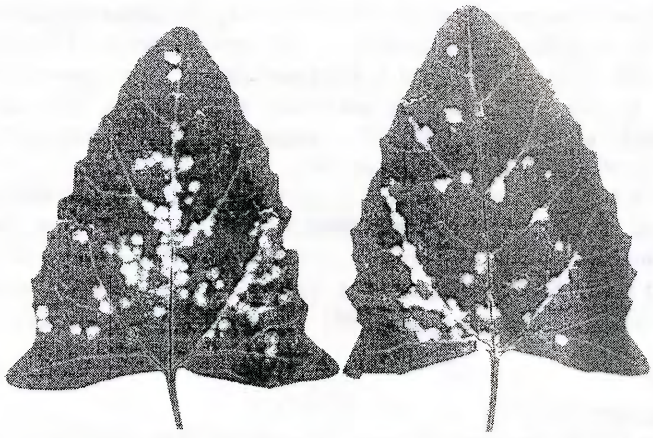

Fig. 2. Local reaction induced by CMV in inoculated leaves of *Atriplex hortensis*

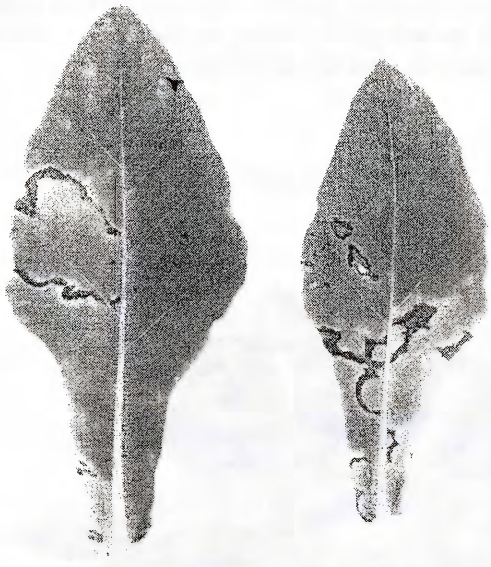

Fig. 3. Systemic reaction induced by CMV in leaves of *Nicotiana alata*

On the basis of particle morphology data, symptom expression on host plants and inoculated test-plants, positive reaction in serological test and according CMV descriptions in literature (Francki et al., 1979; Brunt et al., 1996) virus isolated from delphinium was identified as CMV.

CMV has an extremely wide host range, infects more than 190 plant species in 40 families. The virus is transmissible by inoculation with sap, inducing a variety of symptoms depending on virus strain and host cultivar and in non persistent manner by more than 60 species of aphid (Francki et al., 1979). CMV preserves in diseased delphinium plants and spreads due to vegetative propagation of these flowers.

Tomato ringspot nepovirus (ToRSV)

Young leaves of diseased delphinium plants showed light green or yellowish ringspots (Fig. 4). Later ringspots became more pronounced, necrosis appeared. Leaves became distorted. Symptoms resembled those induced by CMV infection.

ToRSV was identified on the basis of symptom expression on mechanically inoculated test-plants. The results are presented in Table 1. *Amaranthus caudatus, A. paniculatus, Celosia argentea* (Fig. 5), *Chenopodium amaranticolor, C. quinoa* expressed the most characteristic for ToRSV local and systemic reaction. EM revealed isometric virus particles, 28 nm in diameter, in the preparations made from naturally infected delphinium plants and from inoculated test-plants. ToRSV is transmissible by sap inoculation (from delphinium with difficulty).

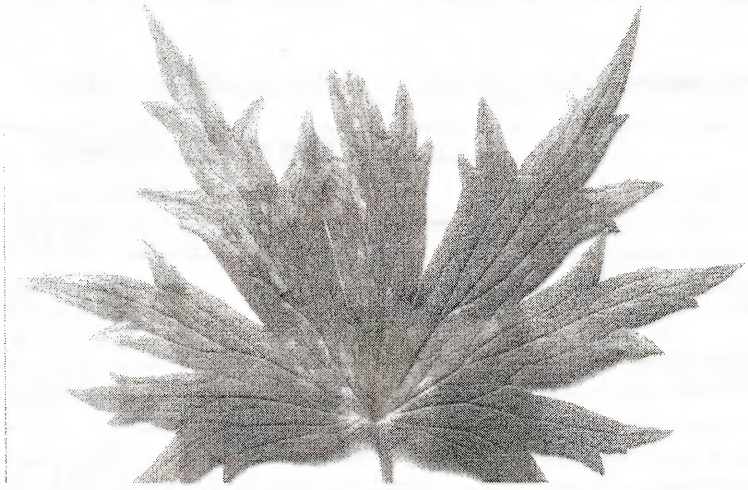

Fig. 4. Symptoms of ToRSV in delphinium

Fig. 5. Local reaction induced by ToRSV in inoculated leaves of *Celosia argentea*

On the basis of the results of test-plant reaction, morphology of virus particles also according to literature (Stace-Smith, 1984; Brunt et al., 1996) we concluded that delphinium plants were infected by ToRSV.

ToRSV is a type member of a nepovirus group and causes economically important diseases in a range of crops. ToRSV has been added to the EPPO list and is considered as a quarantine pest. The virus has isometric particles about 28 nm in diameter, sedimenting as three components and containing RNA as a bipartite genome. It is readily transmissible by inoculation of sap and has a wide host range, including both woody and herbaceous plants. It is transmitted by the nematode *Xiphinema spp.* Seed transmission has been reported in several crops. The virus is also transmitted by vegetative propagation and pollen. Most infected plants show distinctive symptoms as a shock reaction, chronically infected plants usually exhibit no obvious symptoms but show a general decline in productivity (Stace-Smith, 1984; Brunt et al., 1996). The virus occurs in nature mostly in perennial crops. Ornamental hosts have been found naturally infected by ToRSV including: *Anemone* L., *Gladiolus* L., *Hydrangea L, Iris* L., *Narcissus L., Pelargonium* L'Her., *Petunia* Juss. (Loebenstein et al., 1995).

The results of investigation of delphinium viral diseases revealed two causal agents CMV and ToRSV affecting this crop. Previously CMV was isolated and identified affecting 25 ornamental plant species in Lithuania (Navalinskienė, 1994) and earlier this virus was established being one of the most prevalent in this country. But situation has changed during the last decade and CMV occurred not so frequently on ornamental plants. On the contrary, another causal agent of delphinium viral disease, ToRSV, occurred more commonly and was isolated and identified recently affecting 43 ornamental species belonging to 13 botanical families (Navalinskiene et al., 2000; Samuitienė et al., 2002). Such wide distribution of ToRSV can be explained by efficient action of virus vectors — nematodes and high soil infestation by them. Methods of controlling virus diseases consist of growing for propagation selected healthy planting material, survey of plants during the vegetation for symptoms presence and elimination of affected plants. The losses by nematode transmitted viruses can be reduced by soil fumigation with fumigant nematicides to control nematodes. Diseases problems can be sometimes minimised by employing crop rotations that

diminish nematode population. Virus free stocks of valuable cultivars can be produced by heat treatment and meristem culture (Murant, 1981).

Table 1

Test-plants reaction to inoculation by viruses isolated from delphinium

Test-plant	Virus and test-plant reaction	
	CMV	ToRSV
Amaranthus caudatus	L:NLL	L: LLN; S: Sp, LeDis
A. paniculatus		L: LLRiSpN; S: LeDis
Atriplex hortensis	L: NLL	
Celosia argentea f. cristata		L: LL ChrRiSp, LeRu; S: LeDis, ChrSp, Ln, BrV
Chenopodium amaranticolor		L:ClLL; S: VStu,TR, NT
C. ambrosioides		L: NLL
C. hybridum	L: NLL	
C. quinoa	L: LL	L: ClLL; S: ClDot, LeDis, NAp
C. urbicum		L: ClLL; S: MoLeDis
Cucumis sativus 'Delicates'	L: DifSp; S: YSp, Mo	
C. sativus 'Rodničok'	L: ClDifSp; S: ClRi	
Datura stramonium		L: SmLL
Gomphrena globosa	L: LL	L: GRiN; S: LeDis, M
Lycopersicon esculentum		S: Mo, Cl, VN
Nicandra physalodes	L: NLLSp; S: LeDis,ClSp, NDot, LeTN	
Nicotiana alata	L: NSp; S: ClGrRiSp, NRi	
N. debneyi	L: DifClSp; S: ClGrRiSp, NRi	
N. glutinosa	S: ClGrMo, LeDis	
N. rustica	L: NSpRi; S: MoClGr	L: NRiSp
N. tabacum 'Samsun'	S: Mo, ClGRiSp, N	L: NRiSp; S: NSp, Str
N. tabacum 'Xanthi'		L: NRiSp; S: NSp
Petunia hybrida		L: GNRi; S: LeDis, ClSp
Tetragonia expansa		L: Dif ClSp; S: LeDis, ClDot
Zinnia elegans	S: Mo	

Abbreviations: L — local reaction, LL — local lesions, M — mosaic, Mo — mottling, Cl — chlorosis, N — necrosis, S — systemic reaction, Le — leaf, NT — top necrosis, Sp — spots, RiSp — ringspots, Ru — rugosity, Dis — deformation, Str — streaking, Stu — stunting, Sm — small, VC — vein clearing, VN — vein necrosis, Ap — apical, Dif — diffuse, Dot — dots, Ln — line pattern, Sp — spotting, Br — brown, G — grey, Y — yellow, Chr — cherry, Gr — green.

Subgroup 16SrI-B (aster yellows phytoplasma subgroup)

The diseased delphinium plants exhibiting symptoms of general yellows and stunting, and virescence of flowers were found at the Experimental Station of Field Floriculture (Fig. 6). Symptoms of the disease resembled those known to be caused by phytoplasmas. Phytoplasma detection was carried out by PCRs. Phytoplasma characteristic 1.2 kbp 16S rDNA was amplified in nested PCR primed by primer pair R16F2n/R2, confirming that the plants were infected by phytoplasma (data not shown). Phytoplasma was named delphinium virescence (DelVir).

The 1.2 kbp product was subjected to single digestions with 10 different endonucleases. The RFLP patterns of DelVir phytoplasma 16S rDNA (Fig. 7) were indistinguishable from patterns of 16S rDNA of phytoplasma classified in group 16SrI (aster yellows phytoplasma group) and subgroup 16SrI-B (aster yellows subgroup) (Lee et al., 1998; Marcone et al., 2000; Valiūnas, 2003). This phytoplasma subgroup was identified on diseased *Delphinium* hybrid in Germany (Schneider et al., 1993).

Fig. 7. Virescence symptoms of infected *Delphinium cultorum* Voss. 'Blue Bird' plants. Healthy flower on the right

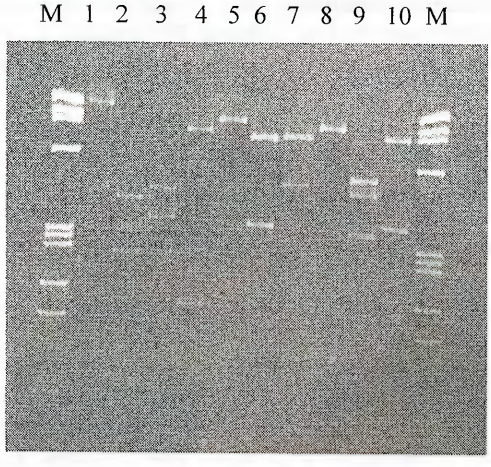

Fig. 8. RFLP analysis of DelVir phytoplasma 16S rDNA, amplified in nested PCR primed by oligonucleotide pair R16F2n/R2. Lanes M, PhiX174 RFI DNA *Hae*III digest, fragment sizes (bp) from top to bottom: 1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, 72. 1 — *Alu*I, 2 — *Mse*I, 3 — *Rsa*I, 4 — *Hpa*II, 5 — *Hae*III, 6 — *Hinf*I, 7 — S*au3A*I, 8 — *Hha*I, 9 — *Kpn*I, 10 — *Taq*I

Phytoplasmas belonging to subgroup 16SrI-B are spread worldwide, mostly in herbaceous plants (Lee et al., 1998; Marcone et al., 2000) but have also been reported in woody plants (Marcone et al., 2000). This phytoplasma subgroup is not widely spread in Lithuania, it has been found in woody plants willow (*Salix* L.), pear (*Pyrus communis* L.) and herbaceous plant valeriana (*Valeriana officinalis* L.) (Valiūnas, 2003).

Preventive measures against the spread of phytoplasmal infection should be aimed to interfere with or prevent the landing of vectors — leafhoppers on susceptible plants during the most sensitive period of their inoculation. Plant tissue culture of meristem tips and heat therapy, known as satisfactory methods, have not yet been used commercially to obtain vegetatively propagated crops free of mollicute infection (Loebenstein et al., 1995).

References

1. Ahmed, A.H., Bailiss, K.W. 1975. Virus infection of delphinium in Britain. J. Hort. Sci., 50(1), 47—54.
2. Alminaite, A., Valiunas, D., Navalinskienė, M., Staniulis, J., Jomantienė, R. 2001. *Hyacinthus orientalis* is the host plant for a new phytoplasma exhibiting ribosomal interperon sequence heterogeneity. Biologija (Vilnius), Vol. 4, 37—39.
3. Bos, L. 1983. Introduction to plant virology. PUDOC, Wageningen, 160.
4. Brunt, A.A., Crabtree, K., Dallwitz, M.J., Gibbs, A.J., Watson, L. (eds.) 1996. Viruses of Plants. Descriptions and Lists from the VIDE Database. Cambridge Univ. Press, Cambridge, 1484.
5. Deng, S., Hiruki, C. 1991. Genetic relatedness between two non-culturable mycoplasmalike organisms reveales by nucleic acid hybridization and polymerase chain reaction. Phytopathology, Vol. 81, 1475—1479.

6. Dijkstra, J., de Jager, C.P. 1998. Practical Plant Virology. Protocols and Exercices. Springer, 459.
7. Francki, R.I.B., Mossop, D.W., Hatta, T. 1979. Cucumber mosaic virus. CMI/AAB Descriptions of Plant Viruses. No. 213 (No 1 revised), p. 6.
8. Gundersen, D.E., Lee, I.-M. 1996. Ultrasensitive detection of phytoplasmas by nested PCR assays using two universal primers. Phytopathol. Mediterr., Vol. 35, 144—151.
9. Jomantiene, R., Davis, R.E., Dally, E.L., Maas, J.L. 1998a. The distinct morphology of *Fragaria multiplicata* is due to phytoplasma. Hort. Science, Vol. 33, 1069—1072.
10. Jomantiene, R., Davis, R.E., Maas, J.L., Dally, E.L. 1998b. Classification of new phytoplasmas associated with diseases of stawberry in Florida, based on analysis of 16 rRNA and ribosomal protein gene operon sequences. Int. J. Syst. Bacteriol., Vol. 48, 269—277.
11. Jomantiene, R., Davis, R.E., Antoniuk, L., Staniulis, J. 2000. First report of phytoplasmas in soybean, alfalfa, and *Lupinus* sp. in Lithuania. Plant Dis. Vol., 84, 198.
12. Jomantiene, R., Davis, R.E., Alminaite, A., Valiunas, D., Jasinskaite, R. 2002. First report of oat (*Avena sativa* L.) as host of phytoplasma belonging to group 16SrI, subgroup A. Plant Dis. Vol. 86(4), 443.
13. Lee, I.-M., Gundersen-Rindal, D.E., Davis, R.E., Bartoszyk, I.M. 1998. Revised classification scheme of phytoplasmas based on RFLP analyses of 16rRNA and ribosomal protein gene sequences. Int. J. Syst. Bacteriol. Vol. 48, 1153—1169.
14. Loebenstein, G., Lawson, R.H., Brunt, A.A. (eds.) 1995. Virus and virus-like disease of bulb and flower crops. Chichester, 876.
15. Marcone, C., Lee, I.-M., Davis, R.E., Ragozzino, A., Seemüller, E. 2000. Classification of aster yellows — group phytoplasmas based on combined analyses of RNA and *tuf* gene sequences. Int. J. Syst.. Evol. Microbiol., Vol. 50, 1703—1713.
16. Marwitz, R., Petzold, H. 1976. Elektronenmikroskopisher Nachweis mykoplasmaähulicher Organismen in Delphinium — Hybriden mit Blütenvergrünung nud-verlaubung. Phytopathol. Z., 87(1), 1—11.
17. Murant, A.F. 1981. Nepoviruses. In: Kurstak E. (ed.) Handbook of Plant Infection. Comparative diagnosis. Amsterdam, New York, p. 943.
18. Navalinskienė, M., 1994. Gėlių virusai (identifikavimas, biologija ir ligų profilaktika). Vilnius, 83.
19. Navalinskiene, M., Samuitiene, M. 2000. Natural occurrence of *Tomato ringspot nepovirus* on ornamental plants in Lithuania. Transactions of the Estonian Agricultural University, Vol. 209, 140–143.
20. Navalinskienė, M., Samuitienė, M., Jomantienė, R. 2003. Identification of *Tomato ringspot nepovirus* and subgroup 16SrI-A of phytoplasmas infecting hellebore plants in Lithuania. Sodininkystė ir daržininkystė. Mokslo darbai, Vol. 22(3), 259—267.
21. Robinson, D.G., Ehlers, U., Herken, R., Hermann, B., Mayer, F., Schürmann, F.-W. 1987. Methods for Electron Microscopy. An introduction for the Biomedical Sciences. Berlin, 190.
22. Samuitiene, M., Navalinskiene, M., Jomantiene, R., Davis, R.E. 2002. Molecular detection and characterization of phytoplasma infecting daisy (*Bellis parennis* L.) plants in Lithuania. Botanica Lithuanica, Vol. 8(2), 195—200.
23. Samuitiene, M., Navalinskiene, M. 2002. New natural hosts of Tomato ringspot nepovirus in Lithuania. VIIIth International Plant Virus Epidemiology Symposium. Plant Virus Epidemiology: First steps into the new millenium. Abstracts. Aschersleben, May 12—17, 2002, p. 114.
24. Schneider, B., Ahrens, U., Kirkpatrick, B.C., Seemüller, E. 1993. Classification of plant-pathogenic mycoplasma – like organisms using restriction-site analysis of PCR-amplified 16S rDNA. J. Gen. Microbiol. Vol. 139, 519—527.
25. Stace-Smith, R. 1984. Tomato ringspot virus. CMI/AAB. Descriptions of Plant Viruses, No. 290, p. 6.
26. Staniulis, J. 1988. Augalų gelta ir jos sukėlėjai. Vilnius, 121.
27. Staniulis, J.B., Davis, R.J., Jomantiene, R., Kalvelyte, A., Dally, E.L. 2000. Single and mixed phytoplasma infections in phyllody — and dwarf-diseased clover plants in Lithuania. Plant Dis. Vol. 84, 1061—1066.
28. Valiunas, D., Jomantiene, R., Davis, R.E., Sindaraviciene, I., Alminaite, A., Staniulis, J. 2000. Molecular detection and characterization of phytoplasmas infecting vegetables, legumes, and ornamental plants in Lithuania. Transactions of the Estonian Agricultural University, Vol. 209, 220—223.
29. Valiunas, D., Alminaite, A., Staniulis, J., Jomantiene, R., Davis, R.E. 2001a. First report of aster yellows-related subgroup I-A phytoplasma strains in carrot, sea lavender, aconitum, and hyacinth in Lithuania. Plant Dis. Vol. 85, 804.
30. Valiunas, D., Alminaite, A., Davis, R. E., Staniulis, J., Jomantiene, R. 2001b. Group 16SrV phytoplasma in diseased alder trees (*Alnus glutinosa*) in Lithuania. Abstract of Joint Meeting of APS, MSA, and SON, 2001 08 25—29, Salt Lake City, Utah, USA. Phytopathology, Vol. 91(6), supplement 91: 91.
31. Valiūnas, D. 2003. Identification of phytoplasmas in Lithuania and estimation of their biodiversity and molecular evolutionary relationships. Summary of doctoral thesis. Vilnius, 36.
32. Макутенайте-Навалинскене, М.К. 1981. Вирусные и микоплазменные болезни цветочных растений. Вильнюс, 72.

IDENTIFICATION AND SOME PROPERTIES OF *BARLEY YELLOW DWARF LUTEOVIRUS* IN LITHUANIA

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Abstract

Oat (*Avena sativa* L.), barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) plants bearing symptoms characteristic to barley yellow dwarf disease, expressed by reddening or yellowing of leaves and general plant stunting were collected in Vilnius State Plant Varieties Testing Station and cereal crop fields of Vilnius region in 1998. The virus infection was also observed in the season next year in this place and in other regions of this country. Plants were tested for *Barley yellow dwarf luteovirus* (BYDV) infection and gave positive reaction in double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA). The agent of investigated disease was experimentally transmitted by *Rhopalosiphum padi* L. aphids to oat, barley and wheat plants. The virus was not transmitted by mechanical inoculation to test plants. The BYDV was purified from naturally infected oat plants and the antigen was prepared. The electron microscopy investigation revealed the presence of particles measuring 24 nm in diameter (negatively stained). The virus identity was confirmed in oat plants by RT-PCR using the BYDV specific primers. This is the first report on the identification and occurrence of BYDV in Lithuania.

Key words: BYDV, cereal crops, DAS-ELISA, RT-PCR.

Introduction

Cereal crops are very important in Lithuania. Every year a great area of arable land is sown with these crops. During vegetation period the yield of cereals, especially winter crops and perennial forage grass, is under influence not only by ecological factors but also by diseases. One of them is barley yellow dwarf (BYD). BYD is one of the most economically damaging virus diseases of grass and cereal crops worldwide (Irwin, Thresh, 1990). BYD was detected in cereal crops in Sweden, Latvia (Bisnieks et al., 2004), Russia (Mozhaeva, Kastalyeva, 2002), Hungary (Pocsai et al., 1995), Germany (Huth, 1998) and other countries. *Barley yellow dwarf virus* (BYDV) has a wide host range including all of the major cereal crops, many annual and perennial weeds, pasture and range grasses (Bencharki et al., 2000). More than 100 species of wild grasses in the family *Poaceae* (*Gramineae*) (R. Br.) are susceptible to this virus (Rochow, Duffus, 1981). The symptoms of infection were expressed by stunting, inhibition of root growth, delayed heading and concomitant reduction in yield (Burgess et al., 1999). Color changes are often present, with an intensive bright yellow discoloration in barley and a yellow or reddish color in wheat and oat (Burgess et al., 1999). The spread of BYDV from plant to plant and from field to field depends entirely on aphid movement. The virus is transmitted by more than 20 aphid species in a persistent circulative manner and is mainly limited to the phloem tissue of an infected plant (Gray, 1996). BYDV exists as several different strains, which are differentiated by their ability to be transmitted by various aphid species and their virulence on a selected variety of oats. Some strains of BYDV are transmitted equally well by several aphids, whereas other strains can be transmitted by only one or two aphid species. The BYDV were originally differentiated as five biotypes on the basis of aphid-transmission specificity (Rochow, 1970). The strains RMV, RPV, MAV and SGV are readily transmitted by *Rhopalosiphum padi* (L.), *R. maidis* (Fitch), *Sitobion avenae* (Fabricus), *Schizaphis graminum* (Rondani), respectively, while PAV is transmitted by both *R. padi* and *S. avenae* (Rochow, 1970; Rochow, Muller, 1971). In Europe, three strains have been reported as wide-spread: RPV is most efficiently transmitted by the aphid species *R. padi*, PAV by *R. padi, S. avenae* and *Metopolophium dirhodum* (Walker), and MAV by *S. avenae* and *M. dirhodum* (Sadeghi et al., 1997). Every year, BYDV cause substantial yield losses in cereals wherever these crops are grown. Average yield losses of 15% in barley, 17% in wheat, and 25% in oats are common (Koev et al., 1998).

BYDV particles are isometric with a diameter of 20 to 30 nm, depending on the isolate and mode of preparation (Rochow, 1970; Šutic et al., 1999) and contain a single-stranded, positive-sense RNA genome (McGrath et al., 1996).

The aim of this investigation was to identify the agent of barley yellow dwarf disease, to determine the means of virus transmission, symptoms, morphology of virions, and to evaluate virus occurrence in graminaceous plants.

Materials and Methods

Barley, wheat and oat plants showing specific symptoms of virus disease were collected in Vilnius State Plant Varieties Testing Station, and in fields of the Vilnius and Kaunas regions of Lithuania. Investigating BYD disease of gramineous plants 10 virus samples were collected from 2 barley, 5 oat and 2 wheat fields. The experimental work was carried out in the greenhouse and Laboratory of Plant Viruses of the Institute of Botany. The possibility of transmission of disease agent by mechanical inoculation was investigated inoculating the following test-plants: *Agrostis stolonifera* L. — 'Guoda', *Avena sativa* L. — 'Edit', 'Jak', 'Jaugila', *Dactylis glomerata* L. — 'Asta', *Festuca pratensis* L. — 'Dotnuva', *Hordeum distichon* L. — 'Anni', 'Rataj', *Lolium perenne* L. — 'Sodre', *Triticum aestivum* L. (variety unknown), *Zea mays* L. — 'Pionier' (about 20 plants of each species). Presence of virus particles was observed in the

dip preparations, using a JEM-100S electron microscope (EM) after negative staining with 2% uranyl acetate solution (Dijkstra, de Jager, 1998). Virus was identified by double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) according to standard procedure (Clark, Adams, 1977). The test kit for detection of BYDV (IgG, conjugate, positive control) and protocol were recommended by Diagnostic group of the Institute for Resistance Research and Pathogen Diagnostics (Aschersleben, Germany). Virus was purified from symptomatic tissue (*Avena sativa* L. '*Jaugila*') according to a modified method (Perry et al., 1998). Frozen leaves were ground in 0.5 M phosphate extraction buffer, pH 6.1. The extract was clarified by extraction with 0.2 vol of chloroform. Virus particles were precipitated with 10% w/v polyethylene glycol Mw 6000 and 0.25 M NaCl. Pellets were resuspended in 0.1 M phosphate resuspension buffer, pH 7.0. Virus was purified by two cycles of differential centrifugation. Further purification was accomplished by sedimentation through a 30% sucrose cushion. Virus particles were collected by centrifugation at 28000^{x} g for 3 h at 4 °C. The concentration and purity of the virus preparations were estimated by EM and spectrophotometrically.

Transmission by aphids was tested with *R. padi.* The aphids were collected in the cereal field and identified by J. Turčinavičienė (Vilnius University). Aphids (approximately 150) were distributed on infected oat leaves in plastic petri dishes with tight-fitting lids and kept at room conditions (18 to 20 °C) for the acquisition period. After 3-days of acquisition feeding period, the aphids were transferred to healthy barley, wheat and oat plants, where they fed 3 days prior to being eliminated with insecticide (Decis). The plants were checked for symptoms 15 days after inoculation and for the presence of virus 25 days after inoculation, using DAS-ELISA.

Reverse transcriptase-polymerase chain reaction (RT-PCR) of field isolates (frozen tissues of *A. sativa* plants) was accomplished. Primarily total RNA was extracted using "QuickPrep total RNA extraction kit" (Amersham Pharmacia Biotech) according to manufacture's instructions.

Synthesis of cDNA was performed under the following conditions. Pellets of RNA were resuspended in a solution containing RNAse inhibitor, primer Lu 4 (Robertson et al., 1991) and PCR water. Samples were incubated for 10 min at 70 °C. For synthesis of the first cDNA strand, the RNA pellet solutions were added to the mixture containing reaction buffer, RNAse inhibitor, dNTP mix and M-MLV reverse transcriptase (MBI Fermentas, Vilnius, Lithuania). The first strand cDNA synthesis was carried out for 60 min at 37 °C and terminated by incubation for 10 min at 70 °C.

DNA amplification was carried out in a reaction mixture containing dNTP mix, Lu 1 and Lu 4 primers (Robertson et al., 199l), PCR buffer with magnesium chloride and *Taq* DNA polymerase (MBI Fermentas). Samples were placed in a Eppendorf Mastercycler Personal programmed to give one cycle at 95 °C (1 min), 41 °C (2 min) and 72 °C (20 min), followed by 40 cycles at 94 °C (1 min), 41 °C (1 min) and 72 °C (2 min), with a final cycle of 72 °C (10 min). PCRs products were analysed by electrophoresis through 5% polyacrylamide gel, stained with ethidium bromide and DNA bands visualized using a UV transilluminator. DNA fragment size standard was a *Hae* III digest of Φ × 174 RFI DNA (MBI Fermentas).

Results and Discussion

BYDV isolates used in this study were collected from naturally infected oat, barley and wheat plants. The symptoms of BYDV infection in oats were very conspicuous. The symptoms of early infection were expressed by yellowish-green spots or chlorosis. Later, most varieties developed reddening (crimson-pink) of the leaves from the tips down (Fig. 1). However, some varieties developed only a yellow-orange coloration. The most characteristic symptoms of infection in barley were dwarfing and yellow coloring of the leaves. In wheat, pale yellowing of older leaves was a more typical symptom. Reddening of leaf tips was sometimes visible. These symptoms were similar to those described for BYDV (Brunt et al., 1996; Šutic et al., 1999).

Fig. 1. Yellowish-green spots and reddening (crimson-pink) on oat leaves. Healthy leaf on the left

EM investigation revealed isometric virus particles about 24 nm in diameter in preparations made from naturally infected 10 plant samples (*Avena sativa* 'Jaugila', 'Hetman', CHD 1698', '1313-100', 'Bagač', *Hordeum vulgare* 'Anni', 'Aura', 'LŽI612', *Triticum aestivum* 'Širvinta' and one variety unknown) as have been reported in literature for BYDV (Rochow, 1970; Šutic et al., 1999) and these investigated plant samples reacted positively in DAS-ELISA performed with BYDV antibodies.

Test-plant species of the families *Poaceae* (indicated in materials and methods) were experimentally inoculated with disease agent. However, monocotyledonous test-plants did not react to inoculation. The virus was not transmissible by mechanical inoculation to test-plants, as have been described in literatura (Brunt et al., 1996; Šutic et al., 1999).

The agent of the investigated disease was experimentally transmitted by aphids (*R. padi*) to oat, barley and wheat plants. The incubation period of the virus in the plants growing in the glasshouse varied from 16 to 30 days. BYDV was detected in oat 20 days after inoculation, and in susceptible barley and wheat 25 days after inoculation. Symptoms were expressed by stunting and yellowing with reddening or purpling of leaf tips. The main symptoms of BYDV in oats were the typical red leaves. The plants showing symptoms reacted positively in DAS-ELISA performed with BYDV antibodies. The aphids used for the test were directly taken from the field and we have no guarantee that they were free of virus infection. However, we can confirm that aphides R. *padi* really transmitted this virus because of typical for BYDV symptomotology on test-plants and also due to positive reaction of infected test-plants in DAS-ELISA test.

Results of *R. padi* transmission of barley yellow dwarf agent and literature data (Rochow, 1970; Brunt et al., 1996; Gildow, 1999) suggest that the causal agent is *Barley yellow dwarf virus*.

Purification of BYDV was carried out from frozen *A. sativa* leaves. The purified virus preparations had A_{max} at 260 nm, A_{min} at 240 nm, the A_{260}/A_{280} ratio being 1.0. The yield of purified virus was 16.4 mg from 1 kg of plant tissue. EM of the purified virus revealed isometric particles about 24 nm in diameter, as have been reported in literature (Rochow, 1970; Šutic et al., 1999).

RT-PCR was used for detection of BYDV isolates in oat infected leaf material. A major PCR product of the expected size (530 nucleotides) was obtained from two tested oat samples, but was not observed in the negative controls with healthy tissue or water (Fig. 2). The results from the RT-PCR test show that the oat plants were infected with a luteovirus (could be BYDV-PAV) or a polerovirus (could be *Cereal yellow dwarf virus*-RPV).

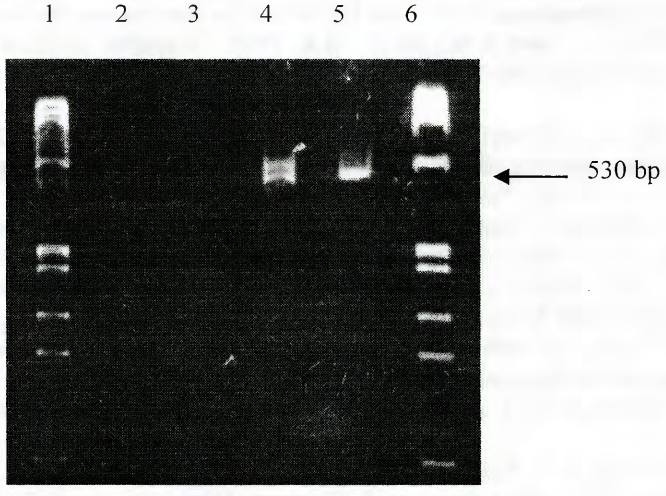

Fig. 2. RT-PCR products amplified from total nucleic acid extracts from oat leaves using luteovirus primers. Lanes 1, 6 — Phix 174 RFI DNA *Hae* III digest size markers; lane 2 — water blank; lane 3 — healthy tissue; lanes 4, 5 — BYDV. DNA fragment size (bp) standard was Phix 174 RFI DNA (*Hae* III) digest (MBI Fermentas), from top to bottom — 1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, 72

On the basis of symptomology of infected cereal plants, length of the incubation period, morphology of virus particles, ability to be transmitted by *R. padi,* positive reaction in serological test, and RT-PCR data, it can be concluded that the virus isolated from barley, oat and wheat is identical to BYDV described in literature (Robertson et al., 1991; Brunt et al., 1996; Šutic et al., 1999).

This is a first report of identification of BYDV in cereal crops of barley, oat and wheat in Lithuania.

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References

1. Bencharki, B., El Yamani, M., Zaoui, D. 2000. Assessment of transmission ability of barley yellow dwarf virus — PAV isolates by different populations of *Rhopalosiphum padi* and *Sitobion avenae*. European Journal of Plant Pathology, 106, 455—464.
2. Bisnieks, M., Kvarnheden, A., Sigvald, R., Valkonen, J.P.T. 2004. Molecular diversity of the coat protein-encoding region of *Barley yellow dwarf virus-PAV*and *Barley yellow dwarf virus — MAV* from Latvia and Sweden. Archives of Virology, 149, 843—853.
3. Brunt, A.A., Crabtree, K., Dallwitz, M.J., Gibbs, A.J., Watson, L. (eds.) 1966. Viruses of Plants. Descriptions and Lists from the VIDE Database. Cambridge Univ. Press, Cambridge, 1484 pp.
4. Burgess, A.J., Harrington, R., Plumb, R.T. 1999. Barley and cereal yellow dwarf virus epidemiology and control strategies. In: Smith H.G., Barker H. (eds.) The *Luteoviridae.* New York, 248—279.
5. Clark, M.F., Adams, A.N. 1977. Characteristic of the microplate method of enzyme linked immunosorbent assay for the detection of plant viruses. Journal of General Virology, 34, 475—483.
6. Dijkstra, J., de Jager, C.P. 1998. Practical Plant Virology. Protocols and Exercices. Berlin, 459 pp.
7. Gildow, F.E. 1999. Luteovirus transmission and mechanismus regulating vector specificity. In: Smith H.G., Barker H. (eds.) The *Luteoviridae* .New York, 88—112.
8. Gray, S.M. 1996. Plant virus proteins involved in natural vector transmission. Trend in Microbiology, 4, 259—264.
9. Huth, W. 1998. 30 Jahre Untersuchungen an Graserviren an der Biologischen Bundesanstalt in Braunschweig. Mitt. Biol. Bundesanst. Land–Forstwirtsh. Berlin-Dahlem, 44—79.
10. Irwin, M.E., Thresh, J.M. 1990. Epidemiology of barley yellow dwarf virus: a study in ecological complexity. Annu. Rev. Phytopathol., 28, 393—424.
11. Koev, G., Mohan, B.R., Dinesh-Kumar, S.P., Tobert, K A., Somers, D.A., Miller, W.A. 1998. Extreme reduction of disease in oats transformed with the 5' half of the Barley Yellow Dwarf Virus-PAV genome. Phytopathology, 88, 1013—1019.
12. McGrath, P.F., Lister, R.M., Hunter, B.G. 1996. A domain of the readthrough protein of barley yellow dwarf virus (NY-RPV isolate) is essential for aphid transmission. European Journal of Plant Pathology, 102, 671—679.
13. Mozhaeva, K.A., Kastalyeva, T.B. 2002. Some aspects of epidemiology of barley yellow dwarf in European Russia. In: VIII th International Plant Virus Epidemiology Symposium. Aschesleben, Germany, 1—187.
14. Perry, K.L., Miller, J., Lister, R.M., Mayo, M.A. 1998. Luteovirus isolation and RNA extraction. In: Foster and Taylor (ed.): Plant virology protocols from virus isolation to transgenic resistance. Methods in Molecular Biology, 81, 231—239.
15. Pocsai, E., Kovacs, G., Muranyi, I., Orosz, A., Papp, M., Szunics, L. 1995. Differentiation of barley yellow dwarf luteovirus serotypes infecting cereals and maize in Hungary. In: VIIth Conference on virus diseases of Poaceae in Europe. AGRNZ, 15 (7—8), 381—516.
16. Robertson, N.L., French, R., Gray, S.M. 1991. Use of group-specific primers and the polymerase chain reaction for the detection and identification of luteoviruses. Journal of General Virology, 72, 1473—1477.
17. Rochow, W.F. 1970. Barley yellow dwarf virus. No. 32. In: Descriptions of Plant Viruses. Commonwealth Mycological Institute and Association of Applied Biologists, Kew, Surrey England.
18. Rochow, W.F., Duffus, J.E. 1981. Luteoviruses and yellows diseases. In: Kurstak E. (ed.) Handbook of plant virus infections. Comparative diagnosis. Amsterdam, New York, 147—170.
19. Rochow, W.F., Muller, I. 1971. A fifth variant of barley yellow dwarf virus in New York. Plant Disease, 55, 874—877.
20. Sadeghi, E., Dedryver, C.A., Riault, G., Gauthier, J.P. 1997. Variation in transmission of two BYDV-MAV isolates by multiple clones of *Rhopalosiphum padi* L. European Journal of Plant Pathology, 103, 515—519.
21. Šutic, D.D., Ford, R.E., Tošic, M.T. 1999. Handbook of Plant Virus Diseases. Boca Raton, London-New-York-Washington, D. C., 1—83.

DETECTION OF PHOMOPSIS CANKER AND DIEBACK OF HIGHBUSH BLUEBERRIES AND CRANBERRIES IN LITHUANIA

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Abstract

Twig canker and blight caused by *Phomopsis vaccinii* Shear in Shear, N. Stevens & H. Bain (telemorph *Diaporthe vaccinii* Shear in Shear, N. Stevens & H. Bain) is a harmful disease of highbush blueberry and cranberry (*Vaccinium* spp.) and is under strong phytosanitary control in Lithuania as well in Europe and Mediterranean region.

In 2002 the *Phomopsis vaccinii* on blueberry was identified for the first time in Lithuania. The diagnosis of this disease using classical phytopathological methods is often difficult because of overgrowing of target fungus by another species mostly necrotrophs or another canker agent *Fusicoccum putrefaciens* Shear. Only in one specimen the sporificated pycnidia with α- and β-conidia were observed on diseased plant. Exact *Phomopsis vaccinii* detection was succeeded using serological method, such as PTA-ELISA. The PTA-ELISA was performed using polyclonal IgG 59/3, specific for the genus *Phomopsis*. First results show positive reaction in 8 specimens. Development of the present serological diagnostic method could be very promising for the fast and exact identification of harmful disease agent *Phomopsis vaccinii.*

Key words: *Phomopsis vaccinii,* twig canker and blight, distribution, Lithuania.

Introduction

Twig blight, stem canker, fruit rot and leaf spots caused by *Phomopsis vaccinii* Shear in Shear, N. Stevens & H. Bain (telemorph *Diaporthe vaccinii* Shear in Shear, N. Stevens & H. Bain) is a serious disease of blueberry and cranberry (*Vaccinium* spp.) crops and is under strict control in Europe as well as in Lithuania. This fungus is indigenous in North America and introducted in Chile (Chao and Glawe, 1985; Guerrero and Godoy, 1989; Weingartner and Klos, 1975), and included into the A1 list of Quarantine Pests of European and Mediterranean Plant Protection Organization (EPPO) and into the list of Quarantine Pests of Lithuania. *P. vaccinii* in the Directive of EU Council 2000/29/EB (Apendix 2, Part A, Section 1) is mentioned as not identified in the EU, and as harmful which introduction and spread within member countries shall be banned if it is present on certain plants or plant products. The identification of the causal agent is an important precognition for a safe diagnosis of a disease, but the realisation is often difficult and lengthy using classical phytopatological methods, because of overgrowing of target fungus by another species mostly necrotrophs. The teleomorph stage of this fungus was described as *Diaporthe vaccinii* Shear in 1931 (Wilcox, 1940). More morphological features and host specifity was described by Chao and Glawe (1985). Exact pathogen detection is possible in short terms by molecular (PCR) or serological methods like DTBIA and PTA-ELISA if specific and sensitive antiserums as well as sufficient antigens are available in the specimens.

Materials and Methods

Plant material and isolation

The plant, consisting of more or less diseased wooden twigs, leaves and fruits (total 30 specimens) originated from 11 locations of Lithuania, was examined by classical phytopatological methods and for 10 of them ELISA test was done (Table 1). Specimens of diseased plant material without sporification structures were incubated in a moist chamber to induce fruitbodies production and sporulation. The isolation of target strains was made according to Waller et al. (1998): plant material (leaves, twigs) showing symptoms was cut into short lengths with a sterile knife and washed in sterile water, dipped for 1 min in 2% NaOCl solution,

for 1 min in 0,01% streptomycin sulphate solution, washed again in sterile water, dried and then placed onto agar medium.

Table 1

The origin, description of symptoms and other fungi associated with analyzed specimens

	Host Specimen No	Origin	Visual symptoms Microscopic analysis	ELISA test
1	*Vaccinium corymbosum* cv. *'Heerma'* 02B	Berry plantation, Šiauliai distr.	Twig blight, sporificated pycnidia (α and β conidia) of *Phomopsis vaccinii* in brownish area	positive
2	*V. corymbosum* cv. *'Heerma'* 02C	-"-	Twig blight without sporificated structure	positive
3	*V. angustifolium* 03B	MAP collection*, Institute of Botany, Vilnius	Twig and stem blight, sporificated pycnidia of *Fusiccoccum putrefaciens*	negative
4	*V. corymbosum* 03H	Berry plantation, Šiauliai distr.	Twig blight, sporificated pycnidia (α and β separately) of *Phomopsis vaccinii*	positive
5	*V. corymbosum* 03K	Berry plantation No 1, Kaunas distr.	Twig blight without sporificated structure	negative
6	*V. corymbosum* 03M	Berry plantation No 2, Kaunas distr.	Twig blight without sporificated structure	positive
7	*V. corymbosum 'Tora'* 03N	VMU Botanical garden**, Kaunas	Twig blight without sporificated structure	positive
8	*V. macrocarpon* cv. *'Washington'* 03C	VMU Botanical garden, Kaunas	Twig blight without sporificated structure	positive
9	*V. palustris* 03D	-"-	-"-	positive
10	*V. macrocarpon* 03F	-"-	-"-	positive
11	*V.corymbosum* cv. *'Patriot'* 234	Tauragė distr.	Twig blight without sporificated structure, agent unknown	NT***
12	*V. corymbosum* 'EarlyBlue' 235	Tauragė distr.	Twig blight without sporificated structure, agent unknown	NT
13	*V. corymbosum* 704	Vilnius city	Twig and stem blight, sporificated pycnidia of *Phyllostycta vaccinii*	NT
14	*V. corynbosum* 287	Panevėžys distr.	Twig blight without sporificated structure, agent unknown	NT
15	*V. corymbosum* 956	Kaunas distr.	Twig blight without sporificated structure, agent unknown	NT
16	*V. corymbosum* cv. *'Heerma'* 300	Berry plantation, Šiauliai distr.	Twig blight with sporificated structure of *Colletotrichum gloeosporioides*	NT
17	*V. corymbosum* cv. *'Ama'* 03P	MAP collection*, Institute of Botany, Vilnius	Twig and stem blight, sporificated pycnidia of *Fusiccoccum putrefaciens*	NT
18	*V. macrocarpon* 03R	-"-	Twig and stem blight, sporificated pycnidia of *Fusiccoccum putrefaciens*	NT
19	*V. corymbosum* cv. *'Ama'* 965	VMU Botanical garden, Kaunas	Twig blight without sporificated structure, agent unknown	NT
20	*V. corymbosum* cv. *'Atlantic'* 969	-"-	Twig blight without sporificated structure, agent unknown	NT
21	*V. corymbosum* cv. *'Bluegold'* 967	-"-	Twig blight without sporificated structure, agent unknown	NT
22	*V. corymbosum* cv. *'Coville'* 966	-"-	Twig blight without sporificated structure, agent unknown	NT

Table 1 (continued)

	Host Specimen No	Origin	Visual symptoms Microscopic analysis	ELISA test
23	*V. macrocarpon* 959	-"-	Twig and stem blight, sporificated pycnidia of *Phyllostycta vaccinii* and *F. putrefaciens*	NT
24	*V. macrocarpon* 958	-"-	Twig blight without sporificated structure, agent unknown	NT
25	*V. macrocarpon* 960	-"-	Twig and stem blight, sporificated conidiomata of *Phomopsis vaccinii, Phyllostycta vaccinii* and *Fusicoccum putrefaciens*	NT
26	*V. macrocarpon* 961	-"-	Twig and stem blight, sporificated conidiomata of *Phomopsis vaccinii, Phyllostycta vaccinii, Fusiccocum putrefaciens, Physalospora vaccinii*	NT
27	*V. macrocarpon* 962	-"-	Twig blight, sporificated pycnidia (α and β conidia) of *Phomopsis vaccinii*	NT
28	*V. myrtyllus* 125	Varėna distr. Natural location	Twig blight without sporificated structure, agent unknown	NT
29	*V. vitis-idaea*	-"-	-"-	NT
30	*V. palustris*	-"-	-"-	NT

* Medicinal and Aromatic Plant Collection of the Institute of Botany, Vilnius
** Botanical Garden of Vytautas Magnus University, Kaunas
*** Not tested

Inoculation

The inoculation of highbush blueberry and cranberry cultures was made according to methods described by Daykin and Milholland (1990): the 2—3-year old twigs of *V. corymbosum* were inoculated with pure sporificated culture of *P. vaccinii*, the berries of *V. macrocarpon* were inoculated with conidia suspension (10^6—10^7 c/ml).

Detailed PTA-ELISA was described previously (Gabler and Ehrig, 2000; Gabler et al., 2004; Gabler and Urban, 1995; Hsu et al., 1995).

Results and Discussion

At first *P. vaccinii* was detected by microscopic examination only in one of specimens having typical symptoms. Pure culture was isolated from brownish area of a two-year old stem of *Vaccinium corymbosum* cv. *'Heerma'*. It was described for the first time in Lithuania (Kačergius et al., 2004). In the absence of fungal sporificated structures on diseased twigs, leaves or fruits the PTA-ELISA test was done. The PTA-ELISA was performed in polysorb microtiter plates (Greiner) using IgG 59/3. The fungus was detected in 8 examined specimens, collected from 3 sites. The highest ELISA values (A_{405} = 1.46 and 1.05) as well as the highest rate of positive detection were estimated among specimens of *V. corymbosum* cv. *'Tora'*, and *V. macrocarpon* Ait. from VMU Botanical Garden (Gabler et al., 2004). Taking it into consideration, the isolation into pure culture was repeated and *P. vaccinii* culture from cultivated cranberry (*V. macrocarpon*) was isolated.

Symptoms and isolation

The first symptoms appear on the tips of non-woody shoots. Infected shoots decline, becoming brown. The fungus can also infect leaves, buds, and fruits of blueberries and cranberries. The diseased part of a stem containing developed fruiting bodies never has purple margin.

After 10—14 days incubation in moist chamber the pycnidial conidiomata appeared. The conidiomata were 0.5—1 mm in the diameter, dark, spherical, they also were flat near the base, partially subcuticular, scattered to confluent, uniostiolate. Riped pycnidia exuded a cream coloured mass of conidia (Fig. 1).

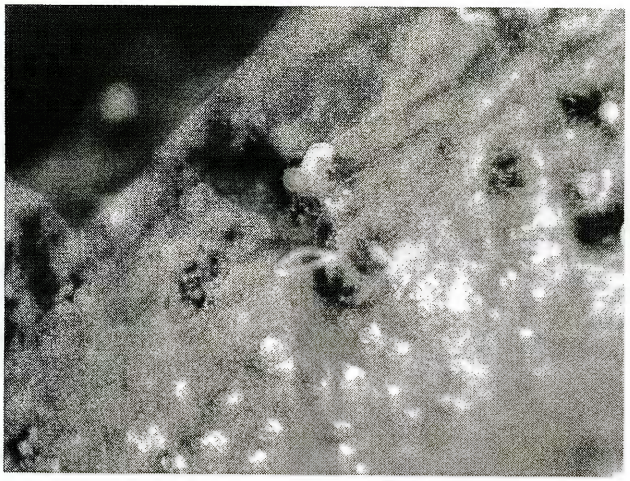

Fig. 1. Pycnidial conidiomata of *P. vaccinii* on diseased stem of highbush blueberry (*V. corymbosum* cv. *'Heerma'*)

Two types of conidia were produced: alpha (α) conidia — hyaline, unicellular, ellipsoid, biguttulate 7—8 × 2.5 μm (according to Ramsdell (1995) 6—11 × 2.5 μm; Farr et all. (2002) 5.5—8.7 × 1.7—2.8), and beta (β) conidia — unicellular, filiform, uncinate, hyaline 18—25 × 1.0 μm, (according to Ramsdell 12—18 × 0.75 μm). *P. vaccinii* grows well on Potato Dextrose Agar (PDA), prepared from fresh potato (PDA natural), Malt Extract Agar (MEA), and MIX (Czapek 16,5 g, PDA 14 g, Orange serum agar 12 g, Chloramphenicol 0,5 g/l).

Morphological identification *in vitro*

Colonies on PDA (natural) grow up to 12.5 mm/day, on MEA (Oxoid) 11.2 mm/day, on MIX and PDA (Oxoid) 11 mm/day. On PDA (Oxoid) fruiting bodies form only slowly and not abundantly. Pycnidia appear first in the centre of colonies on MEA and PDA but later on MIX. They form concentric circles on PDA, but are scattered on MEA. *P. vaccinii* colonies are white, circular and grow up to 35 mm in diameter over three days. Aerial mycelium is not compact, mellow, colony regular in outline with a thinner margin (Fig. 2).

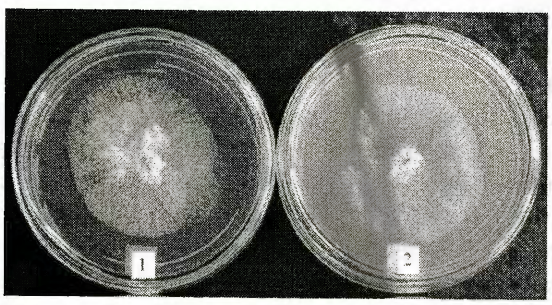

Fig 2. Three days old colonies of *P. vaccinii*: 1 — on MEA (Oxoid); 2 — on PDA (natural)

After 7 days colonies reach 62 mm diameter, become slightly floccose with indistinct concentric rings. At senescence after 3—4 weeks the colors of the mycelium is the same — greyish white, but becoming thinner, more heterogeneous and noticeably floccose. The fungus grows well between 20—28 °C (optimum 25 °C) in the light as well as in the dark. For fructification cultures should be cultivated under a 12/12 hours light/dark regime. Pycnidia start to form after 7—10 days, maturing within 20—28 days. Conidiomata pycnidial are black, spherical, and large, with broader base, with two thirds protruding above the surface of the medium. The pycnidia are 1—3 mm in the diameter, with sclerotic walls. They form one at a time or sometimes close together in time and place. Cream colored spore masses erupt from the broad irregular pore in the top of the mature pycnidia (Fig. 3).

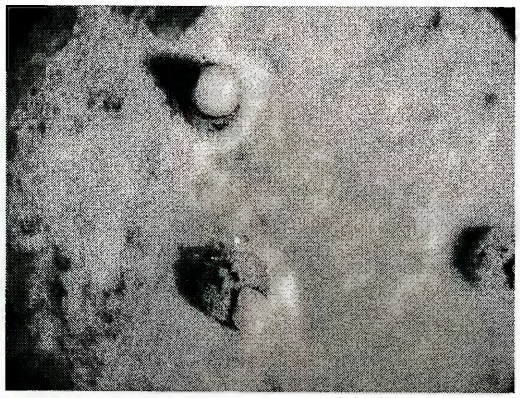

Fig. 3. Pycnidial conidiomata of *P. vaccinii* on PDA (natural) after 24 days of cultivation

Alpha (α) conidia being dominate in these exudates and are unicellular, ellipsoid, hyaline, pointed at both ends, biguttulate, 6—11 × 2,5—3,2 μm. beta (β) conidia — unicellular, hyaline, filiform, uncinate, 16—24 × 1—2 μm (Fig. 4). Conidiophores are simple, narrow at top, sometimes spindle-shaped, 15—25 μm long in young pycnidia but longer in the old. With repeated subculturing only α-conidia may be produced in some isolates.

Fig. 4. Alpha (α) and beta (β) conidia of *P. vaccinii*

Comparison with similar species

Sometimes similar symptoms can be associated with other fungi such as *Godronia cassandrae* Peck (anamorph *Fusicoccum putrefaciens* Shear), *Colletotrichum* sp., *Gloeosporium minus* Shear and *Fusarium* sp. In the presence of reproductive structures these fungi can be separated from *P. vaccinii* very easily by checking sporulating structures under a stereoscopic microscope (20X) or compound microscope (400X—1000X). In the absence of any sporulating structures identification should be done using a moist chamber. For example, *Fusarium* sp. forms yellow conidiomata (sporodochial) after 7—14 days and the structures are easily confirmed by microscopic examination. *Colletotrichum* sp. also produce conidiomata (acervuli), but spores, 15—21 × 2—3 μm, are unicellular, cylindrical, obtuse, granular, pink in mass, and without oil drops. Lesions and conidiomata (pycnidia) produced by *F. putrefaciens* are very similar to *P. vaccinii.* Sometimes both fungi are found on the same host, making identification difficult. If this fungus is already mature, its spores are easily separated by microscope. However, the best way for keeping of lesions host parts of *F. putrefaciens* is in moist chambers or to isolate in pure culture for producing sporulating structures. Cultures on PDA appear white at the margins and pale lemon yellow and yellow-chrome to orange at the centre. Pycnidial conidiomata of *F. putrefaciens* are brown or greyish-yellow, irregular with thick walls and they erupt through the surface when mature. They produce conidia that are thin, usually arched, hyaline, unicellular or pseudoseptate, 9—13,5 × 1,5—2,4 μm.

Inoculation

The lesions caused by *P. vaccinii* isolate PH-03A (Fig. 5) were similar on 2—3 year old stems of highbush blueberry cv. *'Top Hat'* to those described by Weingartner and Klos (1975) and Daykin and Milholland (1990). In our studies, pycnidia developed on leaves of infected shoots 3—4 weeks after artificial

inoculation. The pycnidia on infected of cranberry berries were developed 4—5 weeks after inoculation. Berries became brownish red, inflated and shiny (Fig. 6).

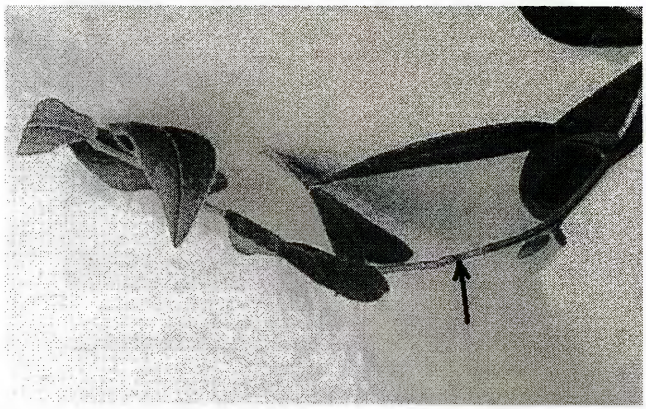

Fig. 5. Twig blight lesion two weeks after inoculation. Dark brown region of tissue developed between the light brown necrotic tissue on the top of injury

Distribution

The thirty specimens having Phomopsis canker symptoms were examined from 11 different locations in Lithuania. Only in five specimens from two sites were identified *P. vaccinii* by microscopic analysis and in eight cases (sampled in 3 locations) by ELISA test. Sites of sampling and exact *P. vaccinii* locations in Lithuania are presented in Fig. 7.

Fig. 6. Infected berries of cultivated cranberries after 4 weeks of artificial inoculation. Berries became brownish red, inflated and shiny (on the left control berries — not inoculated)

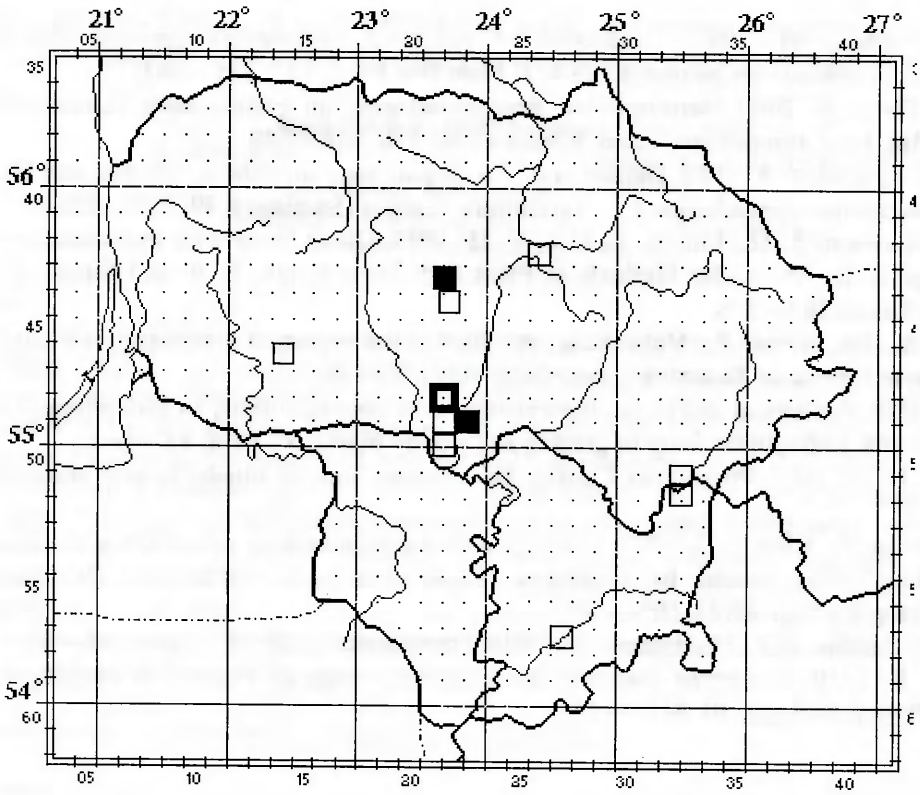

Fig.7. Sites of sampling and exact locations of *P. vaccinii* in Lithuania (□ — *P. vaccinii* not detected; ■ — *P. vaccinii* detected by both methods (morphological and ELISA); ◘ — *P. vaccinii* detected only by ELISA

In addition, the detection of *P. vaccinii* on diseased specimens of blueberries and cranberries in Lithuania indicates that the dangerous quarantine pathogen already occurs in Europe.

The present results highlight the need for investigations on host range and geographic distribution of this pathogen in Europe. For this reason, accurate and rigorous diagnostic methods are required. One of them is molecular characterisation based on sequences of ITS region of rDNA. Far et al. (2002) described *P. vaccinii* and additional isolates from blueberry and cranberry in the USA. They found some isolates differed from *P. vaccinii* and named it *Phomopsis* sp. It is possible that the non *P. vaccinii* isolates are endophytes and not active participants in the blueberry and cranberry diseases. But, as has been summarized by Petrini (1996), there are fungal endophytes that under some circumstances behave as pathogens, while under other conditions will remain innocuous.

References

1. Ames, G. K., Gergerich, R. C., Weidemann, G. J., Patterson, C. A. 1988. First report of *Diaporthe vaccinii* on blueberry in Arkansas. Plant Dis., 72, p. 362.
2. Anonymous. 1997. *Diaporthe vaccinii* Shear. CABI/EPPO. Quarantine Pests of Europe. Wallingford, Paris, 737—741.
3. Chao, C. P., Glawe, D. A. 1985. Studies on the taxonomy of *Diaporthe vaccinii*. Mycotaxon, 23, 371—381.
4. Daykin, M. E., Milholland, R. D. 1990. Histopathology of blueberry twig blight caused by *Phomopsis vaccinii.* Phytopathology, 80(8), 736—740.
5. Far, D. F., Castlebury, L. A., Rossman, A. Y. 2002. Morphological and molecular characterization of *Phomopsis vaccinii* and additional isolates of *Phomopsis* from blueberry and cranberry in the eastern United States. Mycologia, 94(3), 494—504.
6. Far, D. F., Castlebury, L. A., Rossman, A. Y., Putman, M. L. 2002. A new species of *Phomopsis* causing twig dieback of *Vaccinium vitis-idea* (lingonberry). Mycol. Res., 106(6), 745—752.
7. Gabler, J., Kacergius, A., Jovaisiene, Z. 2004. First report of Phomopsis vaccinii on blueberry and cramberry in Europe by direct tissue blot immunoassay and plate trapped antigen ELISA. Journal of phytopatology, 0(0): 00-00. (in press)

8. Gabler, J., Urban, M. 1995. Evaluation of resistance differences between tomato cultivars to *Phytophthora nicotianae* by indirect ELISA. J. Plant Dis. Prot., 102, 275—283.
9. Gabler, J., Ehrig, F. 2000. Serologischer Erregernachweis im Pathosystem *Carum carvi/ Phomopsis diachenii.* Mitt. Biol. Bundesanst. Land- Forstwirtsch, 376, 548—549.
10. Guerrero, C. J., Godoy, A. 1989. Detection of *Phomopsis vaccinii* (Shear, Stevens and Bain) in highbush blueberry (*Vaccinium corymbosum* L.). Agricultura Técnica (Santiago), 49, 220—223.
11. Hsu, H. T., Lawson, R. H., Lin, N. S., Hsu, Y. H. 1995. Direct tissue blot immunoassay for analysis of plant pathogens. In: Molecular Methods in Plant Pathology, Singh, R. P. and Singh, U. S., Eds., CRC Press, Boca Raton, 367—376.
12. Kacergius, A., Jovaisiene, Z., Valiuskaite, A. 2004. First report of *Phomopsis vaccinii* on *Vaccinium corymbosum* in Lithuania. Botanica Lithuaniaca, 10(1): 75—80.
13. Petrini, O. 1985. Ecological and physiological aspects of host-specificity in endophytic fungi. In: Redheat S., Carris L., eds. Endophytic fungi in grasses and woody plants. St. Paul, 87—100.
14. Rammsdell, D. C. 1995. Phomopsis Canker. In: Compendium of blueberry and cranberry diseases. St. Paul, 14—15.
15. Weingartner, D. P., Klos, E. J. 1975. Etiology and symptomatology of canker and dieback diseases on hightbush blueberries caused by *Godronia (Fusicoccum) cassandrae* and *Diaporthe (Phomopsis) vaccinii*. Phytopathology 65(2), 105—110.
16. Waler, J. M., Ritchie, B. J., Holderness, M. 1998. Plant clinic handbook. Surrey, 45—49.
17. Wilcox, M. S. 1940. *Diaporthe vaccinii*, the ascigerous stage of *Phomopsis* causing a twig blight of blueberry. Phytopathology, 30, 441—443.

TRENDS OF THE CHANGES OF THE VIRULENCE GENES FREQUENCIES IN THE LATVIAN POPULATION OF *BLUMERIA GRAMINIS* F.SP. *HORDEI*

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Abstract

Samples of *Blumeria graminis* f.sp. *hordei* were collected in two different part of Latvia during 1996—2002. Frequencies of virulence genes *Va6, Va7, Va9, Va12, Vk* and *Vla* were high in both places. All years of investigation very low frequencies were found for virulences against resistance factors in the barley line *SI1* and variety 'Steffi'. Not any isolate virulent to *mlo* gene was found. During 1996—2002 considerable changes of frequencies of some virulence genes were detected. Firstly virulence genes *Va1, Va3* and *Va13* appeared only in the Central part of Latvia with low frequencies and later their frequencies increased from 4—14% in 1996 to 33—53% in 2000. About 2—3 years later those virulence genes appeared in the South-eastern part of Latvia. In 2001—2002, considerable increasing of *Va1, Va3* and *Va13* were detected also in this region: virulence frequencies against barley lines with *Mla1, Mla3* and *Mla13* resistance genes reached 23—30%. We postulated that mentioned changes of the population structure are result of spore spread which for the barley powdery mildew agent occurs generally in the West-East direction (Limpert et al., 1999). The distance between places of the investigation (about 200 km) and difference in time of the virulence appearing corresponds to the approximately speed of pathogen spread 100 km per year

Key words: powdery mildew, barley, virulence genes, resistance.

Introduction

Barley (*Hordeum vulgare* L.) is one of the most important cereals in Europe (Bousset et al., 2002). There are many dangerous pathogens of barley, the biotrophic fungus *Blumeria graminis* f.sp. *hordei*, an ascomycete that is one of them. The fungus is the causal agent of barley powdery mildew, which can arise more than 25% of loses in yield (Czembor and Czembor, 2001). The disease spreads by conidiospores, which forms a numerous pustules on the leaf surface. Spores mostly dispersed by wind to neighbouring plants, where new infection establishes. Live spores of the *Blumeria graminis* f.sp. *hordei* were wind-transported over distances of 500 km or more with a speed approximately 100 km per year from West to East (Limpert, 1987; Limpert et al., 1999; Brown, Hovmøller, 2002; Hovmøller et al., 2002).

Long-distance dispersal, mutations and recombinations are the most important reasons for genotype or pathotype diversity in different populations of *Blumeria graminis* f.sp. *hordei*. The need to control of barley powdery mildew is a major stimulus for investigation of virulences of the pathogen in different countries where this disease is a problem.

Virulence genes and their frequencies may vary considerably between regions and years. The composition of virulence genes of the pathogen and their frequencies were detected in different parts of Europe (Torp et al., 1978; Hovmøller et al., 2000; Limpert et al., 2000; Czembor, Blandenopolous, 2001). In Latvia, monitoring of the virulence genes frequencies in *Blumeria graminis* f.sp. *hordei* population was done since 1981, mainly in the central part of country (Rashal et al., 1997). Since 1995, South-eastern part of Latvia was included in the research program. Data about virulence frequencies in Latvia were presented earlier (Rashal et al., 1997; Kokina, Rashal, 2001; Kokina et al., 2002; Kokina, Rashal, 2004;).

The aim of this paper is to accentuate changes in the composition of virulence genes of *Blumeria graminis* f. sp. *hordei* and their frequencies in Latvia during several last years. We discuss here reasons of differences between those frequencies in different parts of Latvia.

Materials and Methods

In 1996—2002 samples of the pathogen were collected in the Southeastern part of Latvia. In the Central part of the country samples were collected in 1996—2000. In both cases the universally susceptible variety 'Otra' were used as a trap plant. Monopustules were isolated from collected samples both in sporulation and cleistothecia phases.

A set of differentials was used for the detection of monopustules (Table 1). Differentials were inoculated by microinoculation technique (Dreiseitl, 1998). Infection types of each isolate on the differentials were scored after 8—9 days according (Torp et al., 1978) on a 0—4 scale.

Table 1

Differentials used for detection of virulences in the population of *Blumeria graminis* f.sp. *hordei* in Latvia in 1996—2002

Differentials	Main resistance genes
P01	*Mla1*
P02	*Mla3*
P03	*Mla6*
P04B	*Mla7*
P08B	*Mla9*
P10	*Mla12*
P11	*Mla13*
P17	*Mlk*
P23	*MlLa*
SI1	*Ml(SI1)*
'Steffi'	*Ml(St1), Ml(St2)*

Results and Discussion

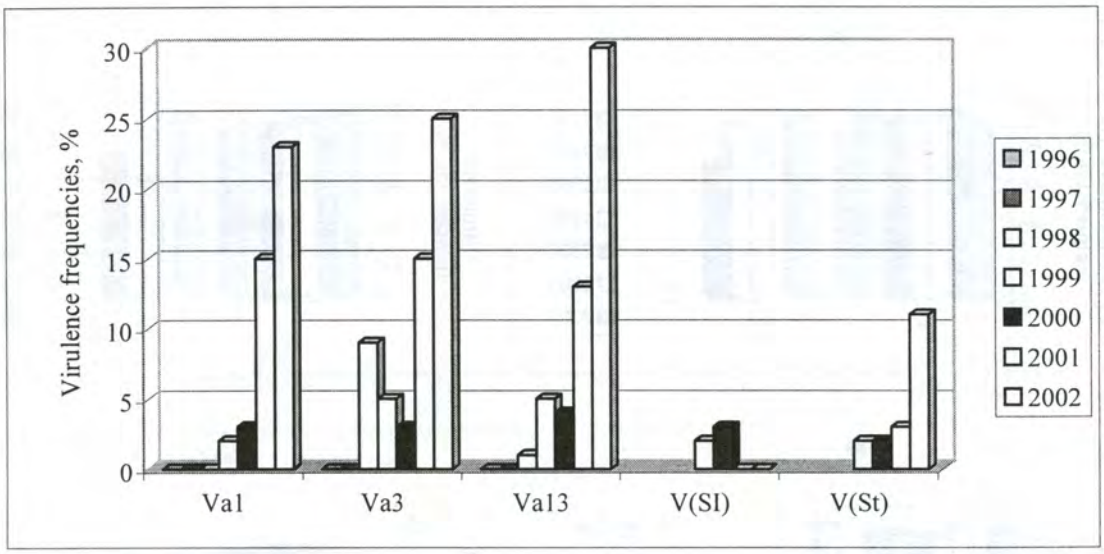
The long-term observation of the pathogen population is necessary to get information about the pathogen spread direction and ability to overcome host resistance factors. This kind of knowledge is very important for resistance breeding programs, because any usage of chemicals for plant protection is increasingly criticized (Czembor, Blandenopoulos, 2001).

During 1996—2002 considerable changes of frequencies of some virulence genes were detected in the pathogen population in Latvia. Particularly interesting are data about virulence genes *Va1, Va3* and *Va13* (Fig. 1). First mentioned virulences appeared in the Central part of Latvia with low frequencies and later their frequencies increased from 4—14% in 1996 to 33—53% in 2000. About 2—3 years later than in the Central part those virulence genes appeared in the Southeastern part of Latvia too. In 2001—2002, considerable increasing of *Va1, Va3* and *Va13* were detected also in this region: virulence frequencies against barley lines with *Mla1, Mla3* and *Mla13* resistance genes reached 23—30%. Most probably, that mentioned changes of virulences are result of spore spread which for the barley powdery mildew agent occur generally in the West-East direction (Limpert et al., 1999). The distance between places of the investigation (about 200 km) and difference in time in virulence appearing corresponds to the approximately speed of pathogen spread 100 km per year (Limpert et al., 1999; Brown, Hovmøller, 2002; Hovmøller et al., 2002).

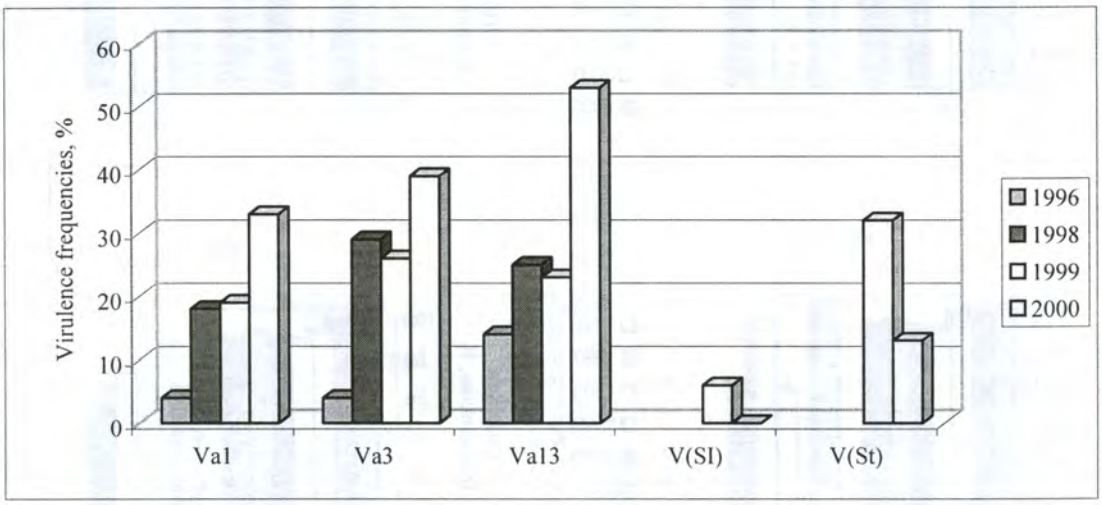
Since 1999, barley line *SI1* and variety 'Steffi' were included in the research programs in Latvia. Some virulent isolates against *SI1* resistance factors were detected in both parts of Latvia in 1999 (Fig. 1). In 2000, such isolates were detected in the Southeastern part of Latvia only. Since 2001, not any virulent isolate against *SI1* was detected here. Gradual increasing of *V(St)* presence in population from 2% to 11% were observed in Southeastern part of Latvia in the opposite from the Central part, where it decreased from 32% in 1999 till 13% in 2000.

There are many virulence genes with high frequencies in Latvian population of barley powdery mildew: *Va6, Va7, Va9, Va12, Vk* and *Vla*. Those virulence genes were presented in the both parts of the population with frequencies higher than 60—90% in all years of investigation. It is not any sense to use correspondent resistance genes in the commercial barley varieties.

Only *mlo* resistance gene was completely effective during all years of the investigation. This gene is very wide exploited in spring barley in the West and Central Europe and it is used also in contemporary barley breeding programs in Latvia. There are attempts to introduce *mlo* resistance into modern winter barley varieties. If *mlo* resistance will be presented on barley fields all year around the natural selection pressure in direction to broken down this resistance type will increase extremely.



a



b

Fig. 1. Virulence genes with low-medium frequencies in the Sourtheastern (a) and Central (b) parts of Latvia in 1996—2002

Taking in account the direction of the spore dispersal in Europe and our results we can predict that any new *Blumeria graminis* f.sp. *hordei* virulence which would occur in the Central or West Europe will come to Latvia in several years. It means that breeding programs should based not only on few resistance sources even they are highly effective at the moment. Therefore it is especially important to looking for the new resistance sources, especially from barley wild relatives, such as *Hordeum spontaneum*.

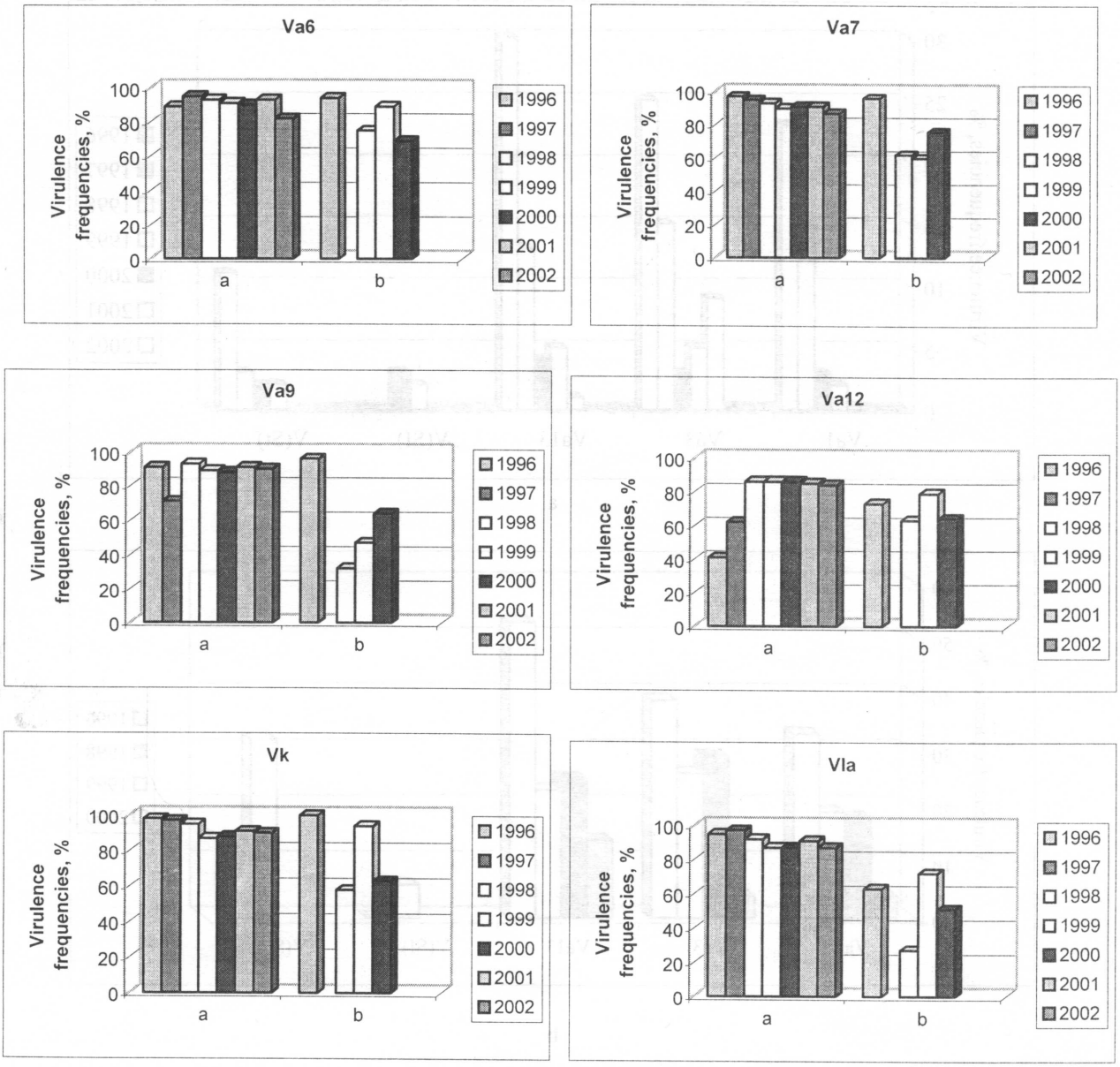


Fig. 2. Virulence genes with high frequencies in the Sourtheastern (a) and Central (b) parts of Latvia in 1996—2002

References

1. Bousset L., Hovmøller M., Caffier V., de Vallavielle-Pope C., Østergard H. (2002) Observed and predicted changes over eight years in frequency of barley powdery mildew avirulent to spring barley in France and Denmark. *Plant Pathology*, 51, 33—44.
2. Brown J., Hovmøller M. (2002) Aerial Dispersal of Pathogens on the Global and Continental Scales and Its Impact on Plant Disease. *Science*, 297, 537—541.
3. Czembor J., Blandenopoulos K. (2001) Genes for resistance to powdery mildew (*Blumeria graminis* f.sp. *hordei*) in cultivars bred in Greece. *Cereal Rusts and Powdery Mildews Bulletin*, http://www.crpmb.org/2001/0316czembor/
4. Czembor J., Czembor H. (2001) Resistance to powdery mildew in barley cultivars and breeding lines included in 1998—2000 Polish registration trials. *Plant Breed. Seed Sci.*, 45(1), 21—41.
5. Dreiseitl A. (1998) Comparison of methods to study powdery mildew and monitor the population of *Erysiphe graminis* f.sp. *hordei* in 1997. *Plant Protection Science*, 34, 33—38.
6. Hovmøller M., Caffier V., Jalli M., Andersen O., Besenhofer G., Czembor J., Dreiseitl A., Felsenstein F., Fleck A., Heinrics F., Jonsson R., Limpert E., Mercerr P., Plesnik S., Rashal I., Skinnes H., Slater S., Vronska O. (2000) The European barley powdery mildew virulence survey and disease nursery 1993-1999. *Agronomy*, 20, 729—743.
7. Hovmøller M., Justensen A., Brown J. (2002) Clonality and long-distance migration of *Puccinia striiformis* f.sp. *tritici* in north-west Europe. *Plant Pathology*, 51, 24—32.
8. Limpert E. Spread of barley mildew by wind and its significance for phytopathology, aerobiology and for barley cultivation in Europe (1987) In: G. Boehm and R. Leuschner (eds.): Advances in Aerobiology. Birkhäuser Verlag, Basel, 331—336.
9. Kokina I., Rashal I. (2001) Genetic structure of barley powdery mildew population in Latgale region. *Acta Biologica Universitas Daugavpiliensis*, 1 (2), 69—72.
10. Kokina I., Rashal I. (2004) Genetical structure of the population of *Blumeria graminis* f.sp. *hordei* in Latgale region of Latvia in 2001-2002. *Acta Biologica Universitas Daugavpiliensis*, in press.
11. Kokina. I. Arāja, I., Rašals I. (2002) Genetic particularities of the causal agent of barley powdery mildew in Latvia. Proceedings of the Conference "Research for Rural Development", May 22—24, 2002, Jelgava, Latvia, 17—18.
12. Limpert E., Godet F., Müller K. (1999) Dispersal of cereal mildews across Europe. *Agric. For. Meteorol.*, 97, 293—308.
13. Limpert E., Bartoš P., Graber WK., Müller K., Fuchs JG. (2000) Increase of Virulence Complexity of Nomadic Airborne Pathogens from West to East Across Europe. *Acta Phytopathologica et Entomologica Hungarica*, 35, 317—322.
14. Rashal I., Tueryapina R., Ornicane D., Kokina I. (1997) Resistance to powdery mildew in barley of Latvian origin: effectiveness and improvement. Proceedings of the Conference "Approaches to Improving Disease Resistance to Meet Future Needs Airborne Pathogens of Wheat and Barley", November 11, 1997, Prague, Czech Rebublic. COST Action 817, Research Institute of Crop Production, Praha-Ruzyne, 145—146.
15. Torp, J., Jensen, H. P., Jørgensen, J. H. (1978) Powdery mildew resistance genes in 106 Northwest European spring barley varieties. *Kgl. Vet.-og. Landbohøjsk. Årsskr.*, 75—102.

FUSARIUM SPP. AS AN IMPORTANT PROBLEM IN CEREAL PRODUCTION IN ESTONIA

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Abstract

An overview of investigations on occurrence and hazardousness of *Fusarium* spp. on Estonian cereal grain and in spoiled grain feeds, and the toxicity of *Fusarium* isolates, was carried out from the year 1973 up to the present. The problems of pure production without *Fusarium* spp. and fusariotoxins are discussed.

Cereal grain samples from seed cultivation farms and from disease control trials were analysed by the moist chamber method. Grain feed samples were investigated by the pour plate method (Harrigan & McCance, 1976). The species and number of *Fusarium* were defined on the basis of the first and second dilution on Nash & Snyder selective medium. The identification of *Fusarium* spp. has been made according to Bilai (1955, 1977) and Gerlach & Nirenberg (1982). The toxicity of *Fusarium* isolates was tested on the basis of the growth inhibition zone of *Bacillus stearothermophilus* (Watson & Lindsay, 1982).

On grain produced within the period of 1973—1981, *Fusarium* spp. were identified in 38—100% of samples with infection level 8—67% of seeds. In 67—100% of the studied wheat samples, the infection was detected on 13—67% of seeds. In the case of rye, *Fusarium* spp. were identified in 38—86% of the studied samples and infection was found in 8—23% of seeds, with barley the figures were 45—97% and 14—46%; and with oats 55—100% and 15—65%, respectively. *F. avenaceum* (Fr.) Sacc., *F. poae* (Pk.) Wr., *F: sporotrichioides* Sherb. *var. minus* Wr., *F. oxysporum* (Schlecht) Snyd. et Hans., *F. verticillioides* (Sacc.) Nirenberg, *F. sambucinum* Fuck., *F. equiseti* (Corda) Sacc. and *F. culmorum* (W.G.Sm.) Sacc. — known as the toxin forming *Fusarium* species (toxicants) — occurred on 50—60% of the studied samples. 90% of samples from wheat grain of the year 1992 were infected with an infection level of 2—21% on spring wheat and 36—59% on winter wheat. The most frequent species were *F. oxysporum, F. semitectum* and *F. sporotrichioides*, and during the years 2002—2003, the species F. *semitectum, F. poae, F.culmorum,* and *F. avenaceum.*

In domestic grain feeds, *Fusarium* spp. were found in 52% of the samples, whereas most of them (78% of the cases) were toxic to *B. stearothermophilus*. 31.3% of the studied *Fusarium* isolates were highly toxic and 37.5% medium toxic. Very toxic isolates belonged to the species *F. verticillioides, F. culmorum,* and *F. tricinctum.*

None of the tried fungicide variants (20) saved the yield from *Fusarium* spp. completely. The best effect on decreasing the number of *Fusarium* spp. was exerted by Tilt (propiconazole) and Corbel (fenpropimorph).

Key words: cereal grain, grain feed, toxicant, *Fusarium* spp., mycotoxin, *Bacillus stearothermophilus*, fungicide.

Introduction

Fusarium species have been a serious problem in production of plant and cattle breeding products already for centuries. Reducing the realisation value of cereals as food, feed and grain cereal, toxins produced by the fungi cause chronic and acute poisoning and allergic signs both to animals and humans. Therefore, throughout the world great attention is paid to investigating *Fusarium* species and elaborating means for controlling them. Contamination of cereals with *Fusarium* toxins is a global problem, occurring in Europe, the Americas, Asia, and Australia (Placinta et al., 1999). A programme initiated by the European Commission "Agriculturally important toxigenic fungi. COST Action 835" focused on the problem of the existence of *Fusarium* species in cereals: disease forms, pathogenicity, species composition, resistance breeding, toxicology, inactivation of toxins. Most European countries participated in the programme (Annual Report 1998. Directorate-General for Research. 2000. EUR 19694).

The Scientific Committee on Plants of the European Commission in its document "Opinion on the relationship between the use of plant protection products on food plants and the occurrence of mycotoxins in food", issued on 30 November 1999, recommends to conduct research to elucidate effects of pesticides for preventing of diseases and production of mycotoxins as well as carry out more monitoring of mycotoxins in foodstuffs used in preparation of food for children and the underaged. Most of the attention should be directed towards toxins produced by *Fusarium, Aspergillus* and *Penicillium* species.

At a session of the cereals workgroup of the European Commission held on 4—5 December 2003 it was admitted that the contamination of cereals with *Fusarium* toxins is a problem in whole Europe. Tens of millions of cereals lay unused until elucidation of their level of contamination, according to which their usage could be determined. It is possible that part of the lots are not fit either as food or feed. At the same time, there was a 10% shortage of necessary grain due to the reduction of the cultivation areas.

Fusarium spp. produce strong toxins, exceeding the toxicity of pesticides used in cereal cultivation hundreds and thousands of times. For example, in peroral administration to mice of LD_{50} mg/kg per liveweight there are toxins: nivalenol (NIV) = 4.1; toxin T-2 = 4.8–5.2; moniliformin = 4.0; fusarenon X = 3.4. Pesticides: seed dressing preparation Baytan Universal 19.5 WS = 3338; the fungicide Tilt 250 EC = 2000; the insecticide Actellic 50 EC = 1522; the herbicide Basagran M = 3200.

Fusarium toxins do not decompose in the animal organism but are transferred into products — eggs, meat and milk, jeopardising thus also human health (Schachermayr & Fried, 2000). It has been found by many scientists that most of the toxins are rather thermostable and do not decompose during thermal processing like boiling, cooking, steaming, and reach our table in brown and white bread (Obenauf, 2002).

This research was carried out to establish the level of contamination of Estonian cereal grain and grain feeds with *Fusarium* species, the potential toxicity of isolates and possibilities to avoid (or reduce) the occurrence of *Fusarium* species in yields and their production of toxic isolates.

For compiling the present overview, the results from author`s long-term studies were used (Lõiveke, 1987; Lõiveke, Laitamm, Sarand, 2003; Lõiveke, Ilumäe, Toome, 2004) as well as data published by other researches from different regions. Other authors have studied neither distribution nor harmfulness of *Fusarium* spp. on grain in Estonia.

Materials and Methods

Mycological survey

Grain (wheat, rye, barley, oats) samples of yields from 1973—1981 and winter and summer wheat samples from 1992 were collected for mycological testing according to the requirements of average sample composition and analysed after 4—5 weeks. The grain samples were taken from the field trials and seed growing farms of the Estonian Research Institute of Agriculture, where general and special agrotechnical requirements, necessary for production of high reproduction seeds, were followed. In mycological analysis the wet chamber method was applied: microfungi were reared up from grains on filter paper in Petri dishes at a temperature of 20—30 °C and 18—22 °C (in the years 1974—1982) or at one regime of 20 °C (in 1992). After two and four weeks the percentage of seed contamination with *Fusarium* species was determined. To identify *Fusarium* species under microscope, preparations were made.

Microbiological survey

Microbiological samples from grains of field trials in 1993—1994 and samples of grain feeds with spoiling signs from 1997—2002 delivered by animal breeders were analysed by the pour plate method (Harrigan & McCance, 1976). The species and number of Fusariums were defined on the basis of the first and second dilution on Nash & Snyder selective medium. The identification of *Fusarium* spp. has been made according to Bilai (1955, 1977) and Gerlach & Nirenberg (1982).

The toxicity of *Fusarium* isolates

It was tested on the basis of the growth inhibition zone of *Bacillus stearothermophilus* (Watson & Lindsay, 1982): 0—1 mm — non-toxic, 2—5 mm — medium toxic, 6—10 mm — highly toxic.

Field trials with fungicides

To control grain diseases were carried out in Üksnurme experimental fields of the Estonian Research Institute of Agriculture by EPPO guidelines PP 1/152(2) in the years 1993 and 1994. In 1993 the trials included 9 variants of the barley `Ida` and the summer wheat `Satu` whereas in 1994 the number of variants was 11. Following 14 fungicides were tested: Alto 400 SC (active substance agent — cyproconazole), Alto Elite (cyproconazole, carbendazim), Alto Combi (cyproconazole, chlorothalonil), Archer 425 EC (propiconazole, fenpropimorph), Calixin (tridemorph), Corbel (fenpropimorph), Folicur BT 225 EC (tebuconazole, triadimefon), Folicur 250 EW (tebuconazole), Rider 400 EC (propiconazole, fenpropidin); Sportak 45 EC (prochloraze); Tango (tridemorph, epoxiconazole); Tilt 250 EC (propiconazole), Tilt Premium (propiconazole), and Tiptor (cyproconazole, prochloraze).

Barley was sprayed for controlling diseases caused by *Cochliobolus sativus, Pyrenophora teres, Rhynchosporium secalis* at the stage GS 37-39 (27.06.1993 and 01.07.1994) and wheat was sprayed for controlling diseases caused by *Mycosphaerella graminicola, Leptosphaeria nodorum* at the stage GS 39-40 (01.07.1993 and 04.07.1994). The trials were harvested at the full-ripening stage on 26.08. and 13.09. in 1993; on 22.08. and 01.09. in 1994, thus 52—73 days following the pesticide application.

Results and discussion

Occurrence and intensity of *Fusarium* infection in 1973—1981

On grain, produced within the period of 1973—1981, *Fusarium* spp. were identified in 38—100% (average 79%) of samples (1065), the infection level being 8—67% (average 29%) of seeds. In 67—100% of the studied wheat samples (57), the infection was detected on 13—67% of seeds. In the case of rye, *Fusarium* spp. were identified in 38—86% of the studied samples (85) and infection was found in 8—23% of seeds, with barley (720 samples) the figures were 45—97% and 14—46%; and oats (203 samples) — 55—100% and 15—65%, respectively. The average figures per year demonstrate that oats was most vulnerable to infection — 87% of the studied samples were infected at an average rate of 33%, with wheat the respective figures were 86% and 29%, in the case of barley — 79% and 29%, whereas rye appeared to be the most resistant — 62% of the samples were infected at an average rate of 14%. The highest average rate of infection (48%) of grain samples was detected in 1978 characterised by the highest amount of precipitation (480 mm) during the growing season (June—September). Harvest time (August and September of 1978) was extremely wet — the amount of precipitation in different regions of Estonia was 1.5—2.4 times higher than the standard amount (Lõiveke et al., 2003). Finnish scientists (Ylimäki, 1981; Avikainen & Hannukkala, 2001) also refer to the favourable effect of warm and wet late summer on the infection of grain ears and kernels with *Fusarium* fungi.

Composition of *Fusarium* species in 1973—1981

The frequency of the occurrence of *Fusarium* species (by Gerlach & Nirenbeg, 1982) depended on the grain species and the year. In 1973 and 1974, the prevailing species was *F. ventricosum* App. et Wr., whereas in 1976, 1978,

1979 and 1981, *F. avenaceum* (Fr.) Sacc. predominated. In 1975, the driest year, the composition of *Fusarium* species was the smallest: *F. avenaceum* and *F. sporotrichioides* Sherb. *var. minus* Wr. did not occur at all. In 1978, the year of the highest amount of precipitation, *F. avenaceum* occurred more frequently than ever — in 62% of the studied samples. The most common species were *F. avenaceum, F. poae* (Pk.) Wr., *F. oxysporum* (Schlecht) Snyd. et Hans, *F. ventricosum, F. sporotrichioides, F. verticillioides* (Sacc.) Nirenberg, and *F. culmorum* (W.G.Sm.) Sacc. In about 40% of the samples, the occurrence of two or more *Fusarium* species was detected simultaneously. *F. avenaceum, F. poae, F. sporotrichioides, F. oxysporum, F. verticillioides, F. sambucinum* Fuck., *F. equiseti* (Corda) Sacc. and *F. culmorum*, known as the toxin forming *Fusarium* species (toxicants), were detected on 50—60% of the studied samples (Lõiveke et al., 2003). The mentioned species are capable of producing many toxins — DON (deoxynivalenol), 3-ADON, 15-ADON, NIV (nivalenol), ZEN (zearalenone), HT-2, fumonisins, moniliformin, fusarin C, wortmannin, sambutoxin, fusarenon X, T-2, DAS (diacetoxyscirpenol), etc. (Bilai, 1977; Kadis et al., 1971; Miller & Trenholm, 1997).

Compared with *Fusarium* flora detected in cereals in Finland (Ylimäki, 1981; Ylimäki & Jamalainen, 1986), Estonian cereals are mostly infected by the same species. A difference is greater occurrence of *F. graminearum* and more modest occurrence of *F. verticillioides* in Finland than in Estonia.

Fusarium species in wheat in 1992, 2002, and 2003

90% of samples from wheat grain of the year 1992 were infected with an infection level of 2—21% (average 12%) on spring wheat and 36—59% (average 47%) on winter wheat. The most frequent species were (by Gerlach & Nirenbeg, 1982) *F. oxysporum, F. semitectum* and *F. sporotrichioides*, and during the years 2002—2003, the species *F. semitectum, F. poae, F. culmorum,* and *F. avenaceum.* As revealed by a comparison of results from 1992 and 1973—1981, in the case of wheat, contamination with *Fusarium* species has not diminished at all, allowing us to conclude that the contamination of other cereals has not changed either.

Consequently, in Estonia there exist factors favouring *Fusarium* infection in grains, although head blight is very rare. The favouring factors are primarily rainy growing seasons and excessively wet pre-harvest periods. Development of *Fusarium* species in harvested grains can be inhibited only by quick quality drying to 13—14% of moisture and storing in conditions avoiding excessive moisture (Ylimäki et al., 1979). In case grain has been infected with *F. graminearum*, it is considered necessary to dry the grain to moisture of 10—13%, to avoid generation of mycotoxins in the future as well (Jevseyeva, 1992). One cannot always ensure operative drying in production conditions, which has caused grain and feeds to be spoiled because of *Fusarium* species and other microorganisms.

Fusarium species in domestic grain feeds in 1997—2002

In feeds with spoiling signs, *Fusarium* spp. sometimes made up 6.8—8.2% of the total number of fungi, whereas also the known toxic species *F. culmorum, F. tricinctum, F. verticillioides* and *F. sporotrichioides* were present. Fusariums were found in 52% of the 32 studied samples of feed that had caused health problems to animals: decrease in production or, in some cases, even death of animals. Could this be caused by *Fusarium* species in addition to other toxic moulds (*Acremonium, Cladosporium, Aspergillus, Penicillium, Rhizopus, Trichothecium, Paecilomyces*)? To elucidate it, *Fusarium* species were isolated into pure culture and their toxicity was studied.

Toxicity of *Fusarium* isolates in domestic grain feeds

The toxicity of *Fusarium* isolates separated from feeds was controlled by biotest by the size of the growth inhibition zone of *Bacillus stearothermophilus.* 78% of samples contaminated with *Fusarium* spp. contained *Fusarium* isolates toxic to *B. stearothermophilus*. Thus approximately 40% of the samples contained toxic isolates, showing that *Fusarium* species often are contaminators of feeds with mycotoxins. Of the 16 isolates, 31.3% were highly toxic (growth inhibition 6—10 mm) and 37.5% medium toxic (2—5 mm). The highly toxic isolates belonged to the species *F. verticillioides, F. culmorum,* and *F. tricinctum.*

A monitoring conducted by the Agricultural Research Centre in 1998—2002 revealed that there are *Fusarium* toxins in grain feeds and feed mixtures used in Estonia. In 1998, ZEN was found in 20%, in 1999 in 24%, in 2000 in 50%, in 2001 in 19%, and in 2002 in 14% of samples. DON was present in 1999 in 1.6% and in 2000 in 4.5% of samples.

Toxicity of *Fusarium* isolates in grain

68 *Fusarium* isolates were separated from grain of 2002 and 2003 and the toxicity of the isolates was determined. In the case of 26 isolates obtained from wheat, the growth inhibition zone was 2—7 mm, with 24 barley isolates — 2—5 mm, with 15 oats isolates — 2—7 mm, and with 3 rye isolates — 3—4 mm. Of wheat isolates from 2002, the most toxic were *Fusarium* sp., *F. culmorum,* and *F. verticillioides* (4—7 mm). Although the named year had a relatively dry growth period (precipitation in Saku 52 % of the norm), the toxicity of separated isolates was 3—7 (mm). Consequently, the variable *Fusarium* flora on grains contains *Fusarium* strains (forms) of very different toxicity.

The effect of fungicides on the occurrence of *Fusarium* species in yields

To suppress both typical pathogens and saphrophytic mycoflora, fungicides are usually applied in grain cultivation when 1—5% of the surface of the third leave from the top has been damaged. As it may occur 60—70 days prior harvest, it is important that the effect of fungicides is possibly long-lasting to protect grain from becoming infected in rainy autumn with delayed harvest. By analysing microbiologically barley and summer wheat yields of 1993 and 1994, it was tried to elucidate whether it was possible to reduce occurrence of *Fusarium* spp. in yield by using fungicides. Corbel (fenpropimorph) reduced the number of *Fusarium* species in yield up to 10 times, Sportak 45 EC (prochloraze) — up to 7 times, Tilt 250 EC (propiconazole) — up to 6 times, Folicur BT 225 EC (tebuconazole, triadimefon) — up to 5 times, Rider (fenpropimorph) — up to 3 times, and Calixin (tridemorph) — up to 2 times. The

best of the named can be considered Tilt and Corbel that were effective in 75—100% of the tests. The effect of other fungicides was unstable, fluctuating from reducing the number of *Fusarium* species to increasing the number.

According to data in literature, contradictory results have been obtained in experiments with fungicides for controlling fusarioses. D`Mello et al. (1998) suggest on the basis of the *in vitro* experiments that fungicides are often ineffective in controlling the production of mycotoxins by *Fusarium* and *Aspergillus* species. Consequently, fungicides are not effective in restricting the number of the species either. The results obtained cannot, however, be transferred to field conditions, where the *Fusarium* flora consists of many species and strains. *In vitro* experiments have been carried out with one specific *Fusarium* strain. Also in field conditions, fungicides have either reduced or increased infection with *Fusarium* fungi and the production of toxins. In Japan, Topsin M (thiophanate-methyl) reduced damage by *F. graminearum* on wheat and barley as well as the content of trichotecenes, DON, NIV in yield (Ueda, Yoshizawa, 1988). Several authors (Suty et al., 1996; etc.) report the good effect of Folicur (tebuconazole) in controlling head blight and reducing toxin content in yield. Gareis and Ceynova (1994), on the other hand, found that the fungicide Matador (tebuconazole, triadimenol) considerably reduced the incidence of head blight in wheat infected with *F. culmorum* but increased the content of NIV in yield 16 times. An increase of toxin content in yield has been noticed particularly in using strobilurines. Oldenburg et al. (2000) tested comparatively azoles and strobilurines in controlling *F. graminearum* and *F. culmorum* on winter wheat. If fungicides containing tebuconazole and metconazole reduced the DON content compared with control, strobilurines, on the other hand, increased it. Tischner and Doleschel (2003) also noticed similar increase of the DON content in using strobilurines. In the same experiments, azoles reduced both spreading of the disease and the DON content in yield by 40—70%. Therefore, the use of highly effective strobilurines is possible only in combination with azoles. Such a fungicide combination system must, during the growth period, suppress development of all plant diseases, restrict effectively reproduction of saprophytic fungi and also ensure unhindered production of *Fusarium* spp. and fusariotoxins.

Fusarium spp. often occur in Estonian grain — 38—100% of samples, whereas the level of seed infection (8—67%) primarily depends on the weather of the growth period. The most contaminated cereal in 1973—1981 was oats (87% of samples, 33% of seeds), followed by wheat (86% and 29%, respectively) and barley (79% and 29%), the least contaminated being rye (62% and 14%). At present, the infection of grain with *Fusarium* species has not diminished. Infection of grain increases with rainy growing seasons, lodging, and delayed harvest. The most widely spread species are *F. avenaceum, F. poae, F. semitectum, F. oxysporum, F. ventricosum, F. sporotrichioides, F. verticillioides,* and *F. culmorum.* The composition of *Fusarium* flora depends on the weather as well. Species known as toxicant often occur in grain (in 50—60% of samples). Fusariums are a genus of microorganisms contributing to the spoiling process of grain feeds (cereal grain). *Fusarium* species in grain feeds with spoiling signs sometimes made up 6.8—8.2% of the total number of fungi and they were found in 52% of samples, including also the toxicants: *F. culmorum, F. tricinctum,* F. *verticillioides,* and *F. sporotrichioides.* 40% of feed samples contained *Fusarium* isolates toxic to *Bacillus stearothermophilus*. Of the isolates studied, most (69%) were medium or highly toxic. The most toxic ones were *F. verticillioides, F. culmorum* and *F. tricinctum* isolates. When separated from dry grains of quality, the toxicity of *Fusarium* isolates fluctuated from non-toxicity to high toxicity. Of the 14 fungicides tested, Tilt 250 EC (propiconazole) and Corbel (fenpropimorph) reduced most (6—10 times) the number of Fusariums in yield.

To obtain production free of *Fusarium* spp. and fusariotoxins, it is necessary to apply a system of measures where both chemical (dressing of seeds sown, spraying of fields with fungicides, use of retardants to avoid lodging) and agrotechnical (optimum sowing norm and time, balanced fertilisation, timely harvest, etc.) methods are used. It is also necessary to elaborate a system of combining strobilurines and azoles (and other fungicides) that would be effective for controlling the majority of diseases and saphrophytic fungi appearing at the end of growth period. Quick drying and cleaning of harvested grain and its storing in suitable conditions are the most important factors for avoiding subsequent production of toxins and ensuring quality storing of grain.

References

1. Avikainen, H., Hannukkala, A. 2001. Viljojen punahomeet valloillaan. Leipä leveämmäksi, 2, 32—33.
2. D´Mello, J. P. F., Macdonald, A. M. C., Postel, D., Dijksma, W. T. P., Dujardin, A., Placinta, C. M. 1998. Pesticide use and mycotoxin production in *Fusarium* and *Aspergillus* phytopathogenes. Eur. J. Plant. Pathol., 104, 74—151.
3. Gareis, M., Ceynova, J. 1994. Influence of the fungicide Matador (tebuconazole, triadimenol) on mycotoxin production by *Fusarium culmorum*. Z. Lebensm. Unters. Forsch., 198, 244—248.
4. Gerlach, W., Nirenberg, H. 1982. The Genus *Fusarium* — a Pictorial Atlas- Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft. Berlin, Heft 209, 1—406.
5. Harrigan, W. F., McCance, E. M. 1976. Laboratory Methods in Food and Dairy Microbiology. Academic Press, London, New York, San Francisco, 452 pp.
6. Kadis, S., Ciegler, A., Ajl, S. J. 1971. Microbial toxins. Volume VII. Algal and Fungal Toxins. Academic Press, New York and London, 586 pp.
7. Lõiveke, H., Ilumäe, E., Toome, M. 2004. Toxigenic microfungi in cereals. Agronomy 2004. Transactions of EAU, 219, 163—165 (in Estonian).
8. Lõiveke, H., Laitamm, H., Sarand, R.-J. 2003. *Fusarium* fungi as potential toxicants on cereals and grain feed grown in Estonia during 1973—2001. Agronomy Research, 1 (2), 185—196.
9. Miller, J. D., Trenholm, H. L. 1997. Mycotoxins In Grain. Compounds Other Than Aflatoxin. Eagan Press, St. Paul, Minnesota, USA, 552 pp.

10. Obenauf, U. 2002. Fusarien machen auch im Norden Probleme. DLZ Agrarmagazin, 5, 20—25.
11. Oldenburg, E., Weinert, J., Wolf, G. H. 2000. Eifluss von Fungiziden auf den Mykotoxin-Gehalt von Weizen. Bericht des Instituts für Pflanzenbau und Grünlandwirtschaft. Jahresbericht, 22 pp.
12. Placinta, C. M., D´Mello, J. P. F. D., Macdonald, A. M. C. 1999. A review of worldwide contamination of cereal grains and animal feeds with *Fusarium* mycotoxins. Animal Feed Science and Technology, vol. 78, 21—37.
13. Schachermayr, G., Fried, M. P. 2000. Porblemkreis Fusarien und ihre Mykotoxine. AGRAR Forschung, 7 (6), 252—257.
14. Suty, A., Mauler-Machnik, A., Courbon, R. 1996. New findings on the epidemiology of fusarium ear blight on wheat and its control with tebuconazole. In: Proceedings of the Brighton Crop Protection Conference. Pests and Diseases, vol. 2, 511—516.
15. Tichner, H., Doleschel, P. 2003. Einflussfaktoren auf den Befall und Toxinbildung durch Ährenfusarien an Weizen. GetreideMagazin, 8 (2), 68—74.
16. Ueda, S., Yoshizawa, T. 1998. Effect of thiophanate-methyl on the incidence of scab and the mycotoxin contamination in wheat and barley. Ann. Phytopath. Soc.Japan, 54, 476—482.
17. Ylimäki, A., Jamalainen, E. A. 1986. The occurrence of *Fusarium* fungi in Finland. Annales Agriculturae Fenniae, 25, 9-30.
18. Ylimäki, A. 1981. The mycoflora of cereal seeds and some feedstuffs. Annales Agriculturae Fenniae, 20, 74—88.
19. Ylimäki, A., Koponen, H., Hintikka, H.L., Nummi, M., Niku-Paavola, M.-L., Ilus, T., Enari, T. — M. 1979. Mycoflora and occurrence of *Fusarium* toxins in Finnish Grain. Techn. Res. Centre Finl. Mater. and Proc. Techn. Publ., 21, p. 28.
20. Watson, D. H., Lindsay, D. G. 1982. A Critical Review of Biological Methods for the Detection of Fungal Toxins in Food and Foodstuff. Journal of Science of Food and Agriculture, 33, 59—67.
21. Билай, В. Й. 1955. Фусарии (Биология и систематика). Киев, 318.
22. Билай, В. Й. 1977. Фусарии. Киев, 441.
23. Евсеева, Р.П. 1992. Опасность можно предотвратить. Защита растений, 11, 12—13.
24. Лойвеке, Х. 1987. Фусариозная инфекция на семенах зерновых в Эстонской ССР. Защита сельскохозяйственных растений в условиях применения интенсивных технологий. Тезисы докладов научно-практической конференции. Минск, 21—22 октьября 1987 г. Минск, 17—18.

FUSARIUM SPP. INFECTION OF SPRING BARLEY IN THE CZECH REPUBLIC AND POSSIBILITIES OF INTEGRATED CONTROL OF THIS DISEASE

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Abstract

Three hundred and sixty spring barley genotypes from the world gene collection were tested in a field trial under the artificial infection with conidia suspension of *Fusarium culmorum.* Six barley cultivars registered for growing in the Czech Republic were tested for DON-toxin accumulation after strong scab inoculation. The effect of plant treatment with a fungicide (tebuconazole 250 g/ha) was assessed.

An infection level as well as DON content were significantly reduced by the application of the fungicide. There was significant influence of barley genotype on final reaction under conditions of inoculation and also with or without the fungicide spraying.

The mean level of DON in the trial was 9.2 mg/kg, minimum concentration was 0.36 mg/kg DON and maximum reached 35.4 mg/kg DON. The lowest DON accumulation combined with zero incidence of powdery mildew (*Blumeria graminis* f. sp. *hordei*) and low and medium net blotch (*Drechslera teres*) infection were found in cvs Princesse and Union Firlbecks.

The possibilities of combining genotypically based resistance with the application of effective fungicides are discussed.

Key words: barley, scab, *Fusarium culmorum,* resistance, fungicides, integrated protection systems.

Introduction

In some years, malting barley harvested in Finland, Sweden, Denmark and Scotland has a very heavy infection by *Fusarium*, and thus not all of the harvest will be accepted by the brewing industry (Larsen, 2000). The main cause of the economic loss in malting barley is the presence of deoxynivalenol (DON) or vomitoxin, a mycotoxin produced by the fungus. Studies in Germany revealed that DON has the highest frequency of occurrence in wheat, oats and barley, with a contamination rate of 30—90% (Drochner, Lauber, 2001). In Europe, *Fusarium* infection is responsible for beer gushing, but in North America the presence of mycotoxins discriminates the barley for use in the brewing industry.

Breeding firms develop extensive phytopathological programmes aiming at the detection of donors with higher resistance to FHB. Fruitful international cooperation among gene banks and research centres is one of preconditions of success in this field.

Producers of agrochemicals have developed and registered on the market some fungicidal active ingredients which are effective against FHB (*Fusarium* head blights). One of them is triazole molecule tebuconazole, which has been used as an effective standard in many studies (Jones, 2000; Hudec et al., 2003; Jorgensen, Jensen, 2003).

On the other hand, the FHB epidemic development is controlled by many independent factors including weather conditions and therefore the optimal timing of fungicide application is very difficult to determine (Kaminski, 2003).

Barley growing and breeding have a long-term tradition in the Czech Republic. The collection of genetic resources housed at the Agricultural Research Institute Kromeriz, Ltd., comprises a large number of foreign genotypes but as well as local varieties and landraces. These accessions are assumed to be well adapted to the conditions where they were developed. Therefore, it is useful to recognize their reactions to current biotic stresses that also include infection by the most frequent diseases.

The objective of this programme was to detect cultivars and genotypes within the world barley collection that are less infected by FHB and to assess genotype-dependent reaction of spring barley cultivars to fungicide treatment under the conditions of strong FHB epidemic.

Materials and Methods

1. Reaction of spring barley genotypes to FHB

The trials were conducted in fields of the Agricultural Research Institute Kroměříž, Ltd., in the season of 2003 (average annual temperature 8.7 °C, average annual precipitation 599 mm).

Ninety-eight spring barley genotypes were sown in two replications using a small-plot drill. Each replication was 1 m^2 in size.

The heading date and plant height were assessed in all genotypes.

The trials were artificially inoculated with spores of *Fusarium culmorum*. The concentration of the inoculum was adjusted to 6 million conidia per ml. Inoculation was carried out at full anthesis (DC 65) in five terms depending on genotypic differences. The assessment was carried out in 2—5 weeks after inoculation in the field as the necrotic area of spikes. We assessed twenty spikes for each genotype.

The DON-toxin content in harvested grains of the barley genotypes was detected using an ELISA method. Data on *Fusarium* infection were completed with field reactions to other leaf diseases. Powdery mildew (*Blumeria graminis*

f. sp. *hordei*), net blotch (*Pyrenophora teres*) and leaf stripe disease (*Helminthosporium gramineum*) were assessed during the whole growing season. The results were statistically compared.

2. Reaction of spring barley genotypes to *tebuconazole* treatment under the conditions of strong FHB infection

Six spring barley genotypes registered for growing in the Czech Republic were sown in twelve replications using a micro-plot seeder in years 2002 and 2003. Each replication was 1 m^2 in size.

The trials were artificially inoculated with spores of *Fusarium culmorum*. The concentration of the inoculum was adjusted to 6 million conidia per ml. Six replications were sprayed with the fungicide Horizon 250 EC (a.i. tebuconazole 250 g/l) and three of them 24 hours later inoculated with *F. culmorum.* Four variants were established:

- A: no FHB infection,
- B: FHB infection,
- C: no FHB infection +treatment with tebuconazole 250 g/ha,
- D: FHB infection + treatment with tebuconazole 250 g/ha.

The content of DON mycotoxin in harvested grains was detected using an ELISA method in all variants.

Results and Discussion

Experiment 1:

ANOVA confirmed highly significant differences in grain contamination by DON mycotoxin (Table 1). The mean value of DON was 9.24 mg/kg. The highest level was found in the cultivar Philadelphia (25.4 mg/kg). Another cultivars exhibiting significantly high DON concentrations were: Early Chevalier, Ceres, Morgenrot, Ymer, Isaria Nova and Provost.

Table 1

ANOVA of DON content (mg/kg) in infected grains of the genotypes assessed

Source of variation	d.f.	Mean square	Significance
genotype	97	113.973	hs
residual	196	0.0186	

Note: hs = significant at α 0.01.

Among 11 cultivars with the lowest DON content, there were seven cultivars whose percentage of visual infection by FHB assessed in the field was low, too. Two genotypes displayed medium and two genotypes — high infection. The important traits of genotypes with low DON in grains are summarized in Table 2.

However, the non-significant correlation was found between grain infection and DON contamination after harvest for all tested cultivars (Table 3). Another analyses indicate that the heading date did not affect final fusarium infection, but plant height at the heading stage was in highly significant negative correlation with an infection level. Higher susceptibility to FHB was accompanied by increased susceptibility to net blotch at high significance. No relationship was found between the susceptibility to FHB and that to powdery mildew and leaf stripe disease.

Table 2

Agronomically important traits of barley genotypes with low DON accumulation in grains

Genotype	DON (mg/kg)	Fusarium head blight	Heading date	Height (cm)	*B. graminis*	*P. teres*	*H. gramineum*
PRINCESSE	0.357	LS	06.06.	80	R	LS	HS
SELECTA HANAK. 1	0.766	MS	10.06.	105	LS	LS	LS
UNION FIRLBECKS	1.058	LS	10.06.	80	R	MS	R
SPARTAN	1.226	LS	10.06.	85	MS	LS	R
JERSEY	1.300	HS	06.06.	80	R	HS	R
KM 1192	1.326	LS	10.06.	75	R	LS	R
DIGGER	1.385	LS	10.06.	75	LS	LS	LS
DIPLOM	1.500	HS	06.06.	87	LS	HS	HS
DOMEN	1.538	LS	10.06.	90	LS	LS	LS
HODONÍNSKÝ KVAS	1.771	MS	06.06.	105	MS	LS	LS
OPÁL	1.959	LS	06.06.	95	R	LS	LS

Note: field disease assessment key: R — resistant, LS — low susceptible, MS — medium susceptible, HS — highly susceptible.

Table 3

Correlation coefficients between *Fusarium culmorum* infection traits (spike infection and DON content) and other characteristics

	Spike infection	Significance	DON (mg/kg)	Significance
Spike infection		ns	0.07	ns
DON (mg/kg)	0.07	ns		ns
Heading date	0.00	ns	–0.18	ns
Plant height (cm)	0.02	ns	–0.27	ns
B. graminis	0.00	ns	–0.17	ns
P. teres	–0.13	ns	0.39	hs
H. graminearum	0.08	ns	–0.07	ns

Note: ns = non-significant, hs = significant at α 0.01.

We assessed the field reaction to FHB in a large collection of genebank accessions which are variable in many agronomic and growth parameters. The main attention was given to resistance to fungal diseases.

There are two cultivars with low DON accumulation which were registered in former Czechoslovakia before the Second World War. 'Selecta Hanak 1', registered in 1926, is a midlate cultivar (111 days), susceptible to powdery mildew, medium susceptible to net blotch and rust. The spike has a high grain number (26) with relatively high TKW. 'Hodonínský Kvas', registered in 1937, is also a midlate cultivar (105—115 days), susceptible to powdery mildew, medium susceptible to rust, but highly resistant to net blotch.

There are two cultivars with a low DON grain content which have been registered for growing in the Czech Republic after 2000 and which are now planted on a large area of malting barley fields. The first of them is 'Jersey' (Cebeco Zaden, B.V. Vlijmen, The Netherlands). The midlate genotype with medium height of stem (76 cm), high resistance to powdery mildew (Mlo gene), medium susceptibility to scald and high susceptibility to net blotch, and a high level of malting quality. The second one is 'Diplom' (Nordsaat Saatzucht, Germany). Medium resistance to powdery mildew, susceptibility to net blotch and rust is combined with high malting quality.

The genotypes with low DON accumulation include 'KM 1162' which was bred by the former Cereal Research Institute Kromeriz. The midlate cultivar has medium plant height (71—80 cm), medium resistance to resistance to powdery mildew, and it could be used as a resistance source to net blotch and rust.

This short review of some accessions from our experiment with low DON accumulation evidences that the higher resistance to FHB can be found on variable genetic background and can be influenced by different factors. We did not find any significant correlation between traits of FHB resistance and agronomic parameters: plant height or heading date. The influence of both these factors, which can significantly change the disease rate under natural epidemic conditions, is completely eliminated under conditions of artificial infection with conidia suspension, which was made regularly when particular genotypes coming to the flowering stage. On the other hand, the significant correlation was found between DON content and net blotch resistance. This finding could probably stand for the set of genotypes tested in this project only.

Experiment 2:

ANOVA showed a highly significant effect of the genotype and fungicide treatment on final DON content (Table 4). The highest level of mycotoxin content was found in cv. TOLAR under artificial inoculation with FHB (Table 5). *Tebuconazole* reduced grain contamination with DON to 60% compared with the non-treated control. High infection by FHB resulting in a high DON level was also found in cv. AKCENT. On the contrary, the lowest DON content was found in both treated and non-treated variants of cv. JERSEY.

Table 4

ANOVA of DON (mg/kg) grain content

Source of variation	d.f.	f-Ratio	Significance
Treatment	3	54.96	hs
Year	1	0.08	ns
Genotype	5	13.34	hs

Note: hs — significant at 0.01, ns — nonsignificant.

Table 5

Two years mean DON level (mg/kg) in selected spring barley cultivars

	A	B	C	D
OLBRAM	0.14	12.20	0.12	6.78
TOLAR	0.59	27.15	0.21	15.80
KOMPAKT	0.48	15.45	0.17	11.64
SCARLETT	0.46	16.60	0.18	12.68
AKCENT	2.37	19.55	0.74	18.37
JERSEY	0.16	1.35	0.30	1.14
Mean	0.70	17.41	0.29	11.56

Note: - A: no FHB infection,
- B: FHB infection,
- C: no FHB infection + treatment with tebuconazole, 250 g/ha,
- D: FHB infection + treatment with tebuconazole, 250 g/ha.

The possibility to reduce the risk of high DON occurrence in harvested grain is strongly influenced by genetic backround of grown cultivars. The efficacy of fungicide treatment against FHB can reduce DON content under the level acceptable for human consumption in moderately or highly resistant genotypes only. The combination of both approaches is therefore the strategy of protection against FHB in spring barley growing.

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References

1. Drochner, W., Lauber, U. 2001. Occurence of the three important Fusarium-toxins deoxynivalenol, nivalenol and zearalenone in grains in central Europe and effects in farm animals. Ubersichtsreferat, Kurzfassungen der Originalmitteilungen und workshop Beitrage der 55. Tagung vom 06.—08.03.2001 in Gottingen, Proceedings of the Society of Nutrition Physiology, 10, 163—168.
2. Hudec, K., Muchova, D., Ondrejcak, F. 2003. Chemical control of barley leaf pathogens and influence of fungicides on Fusarium spp. occurrence in grains. Acta Fytotechnica et Zootechnica, 6, 1, 24—27.
3. Jones, R.K. 2000. Assessments of Fusarium head blight of wheat and barley in response to fungicide treatment. Plant Disease, 84, 9, 1021—1030.
4. Jorgensen, L.N., Jensen, K.F. 2003. New fungicides and strategies for disease control in cereals. 20th Danish Plant Protection Conference 'Cereal, potatoes, pests, environment and posters', February 2003. DJF-Rapport, Markbrug., No. 89, 289—298.
5. Kaminski, D. 2003. Progress in forecasting: FHB risk forecasts in Manitoba. Proceedings of 3rd Canadian Workshop on Fusarium head blight, Delta Winnipeg, Manitoba, December 9th—12th, 2003, p. 91.
6. Larsen, L.J. 2000. Fusarium in malting barley. Proceedings of the 17th Danish Plant Protection Conference II, Site-specific crop protection, decision support, pests and diseases, ear blight. DJF Rapport, Markbrug, No. 24, 179—181.

COMPARISON OF FUNGICIDAL PROGRAMMES IN CEREALS, DIFFERENT IN THEIR INTENSITY, CURRENTLY USED IN THE CZECH REPUBLIC

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Abstract
Three separate field experiments were established to screen fungicidal efficacy of selected, widely used fungicides against leaf diseases of winter wheat, to compare the possibilities of controlling Fusarium head blight (FHB) and DON mycotoxin accumulation and to look for relationship between fungicidal effects and breadmaking quality parameters. The leaf rust (*Puccinia recondita*), septoria leaf blotch (*Septoria tritici*) and powdery mildew (*Blumeria graminis*) development was compared in variants protected with one, two or three treatments differing in the dose of active ingredient. There were highly significant yield differences between the models of one or two treatments and the best septoria leaf blotch control after three treatments made with reduced rates of fungicides. The use or avoidance of treatment with a fungicide at the growth stages DC 37–DC 47 was more important than the type of preparations (active ingredients). A good possibility of combining special products against FHB (triazoles) with some active ingredients from strobilurin group (trifloxistrobin) was effective in suppression of diseases as well as in reduction of DON mycotoxin. A significant influence of weather conditions in particular years on parameters of breadmaking quality was found: falling number, test weight, and final protein content. The test weight was higher in variants treated with fungicides than in the non-treated check in all experimental years.

Key words: winter wheat, *Blumeria graminis, Puccinia recondita, Septoria tritici, Fusarium culmorum,* mycotoxins, fungicides, breadmaking quality.

Introduction

The main aim of our research was to develop efficient, economically acceptable and environmentally safe application schedules of fungicides. Current fungicidal control is characterized by the following trends:

- development and wide use of new active ingredients and substances with a new mode of action;
- use of fungicides with a wide efficacy spectrum and combined control of more pathogens by one treatment;
- right timing of fungicidal treatment and assessment of the dose of applied preparations based on threshold values of disease infection;
- minimizing epidemiological risks of pathogen resistance inception to widely used active ingredients of preparations.

Materials and methods

1. Field experiment for the assessment of leaf diseases under natural infection

Winter wheat cv. Ebi, susceptible to fungal pathogens, was sown in randomized blocks of 10 m^2 in 4 replicates of each in the season of 2003.

The efficacy of one, two and three treatments was assessed. The following fungicides and spraying programmes were used:

Programme A:
T1 Alert 1.0 l/ha (a.i. flusilazole 125 g/l + carbendazim 250 g/l),
T2 Cerelux plus 0.4 l/ha (a.i. flusilazole 160 g/l + phenpropimorph 375 g/l),
T3 Charisma 0.75 l/ha (a.i. famoxadone 100 g/l + flusilazole 107 g/l);
Programme B:
T1 Alert 1.0 l/ha (a.i. flusilazole 125 g/l + carbendazim 250 g/l),
T2 Charisma 0.75 l/ha (a.i. famoxadone 100 g/l + flusilazole 107 g/l),
T3 Cerelux plus 0.4 l/ha (a.i. flusilazole 160 g/l + phenpropimorph 375 g/l);
Programme C:
T1 Alert 1.0 l/ha (a.i. flusilazole 125 g/l + carbendazim 250 g/l),
T3 Charisma 1.0 l/ha (a.i. famoxadone 100 g/l + flusilazole 107 g/l);
Programme D:
T2 Alert 1.0 l/ha (a.i. flusilazole 125 g/l + carbendazim 250 g/l).

The periods between treatments were 10—15 days long, first treatment (T1) was made at DC 31, second (T2) — at DC 37, and third (T3) — at DC 55 (TOTTMAN and BROAD, 1987). The plots were sprayed using an apparatus Gloria, BASF firm.

All programmes were compared with the non-treated control. The standard estimation of the percentage of leaf area covered by pathogens (powdery mildew, septoria leaf blotch and leaf rust) was assessed. After the harvest, yield differences were analyzed by ANOVA.

2. Field experiment under artificial inoculation with *Fusarium culmorum*

Winter wheat cv. Ebi was sown in randomized blocks of 10 m^2 in 4 replicates of each in the season of 2003. The treatment with fungicide Sphere 267.5 EC (trifloxistrobin 187.5 g/l + cyproconazole 80 g/l) at the rate of 0.8 l/ha was carried out at DC 55 (heading).

Second treatments with fungicides were applied one day before inoculation. The following triazoles and doses were used:

- tebuconazole (200 g/ha);
- prothioconazole (200) g/ha;
- tebuconazole + prothioconazole (125 + 125) g/ha.

The whole experiment was inoculated with 6.10^6 conidia/ml of *F. culmorum* isolate with a high level of aggressiveness. The inoculation was carried out at DC 65 (full flowering).

The DON content (mg/kg) in harvested grains was assessed immunologically by ELISA. The results were compared statistically by ANOVA.

3. The influence of fungicides on breadmaking quality

This programme was conducted during 2000—2003. Winter wheat cv. Ebi was sown in randomized blocks of 10 m^2 in 4 replicates of each. The following fungicide programme was used:

T1 Alert 1.0 l/ha (a.i. flusilazole 125 g/l + carbendazim 250 g/l);

T3 Charisma 1.0 l/ha (a.i. famoxadone 100 g/l + flusilazole 107 g/l).

Terms of treatment (T1—T3) were used according to description in part 1. The levels of the following grain quality parameters were assessed: falling number (sec), test weight (g/l) and protein content (%) and compared with results achieved in the non-treated control.

Results and Discussion

Experiment 1:

Biological evaluation of this experiment was aimed at the three most important leaf diseases in the climatic region of the Czech Republic. The highest efficacy was found in programmes A and B (Table 1). Neither two nor one treatment programme reached 50% efficacy against septoria leaf blotch. It is evident that the avoidance of fungicide treatment in T2 caused important decrease in disease suppression, especially in septoria leaf blotch. Wheat crops are at the greatest risk during stem extension, when the final three leaves emerge in close proximity to infected leaves lower in the canopy (Armour et al., 2003). Preventive type of fungicidal influence of some active ingredients (strobilurins) especially needs to be used as soon as possible of the epidemic development.

Table 1

Fungicidal effect of application programmes different in their intensity, DC 77 (late milk)

Programme	Powdery mildew		Septoria leaf blotch		Leaf rust	
	%C	effect. (%)	%C	effect. (%)	%C	effect. (%)
A	23.8	97.5	26.6	97.9	45.9	99.3
B		94.7		100.0		100.0
C		97.4		41.0		85.0
D		73.1		41.6		64.7

Note: %C — infection level in non-treated plot; effect. (%) — fungicidal effectiveness.

The yield reached up to 6000 kg/ha in the non-treated control variant (Table 2). All variants treated with the fungicides exceeded this yield on a high level of significance. The difference of about 500 kg/ha was assessed between the mean yield of variants treated three times or twice and 750 kg/ha between variants treated three times or once.

Table 2

Results of ANOVA — yield analysis

Programme	Mean yield (kg/ha)	Difference to control (kg/ha)	Difference to control (%)	Significant difference
control	6000			
A	8100	2100	135.0	xx
B	8250	2250	137.5	xx
C	7700	1700	128.3	xx
D	7400	1400	123.0	xx

Note: xx = significant at 0.01.

Experiment 2:

The DON content (mg/kg) in harvested grains was significantly lower after all fungicide treatments (Table 3). Only one preventive treatment with Sphere 267.5 EC reduced DON content to about 50% in comparison with the non-treated control. All two-spraying programmes showed final DON-concentration around 1 mg/kg. Tebuconazole was selected because this active ingredient reduced both FHB and the level of DON in many trials (Jones, 2000). Prothioconazole is one of new triazoles, brought on the market by Bayer company. The influence of this fungicide against FHB is very good, too. The fact that trifloxistrobin in mixture with cyproconazole showed positive effects against FHB opened other possibilities of effective control of leaf and spike diseases aggregated in one treatment.

Table 3

Spike infection and DON mycotoxin content in winter wheat after FHB infection and treatments with fungicides

Treatment	Spike infection (%)	DON (mg/kg)
Nontreated	59.1	15.47
Sphere	22.9	7.38
Sphere — Tebuconazole	6.3	1.58
Sphere— Prothioconazole	10.5	1.13
Sphere — (Tebuconazole + Prothioconazole	14.5	1.34

Experiment 3:

There were significant differences between particular years in the levels of falling number and test weight as well as in grain protein content (Table 4). The treatment with fungicides highly significantly influenced the level of test weight in all experimental years. The data of compared traits are summarized in Figures 1—3.

It is widely accepted that the effect of fungicides can prolong the grain filling period as well as the period of active assimilation and production of carbohydrates. We used fungicide Charisma in the second application term. This product is based on active ingredient famoxadone, which belongs to the group of strobilurin fungicides. Strobilurin fungicides have a broad spectrum activity against all major foliar pathogens of wheat. In addition to this extraordinary fungicidal activity, side effects have been reported which result in higher yields of cereals, e.g. the reduction of respiration, delayed leaf senescence, activation of nitrogen metabolism as well as increased tolerance against abiotic stress factors (Beck et al., 2002). Higher levels of test weight are in good relationship with an increased final yield after the treatment with fungicides.

Saunders and Salmon (2000) confirmed clear differences between cultivars for the majority of the carried out quality tests. The authors mentioned that the majority of wheat Hagberg falling number values were below 250s for Hereward and Rialto. This would cause rejection at mill intake and reduce subsequent processing options. We found in our experiments causal influence of the weather on the falling number in particular years, which was not changed by fungicide treatment on a significant level. The years 2002 and 2003 were relatively dry and hot during ripening and falling number values were very high. On the contrary, rainy start of summer in 2000 and 2001 resulted in extremely low levels of this parameter.

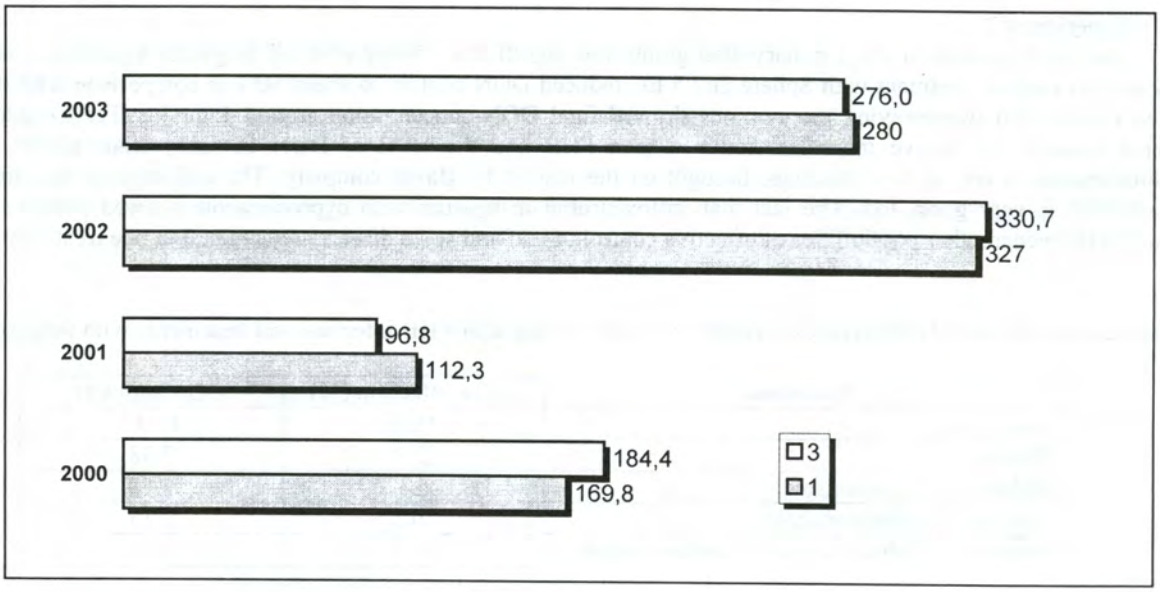
The treatments with fungicides had no major influence on the crude protein content of winter wheat grain (Brzowski et al., 1998). We confirmed these findings and concluded that the effect of the year and cultivar is more important for the final protein content than the use of fungicides. It is important to suggest using some additional N fertilization to winter wheat stands grown with high intensity of protection with fungicides because the disease-free plants have higher requirements for nutrition elements. In these conditions the grain gluten content increased significantly after the combination of N fertilizer, herbicide and fungicide (Surovcik, 1999).

Table 4

ANOVA of grain quality parameters

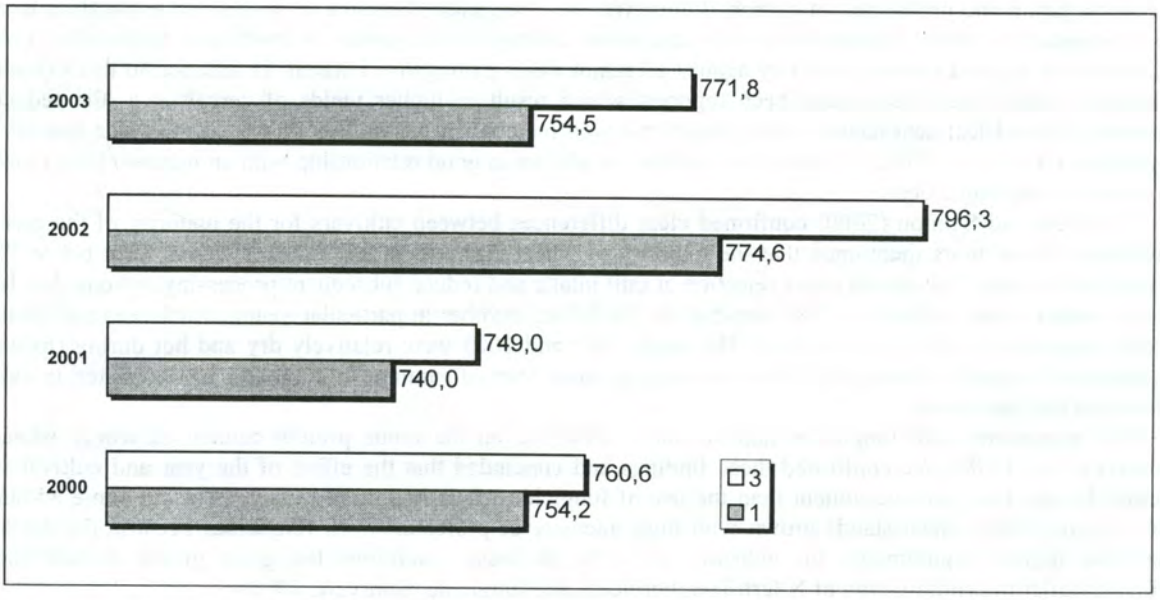
Source	Df	f-Ratio	Significance
Falling Number			
Year	3	1351.44	hs
treatment	1	0.090	ns
Test Weight			
Year	3	187.560	hs
treatment	1	117.710	hs
Protein Content			
Year	3	0.010	s
treatment	1	0.412	ns

Note: hs — significant at 0.01; s — significant at 0.05; ns — non-significant.



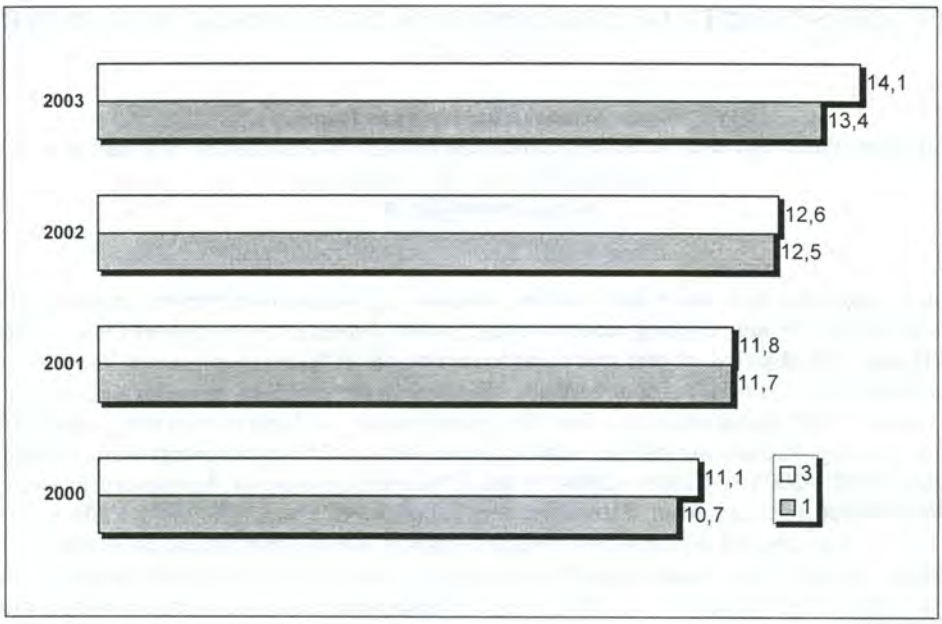
Note: 1 — non-treated with fungicide; 3 — treated with fungicide.

Fig. 1. Winter wheat cv. Ebi: falling number (sec) in 2000—2003



Note: 1 — non-treated with fungicide; 3 — treated with fungicide.

Fig. 2. Winter wheat cv. Ebi: test weight (g/l) in 2000—2003



Note: 1 — non-treated with fungicide; 3 — treated with fungicide.

Fig. 3. Winter wheat cv. Ebi: grain protein content (%) in 2000—2003

The review of quite different protection systems shows that good efficacy can be obtained by permanent fungicidal screen. More intensive (considering particularly the duration of the assumed effect) treatments at the previous term enable a later successive application. The treatments should be wide-spectral, controlling as high the number of diseases as possible. The spectrum of registered preparations provides such an option.

The incidence, dynamics of epidemic development of fungal disease pathogens and infection (damage) severity in winter wheat stands are markedly determined by natural conditions of the given growing season. Multiyear regular evaluations of diseases incidence allow us, to a certain extent, to foresee the epidemics and/or to monitor longer-term developmental trends. However, a major problem is a large variability within populations of fungal disease pathogens, which considerably limits the possibilities of the development of long-term strategies or generally valid methodologies for fungicidal protection. Similarly, reactions of cultivars also change over the time and if they become very susceptible, farmers can gradually lose interest in growing them.

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References

1. Armour, T., Viljanen–Rollinson, S.L.H., Chang, S.F., Butler, R.C., Cromey, M.G., Jamieson, P.D., Zyskowski, R.F., Zydenbos, S.M. 2003. Influence of crop growth and weather conditions on speckled leaf blotch in winter wheat. New Zealand Plant Protection, Volume 56, Proceedings of a conference, Christchurch, New Zealand, 12—14 August, 246—250.
2. Beck, C., Oerke, E.C., Dehne, H.W. 2002. Impact of strobilurins on physiology and yield formation of wheat. 54th International Symposium on Crop Protection, Part I, Gent, 7 May, 2002. Mededelingen Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen, Universiteit Gent, 67, 2, 181—187.
3. Brzowski, J., Brzowska, I., Jastrzebska, M. 1998. Efficiency of fungicide and fungicide-fertilizer treatments applied in winter wheat conditions. Acta Academiae Agriculturae ac Technicae Olstenensis, Agricultura, No. 65, 129—138.
4. Jones, R.K. 2000. Assessments of Fusarium Head Blight of wheat and barley in response to fungicide treatment. Plant Dis., 84, 1021—1030.
5. Saunders, N., Salmon, S. 2000. Effects of strobilurin fungicides on the milling quality of breadmaking winter wheat varieties. HGCA–Project–Report, No. 239, 33 pp.
6. Surovcik, J. 1999. Effect of fertilization and agrochemicals on some qualitative characters of winter wheat. Vedecke Prace Vyskumneho Ustavu Rastlinnej Vyroby v Piestany, No. 29, 39—44.
7. Tottman, D.R., Broad, H. 1987. Decimal code for the growth stages of cereals. Annals of Applied Biology, 110, 683—687.

INCIDENCE AND SEVERITY OF LEAF SPOTTING DISEASES OF WINTER WHEAT IN LITHUANIA

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Abstract

Residue-borne pathogens that cause leaf spotting diseases of wheat have become increasingly important in Lithuania because more growers are adopting reduced tillage, which leaves crop residue on the soil surface compared with conventional tillage. The objective of this study was to investigate differences in susceptibility to leaf infection by *Drechslera tritici-repentis* and septoria leaf spot complex (*Septoria tritici and Stagonospora nodorum*) among some of the currently registered winter wheat cultivars. We also examined the influence of primary field infection on the development of leaf spotting diseases during the whole growing season. The experiments were conducted during the two growing seasons (2002—2003 and 2003—2004) at the Lithuanian Institute of Agriculture in Dotnuva, Kedainiai district. Three winter wheat cultivars Aron, Hereward and Tauras were sown. The field trials were made on two backgrounds: soil surface was covered with naturally infected straw in autumn and natural field infection without straw. Observations of disease severity were made approximately every 7—10 days on each plot starting at the beginning of stem elongation and continuing through the late milk stage. Results showed that the most common disease in winter wheat during the growing seasons 2002—2003 and 2003—2004 in our experiment was tan spot. Septoria leaf spot complex was less prevalent. At the beginning of stem elongation in inoculated with straw and in naturally infected plots the severity of tan spot was similar for all cultivars. Until winter wheat flowering stage, no significant differences between the severity of tan spot in inoculated and naturally infested wheat were found in both years. At late growth stages straw on soil surface had a significant effect on tan spot severity increase. The reaction of test cultivars to leaf spotting disease was different and depended on growing conditions (with or without straw) and growing season.

Key words: winter wheat, tan spot, septoria leaf spot complex, disease severity.

Introduction

Residue-borne pathogens that cause leaf spotting diseases of wheat have become increasingly important in Lithuania because more growers are adopting reduced tillage, which leaves crop residue on the soil surface compared with conventional tillage. The pathogens which cause leaf-spotting disease on wheat include *Pyrenophora tritici-repentis* (Died.) Drechs. (anamorph *Drechslera tritici-repentis* (Died.) Shoemaker) which causes tan spot, and the septoria leaf spot complex, which includes *Phaeosphaeria nodorum* (E. Müller) Hedjaroude (syn. *Leptosphaeria nodorum* E. Müller, anamorph *Stagonospora nodorum* ([Berk.] Berk. in Berk. and Broome) and *Mycosphaerella graminicola* (Fückel) Schroeter (anamorph *Septoria tritici* Rob. ex Desm.). Air-borne ascospores discharged from mature pseudothecia serve as an effective source of primary inoculum for *S. tritici* and. *S. nodorum*. However, there are many instances where the sexual stage was not found and it is, therefore, assumed that infected wheat debris and rain splashed pycnidiosporas serve as a primary source of inoculum (Eyal, 1999). Investigations on epidemiology of tan spot indicate that the main source of primary infection is ascospores produced in pseudothecia developing on dead leaves, leaf sheaths and culms (Maraite et al., 1992; Zamorski and Schollenberg, 1994). Currently *D. tritici repentis* is one of the most important wheat leaves spotting pathogens in many European countries (Bankina, 2002; Gustsson and Berg, 2003; Jorgensen and Jensen, 2003; Sarova et al., 2002; Zamorski and Schollenberg, 1994) and in Lithuania too. Tan spot as winter wheat disease was first reported in Lithuania in 1995.

The objective of this study was to investigate differences in susceptibility to leaf infection by *D. tritici-repentis* and septoria leaf spot complex (*S. tritici and S. nodorum*) among some of the currently registered winter wheat cultivars. We also examined the influence of primary field infection on the development of leaf spotting diseases during the whole growing season.

Materials and Methods

The investigations were conducted during the two growing seasons (2002—2003 and 2003—2004) at the Lithuanian Institute of Agriculture in Dotnuva, Kedainiai district. Three winter wheat cultivars Aron, Hereward and Tauras were sown. A plot size of 300 m^2 was inoculated for each cultivar. A source of inoculum was provided by wheat straw, spread over plants in late autumn. Straw was collected after harvest from the winter wheat field. It was naturally infested with *D. tritici-repentis* and other residue borne pathogens and stored under natural conditions. At 100 m distance from the inoculated plot we marked plots for leaf spotting disease assessments in winter wheat grown on natural field infection background. The development of diseases was monitored by sampling four replications of 15 plants per plots. Random tillers were selected and the top three leaves were scored for disease severity by using the 0—100% scale (Guidelines..., 1999). Observations of disease severity were made approximately every 7—10 days on each plot starting at the beginning of stem elongation and continuing through the late milk stage. Growth stage was recorded when disease severity was assessed (Meier, 1997).

Temperature, relative humidity (RH) and rainfall were recorded from May to July.

The data were analysed by Duncan's multiple range test and significance is reported at $P < 0.05$.

Results and Discussion

In Dotnuva, the winter wheat growing season was little drier in 2003 than in 2004. The total rainfall from 1st May before last assessment in July in 2003 was 105 mm, in 2004 — 122 mm. Frequent rainfall, higher relative humidity in July resulted in more severe infection of leaf spotting diseases in 2004. Results showed that the most common disease in winter wheat during the growing seasons 2002—2003 and 2003—2004 in our experiment was tan spot (*P. tritici-repentis)* (Table1). Septoria leaf spot complex was less prevalent.

Table 1

Leaf spotting disease severity on flag leaf at late milk development in different cultivars of winter wheat in Dotnuva, 2003—2004

Cultivar	2003		2004	
	Tan spot	Septoria leaf spot complex	Tan spot	Septoria leaf spot complex
Natural field infection				
Aron	12.42a*	0.08	9.75a	14.55c
Hereward	13.67a	0	19.58b	0a
Tauras	11.58a	0	8.77a	3.40b
Soil surface inoculated with straw				
Aron	25.17c	0.13	7.75a	0
Hereward	14.08b	0	65.00c	0
Tauras	7.10a	0	59.58c	0

* do not differ (P = 0.05) by the same letter according to Duncan's multiple range test.

At the beginning of stem elongation, the severity of tan spot was similar for all cultivars in inoculated with straw and in naturally infected plots (Figures 1—3). Until winter wheat flowering stage, no significant differences between severity of tan spot in inoculated and naturally infested wheat were found in both years. Tan spot severity in 2003 began to increase at the beginning of ripening (BBCH 71 — milk ripening). More intensive development of tan spot in inoculated plots in 2003 was recorded in cvs. Hereward and Aron compared with naturally infected wheat. In the middle of milk development, the severity of tan spot in the two cultivars in inoculated with straw plots was significantly higher than that in naturally infected plots. In cv. Tauras, significant differences between tan spot severity in inoculated and natural infected plots were not found.

When the weather conditions were more conducive to tan spot development (in 2004) the disease severity began to increase at heading. An increase in tan spot severity was found in both inoculated and not inoculated plots. In winter wheat cv. Aron, development of tan spot was on a similar level in inoculated with straw and naturally infected plots. Significant differences were not found.

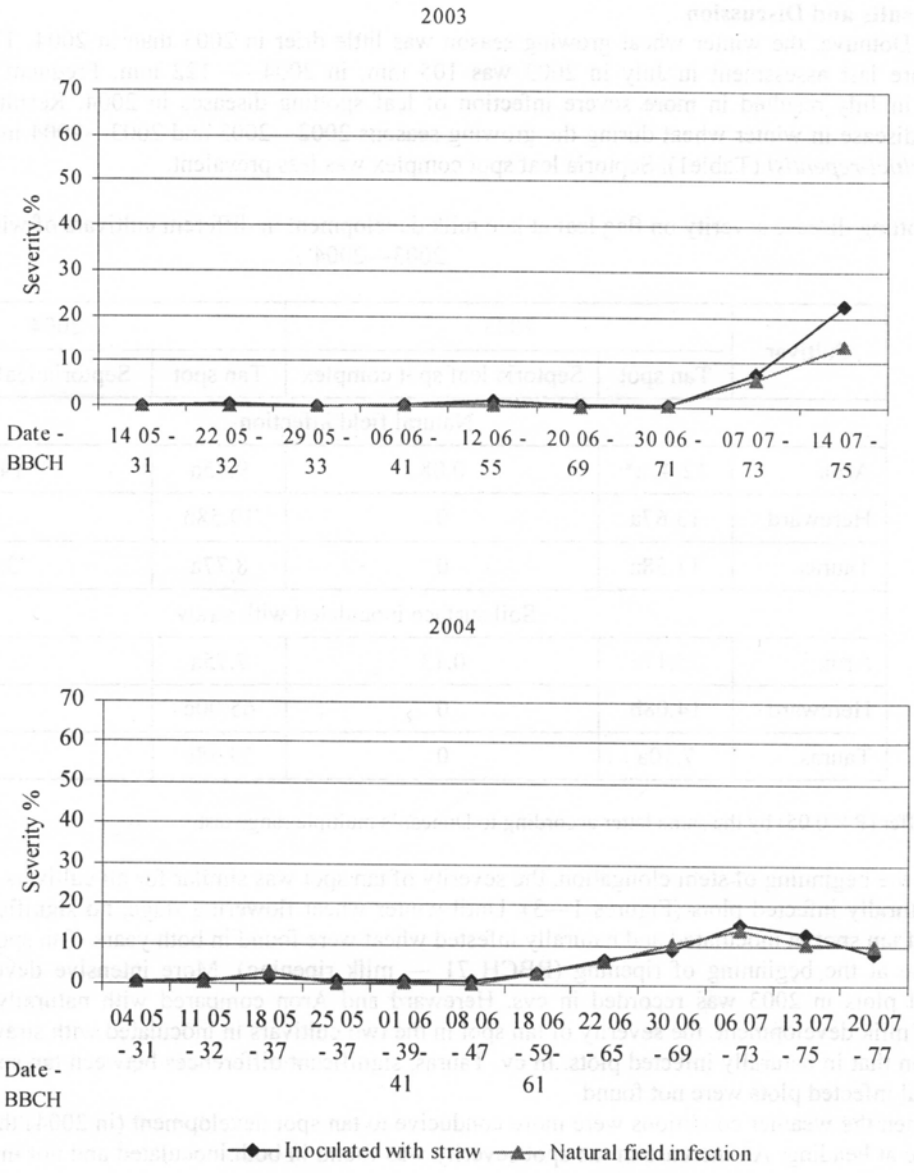

Fig. 1. Tan spot *(Drechslera tritici-repentis)* progress curves of winter wheat cv. Aron in Dotnuva, 2003—2004

In winter wheat cvs. Hereward and Tauras, tan spot developed more intensively in inoculated with straw plots. An extremely severe attack of this disease was recorded at milk growth stage. Our experimental results showed that the most rapid development of tan spot in both years was at late growth stages for all cultivars. We can assume that the severe attack depended not only on the level of primary infection and genotype, but also on the age of plants, too, because older leaves have been found to be more susceptible to tan spot than younger leaves (Cox and Hosford, 1987).

Tan spot severity in cvs. Hereward and Aron at the end of milk development was significantly higher in inoculated with straw plots compared with the plot without inoculation. Some authors have reported that retention of wheat residue on the soil surface generally results in increased tan spot severity, while the incorporation of wheat debris reduces severity (Bockus and Claassen, 1992; Stover et al., 1996; Duveiler et al., 2002).

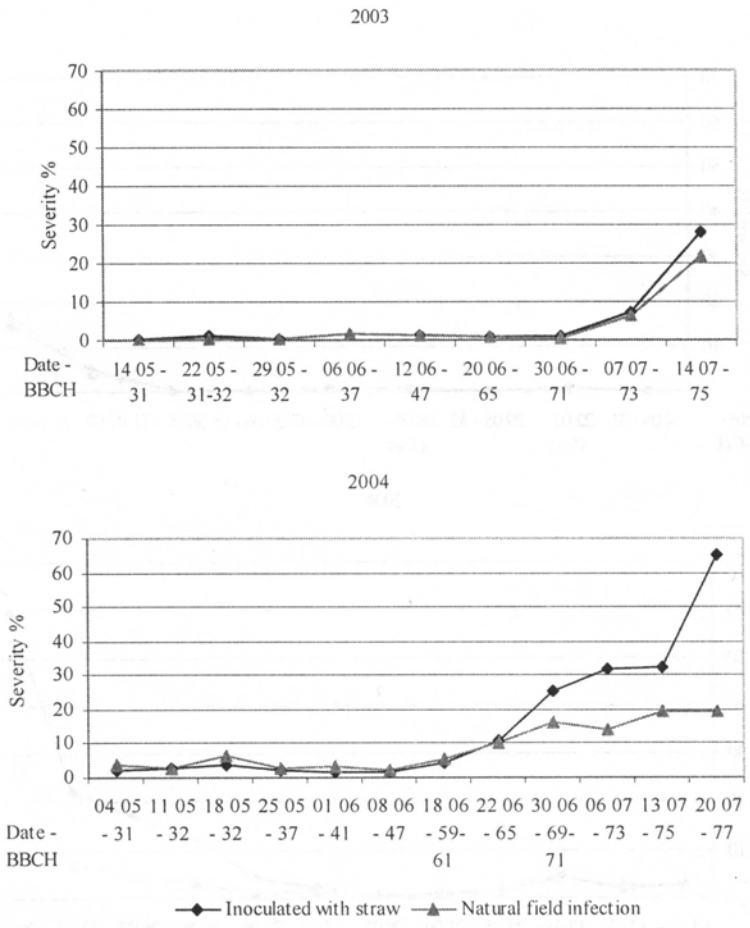

Fig. 2. Tan spot (*Drechslera tritici-repentis*) progress curves of winter wheat Hereward in Dotnuva, 2003—2004

In our study we found that not only straw residues but also the genotype had significant effect on tan spot severity increase. The susceptibility to tan spot at a late milk growth stage of test winter wheat cultivars is presented in Table 1. The leaf area affected by tan spot and septoria leaf spot complex at this growth stage was estimated on flag leaves because lower leaves had died. Under less favourable weather conditions for leaf spotting development in 2003, tan spot severity in naturally infected plots was on the same level. Any significant differences were not found. In inoculated with straw plots the highest severity at milk development was for cv. Aron. This cultivar was significantly more susceptible than other test cultivars. Cv. Tauras was significantly less susceptible to tan spot compared with cvs. Aron and Hereward.

In 2004, when more favourable weather conditions for leaf spotting diseases prevailed, significantly highest susceptibility to tan spot was exhibited by cv. Hereward in naturally infected plots. When soil surface was inoculated with straw, in 2004 significantly lesser tan spot severity was for cv. Aron. Susceptibly of cvs. Aron and Tauras to tan spot was found to differ between the years. This suggests that different race of tan spot might have prevailed in 2003 and 2004. Eight races have been identified in the fungal population based on their ability to produce necrosis and chlorosis symptoms on appropriate wheat differentials (Ali and Francl, 2002). Unfortunately, race populations in Lithuania have not been identified yet.

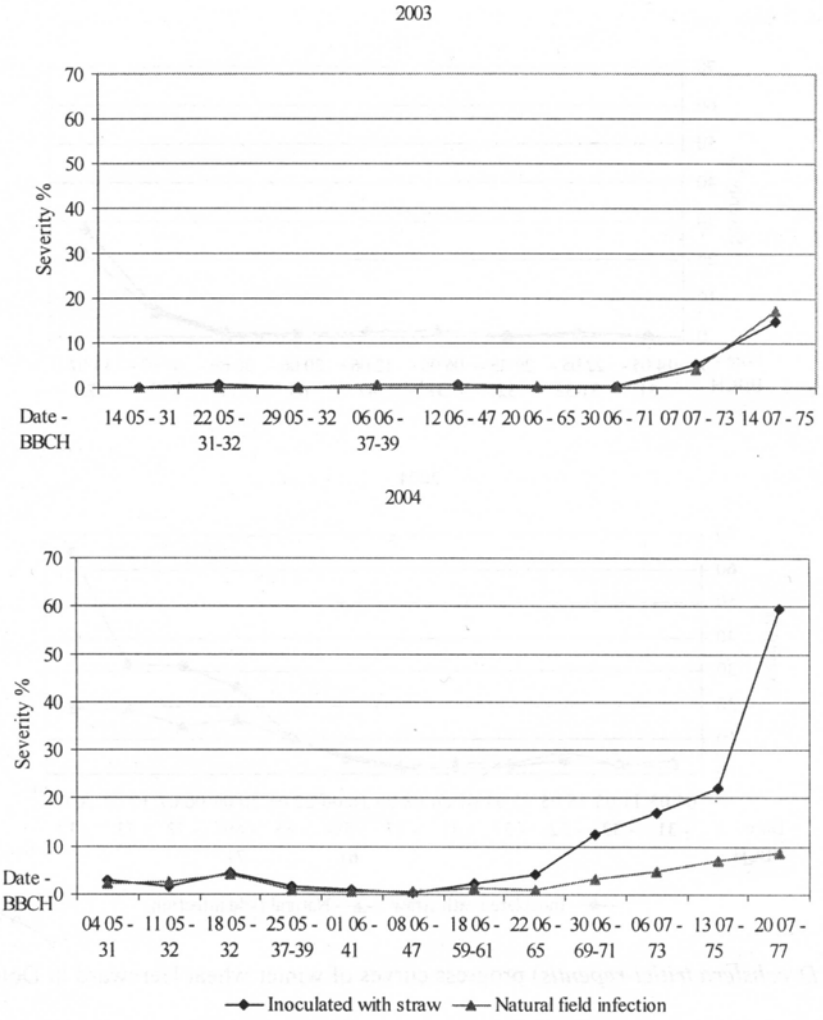

Fig. 3. Tan spot *(Drechslera tritici-repentis)* progress curves of winter wheat Tauras in Dotnuva, 2003—2004

The results presented in this paper highlight the importance of primary infection of soil surface on tan spot development during the growth seasons and genotype response to pathogens in different environmental conditions. The conclusions and assumptions made in this study provide a base for further research.

References

1. Ali, S., Francl, L.J. 2002. Aggressiveness variation within race 1 and 2 isolates of *Pyrenophora tritici-repentis*. 4th International Wheat Tan Spot and spot Blotch Workshop, p. 5.
2. Bankina, B. 2002. Tan spot development peculiarities in Latvia. Plant Protection Science, vol. 2, 381—383.
3. Bockus, W. W., Claassen, M. M. 1992. Effect of crop rotation and residue management practices on severity of tan spot of winter wheat. Plant disease, vol. 76, 633—636.
4. Cox, D. J., Hosford, R. M. 1987. Resistant winter wheats compared at differing growth stages and leaf position for tan spot secerity. Plant diseases, vol. 71, 883—886.
5. Duveiller, E., Garcia, A., Fisher, R. A., Budhathoki, C. 2002. Effect of crop rotation and residue management on tan spot in the sub humid tropical highlands. 4th International Wheat Tan Spot and spot Blotch Workshop, p. 12.
6. Eyal, Z. 1999. *Septoria* and *Stagosnospora* Diseases of Cereals: A Comparative Perspective. In: Septoria and Stagonospora Diseases of Cereals, 1—25.
7. Jorgensen, L. N., Jensen, K. F. 2003. Hvedebladplet — Resultater med fungicidbekæmppelse i Danmark. DJF Rapport nr 89, 329—336.
8. Guidelines for the efficacy evaluation of plant protection products. 1997. PP1/26(3) Foliar diseases on cereals. Vol. 1, 187—195.
9. Gustsson, G., Berg, G. 2003. Vetets bladflacksjuka (DTR) erfarenheter fran Sverige. DJF Rapport nr 89, 337—346.
10. Maraite, H., Berny, J.F., Goffin, A. 1992. Epidemiology of tan spot in Belgium. Advantages in tan spot research. Proceeding of the Second International Tan Spot Workshop, 73—79.
11. Meier, U. (ed.) 1997. Growth Stages of Mono- and Dicotyledonous Plants. BBCH-Monograph, 5—17, 569—572.
12. Sarova, J., Hanzalova, A., Bartos, P. 2002. *Pyrenophora tritici repentis* — one of the most important leaf spot pathogens in the Czech Republic. 4th International Wheat Tan Spot and spot Blotch Workshop, p. 26.
13. Stower, R. W., Francl, L. J., Jordahl, J. G. 1996. Tillage and fungicide management of foliar diseases in a spring wheat monoculture. J. Production Agriculture, vol. 9, 261—265.
14. Zamorski, Cz., Schollenberger, M. 1994. The occurrence of tan spot on wheat and triticale in Poland. Genetic Polish, ser. B, vol. 35, 375—378.

THE OCCURRENCE OF *SEPTORIA* SPP., *PUCCINIA RECONDITA* AND *CLAVICEPS PURPUREA* ON DIFFERENT VARIETIES OF WINTER TRITICALE (*TRITICOSECALE* WITM. EX A. CAMUS) IN LITHUANIA

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Abstract

Resistance of the Lithuania-recommended triticale varieties to septoria leaf blotch, brown rust and ergot was studied at the Botanic Garden of Šiauliai University in 2002 and 2003.

Septoria leaf blotch and brown rust tests were conducted on the background of natural infection. The incidence and severity of these diseases were assessed at plant booting (BBCH 37) and milk ripe (BBCH 75) growth stages. The experimental evidence suggests that due to the dry weather the incidence of septoria leaf blotch in the triticale crops was lower in 2002. At booting stage, the disease severity was 0.04—0.32, at milk ripe 0.81—6.77%. In 2003, when there was more moisture and cooler weather prevailed, the disease severity at milk ripe was as high as 16.06—39.29%. In both experimental years, 'Tewo', 'Tornado' and 'Marko' varieties were found to be more infested with septoria leaf blotch. The spread of brown rust was similar in both experimental years, and it was identified only at the stage of milk ripe. Although the disease incidence ranged from 6.3 to 91.0%, the disease severity was as low as 0.06—3.32%. 'Tewo' and 'Marko' varieties were found to be more susceptible to brown rust.

Ergot tests were carried out on the background of artificial infection. Per cent of ergot-affected ears and the number of ergot-infested ears and sclerotia per 1 m^2 were estimated. Warm and dry weather in 2002 determined a very early and short flowering period of triticale, therefore only in three varieties ergot-affected ears accounted for as little as 0.07—0.20%. In 2003, triticale flowering period started two weeks later compared with 2002, and ergot-affected ears accounted for 0.67 to 3.56%. 'Tewo', 'Tornado', 'Lupus' and 'Marko' varieties with a longer flowering period were more ergot-infested. 'Alzo' and 'Fidelio' varieties with a shorter growing season were most resistant to all investigated diseases.

Key words: triticale, brown rust, septoria leaf blotch, ergot, *Triticosecale, Puccinia recondita, Septoria* spp., *Claviceps purpurea.*

Introduction

In Lithuania, the area sown with winter triticale is on the increase every year. In 1990, winter triticale was grown only in a few plots in Lithuania, whereas in 1999 the area under triticale amounted to 25.9 thousand ha. In 2002, the crop was grown on an area of 40.5 thousand ha and this accounted for 10.4% of the total winter cereals cultivation area (Statistical..., 2000, 2003). One of the problems that triticale growers encounter is diseases. Winter triticale is affected by septoria leaf blotch, brown rust, ergot, head blight, that can cause heavy yield losses (Gaurilčikienė, 1998).

Most triticale varieties are resistant to mildews (*Blumeria graminis*), less susceptible to yellow rust (*Puccinia striiformis*), moderately susceptible to brown rust (*Puccinia recondita*), however, they are very susceptible to septoria leaf blotch (*Septoria tritici*) and ergot (*Claviceps purpurea*). The most common triticale diseases in Lithuania are brown rust, septoria leaf blotch, and ergot (Dabkevičius, Semaškienė, 2001; Gaurilčikienė, 1997).

Septoria leaf blotch is caused by *Septoria tritici* Rob.in Desm. (teleomorfa *Mycosphaerella graminicola* (Fuckel) J. Schröt in Cohn.), septoria glume blotch by *Stagonospora nodorum* (Berk.) E. Castell et Germano=*Septoria nodorum* (Berk.) Berk., teleomorfa *Phaeosphaeria nodorum* (E. Müll.) Hejdar. sin. *Leptosphaeria nodorum* E. Müll.). *S. tritici* is more liable to occur early in spring on lower leaves. *S. nodorum* affects plants later and is the chief pathogen of triticale. In Poland having cross-infected six winter triticale and five winter wheat varieties with the *S. nodorum* pathogen isolated from wheat and triticale it was identified that isolates from triticale are more aggressive to triticale and wheat than those isolated from wheat (Wos, Mackowiak, 1994). In Lithuania, *Septoria spp.* affects triticale annually (Gaurilčikienė, 2001). Weather conditions have the greatest effect on the spread of the disease. Cool and wet weather is especially conducive to the occurrence of the disease (Gaurilčikienė, 1998; Janušauskaitė, Dabkevičius, 2002; Dabkevičius, Semaškienė, 2001).

Brown rust is caused by *Puccinia recondita* Rob. ex Desm. F. sp. *tritici*. Triticale is less susceptible to this pathogen than wheat. The signs of this disease in triticale crops in Poland can be spotted at the end of June and it always occurs in triticale crops in July (Zamorski et al., 1997). The winter triticale variety 'Brio' is characterised by high resistance to brown rust (Fossati et al., 1992). The spring triticale variety 'Gabo' is resistant to this disease, its severity at milk ripe stage is as low as 4.12—4.16% (Janušauskaitė, 2002).

An increase in the occurrence of ergot in winter triticale crops has been noticed in recent years. The causal agent of this disease is *Claviceps purpurea* (Fr.) Tul. During the period 1997—2000, on average 40.3% of the winter triticale cultivation area was infected with ergot in Lithuania, ergot-affected ears accounted for 4.7% (Dabkevičius, Semaškienė, 2001). Ergot assumed an epiphytotic character of occurrence in triticale crops in the neighbouring Belarus, during

1996—1999 the ergot-affected area accounted for 29 to 100% of the total area sown with triticale (Prochorov et al., 2000). Yield losses resulting from this disease can be as high as 13% /Niemkovich, 1999/. In Poland, ergot was observed sporadically (Zamorski et al., 1997). Initial infection with ascospores occurs on triticale crops during flowering. Since cereal flowering period is longer, fungus conidia formed in infected flowers, repeatedly infect a larger number of flowers (Wiese, 1991). Triticale varieties differ in their resistance to ergot, therefore it is recommended growing more resistant varieties in the areas with a severe disease occurrence (Pageau et al., 1994). The crops tend to be less infected with ergot when their development is even, and the flowering period is shorter (Engelke et al., 2001).

The objective of the present study was to estimate the resistance of winter triticale varieties recommended for cultivation in Lithuania to the chief foliar and ear diseases: septoria leaf blotch, brown rust and ergot, and to identify the regularities of the spread of these diseases in relation to the peculiarities of the investigated varieties.

Materials and Methods

Experiments were carried out at Šiauliai University's Botanic Garden during the period 2001—2003 with six winter triticale varieties recommended for cultivation in Lithuania (Table 1).

Table 1

Characteristics of winter triticale varieties according to the data from the Lithuanian State Variety Testing Centre

Variety	Breeder	Recommended for cultivation in Lithuania since:	Average plant height, cm	Average length of growing season, days	Average score of overwinter survival, 9 point scale	Lodging resistance score, 9 point scale
Tewo	Danko H.R., Poland	1995	96	207	8.8	8.4
Alzo	IHAR, Poland	2000	96	210	8.3	7.3
Tornado	IHAR, Poland	2000	100	207	8.5	8.3
Fidelio	Danko H.R., Poland	2001	90	205	8.5	8.1
Lupus	Nordsaat,Saaten — Unijon, Germany	2001	105	204	8.8	6.8
Marko	H.R.Strzelce, Poland	2001	103	206	8.6	8.0

Winter triticale was sown after early potatoes. $P_2O_{5\ 60}$ and K_2O_{-60} were applied before sowing. After renewal of vegetation ammonium nitrate N_{-60} and at booting stage N_{30} were applied. Before sowing the seed was treated with triadimenol 15 g kg^{-1} + fuberidazole 2,0 g kg^{-1} + imazalil 2,5 g kg^{-1} a rate of 2.0 kg t^{-1}. The seed rate was 4.5 million viable seed per hectare. The size of record plots was 1 m^2. The trial included four replications. The plots were arranged in one line systematically. The occurrence of septoria leaf blotch (*Septoria* spp.) and brown rust (*Puccinia recondita*) on triticale were identified at booting (BBCH 37) and milk ripe (BBCH 75) stages. Three top leaves on 25 stems were estimated per plot. Per cent of affected leaves and disease severity, expressed in per cent were assessed. Infection background of ergot was formed by inserting 10 g sclerotia at 2—3 cm depth in the gaps between each plot.

Special test on the dynamics of ergot sclerotia germination and stroma formation were conducted with unbroken 8—14 mm in length sclerotia. On the 21st (2001) and 23rd (2002) of September, 25 sclerotia were sown at a depth of 2—3 cm in a one meter row. In total 16 rows, i. e. 400 sclerotia were sown.

Plant flowering dynamics was estimated by assessing 25 ears per each plot. Assessments were started in May after first flowering ears had emerged and were completed after all ears had finished flowering. Per cent of flowering ears was identified every three days at the same time — from 7 a.m. to 9 a.m.

Assessments of the dynamics of sclerotia germination and stroma formation were started after the first stroma of ergot had appeared on the soil surface. Every three days until stroma had stopped formation we identified the number and per cent of germinated sclerotia, the amount of stroma formed and the number of stroma per one germinated sclerotium.

At triticale hard ripe stage (BBCH 91), before harvesting ergot-affected and healthy ears and the number of sclerotia in each record plot were counted. Per cent of ergot-affected ears and the number of sclerotia per 1 m^2 were estimated.

The experimental data were processed by analysis of variance.

The weather conditions during the experimental years were diverse. In 2001, the autumn was sufficiently humid, long and winter triticale was able to tiller well before winter. In 2002, the spring was early, due to the prevailing warm weather development of cereals started early, unusually hot and dry weather in May influenced an early flowering of triticale crops. In 2002, the summer was warm, there was more rainfall in the second half of June and first half of July. The rainy weather in July delayed triticale ripening. In 2002, the autumn conditions were not favourable for triticale emergence and tillering: dry weather dominated in September, in October it was wet and rather chilly. In 2003, the cool weather at the beginning of spring inhibited triticale development. The weather in the summer was changeable: cool, rainy June and dry, hot July. Such weather delayed triticale flowering, however, the settled warm weather later in the season accelerated its ripening.

Results and Discussion

Septoria leaf blotch

The growing season of winter triticale started early in the spring of 2002. The first spots of septoria leaf blotch were identified on the third leaf from the top at the beginning of triticale booting stage (BBCH 30–32). Although at the end of May the air temperature had reached the optimum limit for septoria leaf blotch development (15 °C), the shortage of moisture inhibited its spread. Consequently, at the end of triticale booting stage (BBCH — 37) the disease affected as little as 3.7—16.3% of three upper leaves, and the disease severity was as low as 0.04—0.32% (Table 2). The smallest number of affected leaves was identified in 'Alzo' (3.7%) and 'Fidelio' (4.3%), while the most heavily affected were the leaves of 'Marko' (16.34%). The disease severity was the highest in the variety 'Marko'. At milk ripe stage (BBCH — 75) the disease affected 40.0—92.0% of leaves, and the disease severity was 0.81—6.67%. Already at this period it became obvious that 'Alzo' and 'Fidelio' were significantly more resistant to septoria leaf blotch, while 'Tornado' and 'Marko' were found to be most susceptible to septoria leaf blotch.

The spring of 2003 was late and cool compared with that of 2002. The weather in the summer was changeable: cool and rainy June, and dry and hot July. Rather cool and wet weather in spring created good conditions for the spread of septoria leaf blotch. At booting stage, the incidence and severity of the disease were similar to those in 2002. The same trend was revealed: 'Alzo' and 'Fidelio' were more resistant to the disease. However, heavy rainfall in the first and second ten-days period of May (in total 65 mm) promoted the spread of septoria, and at the stage of milk ripe the disease affected 94.3—96.7% of leaves. Although the per cent of affected leaves differed little between the tested varieties, the disease severity was rather different. Like in previous years, 'Alzo' and 'Fidelio' were noted for the highest resistance to septoria leaf blotch, the disease affected 18.09 and 16.06% of leaf area, respectively, while 'Tornado' was found to be the most heavily affected — 39.29%. The disease severity for the other varieties was similar — 24.33—28.37%.

Table 2

Incidence and severity of septoria leaf bloch in winter triticale varieties, 2002/2003

Variety	2002				2003			
	BBCH – 37		BBCH – 75		BBCH – 37		BBCH – 75	
	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity
'Tewo'	9.3	0.11	56.7	2.18	10.0	0.10	92.0	28.37
'Alzo'	5.7	0.06	40.0	0.81	3.7	0.06	92.3	18.01
'Tornado'	6.3	0.06	92.0	6.77	13.0	0.19	94.3	39.29
'Fidelio'	4.3	0.04	63.3	0.88	0.8	0.04	86.7	16.06
'Lupus'	7.7	0.08	80.3	2.25	8.7	0.14	91.7	24.33
'Marko'	16.4	0.32	76.0	5.51	12.7	0.16	87.3	25.15
LSD_{05}	5.01	0.058	12.99	3.001	3.31	0.066	7.01	6.184

Brown rust

Brown rust was not found at the stage of booting. It started to spread later, the first pustules appeared at the end of triticale flowering. At milk ripe stage the incidence of brown rust varied more between the varieties: from 6.4 to 91.0% affected leaves (Table 3), in 2003 this difference was smaller — from 48.0 to 75.3%. The disease severity during the experimental years was low — in 2002 up to 2.01% and in 2003 up to 3.32%. In both years the varieties 'Alzo' and 'Fidelio' were also found to be more resistant to brown rust. It is interesting to note that 'Tornado' variety is susceptible to septoria, however, it was rather resistant to brown rust. 'Tewo' and 'Marko' were found to be most susceptible to brown rust. Other researchers have also reported that the winter triticale variety 'Fidelio' is characterised by a complex resistance to crown, leaf and head diseases (Pojmaj et al., 1997).

Table 3

Incidence and severity of brown rust on winter triticale varieties, 2002/2003

Variety	2002		2003	
	BBCH – 75		BBCH – 75	
	Incidence	Severity	Incidence	Severity
'Tewo'	91.0	2.01	73.0	3.32
'Alzo'	43.3	0.43	48.8	0.61
'Tornado'	27.4	0.31	50.3	1.03
'Fidelio'	6.4	0.06	48.0	0.59
'Lupus'	73.1	1.20	69.0	2.03
'Marko'	86.4	2.01	75.3	2.82
LSD_{05}	25.62	0.918	16.91	1.545

Ergot

In 2002, ergot sclerotia started to germinate and form stromas on May 15 and continued until June 20. This process was promoted by the warm weather. In 2003, ergot started to germinate 9 days later, i.e. on May 24, and the germination continued until the end of June (Fig. 1). In 2003, ergot germinated not only later but also a smaller per cent of sclerotia germinated and formed a smaller amount of stromas compared with 2002 (Fig. 2).

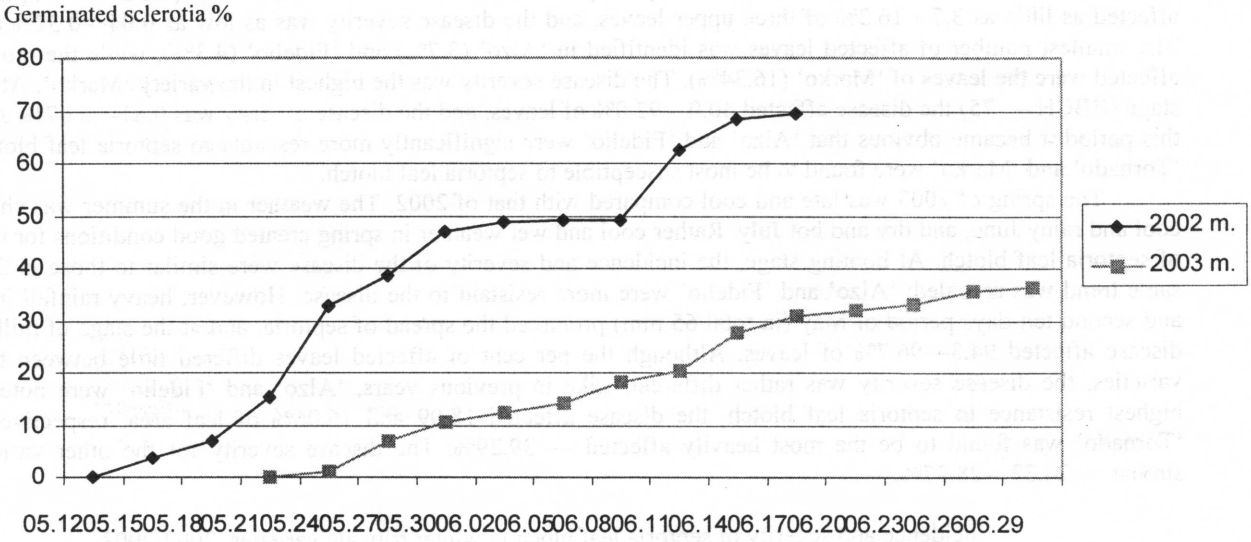

Fig. 1. Germination dynamics of ergot sclerotia 2002—2003

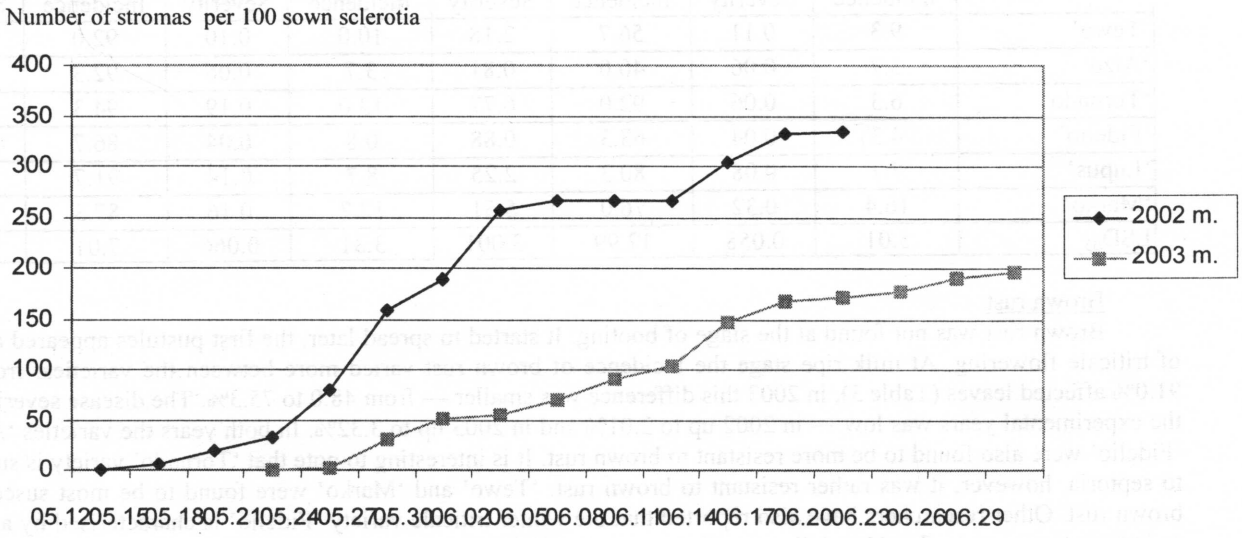

Fig. 2. Dynamics of the formation of ergot sclerotia stromas, 2002—2003

In 2002, triticale flowering started at the beginning of June, when more than half of ergot sclerotia had germinated. Most varieties reached the stage of mass flowering on June 8, only the peak of flowering for the variety 'Tewo' was 3 days later (Fig. 3). The largest part of plants finished flowering on June 14, and the flowering period lasted for about 15 days. In 2003, the plants started flowering as late as on June 13—14 and continued until the end of June (Fig. 4). The flowering period in 2003 was more prolonged than in 2002.

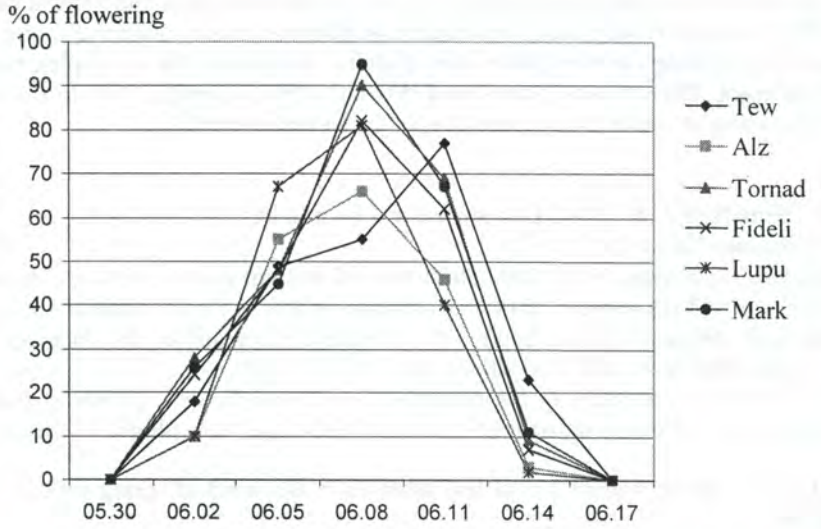

Fig. 3. Flowering of winter triticale varieties recommended for cultivation in Lithuania, 2002

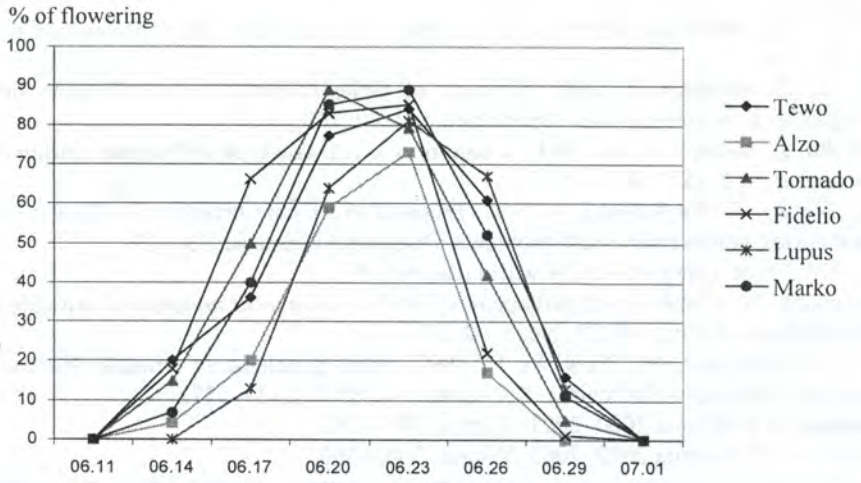

Fig. 4. Flowering of winter triticale varieties recommended for cultivation in Lithuania, 2003

Although provocative background was formed, drier and warmer weather and more rapid flowering in 2002 did not promote triticale infection with ergot. Two winter triticale varieties were not affected by ergot, in the other varieties the affected ears amounted to as little as 0.07—0.20%, the amount of sclerotia per 1 m^2 was as low as 0.25—2.50% (Table 3). Like for the other diseases, 'Alzo' and 'Fidelio' varieties were more resistant to ergot since they had the shortest flowering period.

In 2003, the ergot-affected ears accounted for 0.67—3.56%, and from 6,0 to 20.0 sclerotia were found per 1 m^2. The spread of ergot was promoted more by cool and rainy weather, prolonged and late flowering. 'Lupus', 'Tewo' and 'Marko' varieties that were most heavily affected by ergot had an especially long flowering period.

Table 4

Ergot infection of triticale varieties, 2002/2003

Variety	Ergot-infested ears %		Number of ergot-infested ears per 1 m^2		Number of sclerotia per 1 m^2	
	2002	2003	2002	2003	2002	2003
'Tewo'	0.07	1.65	0.25	8.50	0.75	16.75
'Alzo'	0.00	0.67	0.00	3.75	0.00	6.00
'Tornado'	0.07	1.88	0.25	11.00	0.25	15.25
'Fidelio'	0.00	1.07	0.00	5.50	0.00	9.25
'Lupus'	0.00	3.56	0.00	18.25	0.00	19.50
'Marko'	0.20	2.80	2.50	13.00	2.75	20.00
LSD_{05}	0.205	1.963	1.967	10.181	2.273	10.717

The spread of winter triticale diseases depended on the weather conditions and varietal peculiarities of the grown varieties. The year 2003 with prevailing cooler and wetter weather was more conducive to the occurrence of diseases. The shorter season winter triticale varieties 'Alzo' and 'Fidelio' were noted for a complex resistance to septoria leaf blotch, brown rust and ergot. The varieties 'Tewo' and 'Marko' were susceptible both to foliar diseases and to ergot. Late and prolonged flowering of winter triticale encourages the spread of ergot.

References

1. Dabkevičius, Z., Semaškienė, R. 2001. Occurrence and harmfulness of ergot (*Claviceps purpurea* (Fr.) Tul.) in cereal crops of Lithuania. Biologija, 3, 8—10.
2. Engelke, T., Mielke, H., Hoppe, H.-H. 2002. Influence of cultural control methods on the occurrence of ergot (*Claviceps purpurea* (Fr.) Tul.) in rye. 1st Baltic Conference on Rye in the EU contex. Kaunas, 53—55.
3. Fossati, A., Fossati, D., Weilenmann, F., Saurer, W., Winzeler, M., Winzeler, M., Jaquiery, R. 1992. Brio, a Swiss winter triticale variety. Revue Suise d'Agriculture, vol. 24, 1, 13—15.
4. Gaurilčikienė, I. (1998) Investigation of the occurrence of fungal diseases on winter triticale stands of various sowing time and density in different agroclimatic zones of Lithuania. Agriculture, Scientific articles, vol. 62, 155—165.
5. Gaurilčikienė, I. 2002. The spread of fungal leaf diseases in the stand of spring triticale. Agriculture, Scientific articles, vol. 80, 70—77.
6. Gaurilčikienė, I. 2001. The spread of Septoria leaf blotch in spring triticale stands of Lithuania. Biologija, No. 3, 8—10.
7. Gaurilčikienė, I. 1997. Winter triticale protection against foliar and root rot diseases. Agriculture, Scientific articles, vol. 59, 152—161.
8. Janušauskaitė, D. 2002. Incidence of *Puccinia recondita* Rob. ex Desm. on different cultivars of spring triticale. Vagos, 59 (9), 12—16
9. Janušauskaitė, D., Dabkevičius, Z. 2002. Efficacy of different doses and application timing of triazole and strobilurine fungicides in winter triticale. Agriculture, vol. 80, 109—124.
10. Pageau, D., Collin, J., Wauthy, J. M. 1994. A note on the resistance of soft wheat, durum wheat and triticale to ergot. Phytoprotection, vol. 75, 1, 45—49.
11. Pojmaj, M. S., Wolski, T., Szołkowski, A. 1997. Progress in triticale breeding in "Danko" Hodowla rosiłn LTD. Plant breeding theories, achievements and problems. Dotnuva-Akademija, 23—29.
12. Wiese, M. V. 1991. Ergot. Compendium of wheat diseases. 14—15.
13. Wos, H., Mackowiak, W. (1994) Cross pathogenicity of *Phaeosphaeria nodorum* to triticale and wheat. Rosziniki Nauk Rolniczych Ohrona Roslin, vol. 23, No. 1—2, 27—33.
14. Zamorski, C., Schollenberger, M., Nowicki, B. 1997. Some problems of triticale diseases in Poland. Zescyty Naukowe Akademii Rolniczejw Szczecinie, Rolnictwo, vol. 65, 2, 533—537.
15. Statistical Yearbook of Lithuania 2000. 2001. Vilnius, 391—395.
16. Statistical Yearbook of Lithuania 2002. 2003. Vilnius, 364—366.
17. Немкович, А.И. 1999. Биологическое обоснование защиты озимой ржи от спорыньи. Автореферат дисертации, п. Прилуки, Минской области, 18 pp.
18. Прохорова, С.В., Терещук, В.С., Немкович, А. И. 2000. Фитосанитарное состояние посевов тритикале. Известия Академии аграрных наук Республики Беларусь, № 2, 51—56.

BIOLOGICAL EFFICACY OF DIFFERENT FUNGICIDE DOSAGES TO CONTROL TAN SPOT (*DRECHSLERA TRITICI-REPENTIS* (DIED.) SHOEMAKER) IN LATVIA

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Abstract

Three different strategies were used to control disease development in winter wheat: application of a full dose in GS 51-55, split doses in GS 37-39 and GS 51-55, and treatment according to the recommendation of PC program PC-P Diseases (worked out at the Danish Institute of Agricultural Sciences and developed in collaboration with the Latvia University of Agriculture, Latvian State Centre of Plant Protection, State Plant Protection Service, and Latvian Agricultural Advisory Centre). Evaluation of tan spot development was done assessing disease incidence and severity on the whole plant until GS 31, at GS 32-69 — on 3 upper leaves, at later stages — on 2 upper leaves. 50 leaves from main shoots were randomly taken from each plot. Disease scores made through the season — from detection of first symptoms to GS 85 — were taken and used to evaluate the tan spot development dynamics and rate of infection, to calculate the area under the disease progress curve (AUDPC), characterizing mean infection level and biological efficacy of different control strategies.

In trials, development of tan spot was observed late in the season — GS 61-69. The disease development varied between the years depending on climatic conditions. In most cases, the rate of infection increased sharply after flowering. Volume of AUDPC only slightly correlated with the efficacy of fungicide treatment (correlation coefficient 0.19). Results of calculation of biological efficacy do not suggest any relevant differences between the efficacy of split applications (S2; PC-P) and one application later in the season (GS 51-69).

Key words: winter wheat, tan spot, fungicides, timing and dosages, efficacy.

Introduction

In last years, tan spot caused by fungus *Pyrenophora tritici repentis* (Died.) Drechs., anamorph *Drechslera tritici-repentis* (Died.) Shoemaker, has been distinguished as a very serious wheat disease in Latvia (Bankina, 2002; Resnais, Guste, 2000). The increase of tan spot spreading is linked with the change in the growing technology — increase of wheat growing without crop rotation, which allows to build up inoculum source, i.e. infected wheat plant debris.

Management of tan spot is based on estimated disease severity effects on the yield. Fungicide application is an important tool for disease control in the vegetation period. Important question is the right application timing. The data in literature suggest that it is not necessary to use fungicides as preventive measure in early growth stages to anthesis, but effective can be one-time application in later stages (De Wolf, Effertz et al., 1998; Duveiler, Dubin et al., 1998).

In last years, new technologies of IPM have been developing throughout the world — Decision Support Systems based on PC programs. The Danish model for disease control in cereals was tested in Latvia (Turka, Priekule, 2003). Calculation of advice, i.e. timing of treatment, choice of fungicide and appropriate dosage (in many cases, reduced), is based on epidemical situation in a field and meteorological conditions (Hossy et al., 2000; Henriksen et al., 2000).

The present investigation was undertaken: 1) to evaluate the tan spot development dynamics and rate of infection depending on fungicide treatments (different timing); 2) to compare the AUDPC (area under the disease progress curve); 3) to evaluate the biological efficacy of fungicide treatments.

Materials and Methods

Trials were carried out in the south region of Latvia, an intensive wheat growing area — in the Teaching and Research Farm "Pēterlauki" (Jelgava, Latvia University of Agriculture (LLU)), in the Teaching and Research Farm "Vecauce" (Vecauce, LLU), and in the west — in the State Stende Plant Breeding Station (LSCPP). 14 field trials in winter wheat were carried out in 1999—2002 to test and compare efficacy of fungicide doses according to the Official Register (registered doses in Latvia) and dose(-s) recommended by PC-P to control mildew, septoria blotch and, simultaneously in the complex, tan spot.

The product used in all trials was Tango Super (epoxiconazole 84 g l^{-1}, fenpropimorf 250 g l^{-1}, BASF). The following doses of fungicide were used in the trials:

1. A full dose (S1) — 1.5 l ha^{-1} (GS 51-55), (1.25 l ha^{-1} in 2 trials — 2000, 2001);
2. split doses (S2) — 0.75 l ha^{-1} (GS 37-39), 0.75 l ha^{-1} (GS 51-55), (0.65 l ha^{-1} × 2 in 2 trials — 2000, 2001);
3. treatment(-s) according to the PC-P Diseases recommendation (PC-P).

Different winter wheat cultivars were included in the trials (Table 1). Plots in size 25—28 m^2 were arranged following a randomized completed block design in four replicates. All agronomic requirements were observed. Seed dressing, herbicides and high dosages of nitrogen were used.

Table 1

Localities and winter wheat cultivars used in the trials

Locality	Cultivar	1999	2000	2001	2002
Jelgava	'Donskaya Polukarlikovaya'[1]	×	×	×	×
	'Stava'[2]		×	×	×
Vecauce	'Donskaya Polukarlikovaya'			×	×
	'Kontrast'[3]			×	×
Stende	'Krista'[4]		×	×	×

[1] — susceptible, very early cultivar, split treatment is not necessary;
[2] — quite resistant, late cultivar;
[3] — moderately resistant, middle late cultivar;
[4] — moderately susceptible, middle early cultivar.

Assessments of disease incidence (number of infected plants or leaves/total plants or leaves) and severity (percentage of leaf area covered by tan spot lesions) were carried out on the whole plant until GS 31, at GS 32-69 — on 3 upper leaves, at later stages — on 2 upper leaves. 50 leaves from main shoots were randomly taken from each plot. Causal agents of diseases were determined in the laboratory by investigating pycnidias and morphology of conidiophores and conidia. Moist chambers were used for development of conidia.

Rate of infection was calculated according to Hughes (Hughes, 2003):

$$r = \frac{1}{t2 - t1} * \left(\log \frac{X2}{1 - X2} - \log \frac{X1}{1 - X1} \right),$$

where
r — apparent infection rate;
t1 — time of previous disease assessment;
t2 — time of disease assessment;
X1 — disease severity at the time of first disease assessment;
X2 — disease severity at the time of second disease assessment.

Disease scores made through the season — from detection of first symptoms to GS 85 — were taken and used to calculate the area under the disease progress curve (AUDPC), characterizing the mean infection level (Campbell, Madden, 1990).

Meteorological conditions were rather different during the experimental period. The season was extremely dry in 1999 restricting the development of the disease. A similar situation was in 2002. The vegetation seasons of 2000 and especially of 2001 were favourable for disease development, with wet and rainy summer developing high pressure of leaf spot diseases.

Results and Discussion

The rate of disease progression depends upon host and environmental components of the pathosystem. For tan spot development very important is a favourable temperature regime (not higher than 28 °C) and moisture (free moisture for conidia germination).

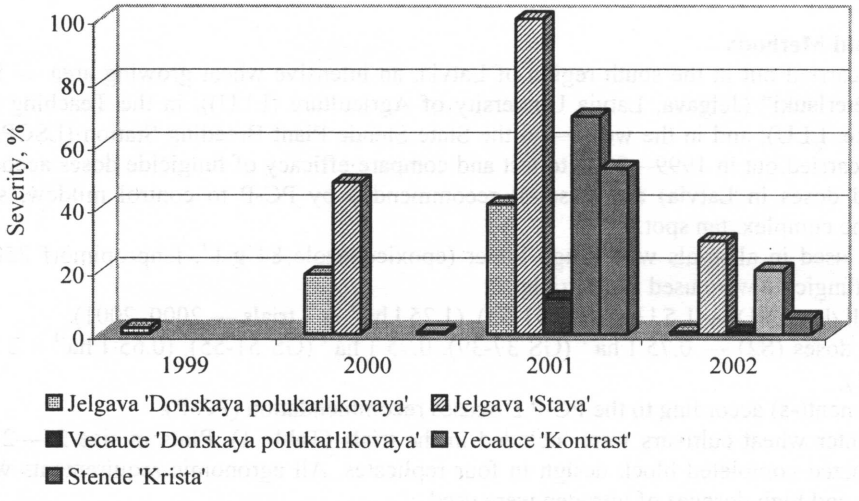


Fig. 1. Severity of tan spot at the time of milk ripening in an untreated area

Results of disease assessments on untreated plots showed very different epidemiological situation of tan spot in the research period. Severity of tan spot fluctuated between the years and localities within the range 0.7—100% (Fig. 1).

Table 2

Application time according to PC-P calculation, 2001

'Donskaya Polukarlikovaya'[1]		'Stava'		'Donskaya Polukarlikovaya'[2]		'Kontrast'		'Krista'	
GS	Dose, l ha^{-1}	GS	Dose, l ha^{-1}	GS	Dose, l ha^{-1}	GS	Dose, l ha^{-1}	GS	Dose, l ha^{-1}
35	0.45	55	0.56	32	0.51	31	0.38	31	0.36
69	0.55	59	0.70	38	0.43	47	0.60	33	0.38
				69	0.51	69	0.54	47	0.53

[1] — Jelgava;
[2] — Vecauce.

Because of more favourable conditions for tan spot development, data from 2001 were used for analysing efficacy of different disease control strategies. In 2001, 5 trials were carried out (Table 1). Calculations for the PC-P recommendation were done taking into account the infection incidence of *Blumeria graminis*, the number of days with precipitation >1 mm after GS 32, and algorithm for other spot disease *Septoria tritici*. According to these advices, different times and doses were used for disease control (Table 2). Results of field assessments (severity) showed that doses and time of treatment did not influence the tan spot development (Fig. 2).

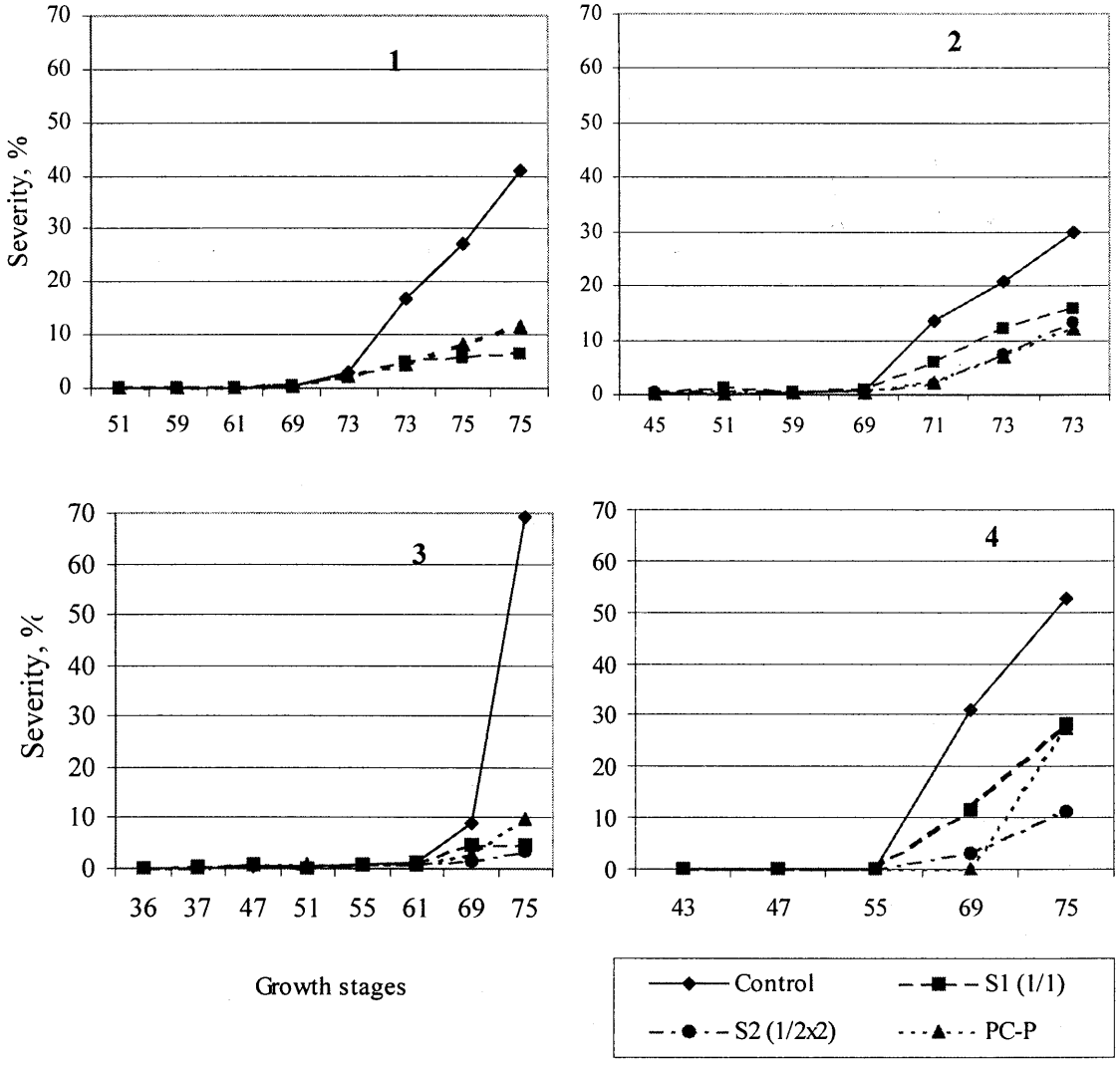
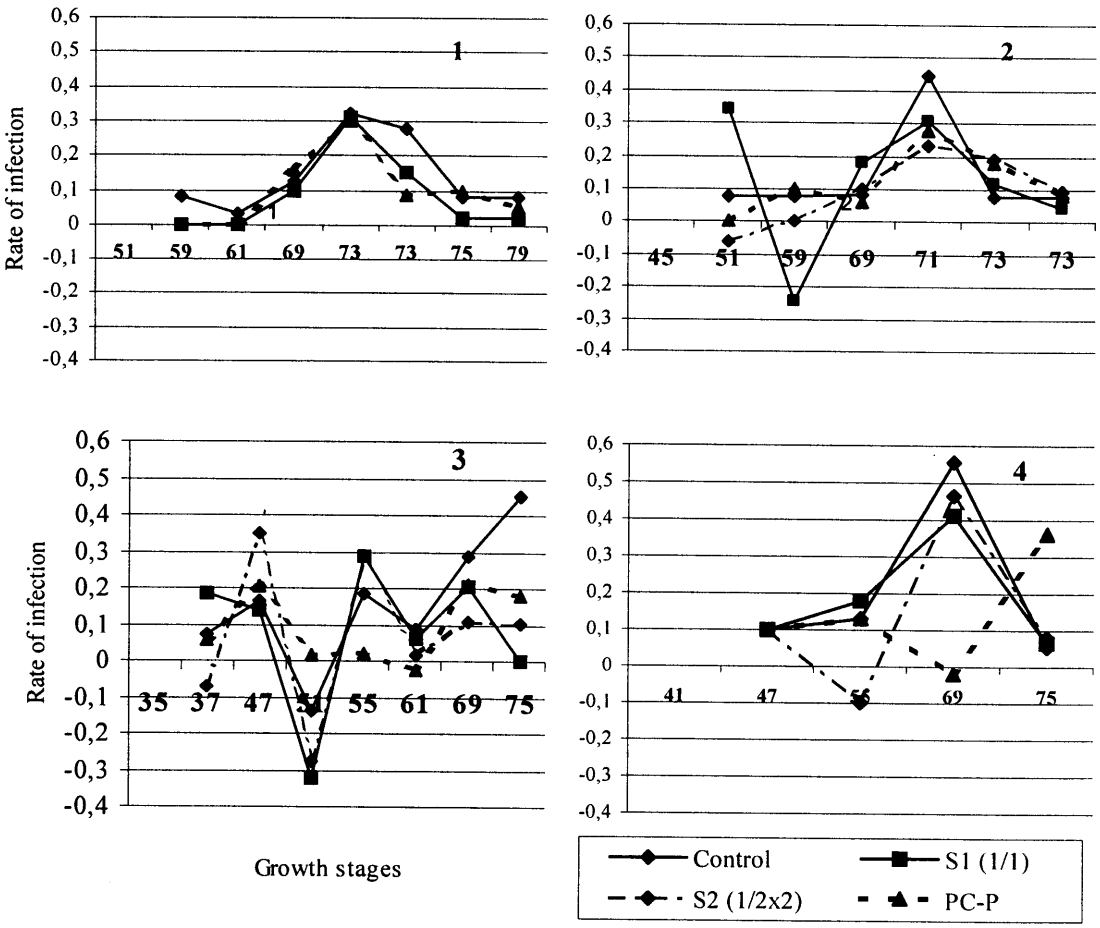


Fig. 2. Development of tan spot depending on the treatment, 2001: 1 — Jelgava 'Donskaya Polukarlikovaya', 2 — 'Stava', 3 — 'Kontrast', 4 — Krista

The rate of infection describes peculiarities of disease development depending on the treatment (Fig. 3). In most cases, the infection rate sharply increased after flowering (the exception was in Vecauce, 'Kontrast', where early infection was observed — GS 37-47), but later severity decreased in all treatments.

Tendencies of rate infection changes were similar in all treatments and all sites. A radical decrease in the rate was noted after fungicide application; rapid extension of disease development was observed in the following weeks in respect of the times and dosages of fungicide application.

In all cultivars the increase in tan spot severity was observed quite late in the season — after flowering (GS 69) in untreated plots. According to this, most effective control was reached when fungicide application was carried out later in the season — in trials with 'Donskaya Polukarlikovaya' and 'Kontrast'. Sprayings were done in GS 69 according to calculations of PC-P. A similar effect was observed when a full dose of fungicide was used in GS 53-55, which gave a long-lasting efficacy compared to the ½ dose applied at the same time.



The results of trials confirm the data on disease development from the Department of Warning and Prognoses of Diseases and Pests (State Plant Protection Service). Analysis of observation results shows that first symptoms were observed in conventional farming fields in tillering-stem elongation period (GS 31-33), in the heading-flowering (GS 51-69) spreading can reach up to 50%, severity — 2—5%. Disease development reached its maximum in the middle-end of milk ripeness (GS 75-79) — spreading up to 100%, severity 10—50%.

To evaluate the efficacy of different treatment strategies, the mean infection level was used (AUDPC). No relevant differences of tan spot development were observed between treatment strategies (Fig. 4).

The volume of AUDPC only slightly correlated with the efficacy of fungicide treatment (correlation coefficient 0.19). Better results were obtained where fungicide applications were done 2 times with split dosages (S2), last of which was done before — in early flowering. This tendency was observed in sowings of 'Kontrast' and 'Krista', medium and medium-late ripening cultivars. Mean infection level was higher on 'Stava', late ripening cultivar with a prolonged period from flowering to full ripeness. According to research results (De Wolf, Effertz et al., 1998), there are more spores of fungus in the air later in the season, thus explaining a higher and not affected by fungicide application AUDPC for 'Stava'.

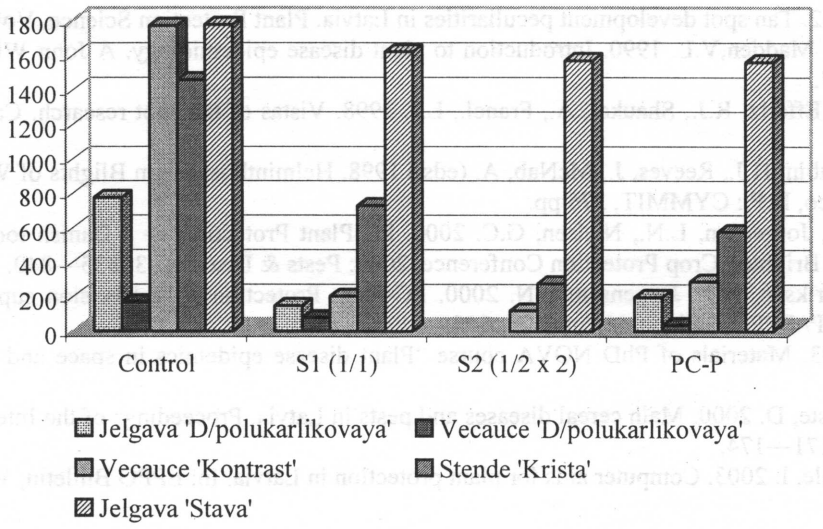


Fig. 4. The AUDPC of tan spot depending on different treatment strategies, 2001

Usefulness of fungicide application characterises biological efficacy — the disease control level in % to untreated area. In 2001, there was a variable level of biological efficacy between treatments and varieties (Fig. 5). On average, the highest efficacy was obtained if fungicide was applied two times with spilt dosages (S2) — 76.0%. Efficacy of one application with a full dose (S1) was 65.9%, of the treatment based on PC-P calculations — 71.7%. Similar figures were obtained if 3-year average (2000, 2001, 2002) was calculated: S1 (application in GS 51-55) — 70.7%, S2 (GS 37, 51-55) — 82.8%, PC-P — 71.5%.

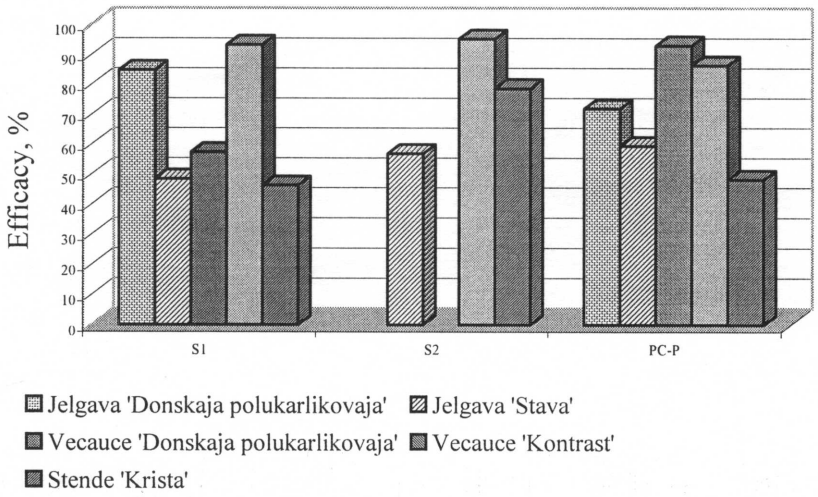


Fig. 5. Biological efficacy of different fungicide treatment strategies to control tan spot, 2001

Basing on the results it can be assumed that there is no clear difference between the efficacy of split applications (S2; PC-P) and one application later in the season (GS 51-69).

It can be concluded that development of tan spot varies between the years depending on climatic conditions, though the reasons for very rapid increase of development in GS 61-69 are not clear. There is speculation that peculiarities of ascospore development or some conditions are promoting conidias spreading. It is important to make a research on conditions which might be favourable for disease development — humidity of debries, humidity on leaves, number of rainy days after specific growth stage of plant or sum of precipitation in the vegetation period of the crop.

The research results suggest that there is no difference between one application in GS 51-55 and split dosages in different times. It is necessary to develop more precise control strategies, using knowledge about tan spot epidemiology in Latvian conditions, based on meteorological information and field description data — previous crop, disease development.

References

1. Bankina, B. 2002. Tan spot development peculiarities in Latvia. Plant Protection Science, Vol. 2, 381—383.
2. Campbell, C.L., Madden,V.L. 1990. Introduction to plant disease epidemiology. A John Wiley& Sons. INC New York, 161—187.
3. De Wolf, E.D., Effertz, R.J., Shaukat, A., Francl., L.J. 1998. Vistas of tan spot research. Can. J. Plant Pthol., 20, 349—370.
4. Duveiler, E., Dubin, H.J., Reeves, J., McNab, A. (eds.) 1998. Helminthosporium Blights of Wheat: SpotBlotch and Tan Spot. Mexico, D.F.: CYMMIT, 376 pp.
5. Henriksen, K.J., Jorgensen, L.N., Nielsen, G.C. 2000. PC-Plant Protection — a Danish tool to reduce fungicide input in cereals. Brighton Crop Protection Conference, 2000: Pests & Diseases, 3, 835—840.
6. Hossy, H., Henriksen, K.J., Jorgensen, L.N. 2000. PC-Plant Protection — a Decision support system for plant protection. OEPP/ EPPO Bulletin, Volume 26, 645—649.
7. Hughes, G. 2003. Materials of PhD NOVA course "Plant disease epidemics in space and time", 2003, Jelgava, Latvia.
8. Resnais, A., Guste, D. 2000. Main cereal diseases and pests in Latvia. Proceedings of the International Conference, Tartu, Estonia, 171—174.
9. Turka, I., Priekule, I. 2003. Computer aids for plant protection in Latvia. In: EPPO Bulletin, Volume 33, 509—513.

THE EFFECT OF FUNGICIDES ON THE MICROFLORA OF GRAINS

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Abstract

This study was carried out in order to investigate the effect of fungicides on the microflora of grain yield (the winter wheat variety `Sani`) in the field trials of 2002—2003.

Variants on background N_0 and N_{150} : 1. Untreated, 2. Bumper Super (a.i. propiconazole + prochloraz) 1.0 l ha^{-1}, 3. Tilt 250 EC (a.i. propiconazole) + Sportak 45 EC (a.i. prochloraz) (0.36 + 0.89 l ha^{-1}), 4. Tilt 250 EC (a.i. propiconazole) 0.5 l ha^{-1}. Application for the control of *Leptosphaeria nodorum* E. Müller was made on 23.05.2002 on GS 39 and on 17.06.2003 on GS 47-49. The date of harvest was 25.07. in 2002 and 11.08. in 2003.

The total number of aerobic mesophilic bacteria was estimated on the PCA (Plate Count agar = Casein-peptone-glycose-yeast agar, ICC Standard No. 125, 1978), whereas for fungi and yeasts, RBCA (Rose bengal chloramphenicol agar, ICC Standard No. 146, 1992) was used. The species and number of *Fusarium* Link. ex Fr. were defined on Nash and Snyder selective medium. The classification of *Fusarium* isolates was made according to Gerlach and Nirenberg (1982). The toxicity of *Fusarium* isolates was tested on the basis of the size of the growth inhibition zone of *Bacillus stearothermophilus* (Watson, Lindsay, 1982).

The changes of microflora in the main groups of microorganisms (bacteria, moulds, and yeasts) depended on the N background and weather conditions of the year more than on the application of fungicides. In the untreated control, the number of bacteria, moulds and yeasts increased on the background of N_{150}. In rainier growth periods, the number of bacteria and moulds was bigger in the untreated control but the number of yeasts was bigger in the drier period. On applying fungicides, the number of both bacteria and yeasts, compared to the untreated control, did not change to a firm direction, however, the number of moulds decreased on the background of N_{150} in both years. In the case of the N_{150} background, there were more *Fusarium* spp. in the yield in rainier growth periods than in drier periods. None of the tried fungicide variants saved the yield from Fusariums completely. Of the three variants, Tilt (propiconazole) 0.5 l ha^{-1} had the best effect on decreasing the number of *Fusarium* spp. Mainly *F. culmorum* (W.G.Sm.) Sacc. and *F. avenaceum* (Fr.) Sacc. occurred in the yield. The toxicity of *Fusarium* spp. isolates towards *B. stearothermophilus* was 3—7 points on a 10-point scale. The changes in microflora when applying fungicides do not generally impair the quality of grain, but the increase of the number of *Fusarium* spp. is unadvisable in certain cases.

Key words: grain, winter wheat, nitrogen, precipitation, microflora, fungicides, *Fusarium* spp.

Introduction

In assessing both food and feed grain quality, its microbiological indicators are important. A considerably higher number of microorganisms (fungi, bacteria, yeasts) than the standards of quality grain is one of the signs of deteriorating grain quality and characteristic of tainted grain. Moreover, many microorganisms, including a number of moulds and part of bacteria and yeasts, are able to produce toxins in certain conditions. *Fusarium, Aspergillus, Penicillium* and *Alternaria* species are most common toxicants in stored grain (Miller, Trenholm, 1997). Unfortunately, in Estonia there are still no microbiological standards for food and feed grain, causing a situation, where cereal growers are not interested in producing grain with the best microbiological properties. In Estonia, no-one has paid attention to the problem yet. At the same time, in several countries the microbiological quality of cereals is observed, for example, in Germany microbiological standards have been established both for food (Baumgart, Firnhaber, 1993) and feed grain (Schmidt-Lorenz, 1980). There are several methods for optimising the microbiological composition of yields: including change of N fertiliser doses, use of fungicides during plant growth to suppress pathogens and saphrophytic flora, acceleration of grain ripening, and possibly quick harvest.

Materials and Methods

Experiments for controlling diseases in the winter wheat `Sani` with 4 variants on 2 N backgrounds (N_0, N_{150}) were carried out at Olustvere experimental station in Viljandi County in 2002 and 2003, on a fertile sod calcareous soil, with humus horizon thickness 23—25 cm, grading heavy sandy clay, humus content 1.7—2.2%, pH_{KCl} 6.4—6.7, P determined by the Mechlich III method very high — 140—160 mg kg^{-1} and medium K level — 110—145 mg kg^{-1}. Prior to the sowing, seeds were dressed with Baytan Universal 19.5 WS 2.0 kg t^{-1} or Maxim Star 025 FS 1.0 l t^{-1}, and sown on 18.09.2001 and 13.09.2002, respectively. Fertilisers (SKALSA 0-11-20 300 kg ha^{-1}) were applied during the sowing, N upper fertiliser as ammonium salpeter before the beginning of shoot growing on 11.04.2002 and 22.04.2003, respectively. For chemical weed control, Granstar 13 g ha^{-1} + Kemiwett 175 ml ha^{-1} on 06.05.2002 and Granstar 20 g ha^{-1} + Banvel 0.3 l ha^{-1}, + Kemiwett 200 ml ha^{-1} were used on 26.05.2003. The plants were sprayed with the growth regulator Kemira CCC 1.4 l ha^{-1} on 09.05.2002, and with Terpal 1.25 l ha^{-1} on 02.06.2003. The tested variants were as follows: 1. Untreated, 2. Bumper Super (a.i. propiconazole + prochloraz) 1.0 l ha^{-1}, 3. Tilt 250 EC (a.i. propiconazole) + Sportak 45 EC (a.i. prochloraz) (0.36 + 0.89 l ha^{-1}), 4. Tilt 250 EC (a.i. propiconazole) 0.5 l ha^{-1}. Application for the control of *Leptosphaeria nodorum* was made at GS 39 on 23.05.2002 and at GS 47-49 on 17.06.2003.

Weather conditions of the vegetation period were fixed by a local weather observation station and are presented in Table 1.

The experiments were harvested at the full-ripening stage on 25 July in 2002 and on 11 August in 2003, and the yields were dried immediately. In mean samples weighing 2.5 kg microbiological composition of seeds was determined at the microbiological laboratory of the Agricultural Research Centre. The preparation of samples and analysis was carried out according to the standard of EVS-EN ISO 6887-1:2001 (Preparation of test samples, initial suspensions and decimal dilutions for microbiological examination). The total number of aerobic mesophilic bacteria was estimated on the PCA (Plate Count agar = Casein-peptone-glycose-yeast agar, ICC Standard No. 125, 1978), whereas for fungi and yeasts, RBCA (Rose bengal chloramphenicol agar, ICC Standard No. 146, 1992) was used. Species and number of *Fusarium* were defined on Nash and Snyder selective medium. The classification of *Fusarium* isolates has been made according to Gerlach and Nirenberg (1982). The toxicity of *Fusarium* isolates was tested on the basis of the size of the growth inhibition zone of *Bacillus stearothermophilus* (Watson, Lindsay, 1982).

Results and discussion

Weather conditions.

Weather conditions of the years differed significantly. The vegetation period of 2002 was considerably warmer and drier than that of 2003 (Table 1). In 2002 there was drought (precipitation only 74% of the norm) and the sum of mean daily temperatures was 12.7 °C above the norm, whereas in 2003 precipitation exceeded the norm by 56% and the warmth of the first half of the vegetation period was even below the norm. During the wheat flowering period in June (beginning of flowering on 10.06. in 2002 and on 30.06. in 2003), which is the important period for grain infection with *Fusarium* species, rainy weather was dominating in 2002, and in 2003 the vegetation period was relatively dry. Pre-harvest weather has a considerable impact on the infection of grain with several saphrophytic fungi (*Alternaria, Cladosporium, Aureobasidium, Epicoccum,* etc.) and epiphytic *Fusarium* species. In 2002, grain was harvested on 25 July in dry weather, but in 2003 harvesting was delayed until 11 August due to the high precipitation. This created preconditions for heavier contamination of the yield of 2003 with moulds (compare Tables 2 and 3). Since the total infection with *Fusarium* species depends on the weather of both the flowering period and pre-harvest period, the heavier infection of the yield of 2003 with *Fusarium* species was probably caused by the excessively wet pre-harvest period that became especially apparent on the N_{150} background. The abundance of bacteria was also greater in the yield grown in the weather conditions of 2003 (compare Tables 3 and 2). The abundance of yeasts seems to respond less to diminished moisture, and was even greater after a drier growth period in the control variant than following a more humid growth period. Whether it was occasional or caused by the better competitiveness of yeasts in drier conditions requires further investigations.

Table 1

Weather conditions of the field trial period at Olustvere, 2002 and 2003

Month	Year 2002					
	Air temperature, average °C			Precipitation, mm		
	per month	normal	+ or –	per month	normal	% of normal
April	6.2	3.9	+2.3	12.5	35.0	36
May	13.8	10.4	+3.4	28.8	48.0	60
June	16.1	15.0	+1.1	103.6	67.0	155
July	19.3	16.6	+2.7	73.4	84.0	87
August	18.7	15.5	+3.2	11.6	78.0	15
Sum	–	–	+12.7	229.9	312.0	74
Month	Year 2003					
	Air temperature, average °C			Precipitation, mm		
	per month	normal	+ or –	per month	normal	% of normal
April	3.3	3.9	–0.6	54.3	35.0	155
May	11.4	10.4	+1.0	76.4	48.0	159
June	13.5	15.0	–1.5	48.5	67.0	72
July	19.9	16.6	+3.3	191.0	84.0	227
August	15.7	15.5	+0.2	117.3	78.0	150
Sum	–	–	+2.4	487.5	312.0	156

Table 2

Quantitative and group composition of microorganisms on seeds of wheat in 2002
(number of cells in one gram of dried seeds)

Variant	*Moulds on RBCA	Yeasts on RBCA	*Fusarium* spp. on Nash &Snyder	Aerobic mesophilic bacteria on PCA	pH in H_2O	Moisture, %
	Background N_0					
Untreated	1.5×10^4	2.2×10^4	0	1.1×10^5	7.16	9.8
Bumper Super 1.0 l ha^{-1}	1.5×10^4	3.0×10^4	11	1.2×10^5	7.18	9.8
Tilt + Sportak 0.36 + 0.89 l ha^{-1}	0.8×10^4	3.1×10^4	11	1.6×10^5	7.20	9.8
Tilt 0.5 l ha^{-1}	1.5×10^4	4.5×10^4	5	2.3×10^5	7.20	9.4
	Background N_{150}					
Untreated	2.1×10^4	8.7×10^4	0	2.4×10^5	7.18	9.7
Bumper Super 1.0 l ha^{-1}	1.7×10^4	3.1×10^4	0	1.4×10^5	7.21	10.1
Tilt + Sportak 0.36 + 0.89 l ha^{-1}	1.1×10^4	2.7×10^4	27	2.8×10^5	7.23	9.9
Tilt 0.5 l ha^{-1}	1.6×10^4	3.0×10^4	0	1.3×10^5	7.28	9.7

* Moulds — *Alternaria, Cladosporium, Penicillium, Aspergillus, Fusarium, Trichothecium, Mucor* spp.

The effect of N fertilisation

Increasing of N background increased the number of moulds both in 2002 and 2003. The same tendency was evident in the abundance of *Fusarium* species. Heavier N fertilisation primarily favours the vegetative growth of plants, however, probably it also creates better development conditions for pathogenic and epiphytic microflora. Larger N doses in the drier conditions of 2002 increased the number of moulds and bacteria, as well as yeasts in the control variant. In the wet conditions of 2003 it was particularly evident in all groups of microorganisms, especially in the number of *Fusarium* species.

The effect of fungicides

In using fungicides at stages GS 39 on 23 May 2002 and GS 47-49 on 17 June 2003, yield harvest was done after 64 days in 2002 and after 66 days in 2003. According to the sales firms, the length of the effect of the active substance agents of the fungicides used (propiconazole, prochloraz) is 28—42 days. The spraying was performed in favourable weather conditions in both years, which did not decrease the effect of fungicides. However, could such single use of fungicides affect also the microbiology of yields? Compared with the untreated control, the number of yeasts and bacteria did not change in a firm direction (sometimes it increased, sometimes decreased), however, a certain decrease in the number of moulds on a higher N background due to the effect of fungicides could be noticed during both years.

Table 3

Quantitative and group composition of microorganisms on seeds of wheat in 2003
(number of cells in one gram of dried seeds)

Variant	Moulds on RBCA	Yeasts on RBCA	*Fusarium* spp. on Nash &Snyder	Aerobic mesophilic bacteria on PCA	pH in H_2O	Moisture, %
	Background N_0					
Untreated	2.5×10^4	1.8×10^4	0	2.7×10^5	7.12	9.6
Bumper Super 1.0 l ha^{-1}	3.1×10^4	4.2×10^4	0	6.4×10^5	7.14	9.6
Tilt +Sportak 0.36 + 0.89 l ha^{-1}	2.2×10^4	1.3×10^4	0	6.4×10^5	7.13	9.7
Tilt 0.5 l ha^{-1}	$2.7x10^4$	1.5×10^4	0	5.6×10^5	7.12	9.9
	Background N_{150}					
Untreated	4.0×10^4	3.8×10^4	440	1.3×10^6	7.18	9.6
Bumper Super 1.0 l ha^{-1}	3.1×10^4	$3,7\times10^4$	220	1.6×10^6	7.17	9.5
Tilt + Sportak 0.36 + 0.89 l ha^{-1}	4.0×10^4	3.4×10^4	110	1.0×10^6	7.24	9.9
Tilt 0.5 l ha^{-1}	3.7×10^4	9.1×10^4	55	1.1×10^6	7.30	9.8

With the generally low numbers of *Fusarium* species in 2002, the effect of fungicides on their occurrence cannot be detected, however, in 2003 the use of fungicides decreased the number of *Fusarium* species on the N_{150} background. If in the control the number of Fusariums per 1 gram of dry grains was 440, fungicides decreased the number by 2—8 times, whereas Tilt 0.5 l/ha had the strongest effect. Of *Fusarium* species, *F. culmorum* and *F. avenaceum* occurred most frequently, and *F. semitectum* Berk. et Rav. And *F. poae* (Pk.) Wr. less frequently.

The toxicity of *Fusarium* spp.

The toxicity of *Fusarium* spp. to *Bacillus stearothermophilus* was mainly 3—7 points on a 10-point scale. There were also non-toxic isolates. More toxic isolates were more frequent in the untreated control variant on the N_{150} background. Isolates obtained from variants treated with fungicides had generally lower but similar toxicity.

The microbiological quality of grain

On assessing the quality of the grain according to the German standards for quality feed grain (Schmidt-Lorenz, 1980), all the variants meet the requirements with their contents of moulds, yeasts (4×10^4–8×10^4) and aerobic mesophilic bacteria (up to 6×10^6) in both years. With their moulds content, all of the 8 variants of 2002 meet the requirements for quality food grain (Baumgart, Firnhaber, 1993), for which the number of either moulds or yeasts must not exceed 3×10^4, whereas with their yeasts content only 6 variants meet the requirements. In the more wet year 2003, with their moulds content only 5 of the 8 variants and with their yeasts content only 3 variants meet the standard for quality food grain. For the total number of bacteria (standard — up to 5×10^6) all variants of both years meet the requirements.

The changes of microflora in the main groups of microorganisms (bacteria, moulds, and yeasts) depended on the background of N and weather conditions of the year more than on application of fungicides with active ingradient propiconazole and prochloraz. Incidence of moulds, *Fusarium* species and bacteria increased in more rainy growth periods as well as with greater application of N-fertilisers. Changes in the number of yeasts are not clearly connected with the humidity of the growth period, however, with a combination of greater humidity and a higher N background the number of yeasts increased considerably. In the case of the larger number of *Fusarium* species in more humid growth periods, fungicides decreased their number 2—8 times, whereas the most effective fungicide was Tilt 0.50 l ha^{-1}. *F. culmorum* and *F. avenaceum* were the main species on the seeds. The toxicity of *Fusarium* isolates to *B. stearothermophilus* was 3—7 points in most cases, whereas more toxic isolates were more frequent in the control variant. The changes in microflora when applying fungicides do not generally impair the quality of grain but the increase of the number of *Fusarium* spp. is unadvisable in certain cases.

The results of the research conducted revealed a need to postpone the use of fungicides to a later time to control the development of microflora more effectively.

References

1. Baumgart, J., Firnhaber, N. M. 1993. Mikrobiologische Untersuchungen von Lebensmitteln. Behr`s Verlag, Hamburg.
2. Gerlach, W., Nirenberg, H. 1982. The Genus *Fusarium* — a Pictorial Atlas- Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft. Berlin, Heft 209, 1—406.
3. Miller, J. D., Trenholm, H. L. 1997. Mycotoxins In Grain. Compounds Other Than Aflatoxin. Eagan Press, St. Paul, Minnesota, USA, 552 pp.
4. Schmidt-Lorenz, W. 1980. Sammlung von Vorschriften zur mikrobiologischen Untersuchungen von Lebensmitteln. Produktgruppe 27. Futttergrundstoffe und Mischfutter, Weinheim.
5. Watson, D. H., Lindsay, D. G. 1982. A Critical Review of Biological Methods for the Detection of Fungal Toxins in Food and Foodstuff. Journal of Science of Food and Agriculture, 33, 59—67.

DISTRIBUTION OF FUNGI PRODUCING TOXINS ON PLANT-ORIGIN FOOD STOCK AND PRODUCTS

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Abstract

The diversity of fungi species spread on food products grown and processed under various ecological conditions were estimated during the researches carried out in 1998—2003. Fungi were isolated and identified from grain, seed, vegetables, fruit and other plant-origin products grown in Lithuania and imported from 24 countries. Fungi distribution in various premises of storing, selling and processing was revealed. 312 species of fungi ascribed to 102 genera were isolated and identified out of the investigated plant-origin food products stored and processed in different premises. Majority of the isolated fungal species belonged to genera: *Penicillium* (45,4%), *Aspergillus* (10,8%), *Fusarium* (3,6%), *Mucor* (3,5%), and *Rhizopus* (3,2%). Potentially toxic fungal species prevailed on plant-origin food products: *Penicillium expansum* (detection frequency 13,2%), *Aspergillus niger* (12,8%), *P. granulatum* (8,8%), *P. claviforme* (6,0%), *P. spinulosum* (5,6%). The total number of identified fungal species correlated with the number of toxic species (R = 0,92) and with the variety of investigated products (R = 0,81). Efficiency of 7 standard fungicidal preparations to reduce the contamination of food product storage premises with fungal propagules was evaluated.

Key words: fungi, contamination, grain, seed, vegetables, fruit, berries, toxin.

Introduction

The propagules of micromycetes get on plant-origin food products from soil and air. Ability to adapt to changing environmental conditions enables them to contaminate various vegetative substrata. If requirements of hygiene of the premises are neglected, micromycetes can cause ecological problems, become the agents of plant and even people diseases.

Mycotoxins are complex secondary metabolites produced by various species of fungi. Not all fungi produce mycotoxins. Some species only reproduce one type of mycotoxin, while other produces several. Also, a specific type mycotoxin can be produced by different fungal species. Some of the best-known mycotoxins include aflatoxins, ochratoxins, trichothecenes, fumonisins, zearalenone and patulin. These mycotoxins can be produced by various fungal genera including *Aspergillus, Penicillium, Claviceps, Alternaria, Trichoderma,* etc. Mycotoxins are produced under specific conditions, which in general are independent of those required for fungal growth (Boutrif, Bessy, 2000; Lugauskas et al., 2002; Parshikov et al., 2002).

Interest in the products of micromycete secondary metabolism — mycotoxins increases, new toxic materials produced by microscopic fungi are revealed; new diseases caused by them are diagnosed (Brera et al., 1988; Pieckova, Jesenska, 1999; Samson et al., 2000; Dutkiewich et al., 2001). Mycotoxicoses are not always directly associated with consumption of the contaminated food. Often with contaminated forage mycotoxins get into organism of cattle and later can be recorded in milk, meat of cattle and even in breast milk. Hence mycotoxins get into organisms of animal or people through nutrition chains (Frisvad et Samson, 1991; Miraglia et al., 1995; Sabino et al., 1996; Correa et al., 1997; Keblys et al., 2000; Lugauskas, Stakėnienė, 2001, 2002).

Aiming to evaluate potential hazard to the health of people and animals caused by mycotoxins, scientists from various countries usually investigate the contamination of forage and grain with micromycetes, determined the synthesized mycotoxins. The data on micromycetes damaging vegetables, fruit, nuts, bread products, are seldom associated with the produced mycotoxins (Osipjan, Batikjan, 1993; Robiglio, Lopez, 1995; Jimenecz et al., 1997; Lugauskas, Stakėnienė, 2001; Stakėnienė et al., 2001). These investigations are usually not complex, only microbiological or chemical contamination of products are regarded, preventive measures are rarely indicated, almost no data considering hazard of the consumption of contaminated products to the health of people are provided.

Purpose of the research — to investigate the diversity of micromycete species spread on food products grown in Lithuania and imported from other countries and to search for preventive safety enabling to reduce spreading of toxin-producing micromycetes.

Materials and Methods

In 1998—2003, investigations on mycological state of fruit, berries, vegetables, and other food products of plant origin grown in Lithuania and imported from 24 countries were performed in different premises of storing, sale and processing with differing conditions of production preservation. For mycological investigations, 650 samples of 95 product types were taken. They were analysed according to the methods described by Kudrjasheva (1986), Rabie et al. (1997), Samson et al., (2000). The analysis of each sample was performed in three replications. The methods of direct plating and dilution plating were applied for isolation of micromycetes. The agar medium of malt extract supplied chloramphenicol (50 mg/l) was used. The upside-down Petri dishes were kept for 4 days in thermostat at a temperature of 28 °C, for the next 4 days at a temperature of 20 °C, the regime of light and dark was being changed every 12 hours. Pure micromycete cultures were isolated; they were cultivated in standard agar Czapek, malt and corn extract media at a

temperature of 28 °C for 5—6 days and identified according to their cultural and morphological peculiarities. Systematic subordination of micromycetes was determined following the Ainsworth et Bisby's dictionary of the fungi (Hawksworth et al., 1995). Detection frequency (%) of each identified species was calculated (Mirchink, 1988).

Effectiveness of fungicidal preparations "Lysoformin special", "Sokrena", "Terralin", "Taab-1", "Taab-2", "Incidur", "Cuprinol" (different manufacturers) was estimated by the method of agar diffusion basing on the average diameter of fungicidal zone in Petri dishes where on freshly prepared fungal inoculate paper discs soaked in the preparation had been placed (Bilaj, 1982; Aleman et al., 1993).

Results and Discussion

After analysis of 650 samples of 95 plant origin food product types grown in Lithuania and imported from 24 countries, stored, sold and processed in different premises 312 species of fungi ascribed to 102 genera, 23 families, 16 orders, 3 classes, 6 phyla were isolated and identified. Mitosporic fungi prevailed. In the investigated premises micromycetes of *Penicillium* (45,4%) and *Aspergillus* (10,8%) genera reliably dominated; less frequent were fungi of *Fusarium* (3,6%), *Mucor* (3,5%), *Rhizopus* (3,2%), *Acremonium* (3,0%), *Cladosporium* (2,4%), *Sclerotinia* (2,2%), *Geotrichum* (1,9%), *Alternaria* (1,8%), *Mortierella* (1,8%), *Botrytis* (1,6%), *Verticillium* (%), *Gliocladium* (1,2%), *Trichoderma* (1,0%), *Chrysosporium* (0,9%), *Phoma* (0,7%), *Rhizoctonia* (0,7%) and other genera (Fig. 1).

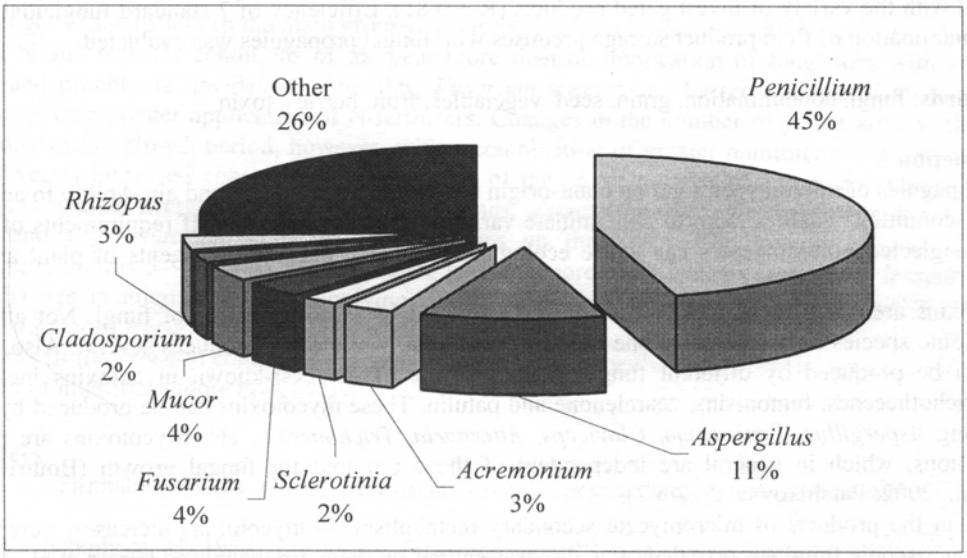

Fig. 1. Distribution of fungi genera infecting the food products of plant origin, %

The most of fungal species isolated from grain, seeds, fruit, berries, vegetable and other products are cosmopolitans, which could grow and develop on various substrates in all continents. Fruits and berries grown in Lithuania were infected most often by such fungi as *Sclerotinia sclerotiorum, Absidia butleri, Alternaria alternata, Drechslera biseptata, Sphaerotheca mors-uvae, Aspergillus niger, Eurotium herbariorum, Geotrichum fermentans* and by many species of the genus *Penicillium.* The predominant species depended on the species of fruit and berries and on the surrounding ecological conditions. But certain fungal species were isolated only from definite fruit imported from a concrete country. For example, *Exophiala mansonii* and *Eupenicillium brefeldianum* were imported together with grapes from Chili, and oranges from Spain, *Verticillium trifidum* — with plums from Hungary, *Anellophorella magdalensis, Fusarium sporotrichioides, Nectria haematococca* — with banana from Ecuador, *Cunnighamella vesiculosa, Thamnidium fulvus* — with plums, and *Leptodontium boreale* — with mandarins from Marocco, *Paecilomyces javanicus* — with apricots from Spain, *Ascochyta pruni* — with peach from Greece. Many fungal species from genera *Penicillium* and *Aspergillus* would be constantly imported with products of plant origin.

The checked potatoes grown in Lithuania and taken from storehouses were infected by the fungi *Sclerotinia sclerotiorum, Rhizoctonia solani, Verticillium alboatrum, Fusarium equiseti, Geotrichum candidum* most often. Fresh new harvest potatoes sold in market were infected by *Rhizoctonia solani, Fusarium merismoides, F. oxysporum*. On carrots damaged by rot most often the following fungi were found: *Verticillium alboatrum, Myrothecium roridum, M. verrucaria,* on dill — *Cladosporium cucumerinum, Acremonium strictum, Penicillium digitatum*. The leek were damaged most severly by *Sclerotinia sclerotiorum, Penicillium granulatum, P. expansum, Rhizopus stolonifer, Fusarium moniliforme*. Beetroots from storehouses were infected most often by fungal species from the *Penicillium* genus and well known parasites: *Verticillium alboatrum, Sclerotinia sclerotiorum,* and *Pythium ultimum*.

From the cabbages widespread micromycetes from the specie *Aspergillus niger* were isolated and they were accompanied by the following fungal species: *Botrytis cinerea, Perenospora brassicae, Sclerotinia sclerotiorum, Penicillium expansum,* and *P. granulatum*. Cucumbers sold in the market were infected by *Alternaria alternata, Botrytis cinerea,* and paprika — by *Sclerotinia sclerotiorum, Fulvia fulva* fungi, which is known as *Cladosporium fulvum* like agents of spot disease tomato's leaves, were spread on the tomatoes sold in the market. Onions in the

storehouses were infected by *Botrytis alii, B. bifurcata, Perenospora destructans, Fusarium moniliforme, F. oxysporum,* and *Penicillium spinulosum.*

The vegetables imported from various countries were infected by propagules of micromycetes. The diversity of determined species of micromycetes was not large. It could be connected with treating vegetables with fungicides during their growth or after gathering. However, this statement should be well grounded by special investigations.

The contamination of matured seeds of various plants grown in the fields by fungi propagules was not significant: wheat contamination was from 328 to 502 cfu/g; barley — from 793 to 1020; rye — from 580 to 867; corn — from 340 to 370; soybean — from 32 to 56 cfu/g. Fungi of *Alternaria* and *Fusarium* genera were prevailing (47,8% and 27,3%, respectively). On the grains stored under various conditions, fungi of *Penicillium* and *Aspergillus* genera were prevailing (32,0% and 27,3%, respectively). Fungal propagules existing in the storage air are one of the grain contamination sources while storing. It is determined that the amount of fungi propagules in the air is related to the works performed in those premises, especially with the grain drying when the intensive air convection is taking place. The composition of micromycetes species in the investigated premises depended upon diversity of the analysed products, their origin, and quality of their storing environment, preventive measures.

It is referred in literature sources that 60—70% of micromycetes spread on food are toxic to the warm-blooded (Hussein, Brasel, 2001). Particularly hazardous are mycotoxins accumulated in fatty fraction that through nutrition chain get into consumers food without any perceivable changes of quality (Sabino et al., 1996; Dragacci, Fremy, 1996; Correa et al., 1997). Micromycetes settled on food are hazardous just for people eating the contaminated food, but also to those working in the premises of its storage, sorting, as well as in flourmills and food-processing establishments (Krysińska-Traczyk et al., 2001).

According to the detection frequency, potentially toxic micromycete species prevailed on the on plant-origin food products: *Penicillium expansum* (detection frequency 13,2%), *Aspergillus niger* (12,8%), *P. granulatum* (8,8%), *P. claviforme* (6,0%), *P. spinulosum* (5,6%), *P. italicum* (5,4%, Fig. 2).

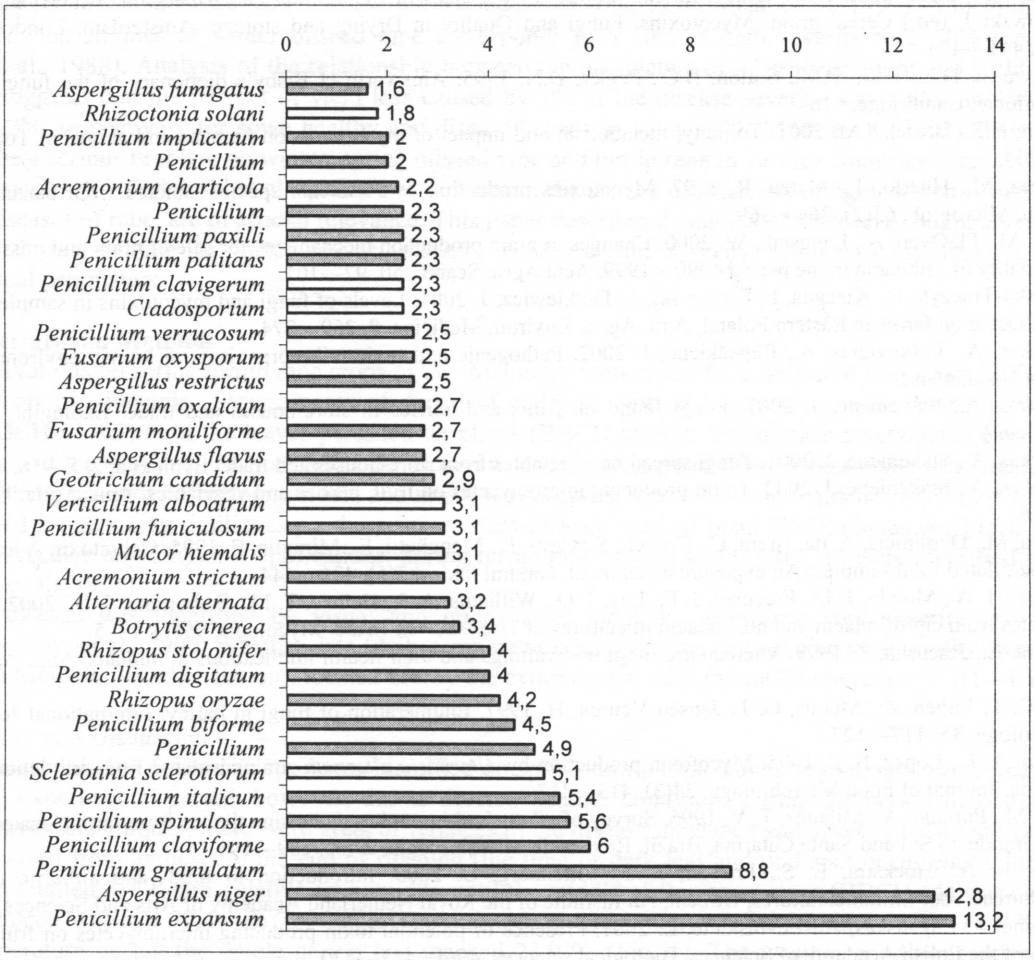

Fig. 2. Detection frequency of fungi on the investigated products of plant origin, % (black — potentially toxic species)

In the investigated premises the total number of identified fungal species strongly correlates with the number of potentially toxic species (R = 0,92). Diversity of food items correlates with the total number (R = 0,81) and the number of potentially toxic micromycete species (R = 0,80).

Resistance of 66 micromycete strains towards 7 fungicidal substances, used in concentrations recommended by the manufacturers, was checked. Statistical analysis of the results revealed that *Rhizopus stolonifer* and *R. oryzae* were

resistant to all preparations, *Aspergillus penicilloides* — sensitive to only one preparation "Terralin". Four mivcromycete strains: *Eurotium niveoglaucus, Fusarium solani, F. sporotrichioides, Penicillium verruculosum* were sensitive to "Lysoformin special" and "Terralin". Micromycetes of six strains: *Mucor hiemalis, Penicillium biforme, P. canescens, P. commune, P. daleae, P. expansum* were sensitive to "Lysoformin special" and "Sokrena". Micromycetes of 60% of tested strains were sensitive to a half of the investigated preparations. Among micromycetes, most resistant to fungicidal substances 54% of strains were toxic. Various fungicidal preparations not equally inhibited the development of the same micromycete strain.

It was revealed that of all tested fungicidal preparations most universal and efficient ($p < 0,05$) was: "Lysoformin special" (effective against 95% of tested strains), its active agent — aldehydes. Therefore, it could be stated that there is no fungicidal preparation effectively inhibiting the development of all micromycete species.

References

1. Alleman, B. C., Logan, B. E., Gilbertson, R.L. 1993. A rapid method to screen fungi for resistance to toxic chemicals. Biodegradation, 4, 125—129.
2. Boutrif, E., Bessy, C., 2000. Global significance of mycotoxins and phytotoxins. Mycotoxins and phycotoxins in perspective at the turn of the Millenium. Proceedings of the 10th International IUPAC symposium on mycotoxins and phytotoxins, 21—25 May, Guaruja (Brazil), 3—16.
3. Brera, C., Miraglia, M., Colatosti, M. 1988. Evaluation of the impact of mycotoxins on human health: Sources of errors. Microchemical Journal, 59(1), 45—49.
4. Correa, B., Galhardo, M., Costa, E.O., Sabino, M. 1997. Distribution of moulds and aflatoxins in dairy cattle feeds and raw milk. Revista de Microbiologia, 28(4), 279—283.
5. Dragacci, S., Fremy, J. M. 1996. Application of immunoaffinity column cleanup to aflatoxin M-1 determination and survey in cheese. Journal of food Protection, 59(9), 1011—1013.
6. Dutkiewicz, J., Krysińska-Traczyk, E., Skóska, C., Sitkowska, J., Prażmo, Z., Golec, M. 2001. Exposure to airborne microorganisms and endotoxin in herb processing plants. Ann. Agric. Environ. Med., 8, 201—211.
7. Frisvad, J.C., Samson, R.A. 1991. Mycotoxins produced by species of *Penicillium* and *Aspergillus* occurring in cereals. In: Chełkowski J. (ed.) Cereal grain. Mycotoxins. Fungi and Quality in Drying and storage. Amsterdam, London, New York, Tokyo, 441—475.
8. Hawskworth, D.L., Kirk, P.M., Sutton, B.C., Pegler, D.N. 1995. Ainsworth et Bisby's dictionary of the fungi. 8th ed. CAB International, Cambridge, 616.
9. Hussein, H.S., Brasel, J.M. 2001. Toxicity, metabolism and impact of mycotoxins on humans and animals. Toxicology, 167, 101—134.
10. Jimenecz, M., Huerto, T., Mateo, R. 1997. Mycotoxins production by *Fusarium* species isolated from bananas. Appl. and Environ. Microbiol., 63(2), 364—369.
11. Keblys, M., FläØyen, A., Langseth, W. 2000. Changes in grain production mechanisms for sale of grain and possible effects on grain quality in Lithuania in the period 1990—1999. Acta Agric Scand., 50, 97—101.
12. Krysińska-Traczyk, E., Kiecana, I., Perkowski, J., Dutkiewicz, J. 2001. Levels of fungi and mycotoxins in samples of grain and dust collected on farms in Eastern Poland. Ann. Agric. Environ. Medicine, 8, 269—274.
13. Lugauskas, A., Paškevičius, A., Repečkienė, J. 2002. Pathogenic and toxic mikroorganisms in human environment. Vilnius, 434. (In Lithuanian.)
14. Lugauskas, A., Stakėnienė, J. 2001. Fungi found on fruits and berries in store-houses and trade. Ekologija, 1, 3—11. (In Lithuanian.)
15. Lugauskas, A., Stakėnienė, J. 2001. Fungi spread on vegetables from store-houses and trade. Ekologija, 2, 8—18.
16. Lugauskas, A., Stakėnienė, J. 2002. Toxin producing micromycetes on fruit, berries and vegetables. Ann. Agric. Environ. Med., 9, 1—16.
17. Miraglia, M., Dominicis, A. de, Brera, C., Corneli, S., Cava, E., Menghetti, E., Miraglia, E. 1995. Ochratoxin A levels in human milk and related food samples: An exposure assessment. Natural toxins, 3(6), 436—444.
18. Parshikov, I. A., Moody, J. D., Freeman, J. P., Lay, J. O., Williams, A. J., Heinze, T. M., Sutherland, J. B., 2002. Formation of conjugates from ciprofloxacin and norfloxacin in cultures of *Trichoderma viride.* Mycologia, 99(1), 1—5.
19. Pieckova, E., Jesenska, Z. 1999. Microsporic fungi in dwellings and their health implications in humans. Ann. Agric. Environ. Med., 6, 1—11.
20. Rabie, C. J., Lűben, A., Marais, G. J., Jansen Vauren, H. 1997. Enumeration of fungi in barley. International Journal of food microbiology, 35: 117—127.
21. Robiglio, A. L., Lopez, S. E. 1995. Mycotoxin production by *Alternaria alternata* strains isolated from red delicious apples in Argentina. Journal of Food Microbiology, 24(3), 413—417.
22. Sabino, M., Purchio, A., Milanez, T. V. 1996. Survey of aflatoxicol in poultry and swine tissues from farms located in the states of Rio Grande do Sul and Santa Catarina, Brazil. Revista de Microbiologia, 27(3), 189—191.
23. Samson, R. A., Hoekstra, E. S., Frisvad, J. C., Filtenborg, O. 2000. Introduction to food- and airborne fungi. 6th ed. Centralbureau voor Shimmelcultures, Utrecht, An Institute of the Royal Netherland Academy of Arts and Sciences, 389.
24. Stakėnienė, J., Lugauskas, A., Levinskaitė, L. 2001. Presence of potential toxin producing micromycetes on fruit and berries. Bulletin of the Polish Academy of Sciences. Biological sciences, 49(4), 413—420.
25. Билай, В. Й. 1982. Методы экспериментальной микологии. Справочник. Киев, 241.
26. Кудряшева, А. А. 1986. Микологические основы сохранения плодов и овощей. Москва.
27. Мирчник, Т.Г. 1988. Почвенная микология. Москва.
28. Осипян, Л. Л., Батыкян, А. Г. 1993. Выдовой состав и некоторые биологические особенности микелиальных микромицетов, контаминирующих баклажановую икру промышленного производства. Микология и фитопатология, 27(6), 25—31.

THE SPREAD OF DARK LEAF AND POD SPOT (*ALTERNARIA* SPP.) ON SPRING TURNIP RAPE (*BRASSICA CAMPESTRIS*) LEAVES AND SILIQUES

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Abstract

Dark leaf and pod spot disease, caused by *Alternaria spp.* is a serious problem on spring turnip rape (*Brassica campestris*) in Lithuania. The effects of weather conditions on the dynamics of dark leaf and pod spot incidence and severity on the spring turnip rape cv. 'Mammut' were assessed during 2000—2003 in the central part of Lithuania. Disease assessments were carried out once a week from BBCH 63 to BBCH 83 on leaves and from BBCH 70 to BBCH 89 on siliques. Every year the incidence and severity of the disease were significantly affected by the meteorological conditions — precipitation, temperature and relative air humidity. The most conducive weather conditions to the occurrence of dark leaf and pod spot disease on spring turnip rape leaves and siliques were in 2000, while the most adverse year was 2002. Weather factors had a greater effect on *Alternaria* blight severity than on incidence. The disease severity positively correlated with the mean daily air temperature, content of precipitation and relative air humidity.

Key words: spring turnip rape, *Alternaria spp.*, incidence and severity, dark leaf and pod spot.

Introduction

Alternaria species are worldwide and attack a large number of *Brassicaceae* crops (Kadian & Saharan, 1983; Tewari & Conn, 1993; Verma & Saharan, 1994). The disease is favoured by moderate temperature, high humidity and splashing rain (Humpherson-Jones & Phelps, 1989; Verma & Saharan, 1994; Meah et al., 1999). A medium *Alternaria* blight severity on siliques of winter oilseed rape corresponds to a 20%, a high severity to a 50% seed yield loss (Daebeler et al., 1988). Analysis of the relationship between the parameters of *Alternaria* blight and yield of *Brassica campestris* suggests that the percent of yield loss caused by 1% of the disease severity was 1.66% in 1997/1998, but 7.2% and 5.2%, respectively for sown healthy and diseased seeds in 1998/1999 (Meah et al., 2002). This disease can sometimes be a serious problem on winter, spring oilseed rape and turnip rape in various countries (Frencel et al., 1991; Clear & Patrick, 1995; Sadowski et al., 2002). For this reason the studies of *Alternaria* blight, which is one of the most important diseases of rape, are of special relevance. This paper describes dynamics of *Alternaria* blight spread on spring turnip rape lower, middle or upper leaves, disease incidence and severity on leaves, siliques in relation to meteorological parameters.

Materials and Methods

Observations on spring turnip rape crops of *cv.* 'Mammut' were made for a period of four years (2000—2003) in the central part of Lithuania. Assessments of dark leaf and pod spot on leaves were made from the beginning of anthesis (BBCH 61–63) until the leaves persisted on plants (BBCH 80-85). The disease assessments on siliques were made throughout the whole silique formation, development and ripening period. The assessments were made weekly on 25 marked plants per plot on bottom, middle and upper leaves and siliques. Disease incidence and severity on siliques was identified by assessing 5 siliques on the primary stem of each marked plant (125 siliques per plot). The sample plots did not receive any fungicide applications. Spring turnip rape growth stages (BBCH) were identified according to (Lancashire et al., 1991).

The disease was identified by visual and microscopic methods. *Alternaria* spp. severity was determined according to Conn et al., 1990. Weather data (daily temperature, precipitation and relative air humidity) were generated by the Hardi Metpole (weather station), located nearby the experimental field (about 25 metres).

Results and Discussion

Alternaria blight (*Alternaria* spp.) was identified on spring turnip rape leaves and siliques every year during the 2000—2003 experimental period, however, due to diverse weather conditions during the period of Alternaria blight spread on leaves and siliques, there were great differences in the disease incidence and severity between years. In 2000, during the period from anthesis to the end of ripening (the time of dark leaf and pod spot occurrence), the amount of precipitation totalled 214.8 mm, relative air humidity until the middle of flowering stage (BBCH 64) was close to 70%, later it fluctuated within a 79.4—86.7% range. The mean daily temperature varied within a 12.7—17.2 °C range, the maxim temperature during the whole period had reached 23.9 °C (Fig. 1). The weather conditions determined slow development of turnip rape (9 weeks elapsed from the beginning of flowering to harvesting) and had a conducive effect on Alternaria blight occurrence.

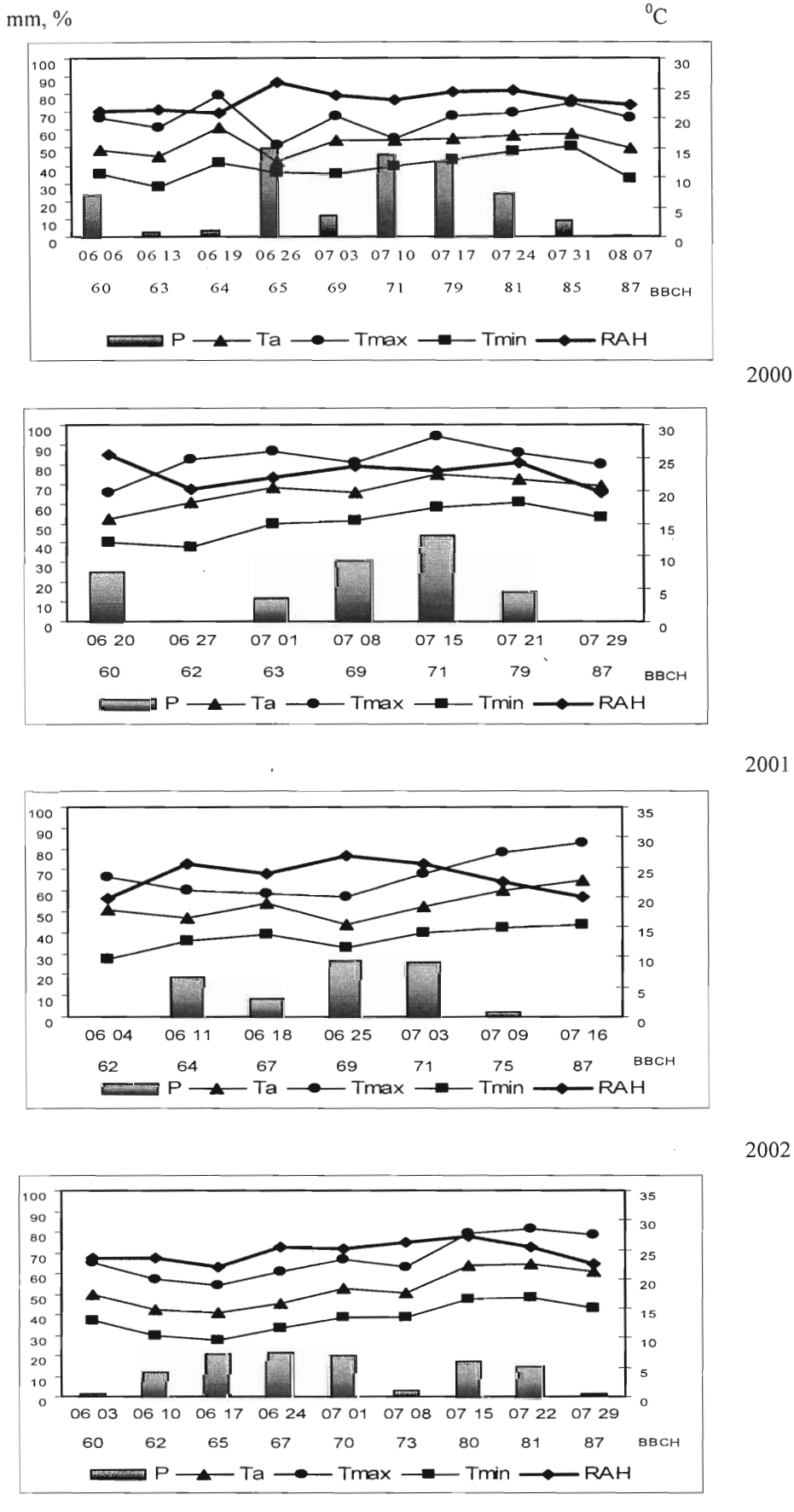
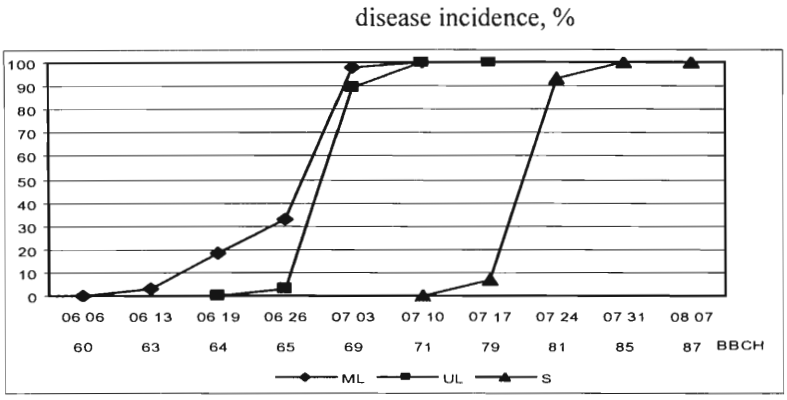
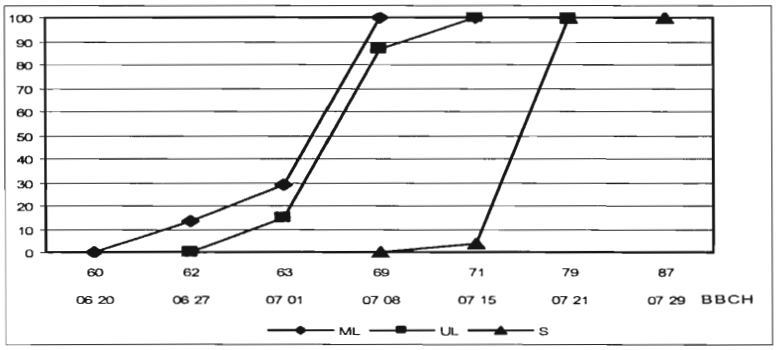


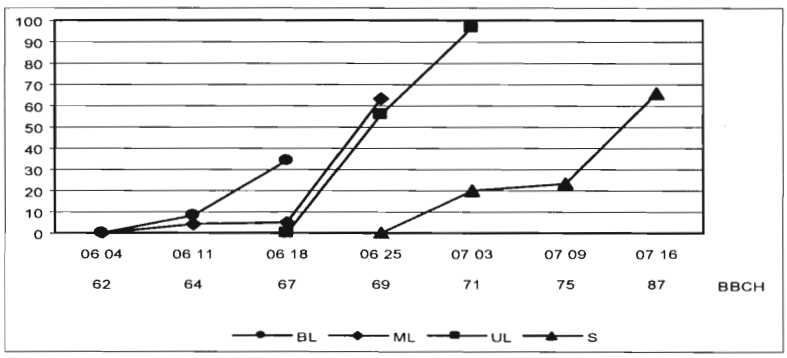
Fig. 1. Weather conditions during the period of dark leaf and pod spot development on spring turnip rape: P — precipitation, mm; Ta — mean air temperature, °C; Tmax — maximum air temperature, °C; T min — minimum air temperature, °C; RAH — relative air humidity, %



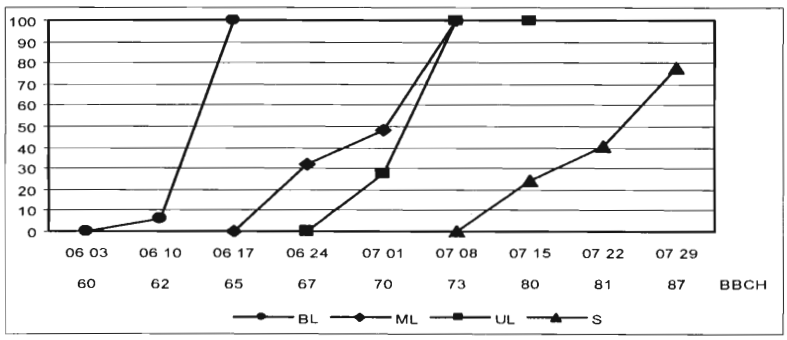
2000



2001



2002



2003

Fig. 2. Alternaria blight incidence (%) on spring turnip rape bottom leaves (BL), middle leaves (ML), upper leaves (UL) and siliques (S) in 2000—2003

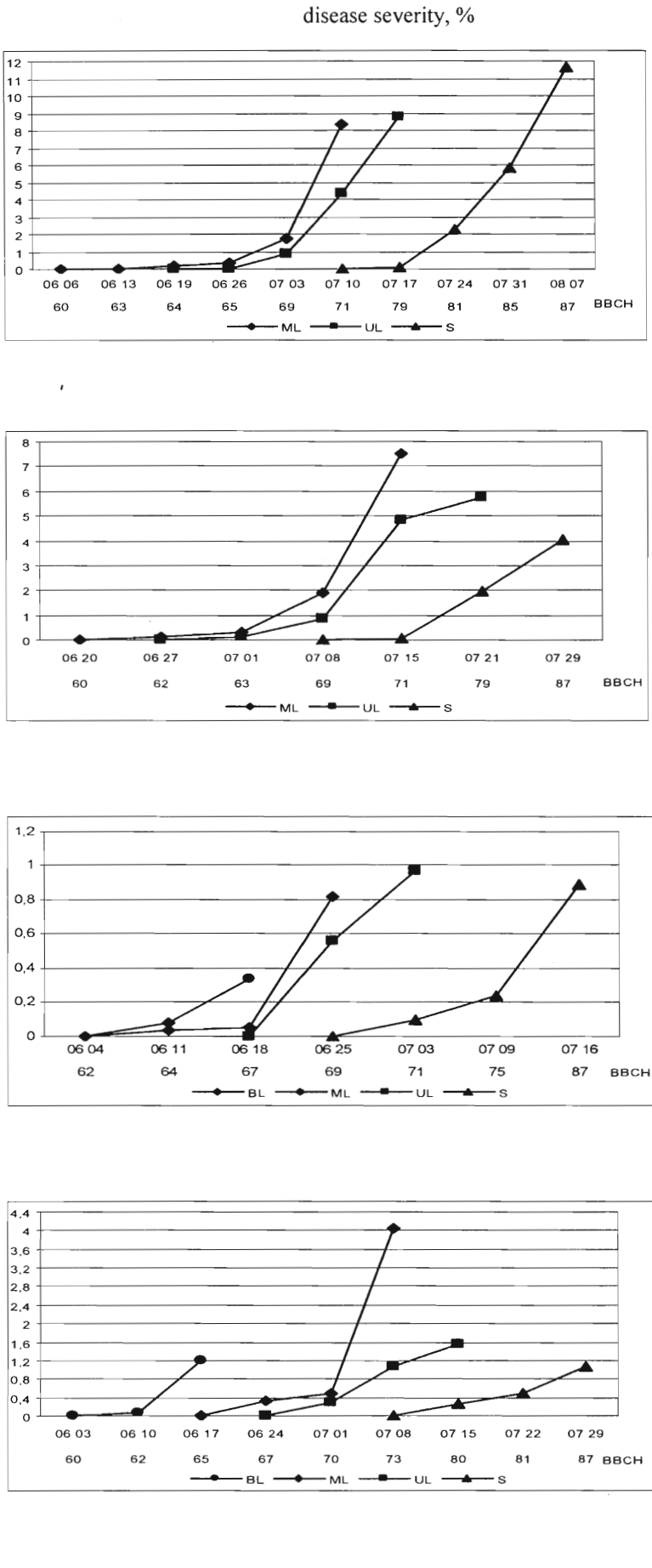


Fig. 3. Alternaria blight severity (%) on spring turnip rape bottom leaves (BL), middle leaves (ML), upper leaves (UL) and siliques (S) in 2000—2003

In 2001, unlike in 2002, only six weeks elapsed from the beginning of spring turnip rape flowering to full ripeness, turnip rape developed much more rapidly due to the shortage of moisture and higher mean daily temperature (15.7—22.4 °C), and maximum air temperature reached 24.0—28.3 °C almost every week. The amount of precipitation, that fell from the beginning of flowering to harvesting, was as low as 127.9 mm, its distribution during the period was uneven; in the periods with no precipitation relative air humidity dropped below 66% but in the middle of the period, from BBCH 63 to BBCH 79, precipitation occurred every week, and relative air humidity ranged within 73.3—80.5%. The conditions for the spread of dark leaf and pod spot were rather conducive.

The year 2002 was the most unfavourable of all experimental years for spring turnip rape development and dark leaf and pod spot occurrence, especially in terms of precipitation. Since BBCH 62, when the first spots of dark leaf and pod spot were identified on lower and middle leaves, till the end of ripening stage, six weeks elapsed, and the amount of precipitation totalled as little as 82.0 mm, i.e. 2.6 times less than in 2000 and 1.6 times less than in 2001. Relative air humidity during the larger part of the described period was below 70% in 2002, and average daily temperature was higher than usual, especially at ripening period, which markedly accelerated ripening of turnip rape and created unfavourable conditions for Alternaria blight development. In 2003, the amount of precipitation that fell during the same period was 111.4 mm and its distribution was rather even, however, relative air humidity at the beginning of the period was below 70%, in the middle of the period it amounted to 72.1—77.7%. Since growth stage 79—80 the mean daily temperature rose to above 22 °C, maximum temperature was 27.6—28.6 °C, which had a negative effect on the disease occurrence on siliques.

As we can see, a great diversity of precipitation, relative air humidity and mean daily temperature was recorded over the four experimental years. Various combinations of these factors had a positive or negative effect on the intensity of Alternaria blight spread, as was also noted by other authors (Hong et al., 1996).

The first spots of dark leaf and pod spot on middle leaves of turnip rape were identified at stage 63 in 2000, at stage 62 in 2001, at stage 64 in 2002, and at stage 67 in 2003. In the year 2000, within two weeks the disease had spread on the upper leaves (BBCH 65). With senescence of spring turnip rape leaves the disease severity tended to increase due to the greater disease susceptibility of older leaves (Conn & Tewari, 1989; Mridha & Wheeler, 1993). After another 3 weeks the first spots were spotted on siliques (BBCH 79) and within two weeks up to stage 85 dark leaf and pod spot had spread on all siliques (Fig. 2). In 2000, the disease severity on siliques was the highest of all experimental years, higher than on leaves, and at the end of ripening period reached 11.7% (Fig. 3).

In 2001, Alternaria blight on middle leaves appeared at growth stage 62, after a week — on upper leaves, after another two weeks the first disease spots were identified on young siliques (71 BBCH) and within a week had spread on all siliques, although disease severity was as low as 1.97%. After another week the disease had covered 4.0% of siliques surface, however, turnip rape had stopped ripening at that time and the disease development stopped. That year the disease severity on siliques was lower than on leaves, since the conditions (precipitation and relative air humidity) for the disease development were more favourable in the middle of the period rather than at the end.

During the 4-year period the year 2002 was extremely unfavourable for the disease development on leaves and siliques. Due to the shortage of moisture the incidence of dark leaf and pod spot on lower and middle leaves was 34—63%, and on upper leaves — 97%, and on siliques — 66%. The disease severity was the lowest of all experimental years and did not exceed 1% on leaves and siliques.

In 2003, although the disease had spread on all lower, middle and upper leaves, the disease spots covered as little as 1.2—4.0% of leaf surface area. On siliques the first disease spots were identified at growth stage 80 and until the end of ripening, within two weeks, the disease had spread on 78% of siliques, but the disease severity on siliques amounted to as little as 1%, like in 2002.

Summarising the experimental results, we can maintain that the period of Alternaria blight occurrence on spring turnip rape leaves and siliques is usually characterised by large variation of the weather factors both between years and within one season. Our observations suggest that precipitation, relative air humidity, and mean air temperature were equally important factors and they had a greater effect on Alternaria blight severity rather than on incidence, therefore it is important that both of these factors are identified in the tests instead of focusing on one of them, especially on the incidence alone. Correlation analysis have shown that the disease severity in 2000—2003 positively correlated with the mean daily temperature (R_{2000} = 0.949*, R_{2001} = 0.742, R_{2002} = 0.708, R_{2003} = 0.956*), in three experimental years it strongly positively correlated with the amount of precipitation (R_{2000} = 0.544, R_{2001} = 0.740, R_{2002} = 0.574). A strong correlation between the disease severity and relative air humidity was identified only in 2001 and 2002 (R_{2001} = 0.667, R_{2002} = 0.582). The identified correlations in most cases were not statistically significant, but we can assert that trends of these correlations were determined.

References

1. Daebeler, F., Amelung, D., Riedel, V. 1988. Untersuchungen über die Schadwirkung der durch *Alternaria spp.* verursachaten Rapsschwärze im Winterraps. Nachrichtenblatt für Pflanzenschutz in der DDR, Vol. 42, N.10, 197—199.
2. Clear, R.M., Patrick S.K. 1995. Frequency and distribution of seedborne fungi infecting canola seed from Ontario and western Canada 1989 to 1993. Canad. Plant Dis. Sur., 75 (1), 9—17.
3. Conn, K.L., Tewari, J.P. 1989. Interactions of *Alternaria brassicae* conidia with leaf epicuticular wax of canola. Mycol. Res., 93, 240—240.
4. Conn, K.L., Tewari, J.P., Awasthi, R.P. 1990. A disease assessment key for *Alternaria* black spot in rape seed and mustard. Canad. Plant Dis. Sur., 70 (1), 19—22.
5. Frencel, I.M., Lewartowska, E., Jedrycka, M. 1991. The spectrum and severity of fungal diseases in field infections of winter oilseed rape in Poland. Bulletin OILB SROP, 14 (6), 137—140.
6. Hong, C.H., Fitt, B.D.L., Welhalm, S.J. 1996. Effects of wetness period and temperature on development of dark pod spot (*Alternaria brassicae*) on oilseed rape (*Brassica napus*). Plant Pathol., 45, 1077—1089.
7. Humpherson-Jones, F.M., Phelps, K. 1989. Climatic factors influencing spore production in *Alternaria brassicae* and *Alternaria brassicicola*. Ann. Appl. Biol., 114, 449—459.
8. Kadian, A.K., Saharan, G.S. 1983. Symptomatology, host range and assessment of yield losses due to *Alternaria brassicae* infection in rapeseed and mustard. Indian J. Mycol. Plant Pathol., 13, 319—323.
9. Lancashire, P.D., Bleiholder, H., Boom, T., Langelüddecke, P., Staugs, R., Weer, E., Witzenberger, A. 1991. A uniform decimal code for growth stages of crops and weeds. Ann. Appl. Biol., 119, 561—601.
10. Meah, M.B., Islam, M.T., Rahman, S.L., Rahma H. 1999. Reducing *Alternaria* infection in seeds of mustard through management practices. Bangladesh J. Pl. Pathol., 15, 5—8.
11. Meah, M.B., Hau, B., Mahbuba, K., Siddiqua, K. 2002. Relationships between disease parameters of *Alternaria* blight (*Alternaria brassicae*) and yield of mustard. Journal. Plant Diseases and Protection, 109(3), 243—251.
12. Mridha, M.A., Wheeler, B.E. 1993. In vitro effects of temperature and wet periods on infection of oilseed rape by *Alternaria brassicae*. Plant Pathol., 42, 671—675.
13. Sadowski, C., Dakowska, S., Lukanowski, A., Jedryczka, M. 2002. Occurrence of fungal diseases on SOSR rape in Poland. IOBC/wprs Bulletin, 25 (2), 1—12.
14. Tewari, J.P., Conn, K.L. 1993. Reactions of some wild crucifers to *Alternaria brassicae*. IOBC/WPRS Bull., 16, 53—58.
15. Verma, P.R., Saharan, G.S. 1994. Monograph on *Alternaria* Disease of Crucifers. Agric. Canada Res. Branch Techn. Bull., 1994-6E, 53—57.

INTERACTION BETWEEN SECONDARY INFECTION OF LATE BLIGHT AND YIELD QUALITY

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Abstract

Potato late blight caused by *Phytophthora infestans* (Mont) de Bary may occur and infect plants at any time during the growing season.

It is well known that potato tubers are the most important first sources of inoculum. Infection of tubers happens when sporangia or zoospores are washed from foliage through the soil to developing tubers. Secondary spread of late blight occurs when spores are produced on infected potato leaves. Spores from infected potato in neighboured fields or gardens can travel through the air.

Potato late blight field trials were carried out in 1999—2002 in Central, Eastern and Western parts of Latvia. The role of potato late blight secondary infection and yield quality in untreated and treated with fungicide fields was compared.

The first symptoms of late blight were observed approximately at the same time in the area and in the trial fields. The year 2001 was the most favourable for development of potato late blight. The disease severity at the end of season was 100% whereas only 1—19,6% in 1999 and 2002. Tuber infection at harvesting time varies 0—12,7% depending on the year.

Comparison of the disease development, tuber infection and yield between the years and between untreated and treated with fungicide fields showed significant differences.

Key words: *Phytophthora infestans,* a potato, late blight.

Introduction

Potato plants in all stages of growth are susceptible to late blight caused by *Phytophthora infestans* (Mont) de Bary (Fry and Apple, 1986). Early infection of the crop can remarkably reduce the yield, but equally infection late in the season can be more destructive because of the increased risk of tuber blight.

Late blight survives from one season to the next season in infected tubers that are placed in storage or left in cull piles. Infected tubers sprout, spores are formed on the sprouts and are carried by wind or precipitation to healthy potato leaves (Andrivon, 1995).

When weather conditions are favourable, the fungus can spread rapidly through the foliage and is able to destroy potato plants in very short time. Late blight epidemic results from asexual reproduction of *P. infestans* in susceptible host tissue (foliage, stems and tubers) (Fry and Goodwin, 1997).

The asexual reproduction of the cycle involved the production of sporangia and the release and then germination of zoospores. Single sporangia or several sporangia often initiate potato late blight, each of which is able to produce leaf lesion. The lesion progress and new sporangia are formed in a few days after infection. The disease cycle (penetration, colonisation, sporulation and dispersal) can occur in less than five days (Harrison and Lowe, 1989). Development of potato late blight primarily depends on weather conditions. During the relatively warm summer with lot of precipitation the disease can be impossible to control. Other, more dry years, the disease can be completely absent. The optimum temperature for both the speed for formation of sporangia and their final quantity can vary from 16 to 22,5 °C (Harrison, 1995).

Aerial dispersal of *P. infestans* is the main process involved in spreading of late blight. Spores from infected potato in neighboured fields or gardens can travel through the air more than 100 kilometres (Mizubuti et al., 2000).

Sporangia produced in the foliage can be washed from leaves to infect the tubers. In the case of serious potato late blight attack in different countries, up to 25—50% of the harvest may be lost (Fry and Mizubuti, 1998; Turka, 1998). Some of the infected tubers may be destroyed before harvest, but other become diseased in storage (Fry and Goodwini, 1997). Soft rot of tubers can occur in storage following late blight tuber infection, in addition to the indirect losses from late blight.

The only way to protect the tubers is to keep the potato foliage free from late blight. Applying fungicides every year mainly does this. The fungicides used against *P. infestans* have a preventive effect and stop the pathogen from infecting the crop. Subsequent sprayings have to be done all through the growing season.

Materials and Methods

Potato late blight field trials were carried out in the growing seasons 1999—2002. The Latvia University of Agriculture Study and Training farm "Vecauce", Stende Plant Breeding and Experimental Station, Research Centre at Skriveri, Latvia State Plant Protection Service Units of Prognosis and Diagnostics at Priekuli, Saldus and Bauska and State Plant Protection Centre in Carnikava were involved in the monitoring of late blight.

Moderately susceptible variety 'Sante' was used in all experimental years, except in Carnikava (1999), when variety 'Asterix' was used. In the year 2002, Carnikava and Skriveri were not included in the experiment.

The role of potato late blight secondary infection on the yield quality in untreated and treated with fungicides fields were compared. Appearance of first symptoms, tuber infection and yield estimation was done during harvesting. Disease assessments during growing seasons were made in the years 2000, 2001 and 2002. Approximately one month after planting, every trial field was observed once a week but after row closing twice a week. ANOVA models were used to assess the effect of potato late blight severity and tuber blight and tuber yield.

Experimental design:

1. untreated control;
2. treated with fungicides — two first treatments with systemic fungicide and subsequent treatments with protective fungicide. First treatment was made during the row closing or according to prognosis when late blight was recorded in the region.

The weather conditions were different in growing seasons 1999—2002 (Fig. 1). The growing season 1999 was not favourable for disease development. The end of growing season 2000 was favourable but growing season 2001 was most favourable for development of late blight. The sum of temperatures and amount of precipitation were in optimum for disease development.

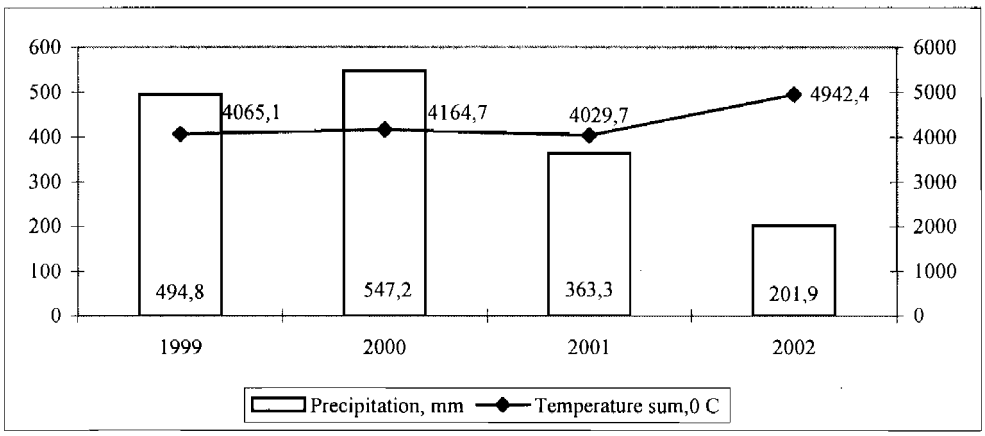


Fig. 1. Temperatures sum and amount of precipitation during growing season, 1999—2002

Whereas, the growing season 2002 was very hot and amounts of precipitation were less than other years. Development of potato late blight was decreased. August was dry and air temperature in some days exceeded 30 °C.

Results and Discussion

Secondary infection of late blight occurs when spores from infected potato leaves in neighboured fields or gardens can travel through the air. The home gardens are a major primary source of inoculum because here potatoes are growing in one place for a long period and certified seed material is not used. Interactions between secondary infection of late blight and yield quality depend on appearance of first symptoms and disease development.

The time of appearance of first symptoms of late blight was not very different between different places within in one year. Mostly, potato late blight spread in country within two weeks. In the years 1999 and 2001, the first symptoms were observed very early — 25th and 29th June and it was in Bauska. In others places in the year 1999 first symptoms appeared 7—28 days later, but in the year 2000 the first symptoms were observed approximately one month later. In 2001 and 2002, observation results of first symptoms were similar (except Bauska). Potato late blight symptoms were appearing at the same time in untreated and treated with fungicide fields (Table 1).

Similar tendencies were observed with disease development in different trial sites. The results from Vecauce were analysed for potato late blight development.

Disease development curves for untreated fields are shown in Fig. 2. The year 2001 was most favourable for disease development. After observation of first symptoms the disease developed very fast and at the end of July disease rating was 94%. In 2000, potato late blight development started at the second half of the growing season. In 2002, the situation was similar.

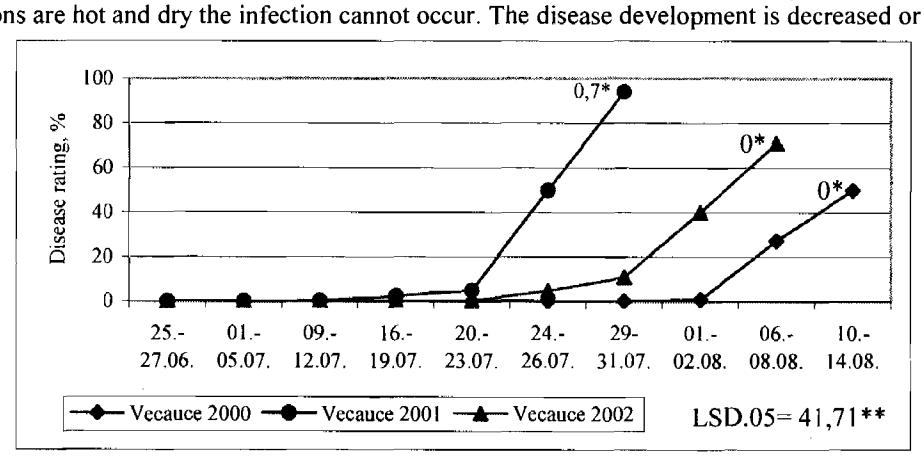
Disease development curves for treated with fungicides fields are shown in Fig. 3. Usage of fungicides was not effective for control of potato late blight in 2001, and disease rating at the end of the season was 83,5%. Whereas in 2000 and 2002, the disease ratings were 0,1 and 2,0%, respectively.

Table 1

Appearance of first symptoms and tuber infection in untreated and treated with fungicides fields, 1999—2002

Location	Appearance of first symptoms	Tuber infection, % at harvesting time	
		Untreated	Treated with fungicide
1999			
Vecauce	05.07.	0	0
Stende	16.07.	0	0
Skriveri	19.07.	0	0
Priekuli	19.07.	0	0
Saldus	27.07.	0,1	0
Bauska	29.06.	0	0
Carnikava	17.07.	0	0
2000			
Vecauce	31.07.	0	0,8
Stende	24.07.	3	2,6
Skriveri	24.07.	0,1	0
Priekuli	21.07.	5,7	4
Saldus	24.07.	1,1	2
Bauska	21.07.	1,1	0,1
Carnikava	19.07.	1,5	0,5
2001			
Vecauce	10.07.	0,7	0,3
Stende	02.07.	5,4	2,1
Skriveri	17.07.	0	0,1
Priekuli	28.07.	3,5	0,5
Saldus	17.07.	7,3	3,7
Bauska	25.06.	4,3	2
Carnikava	10.07.	12,7	14,5
2002			
Vecauce	12.07.	0	0
Stende	14.07.	0	0
Skriveri	–	–	–
Priekuli	02.07.	0	0
Saldus	02.07.	0	0
Bauska	31.07.	0,1	0
Carnikava	–	–	–
LSD		3,45	

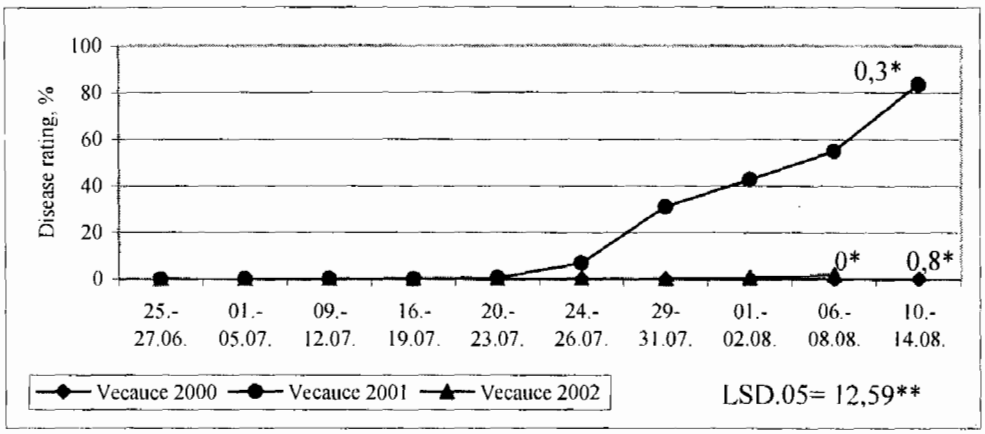
However, tuber infection not always depends on disease rating. In 2001, disease rating was high and tuber infection was 0,8% in untreated variant and 0,3% in treated with fungicides variant. But in the years 2000 and 2002, although disease rating was 50% and 71%, in untreated fields tuber infection was 0%. It could be explained by comparatively late appearance of first symptoms and disease progress. The potato foliage is nearly destroyed and if weather conditions are hot and dry the infection cannot occur. The disease development is decreased or stopped.



* tuber infection at harvesting time, %

** LSD for final disease rating

Fig. 2. Development of potato late blight in untreated fields in Vecauce, 2000—2002



* tuber infection at harvesting time, %
** LSD for final disease rating

Fig. 3. Development of potato late blight in treated with fungicides fields in Vecauce, 2000—2002

Situation was different in the variant treated with fungicides in the year 2000. The disease rating was low but tuber infection level was 0,8%. In this situation, disease level was constant for a long period and that affected the level of tuber infection, because on infected potato foliage *P. infestans* spores were present.

Development of potato late blight in the growing season has shown remarkable influence on the tuber quality and on tuber yield as well.

The total yields are given in Figures 4 and 5. Average lowest yield was obtained in the year 2001 if we compare different growing seasons. The yield was 25—65% less than in other growing seasons.

To compare untreated and treated with fungicides fields there were significant differences between locations and years. Usage of fungicides gave a 8—28% increase of yield in unfavourable year for development of potato late blight and 18—23% in favourable for development of late blight year.

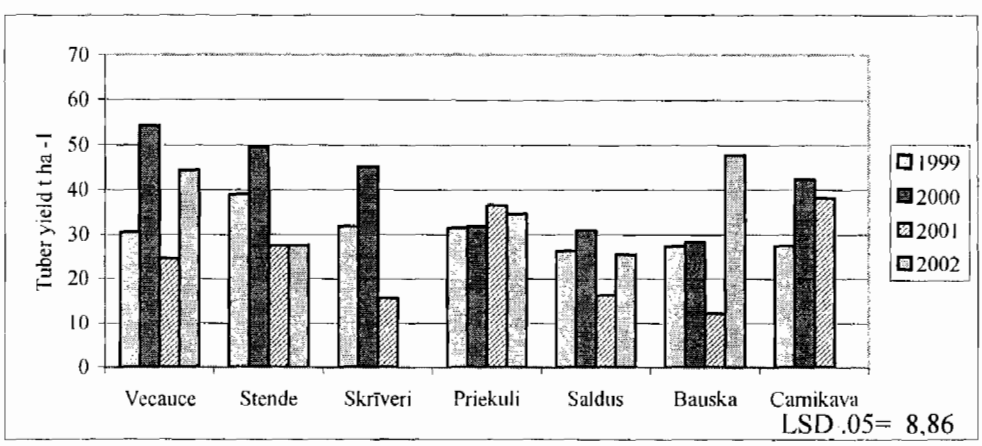


Fig. 4. Tuber yield in untreated fields, 1999—2002

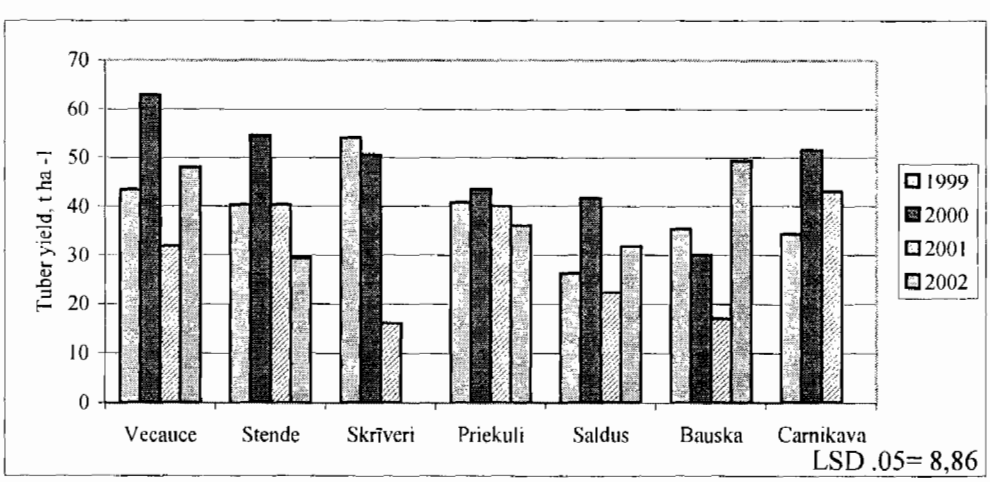


Fig. 5. Tuber yield in treated with fungicides fields, 1999—2002

Although potato late blight is observed every year, the relationships between yield and yield loss by *P. infestans* and weather conditions are still unclear. The biology of *P. infestans* has changed during recent years and the role of sexual reproduction cannot be excluded.

The monitoring of potato late blight may promote disease control, and monitoring information is an important component in all potato late blight forecasting system. This information about an earlier potato late blight appearance in neighbouring regions or countries is used to forecast its earlier appearance also in this country.

References

1. Andrivon, D. 1995. Biology, ecology and epidemiology of the potato late blight pathogen *Phytophthora infestans* in soil. Phytopathology, Vol. 85, No. 10, 1053—1056.
2. Harrison, I.G. 1995. Factors involved in the development of potato late blight disease (*Phytophthora infestans*). Potato ecology and modelling of crops under conditions limiting growth. Eds. A. J. Haverkort and D.K.L. MacKerron. Kluwer Academic Press, Netherlands, 215—236.
3. Harrison, I.G., Lowe, R. 1989. Effects of humidity and windspeed on sporulation of *Phytophthora infestans* on potato leaves. Plant Pathology, Vol. 38, 585—591.
4. Fry, W.E., Apple, A.E. 1986. Disease management implication of age-related changes in susceptibility of potato foliage to *Phytophthora infestans*. American Potato Journal, 63, 47—56.
5. Fry, W.E., Goodwin, S.B. 1997. Resurgence of the Irish potato famine fungus. After 150 years, the late blight fungus is again menacing farmers. Biological Sciences, Vol. 47, No. 6, 363—371.
6. Fry, W.E., Mizubuti, E.S. 1998. Potato late blight. The Epidemiology of Plant Diseases. Ed. by D. Gareth Jones, 371—386.
7. Mizubuti, E.S.G., Aylor, D.E., Fry W.E. 2000. Survival of *Phytophthora infestans* sporangia exposed to solar radiation. Phytopathology, Vol. 90, 78—84.
8. Turka, I. 1998. Potato late blight in Latvia and management of forecasting and warning. Proceedings of fifth workshop of an European network for development of an integrated control strategy of potato late blight. Uppsala, Sweden, 9—13 September 1998, 172—177.

ESTIMATION OF LILIUM RESISTANCE AGAINST GREY MOLD (*BOTRYTIS* MICHELI ex FR.)

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Abstract

Grey mold, caused by fungus *Botrytis* Micheli ex Fr., is especially destructive to lilies (*Lilium* L.) in the second half of summer. It attacks the leaves, flower buds and flowers; in the result lilies loose their general attractiveness. In lily breeding the current activities are directed towards development of disease resistant cultivars to avoid the use of chemical plant protection, to make the growing economically sound and ecologically safe. Resistance to grey mold (*Botrytis elliptica* (Berk.) Cooke) in 7 species and 75 cultivars of lilies was tested visually on natural and artificial infection backgrounds according to method of S. Sidorova and V. Popov in grades 0 to 4 (0 — healthy; 4 — very heavy susceptible). The 10 lily plants selected from each species and cultivars were estimated for their resistance to diseases and the average index of morbidity was calculated. The obtained results show no differences when testing lilies for grey mold resistance under field conditions (1997—1998) and utilizing leaf segments under laboratory conditions. Cultivars of different levels of resistance to diseases were included in our trial. Estimation of parent plants and the produced hybrids was done. The obtained hybrids were resistant to grey mold if parents crossings were resistant to this disease agent. When crossing cultivars susceptible to grey mold with cultivars resistant to this disease agent, the obtained hybrids were either intermediate by this trait or susceptibility to this disease was dominant. 10 perspective hybrids were tested under field conditions by resistance to grey mold (1999—2000). No significant differences were found between the two years results.

Key words: *Botrytis*, estimation, inoculation, fungi, lilies.

Introduction

In moderate climate one of most widely spread diseases of lilies is grey mold, caused by fungus *Botrytis* spp. mold infects various vegetative organs — leaves, flower buds, flowers, stems and seed pods of lilies.

Various wild plants and cultivars infect about 50 species of the genus *Botrytis* Micheli ex Fr. that differ by specialization of host plants. The most important are: clover flowers (*B. anthophila* Bond.) (Van der Plank, 1963), leaves and bulbs of tulips (*B. tulipae* (Lib.) Lind.) (Koster and Meer, 1993), gladioluses (*B. cinerea* Pers.) (Van den Ende, 1996). The grey mold is caused in lilies (*Lilium* L.) by two species of *Botrytis:* mainly *B. elliptica* (Berk.) Cooke and *B. cinerea* Pers (McRae, 1987; Lawson and Hsu, 1996), seldom *B. tulipae* (Lib.) Lind (Doss et al., 1988; Van Aartrijk, 1996). The conidia spores germinate and enter leaves through the epidermal stomates. The first evidence of the disease appears as reddish-brown oval spots on the leaves. During wet weather the spots eventually coalesce and the whole leaf collapses and decays (McRae, 1987). The conidia of the genus *Botrytis* germinates on the plant only if there is sufficient moist environment with drops of water. Injury to the plants, especially frost or mechanical damage, will always make it easier for *Botrytis* spores to enter the leaf. The fungus formed on died tissue small, black sclerotia and over winter in soil (Lawson and Hsu, 1996). These sclerotia produce spores in the next vegetation season, which spread by the wind and are splashed by rain onto the new foliage.

Even if fungus *B. cinerea* Pers. has a lot of food plants, it is possible to establish varieties and races that differ by morphological and biological traits (by form and measure of conidia spores, measure of sclerotia and its formation, virulence to individual food plants). It is observed that these parasites have biological specialization to substrates and biotopical diversity (Рудаков, 1959).

The breeding of the fungus resistant varieties of lilies can help to solve the *Botrytis* problems. Resistant cultivars can play an important role in the control of this disease. Using resistant genotypes as parents give the highest chance of a resistant progeny.

Chemical treatment is not always adequate and it pollutes the environment. In Latvia, lilies are mainly grown under field conditions and in nature flowers are pollinated by useful insects: bees, bumble-bees, night-moths, ants, etc.

The goal of research was to estimate resistance to grey mold in lilies originated from different countries and hybrids in field and laboratory trials for recommendations to variety resistance, to make hybrid selection and evaluation of the perspective hybrids in field trials. Up to now no research on distribution of grey mold and evaluation of resistance of lilies has been done.

Materials and Methods

In autumn 1996, a field trial was established in Saulkrasti of the Riga region. The soil was well cultivated, pH_{KCl} 6.7, P_2O_5 — 850 mg kg^{-1}, K_2O — 324 mg kg^{-1} (determined by Egner-Riehm), Ca —1388 mg kg^{-1}, Mg — 219 mg kg^{-1} (determined by photometry method). Organic matter content — 4.6 g kg^{-1} (by Tyurin's method).

Between 1997 and 1998, 7 species of lilies, 75 different cultivars of lilies were evaluated for resistance to grey mold (*B. elliptica* (Berk.) Cooke). Initial material was obtained in the following way: a lily bulb was vegetatively propagated from bulb scales, and 10 bulbs of the same age (two-year old) from each species and cultivars, 2.5—3.5 cm in diameter, were included in the trial. The seedbed was prepared in early September prior to planting of bulbs. The 25 varieties of parent plants varying in resistance to grey mold were included in 14 cross combinations. The 10 bulbs of

each parent plant and hybrid bulbs were planted in 1 m wide beds; the planting depth was 10 cm, the distance between rows — 20 cm. Two free rows separated parent plants from hybrid populations. Bulbs were grown in the same site for two years.

The level of grey mold resistance of plants was rated visually on natural and artificial backgrounds according to method of S. Sidorova and V. Popov in grades 0 to 4:

0 — healthy, resistant, free from any grey mold symptoms;

1 — slightly susceptible, individual spots are detected on plant leaves, injury of leaves below 25%;

2 — moderately susceptible, injury of leaves below 50%;

3 — heavy susceptible, leaf damage below 75%;

4 — very heavy susceptible, leaf damage exceeds 75% or spots are spreading over the whole leaves.

The 10 perspective lily hybrids selected from 14 cross combinations were estimated for resistance to grey mold under field conditions and the average index of morbidity was calculated (1999—2000). No chemicals were used in the trial. The resistance test of lily varieties was determined using the artificial infection with application of conidia suspension of *B. elliptica*. The segments of lily leaves grown in a glasshouse were used. Five leave segments from each variety were selected at random. Isolates of *B. elliptica* was obtained from infected leaves of Asiatic lily variety 'Roma' collected from a field. The segments of lily leaves were put in Petri dishes on potato-dextrose agar with spore suspension and kept for 48 h, at 22 °C, with moisture present (Doss et al., 1984). After this incubation, Petri dishes with segments of lily leaves were transferred to a growing chamber (12 h day, light 3000 lx, 18 °C, 70% moisture). Segments of lily leaves were examined 6 days after inoculation according to 0—4 grades: 0 — healthy; 4 — very heavy susceptible.

The obtained data were analyzed by heterogeneous complex dispersion method; the hybrids produced were compared to parent plants using Fisher's criterion (F); phenotypic variability of the studied traits was characterized by the coefficient of variation (V %) according to method of J. Guzov: < 10% — low, 10.1—20.0% — medium, > 20.1% — high.

Meteorological conditions

The April and May of 1997, 1999 and 2000 were mostly cold, with often frosts and heavy downpours. The development of lilies was slow. Fast development of lilies was observed in the third decade of May, when air temperature increased. The weather was warm in June with abundant rainfall, speeding up the development of lilies. July was hot, sunny and dry, which influenced the development of lilies and did not promote *Botrytis* Micheli ex Fr. development. The April of 1998 was cold and cloudy with precipitation in the first half, and warm and sunny in the second phase, favouring fast development of lilies. The first two ten-day periods in May were very warm and sunny, but the third ten-day period in May and June was cold with abundant rainfall slowing down the development of lilies. July was hot and sunny in the first ten-day period, but in the second half of the month it was cloudy with much rainfall and cold nights. The parasitic fungus *Botrytis* damaged the leaves and flowers of lilies.

The trial years were characterized by contrasting meteorological conditions. Due to sunny and dry July, the years 1997 and 1999 were most favourable for lily breeding. More unfavourable weather conditions occurred in 1998 and 2000 when lily plants were frost-damaged (2000) and severely attacked by grey mold (1998).

Results and Discussion

Assessment of lily *Botrytis elliptica* (Berk.) Cooke resistance

Resistance to grey mold (*Botrytis elliptica*) in 7 species and 75 cultivars of lilies included in the collection was tested on natural infection background during 1997—1998 (Table 1).

As healthy (grade 0) were assessed 23 (28%) plants of the collection, including 6 species of lilies. 27 (33%) varieties were assessed as slightly susceptible (grade 1) to grey mold. Disease ratings in grades 0 and 1 were given to 24% of lily cultivars from Latvia, 10% — from USA, 7% — from Canada, and to 6%, 4% and 2% of cultivars coming from Holland, Russia and Germany, respectively. As moderately susceptible to grey mold (grade 2) were assessed 20 (24%) cultivars, including 9% of cultivars selected in Latvia, 9% — in Holland, 5% — in Russia, 1% — in USA, and martagonlilies (*L. martagon var. album* West.). 9 (11%) varieties were assessed as heavy susceptible (grade 3), including 'Ča-Ča-Ča' developed in Latvia. As very heavy susceptible (grade 4) to grey mold were recognized 3 (4%) varieties of the collection. Disease ratings in grades 3 and 4 were given to 12% of lily cultivars from Holland, 1% from both USA and Russia, respectively, and 1% from Latvia. The obtained results show no differences when testing lilies for *Botrytis elliptica* resistance under field conditions and utilizing leaf segments under laboratory conditions. Disease resistance test on leaf segment of lilies is recommended for assessment of the initial breeding material and hybrid material to obtain precise results in a comparatively short period of time. Results of resistance testing of different lily groups suggest that the level of infection in tested cultivars varied. The cultivars of Oriental lilies and Trumpet lilies were healthy (grade 0) and slightly susceptible (grade 1) to grey mold but in Asiatic lilies the infection level varied (grade 0 to 4). All species of lilies included in the trial were healthy, except *Lilium martagon* var. *album* West., which was moderately susceptible to grey mold (grade 2). Basing on the trial results, initial material was selected including varieties with different infection level (grade 0 to 3).

Table 1

Results of testing resistance against *Botrytis elliptica* in lilies on natural infection background (on average, 1997—1998)

Level of infection by *Botrytis elliptica* (grades 0—4)	Lily species and varieties		
	Number	%	Name
0 — healthy	23	28	'Baltais Lācis', 'Eksotika', 'Flaming Giant', 'Herrold', 'Jumprava', 'Leslie Woodriff', 'My Joann', 'Olga', 'Orfejs', 'Red Carpet', 'Solstice', 'Teika', 'Tirrol', 'Pārgalvīgā', 'Višenka', 'White Henryi', 'Tetra Oriental' *L. henryi* Bak., *L. henryi* var. *citrinum* hort., *L. monadelphum* Bieb., *L. kesselringianum* Miscz., *L. szovitsianum* Fisch. et Ave- Lall., *L.martagon* L.
1 — slightly susceptible	27	33	'Alisa', 'Banga', 'Apricot Supreme', 'Compass', 'Connecticut King', 'Gardenia', 'Gran Paradiso', 'Honey Pink', 'Liesma', 'Līksna', 'Marsha', 'Marco Polo', 'Mona', 'Miss Alice', 'Nākotne', 'Otello', 'Paija', 'Patrīcija', 'Peachblush', 'Saule', 'Saules Meita', 'Skrīveri', 'Veltījums', Venta', 'Tokyo', 'Zemgale', 'Yellow Blaze'
2 — moderately susceptible	20	24	'Arabeska', 'Brushstroke', 'Corsica', 'Dzintars', 'Evrika', H-7-92, 'Jolanda', 'Lastočka', 'Lolly', 'Nakts Tango', 'Nepal', 'Magic Eye', 'Rotaļa', 'Rosita', 'Sally Girl', 'Saulstarīte', 'Shirley', 'Swing', 'Virineja', *L.martagon* var. *album* West.
3 — heavy susceptible	9	11	'Aristo', 'Chinook', 'Ča-Ča-Ča', 'Nočka', 'Moneymaker', 'Montreux', 'Parisienne', 'Sirocco', Tirreno'
4 — very heavy susceptible	3	4	'Monte Rosa', 'Mont Blanc', 'Roma'
Total	82	100	

Assessment of hybrid resistance to grey mold (*Botrytis elliptica*)

Grey mold, caused by fungus *Botrytis elliptica*, tends to show up in lily plantings in the second half of summer. It attacks the leaves, flower buds and flowers at the result of which plants loose their general attractiveness. Making selections of disease resistant plants was one of the most significant aims of the breeding program. Cultivars with different levels of resistance to diseases were included in the trial. Assessment of parent plants and produced hybrids was done (Table 2).

The result of a cross between resistant cultivars chosen as parents (K-7, K-13, K-14), were hybrids resistant to this disease agent. The produced hybrids didn't show significant differences compared to parent plants ($F_{fakt} < F_{0.05}$). However, in one case (K-7) there was detected increased susceptibility to the disease ($F_{fakt} > F_{0.05}$) suggesting that in chosen parents gene interaction was of specific character resulting in hybrids with partial immunity to grey mold. However, resistant initial material *L. henryi* var. *citrinum* hort. has contributed to the development of more resistant hybrids with perfectly healthy plants among them. This is in agreement with literature findings on the use of this lily species as a resistance donor in lily breeding (Kroell, 1991). The increase in susceptibility to grey mold was observed in reciprocal crossing between slightly susceptible cultivars (K-8). Reciprocal crossing between moderately and moderately heavy susceptible cultivars (K-1, K-2, K-4, 3rd group) produced hybrids which got infected by the fungus as heavy susceptible parents (K-1, K-4), or by susceptibility to the disease the produced hybrids were in medium position between their parent plants (K-2). In these cases, proportion of the effect of combinations accounted for 2.68—18.91%. The major factor that caused variability was the environment. Parent plants contrasting in susceptibility to disease agent (4th, 5th groups) had greater effect on the degree of infection in hybrids. In crosses between resistant and slightly susceptible (K-5, K-12, K-10) parents and vice versa (K-9), the proportion of the effect of combinations accounted for 20.34—40.58%. There exist significant differences ($F_{fakt} > F_{0.05}$) between the average indices of variants (P_1, P_2, F_1). In hybrids, susceptibility to grey mold was dominant (K-5) or prevalent (K-9, K-10, K-12).

Table 2

Estimation of hybrid resistance to grey mold (*Botrytis elliptica*)

Cross	Parents	Level of infection in grades, on average (0 — healthy; 4 — very heavy susceptible)		Level of significance	Influence of variants η^2 %
		Parents	Hybrids		
Group 1 (Healthy / healthy)					
K-7	'Herrold'	0.2	0.8*	$F_{fakt} = 4.32 > F_{0.05} = 3.25$	18.94
	'Solstice'	0.2			
K-13	'Zemgale'	0.5	0.5	$F_{fakt} = 0.93 < F_{0.05} = 3.34$	6.24
	'White Henryi'	0.2			
K-14	'Eksotika'	0.4			
	L. henryi var. *citrinum* hort.	0.0	0.2	$F_{fakt} = 2.70 < F_{0.05} = 3.35$	16.67
Group 2 (Slightly susceptible / slightly susceptible)					
K-8	'Compass'	1.2	1.7	$F_{fakt} = 2.0 < F_{0.05} = 3.33$	12.10
	'Gran Paradiso'	1.2			
Group 3 (Moderately susceptible / heavy susceptible)					
K-1	'Nakts Tango'	1.9	2.2	$F_{fakt} = 0.48 < F_{0.05} = 3.27$	2.68
	H-7-92	2.2			
K-2	'Nakts Tango'	1.9	2.4*	$F_{fakt} = 4.29 > F_{0.05} = 3.19$	15.43
	'Ča-Ča-Ča'	2.7			
K-4	'Ča-Ča-Ča'	2.7	2.7	$F_{fakt} = 2.94 < F_{0.05} = 3.24$	13.11
	'Sally Girl'	2.1			
Group 4 (Healthy / slightly susceptible) and on the contrary					
K-5	'Baltais Lācis'	0.2	1.7*	$F_{fakt} = 18.78 > F_{0.05} = 3.16$	40.58
	'Shirley'	1.5			
K-10	'Patrīcija'	0.5	1.4*	$F_{fakt} = 10.52 > F_{0.05} = 3.28$	38.93
	'Magic Eye'	1.7			
K-12	'Olga'	0.1	0.9*	$F_{fakt} = 8.82 > F_{0.05} = 3.31$	36.27
	'Arabeska'	1.4			
K-9	'Nepal'	1.6	1.3*	$F_{fakt} = 4.72 > F_{0.05} = 3.25$	20.34
	'Alisa'	0.7			
Group 5 (Healthy / very heavy susceptible) and on the contrary					
K-11	'Orfejs'	0.3	0.5*	$F_{fakt} = 44.10 > F_{0.05} = 3.34$	75.91
	'Aristo'	2.5			
K-3	'Ča-Ča-Ča'	2.7	1.7*	$F_{fakt} = 29.92 > F_{0.05} = 3.20$	57.08
	'My Joann'	0.5			
K-6	'Tirreno'	2.5	2.6*	$F_{fakt} = 65.97 > F_{0.05} = 3.20$	74.56
	'Honey Pink'	0.2			

* Difference is significant at $P_{0.05}$.

The result of crossings between cultivars susceptible to this disease agent, such as 'Ča-Ča-Ča' (K-3) and 'Tirreno' (K-6) and resistant varieties ('My Joann', 'Honey Pink'), were the hybrids being an intermediate (K-3) by this trait, or susceptibility was dominant (K-6). In one case (K-11), resistant to grey mold variety 'Orfejs' crossed with susceptible variety 'Aristo' resulted in resistant hybrids (grade 0—1). It suggests that, genetically, variety 'Orfejs' could be used in breeding for disease resistance. Significance of the obtained results is confirmed by the results of mathematical analysis ($F_{fakt} > F_{0.05}$); the noted influence of variants (η^2) is 57.08—75.91%. Within the hybrid populations, selection of resistant forms is possible in each individual combination thus promoting the development of immune cultivars. The hybrids produced were estimated in 1998 too (Fig.). On average, the level of infection was somewhat increased due to the periods of excessive rainfall in July, thus contributing too rapid spread of infection. This disease was greatly favoured by the density of a two-year old plantation. There exist close correlation between two-year (1997, 1998) estimation results ($r = 0.873$, $r_{0.05} = 0.532$, $r_{0.01} = 0.661$) and between these results no significant differences have been found ($F_{fakt} = 3.76 < F_{0.05} = 3.86$).

The research results indicate that the rejection of heavy susceptible genotypes could be realized considering one-year testing results

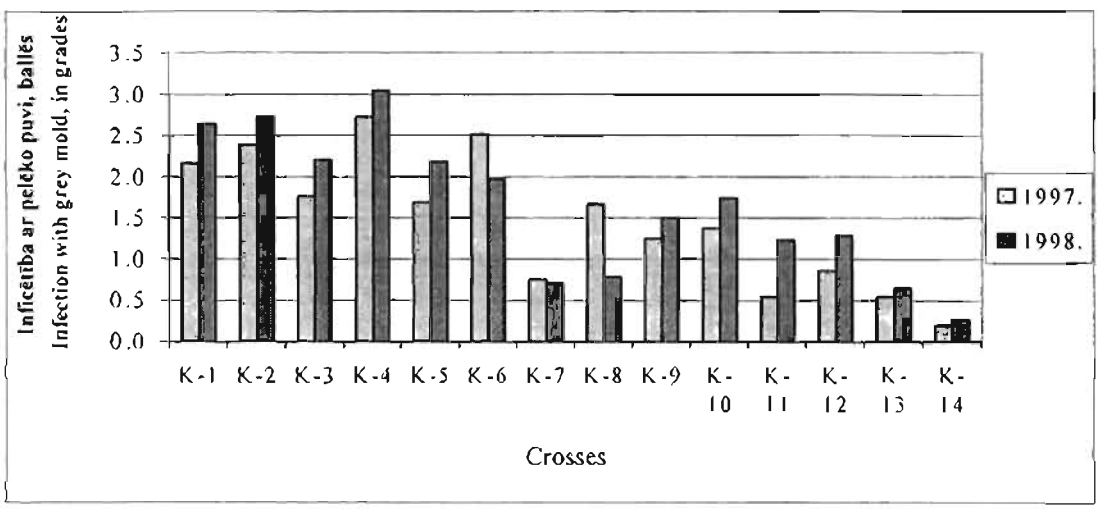

Fig. The level of infection of hybrids (in grades 0–4) with grey mold (*Botrytis elliptica*) in 1997—1998

Evaluation of perspective hybrids (in grades 0—4) to resistance to grey mold

10 perspective hybrids were selected from 14 crosses (Table 3). In both years high level of resistance was shown by the following hybrids: 'Resista 1', 'Arfa', 'Aurēlija', 'Vārsma'. These hybrids come from crosses K-7, K-11, K-13, K-14 in which one or both parent plants were detected as healthy to disease ('Herrold', 'Solstice', 'Orfejs', 'Eksotika', 'Zemgale', 'White Henryi', *L. henryi* var. *citrinum*). From cross K-11 was selected hybrid 'Arfa' which showed resistance. As father plant was used variety 'Aristo' originating from Holland and showing very heavy susceptibility to grey mold, which agrees with data in literature on susceptibility of this variety (Doss, 1986). From cross K-2, hybrid 'Maksis' was selected and assessed as moderately susceptible. The mother plant 'Nakts Tango', included in this cross, was moderately susceptible to grey mold but father plant 'Ča-Ča-Ča' showed heavy susceptibility to grey mold.

Results demonstrated that no significant differences were found between two-year estimations.

Table 3

The level of infection of perspective hybrids (in grades 0–4) to resistance to grey mold (*Botrylis elliptica*)

Cross	Name of the hybrid	Level of infection			
		1999		2000	
		Grades	To control	Grades	To control
	Roma — control	3.8	–	3.9	–
K – 2	Maksis	2.2	–1.6	2.7	–1.2
K – 5	Baltā Brigantīna	1.5	–2.3	1.9	–2.0
K – 6	Menarda	2.5	–1.3	2.7	–1.2
K – 7	Resista 1	0.5	–3.3	0.7	–3.2
K – 8	Kalsnava	1.2	–2.6	1.7	–2.2
K – 9	Šarlote	1.2	–2.6	1.4	–2.5
K – 10	Mēnesnīcas Sonāte	1.2	–2.6	1.6	–2.3
K – 11	Arfa	0.3	–3.5	0.5	–3.4
K – 13	Aurēlija	0.2	–3.6	0.5	–3.4
K - 14	Vārsma	0.1	–3.7	0.2	–3.7
			$\gamma_{0.05} = 0.43$		$\gamma_{0.05} = 0.47$

The obtained hybrids were significantly resistant to grey mold and considerably surpassed control variety 'Roma' in both years: 1999, $F_{fakt.} = 54.11 > F_{0.05} = 1.94$; 2000, $F_{fakt} = 47.10 > F_{0.05} = 1.94$. No significant differences were found between two-year results.

Estimation of the lily collection against grey mold in natural and artificial infection backgrounds did not show any significant differences in the obtained results. Resistance rating (grades 0—4) for grey mold was the following: 0 — healthy with no evidence of the disease, 28%; 1 — slightly susceptible, 33%; 2 — moderately susceptible, 24%; 3 — heavy susceptible, 11%; 4 — very heavy susceptible, 4% of the tested collection material. The following local varieties were assessed as resistant to grey mold infection: 'Baltais Lācis', 'Jumprava', 'Orfejs', 'Teika', 'Venta', 'Eksotika', 'Pārgalvīgā'. The following cultivars coming from Holland were rated as very heavy susceptible to infections: 'Monte Rose', 'Roma', and 'Mont Blanc'. These lily varieties are not recommended for growing outdoors under conditions of Latvia.

The resistance to grey mold characterized the obtained hybrids if parents were resistant to this disease agent. When crossing cultivars susceptible to grey mold with cultivars resistant to this disease agent, the obtained hybrids were intermediate by this trait or susceptibility to this disease was dominant.

10 perspective hybrids showed significant resistance to grey mold and considerably surpassed control variety 'Roma' in both years of the trial.

References

1. Doss, R. P., Chastagner, G., A., Riley, K. L. 1984. Techniques for inoculum production and inoculation of lily leaves with *Botrytis elliptica*. Plant Disease, No. 68, 854—856.
2. Doss, R. P., Chastagner, G. A., Riley, K. L. 1986. Screening ornamental lilies for resistance to *Botrytis elliptica*. Scientia Horticulturae, No. 30, 237—246.
3. Doss, R. P., Chastagner, G. A., Riley, K. L. 1988. Summary of Recent Research on *Botrytis elliptica*. The Lily Yearbook of the North American Lily Society, No. 41, 97—102.
4. Koster, A.Th.J., Meer, L.J. 1993. Control of *Botrytis* ('Fire'). Annual Report. Bulb Research Centre. Lisse, The Neatherlands, 34—37.
5. Kroell, C. 1991. The Yellow Henry. The Lily Yearbook of the North American Lily Society, No. 44, 90—95.
6. Lawson, R.H., Hsu, H.T. 1996. Lily disease and their control. Acta Horticulturae, No. 414, 175—183.
7. McRae, E. A. 1987. Lily disease handbook. The North American Lily Society. Canada, 28 pp.
8. Van Aartrijk, J. 1996. Department of plant disease and crop protection. Annual Report. Bulb Research Centre. Lisse, The Netherlands, 48—49.
9. Van den Ende, J. E. 1996. Towards optimal control of fire (*Botrytis spp.)* in flower bulbs. Annual Report. Bulb Research Centre. Lisse, The Netherlands, 35—38.
10. Van der Plank, J. 1963. Plant diseases epidemics and control. New York and London. Academic Press, 221 pp.
11. Гужов, Ю. Л. 1978. Пути использования в селекции растений закономерностей модификационной изменчивости количественных признаков. Известие АН СССР: серия биологии, N^{0}3, 418—429.
12. Рудаков, О. Л. 1959. Биология и условия паразитизма грибов рода Botritis. Фрунзе, 84.
13. Сидорова, С. Ф., Попов, В. И. 1980. Методические указания по изучению вертициллёзного и фузариозного увядания однолетних сельскохозяйственных растений. Ленинград, Всесоюзный научно -исследовательский институт защиты растений, 27 с.

DISEASES THREATENING PLANTS OF RUNNER BEAN (*PHASEOLUS COCCINEUS* L.) CULTIVATED IN SOUTH-EAST POLAND

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Abstract

Studies of three years were conducted at the Experimental Station at Czesławice in the south-east of Poland. The objects of research were the plants of four cultivars of runner bean: 'Blanka', 'Eureka', 'Kontra', and 'Westa'. Observations were carried out twice each year: at the seedling stage and at anthesis. At each stage the number and health status of plants were determined and diseased plants were collected for mycological analysis. The seed yield and its quality of each cultivar was estimated at the end of the experiment. The best emergence, health status and yield were noticed for cv. Kontra, while cv. Blanka seemed to be the most affected by soil-borne pathogens under the conditions of experiment.

Key words: runner bean, diseases, pathogenic fungi.

Introduction

Runner bean (*Phaseolus coccineus* L.) has been known in Poland since 19th century (Korohoda, 1969). The species is cultivated for seeds as the material for sowing and consumption. The world production of runner bean in 2002 reached 1.18 Mt, including 584 Mt in Asia and 44.3 Mt in Europe. In Poland its production reaches 2.8 Mt, with the mean seed yield of 19.9 Hg·Ha (FAO data 2002).

Because of favorable soil and climatic conditions, the cultivation of runner bean is concentrated mainly in Lublin region (south-eastern Poland), which is the cause of growing runner bean too frequently in the same field. Too short crop rotation is conducive to pathogens accumulation in soil. Earlier research (Pięta et al., 2001) showed that plants of this crop were infected by *Botrytis cinerea, Rhizoctonia solani, Sclerotinia sclerotiorum* and *Fusarium* spp. These fungi infect the runner bean plants at each growth stage causing necrosis of the underground and aboveground parts as well as damping-off and tracheomycosis that reduces the size and quality of the yield (Miklas et al., 2003; Paul, 2002; Pięta, Łabuda, 1993a; Snapp et al., 2003).

The purpose of the present study was to determine the communities of pathogenic fungi dangerous to runner bean cultivated in the south-eastern Poland.

Materials and Methods

Studies were carried out in 2001—2003 at the Experimental Station at Czesławice near Lublin in the field naturally infested with fungi after 10 years of bean monoculture. The objects of the studies were plants of four cultivars of runner bean: 'Blanka', 'Eureka', 'Kontra', and 'Westa'. The experiment was set in four repetitions (4 plots for each cultivar), with the area of each plot of 4.5 m^2. In each year, a field observation was performed on the plots of particular cultivars at the phase of seedlings and at anthesis. At each growth stage, 5 plants with root and stem necrosis were collected from each plot for mycological analysis (Pięta, Łabuda, 1993b). Fungal colonies were identified with available monographies. After the harvest, the size and quality of yield of each cultivar were determined. Percentage of seeds with brown spots was estimated.

The results were statistically analyzed with Tukey's test (Oktaba, 1987).

Results and Discussion

At both growth stages the differences in health status and number of plants were noticed between the plots (Table 1). The lowest number of seedlings was observed on plots sown with cv. 'Blanka', while the highest was noticed for cv. 'Kontra'. The mean number of seedlings on the plot for the other two cultivars ranged from 80 to 83.7. Each year seedlings with inhibited growth and yellowing lower leaves occurred in the experimental plots. The greatest numbers of such seedlings were observed for cv. 'Blanka' (2.2%, on average), while the smallest — for cv. 'Kontra' (0.5%, on average) (Table 1).

The number and healthiness of plants at anthesis were similar to the results obtained in the first observation. The highest number of plants was noticed for cv. 'Kontra' (92.3, on average), while the lowest — for cv. 'Blanka' (74.3, on average). The highest number of stunted plants with yellowing lower leaves and root and stem rot was observed on plots sown with cv. 'Blanka' and cv. 'Kontra' (Table 1). As the result of mycological analysis of runner bean seedlings, 623 fungi isolates belonging to 17 species were obtained (Table 2).

Table 1

The number and healthiness of particular cultivars of runner bean

Cultivars of runner bean	Number of seedlings							
	Number of seedlings per plot				Percentage of infected seedlings			
	2001	2002	2003	mean	2001	2002	2003	mean
BLANKA	72[a*]	78[a]	75[a]	75.0[a]	2.7[c]	1.5[b]	2.5[c]	2.2[c]
EUREKA	79[b]	81[ab]	80[ab]	80.0[ab]	2.0[bc]	1.7[b]	1.5[b]	1.7[b]
KONTRA	93[c]	92[c]	94[c]	93.0[c]	0.5[a]	0.7[a]	0.5[a]	0.5[a]
WESTA	81[b]	85[b]	85[b]	83.7[b]	1.7[b]	1.8[b]	1.5[b]	1.7[b]
	Number of plants at anthesis							
	Number of plants per plot				Percentage of infected plants at anthesis			
	2001	2002	2003	mean	2001	2002	2003	mean
BLANKA	72[a]	76[a]	75[a]	74.3[a]	2.8[c]	1.7[bc]	2.5[c]	2.3[c]
EUREKA	77[ab]	79[ab]	78[ab]	78.0[ab]	2.0[b]	1.5[b]	2.5[c]	2.0[bc]
KONTRA	92[c]	91[c]	94[c]	92.3[c]	0.7[a]	0.7[a]	0.7[a]	0.7[a]
WESTA	80[b]	84[b]	82[b]	82.0[b]	1.8[b]	2.0[c]	1.7[b]	1.8[b]

* mean values in columns differ significantly ($P \leq 0.05$), if they are not marked with the same letter.

Table 2

Fungi isolated from the infected seedlings of particular cultivars of runner bean (total from 2001—2003)

Fungus species	Number of isolates												
	Blanka			Eureka			Kontra			Westa			Total
	r	sb	Σ	r	sb	Σ	r	sb	Σ	r	sb	Σ	
Alternaria alternata (Fr.) Keissler	4	6	10	3	5	8	2	4	6	4	4	8	32
Botrytis cinerea Pers.	2	7	9	2	4	6	1	3	4	3	5	8	27
Cladosporium cladosporioides (Fres) de Vries	3	4	7	3	6	9	4	5	9	2	4	6	31
Epicoccum purpurascens Ehr. ex. Schl.	3	4	7	4	2	6	1	2	3	2	1	3	20
Fusarium culmorum (W.G.Sm.) Sacc.	6	6	12	5	6	11	3	4	7	6	8	14	44
Fusarium oxysporum Schl. f. sp. *phaseoli* Kend. Snyd.	15	19	34	10	12	22	7	11	18	10	13	23	97
Fusarium solani (Mart.) Sacc. f. sp. *phaseoli* (Burk.) Snyd. Hans	10	15	25	9	10	19	5	7	12	8	12	20	76
Fusarium sporotrichioides Scherb	9	13	22	6	8	14	4	5	9	7	11	18	63
Gliocladium catenulatum Gilman Abbott	4	5	9	4	3	7	9	13	22	1	2	3	41
Mucor hiemalis Wehmer	3	3	6	3	1	4	1	3	4	4	2	6	20
Penicillium nigricans Bainier ex Thom	2	2	4	2	4	6	3	5	8	3	4	7	25
Penicillium verrucosum Dierckx var. *verrucosum* Samson Stolk et Hadlok	5	3	8	2	1	3	2	1	3	2	1	3	17
Phoma exigua Desm. var. *exigua*	-	4	4	-	3	3	-	-	-	1	4	5	12
Rhizoctonia solani Kühn	9	14	23	7	9	16	4	5	9	5	8	13	61
Sclerotinia sclerotiorum (Lib.) de Bary	-	3	3	-	4	4	-	1	1	-	5	5	13
Torula herbarum (Pers.) Link ex Fr.	3	4	7	1	1	2	2	2	4	2	3	5	18
Trichoderma koningii Oud.	1	2	3	2	3	5	7	9	16	1	1	2	26
TOTAL	79	114	193	63	82	145	55	80	135	61	88	149	623

r — root, sb — stem base.

Table 3

Fungi isolated from the infected plants of particular cultivars of runner bean (total from 2001—2003)

Fungus species	Number of isolates												
	Blanka			Eureka			Kontra			Westa			Total
	r	sb	Σ	r	sb	Σ	r	sb	Σ	r	sb	Σ	
Alternaria alternata (Fr.) Keissler	4	7	11	4	6	10	2	4	6	5	5	10	37
Botrytis cinerea Pers.	3	8	11	2	6	8	1	4	5	4	7	11	35
Cladosporium cladosporioides (Fres) de Vries	4	6	10	4	8	12	5	6	11	4	5	9	42
Fusarium culmorum (W.G.Sm.) Sacc.	7	9	16	5	8	13	5	5	10	6	8	14	53
Fusarium oxysporum Schl. f. sp. *phaseoli* Kend. Snyd.	15	20	35	12	17	29	9	10	9	11	14	25	98
Fusarium solani (Mart.) Sacc. f. sp. *phaseoli* (Burk.) Snyd. Hans	9	14	23	9	10	19	6	8	19	9	12	21	82
Fusarium sporotrichioides Scherb	10	15	25	7	11	18	5	6	14	8	12	20	77
Gliocladium catenulatum Gilman Abbott	4	5	9	5	4	9	10	15	11	3	4	7	36
Humicola grisea Domsch	7	4	11	3	7	10	2	2	4	6	4	10	35
Penicillium chrysogenum Thom	5	4	9	10	2	12	7	3	10	3	-	3	34
Penicillium nigricans Bainier ex Thom	3	4	7	2	4	6	4	5	25	4	4	8	46
Penicillium verrucosum Dierckx var. *cyclopium* (West.) Samson Stolk et Hadlok	6	8	14	4	4	8	2	6	8	5	5	10	40
Phoma exigua Desm. var. *exigua*	1	5	6	-	4	4	-	1	1	2	6	8	19
Rhizoctonia solani Kühn	5	7	12	4	6	10	2	3	5	5	8	13	40
Sclerotinia sclerotiorum (Lib.) de Bary	-	10	10	-	8	8	-	4	4	-	9	9	31
Torula herbarum (Pers.) Link ex Fr.	4	5	9	2	2	4	3	3	6	4	4	8	27
Trichoderma koningii Oud.	2	3	5	2	4	6	9	10	19	2	3	5	35
TOTAL	89	134	223	75	111	186	72	95	167	81	110	191	767

r — root, sb — stem base.

Fusarium oxysporum f. sp. *phaseoli* and *Fusarium solani* f. sp. *phaseoli* were most frequently isolated from roots of bean seedlings. *F. oxysporum* f.sp. *phaseoli* constitutes the greatest danger to bean at the temperature of 26—30 °C since such temperature is conducive to the germination of conidia, the growth of mycelium and the process of infection (Aloj et al., 1983). On the other hand, *F. solani* f. sp. *phaseoli* can infect plants at lower temperature and heavy rainfalls (Burke et al., 1980). These pathogens were isolated most frequently from the seedlings of 'Blanka', 'Westa' and 'Eureka', which suggests high degree of infection (Table 2). The seedlings of cv. 'Kontra' were much less infected by these pathogens. Moreover, the seedlings of all cultivars were colonized by *Alternaria alternata, Botrytis cinerea, Fusarium culmorum, Phoma exigua* var. *exigua, Rhizoctonia solani,* and *Sclerotinia sclerotiorum.* The highest number of these fungi were isolated from the infected parts of cv. 'Kontra' seedlings, while the lowest — from cv. 'Blanka' seedlings (Table 2). Plants at anthesis were colonized mostly by *F. oxysporum* f. sp. *phaseoli.* The colonies of this pathogen constituted more than 12% of all fungal colonies obtained at this growth phase. Like in the case of seedlings, at anthesis this species was most frequently isolated from the infected plants of cv. 'Blanka', while most rarely — from cv. 'Kontra' plants. Moreover, *F. solani* f. sp. *phaseoli, Rhizoctonia solani, Sclerotinia sclerotiorum* and *Phoma exigua* var. *exigua* were often isolated from the analyzed plants (Table 3). High harmfulness of those pathogens to runner bean plants was observed in earlier research by Pięta et al. (2003) and Shibata (2002). The seed yield from each cultivar was proportional to the number and healthiness of plants. The highest yield (1272 g per m^2) was obtained from the plot sown with the seeds of cv. 'Kontra', while the lowest (877 g per m^2) — from the plants of cv. 'Blanka'. A good yield was also obtained from plots sown with cv. 'Eureka' (1005 g seeds per m^2) (Table 4).

Table 4

The yield and healthiness of seeds of particular cultivars of runner bean (mean from 2001—2003)

Cultivars of runner bean	Yield of seeds in g per m^2	Percentage of seeds with spots
BLANKA	877^{a*}	$10,5^{c}$
EUREKA	1005^{b}	$7,4^{b}$
KONTRA	1272^{c}	$5,4^{a}$
WESTA	919^{ab}	$8,4^{b}$

* mean values in columns differ significantly ($P \leq 0,05$), if they are not marked with the same letter.

The seeds with necrotic spots occurred in each cultivar and their percentage ranged from 5.4% for cv. 'Kontra' to 10.5% for cv. 'Blanka' (Table 4). The research on breeding the runner bean cultivars characterized by high yielding, good seed quality and low susceptibility to fungal diseases is conducted in a number of research centres (Witek, Witek, 1991).

References

1. Aloj, B., Marziano, F., Zoina, A., Noviello, C. 1993. La tracheofusariosi del fagiolo in Italia. Fitopathol., XXIII, 11, 63—66.
2. Burke, D. W., Hiller, D. E., Barker, A. W. 1980. Effects of soil temperature on growth of beans in relation to soil compaction and *Fusarium* root rot. Phytopathol., 70, 1047—1049.
3. Korohoda, J. 1969. Fasola. PWRiL, Warszawa.
4. Miklas, P. N., Delorme, R., Riley, R. 2003. Identification of QTL conditioning resistance to white mold in snap bean. J. American Society for Horticultural Science, 128, 4, 564—570.
5. Oktaba, W. 1987. Metody statystyki matematycznej w doświadczalnictwie. PWN. Warszawa, 488 pp.
6. Paul, Y. S. 2002. Biodeterioration of frenchbean seed and its management. J. Mycol. and Plant Pathol., 32, 2, 167—173.
7. Pięta, D., Łabuda, H. 1993a. Zdrowotność i plonowanie fasoli wielokwiatowej (*Phaseolus coccineus* L.) odmiany Eureka i populacji lokalnych w warunkach zagrożenia chorobowego. Materiały Konf. Nauk. III Ogólnopolskiego Zjazdu Hodowców Roślin Ogrodniczych, SGGW, Warszawa, 181—185.
8. Pięta, D., Łabuda, H. 1993b. Choroby grzybowe korzeni fasoli wielokwiatowej (*Phaseolus coccineus* L.). Roczn. AR w Poznaniu CCXLVII, 239—248.
9. Pięta, D., Pastucha, A., Patkowska, E. 2001. Podatność różnych odmian fasoli wielokwiatowej na porażenie przez grzyby przeżywające w glebie. Progress in Plant Protection/Postępy w Ochronie Roślin, 42, 2, 749—751.
10. Pięta, D., Pastucha, A., Struszczyk, H., Niekraszewicz, A. 2003. The use of chitosan in controlling bean (*Phaseolus coccineus* L.). Ed. Henryk Struszczyk. Polish Chitin Society. Progress on Chemistry and Application of Chitin and its Derivatives. Monograph, IX, 119—127.
11. Shibata, S. 2002. Elucidation of pathogen for the wilting which occurred in scarlet runner bean and kidney bean. Bulletin of the Gunma Agricultural Experiment Station, 7, 21—30.
12. Snapp, S., Kirk, W., Roman-Aviles, B., Kelly, J. 2003. Root traits play a role in integrated management of *Fusarium* root rot insnap beans. Hort. Science, 38, 2, 187—191.
13. Witek, Z., Witek, A. 1991. Kierunki hodowli fasoli wielokwiatowej. Mat. I Ogólnopol. Zjazdu Hodow. Roślin. Ogrodn., Kraków, 171—173.

EFFECT OF SOME HERBS ON POST-HARVEST PATHOGENS OF PEPPER (*CAPSICUM ANNUUM* L.)

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Abstract

Dry biomass of *Angelica archangelica* Linn., *Thymus vulgaris* L. and *Silphium perfoliatum* L. were tested for their effect on the growth of *Alternaria alternata*, *Botrytis cinerea*, *Colletotrichum gleosporioides* and *Rhizopus nigricans*. Thyme had the strongest effect on all fungi. The inhibition of *C. gleosporioides* growth ranged from 56.2% to 100%. The growth of other fungi was inhibited entirely. Angelica reduced significantly the growth of *C. gleosporioides* and *R. nigricans* but had no real effect on *A. alternata* and *B. cinerea*. Cup plant did not inhibit the analyzed fungi and stimulated linear growth of *A. alternata* and *C. gleosporioides*.

Key words: angelica, cup plant, thyme, post-harvest pathogens.

Introduction

Increasing competition in horticultural markets requires fruits and vegetables of highest quality. One of the factors diminishing the value of fresh produce are residues of pesticides used before the harvest. There are many biocides based on antagonistic bacteria and fungi which can be as effective as chemical fungicides (Harman and Bjőrkman, 1990). However, the public is concerned also about antibiotics residues as well. Therefore, many researchers began investigations on the effect of natural substances on plant pathogens and found them effective (Motiejūnaitė and Kalėdienė, 2003). Cuccioni and Guizzardi (1994) proved that oil extracts from various plants, including thyme, inhibited *in vitro* mycelial growth of *Botrytis cinerea.* Vapors of thyme and oregano oils showed strong antifungal activity against *B. cinerea*, *Alternaria arborescens* and *Rhizopus stolonifer* (Plotto et al., 2003). Before testing the abilities of active plant compounds it seems reasonable to check general abilities of plant biomass to inhibit the growth of pathogens. The aim of our work was to test the antifungal ability of dry mass of angelica, thyme and cup plant against some pathogens of pepper fruit.

Materials and Methods

Isolates of *Alternaria alternata, Botrytis cinerea, Colletotrichum gleosporioides* and *Rhizopus* nigricans obtained from diseased fruits of pepper were grown on PDA medium for 10 days. Grounded dry seeds of angelica and grounded leaves of thyme and cup plant were added to PDA medium in 5% and 15% concentrations. Disks of fungal colonies were placed in the center of Petri dishes filled with PDA with amendments or without them (as controls). 10 Petri dishes were used for each experiment combination. The diameter of colonies was measured after 6, 10 and 14 days after inoculation and compared to control colonies. All colonies were analyzed under the microscope. The disks from the combinations where the growth of fungi was inhibited entirely were replaced into Petri dishes with PDA to check the toxicity of amendment.

Results and Discussion

After 6 days, lower (5%) concentration of dry thyme reduced the growth of *A. alternata* by 76.5%, *C.gleosporioides* by 56.2%, *B. cinerea* and *R. nigricans* by 100%. After 10 days *A. alternata* was inhibited by 77.2%, *C. gleosporioides* by 82.3% and other pathogens by 100%. After two weeks the growth of all fungi was entirely inhibited, except for *A. alternata* (82.8%). The higher concentration of thyme (15%) had a fungitoxic effect on all fungi after 6 days except for *C. gleosporioides*. This growth of this pathogen was reduced by 64 % after 6 days and by 84.2% after 10 days. Only after 14 days the growth of pathogen was inhibited by 100% (Tab.). The effect of thyme on all fungi except *C. gleosporioides* was fungitoxic. No conidia of *C. gleosporioides* or *A.alternata* were observed when treated with thyme.

Angelica inhibited *B. cinerea* by 13.5%, *C. gleosporioides* by 64% and *R. nigricans* by 28.8% after 6 days. It had no effect *on A. alternata*. After 10 days a slight inhibition was noticed for *A. alternata* (by 7%) and *R. nigricans* (by 17.7%) and stronger for *C. gleosporioides* (70.1%). There was no effect on the growth of *B. cinerea*. After two weeks the growth of *A. alternata* was inhibited by 5%, *R. nigricans* by 77.7% and *C. gleosporioides* by 80.3%. No effect on *B. cinerea* was noticed. At higher concentration angelica inhibited strongly only *C. gleosporioides* and *R. nigricans*. The colonies of *B. cinerea* were smaller by 5% after 6 days but no effect was observed after 10 and 14 days. At the time of first and second observations angelica stimulated the growth of *A. alternata* by 10.8% and 1.7%, respectively and did not affect the growth of pathogen after 14 days (Tab. 1). The sporulation of *A. alternata* and *B. cinerea* seemed to be similar to that in control colonies.

Cup plant at 5% concentration stimulated the growth of *A. alternata* and *C. gleosporioides*. At higher concentration the herb inhibited only *A. alternata* (by 11.4%) after 14 days and stimulated this pathogen after 6 and 10 days and *C.gleosporioides* from the beginning to the end of test. The growth of *B. cinerea* and *R. nigricans* with the amendment of cup plant was the same as in control combination (Tab. 1). No differences in conidia production were noticed.

Inhibition of the growth of fungi colonies (in %) by herbs amendments

Treatment	*A. alternata*			*B. cinerea*			*C. gleosporioides*			*R. nigricans*		
	6	10	14	6	10	14	6	10	14	6	10	14
Control	0	0	0	0	0	0	0	0	0	0	0	0
Cup plant 5%	–18.9	–40.3	–5.0	0	0	0	9.4	–17.3	–3.9	0	0	0
Cup plant 15%	–51.3	–19.2	11.4	0	0	0	–20.0	–23.0	–7.8	00	0	0
Thyme 5%	76.5	77.2	82.3	100	100	100	56.2	82.8	100	100	100	100
Thyme 15%	100	100	100	100	100	100	64.0	100	84.2	100	100	100
Angelica 5%	0	7.0	5.0	13.5	0	0	60.3	70.1	72.3	28.8	17.7	77.7
Angelica 15%	–10.8	–1.7	0	5.5	0	0	62.8	67.8	68.4	44.4	42.2	14.4

Note: numbers marked with "–" stand for stimulating effect.

The tests confirmed the results of earlier investigations that proved a strong ability of thyme as an inhibitor of fungal growth (Wagner et al., 2003). Also other authors indicate the effectiveness of essential oils of thyme and oregano in plant protection both *in vitro* (Plotto et al., 2003) and *in vivo* (Paster et al., 1995). Even in a natural form thyme was able to inhibit or even kill tested fungi. Motiejūnaitė and Kalėdienė (2003) achieved similar results testing green mass of this herb. As the effect of thyme was prolonged it seems that not only essential oil but also other components might be responsible for inhibiting fungi in our tests. Research of Saniewska (2002) proved that flavonoids can inhibit the growth of some plant pathogens. The fungitoxic effect of thyme suggests the possibility of developing a biocide based on this herb.

Angelica was not so effective but strongly inhibited the growth of storage pathogens: *C. gleosporioides* and *R. nigricans*. The herb gives a very high yield and its production is cheap, so the investigations should continue on the activity of angelica and its components.

Cup plant, regarded as effective natural medicament for several human diseases (Kowalski, 2001) did not show inhibitory effect on tested pathogens. On the contrary, the amendment of this herb stimulated sometimes the fungi. Similar effect was observed by Motiejūnaitė and Kalėdienė (2003) for a green mass of rosemary. The components of cup plant (Kowalski and Wolski, 2003) might serve as additional nutrients for some fungi.

References

1. Caccioni, D. R. L., Gizzardi, M. 1994. Inhibition of germination and growth of fruit and vegetable postharvest pathogenic fungi by essential oil components. J. Essential Oil Research, 6 (2), 173—179.
2. Harman, G. E., Bjőrkman, T. 1998. Potential and existing uses of *Trichoderma* and *Gliocladium* for plant disease control and plant growth enhancement. In: G. E. Harman, C. P. Kubicek (Eds): *Trichoderma & Gliocladium,* Vol. 2, 229—266.
3. Kowalski, R. 2001. Analiza składu chemicznego organów nadziemnych I podziemnych rożnika przerośniętego (*Silphium perfoliatum* L.). Typescript, Ph.D. thesis, Agricultural University, Lublin, Poland.
4. Kowalski, R., Wolski, T. 2003. Evaluation of phenoloc acid content in *Silphium perfoliatum* L. leaves, inflorescences and rhizomes. EJPAU, s. Hort., 6, 1—12.
5. Motiejūnaitė, O., Kalėdienė, L. 2003. Antimicrobial Activity of *Lamiaceae* Plant Essential Oils on *Aspergillus niger* Growth. Bull. Pol. Acad. Sci, Biol. Sci., 51 (3), 237—242.
6. Paster, N., Menasherov, M., Ravid, U., Juven, B. 1995. Antifungal activity of oregano and thyme essential oils applied as fumigants against fungi attacking stored grain. J. Food. Prot., 58 (1), 81—85.
7. Plotto, A., Roberts, D. D., Roberts, R. G. 2003. Evaluation of plant essential oils as natural postharvest disease control of tomato (*Lycopersicon esculentum*). ISHS Acta Horticulture 628: XXVI International Horticultural Congress: Issues and Advances in Postharvest Horticulture, Abstracts.
8. Saniewska, A. 2021. Aktywność grzybowa engogennych flavonoidów grejpfruta (*Citrus paradisi*). Symp. Nauk. Fitopatologia polska w Europie, Streszczenia, 62.
9. Wagner, A., Strudzinska, A., Struszczyk, H. 2003. Effect of Some Natural Compounds on *Alternaria alternata* Keiss. and *Botrytis cinerea* Pers. Bull. Pol. Acad. Sci. Biol. Sci., 51 (3), 287—290.

POSSIBILITY TO CONTROL FUNGAL DISEASES OF WHEAT AND BARLEY BY USE OF PLANT EXTRACTS

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Abstract

The objectives of this work were to detect fungicidal effects in some plant extracts and their mixtures against fungal diseases of wheat and barley, to test various extraction methods, to determine optimum concentration of such extracts, interactions between the effect and environmental conditions, particularly temperature, and if it affects different phytopathogenic fungi. In plant extracts that were efficient in vitro conditions, their fungicidal effect was tested in glasshouse and field experiments.

There were tested 95 extracts obtained from frozen plants occurring in the Czech Republic and 10 extracts from dried plants delivered from two Asian regions and Western part of Africa.

The fungus *Microdochium nivale* Fr. Samuels, I.C.Hallet (the pathogen of snow mould) was suppressed in vitro most effectively by extracts of the plant species: wild garlic *Alllium ursinum* L., garlic *Allium sativum* L., common chickweed *Stellaria media* L. WILL., greater celandine *Chelidonium majus* L., lavender *Lavandula officinalis* Chaix *et* Kitt., and thyme *Thymus serpyllum* L.. The highest fungicidal efficacy among plants originating from distant areas was found in Chinese sumac *Rhus chinensis* Mill, Szechuan lovage root *Ligusticum chanxiong* Hort and schisandra *Schisandra chinensis* (Turcz). The mixture consisting of extracts of wild garlic, forest pine, Szechuan lovage root and schisandra showed the highest fungicidal effect against powdery mildew caused by *Blumeria graminis* f. sp. *hordei* on spring barley. The effectivity was significantly increased by adding surface activator Greemax or olive oil to the plant extract solution.

Treatment of winter wheat plants with extract of wild garlic made at flowering stage significantly reduced the development of leaf rust caused by *Puccinia recondita* epidemy, as well as infection by scab caused *by Fusarium culmorum.*

Key words: medical plants, plant extracts, fungicides, wheat, barley, diseases.

Introduction

Many of currently used fungicides are toxic for people and animals (Sinclair, Pressinger, 2002). A possible solution to this problem is the use of biological protection. The most important method is breeding for resistance, which is, however, a long-term process and the final resistance is not durable in all cases. Another possibility is the use of biological products. Fungicidal activity may be found in natural materials like cultures of microorganisms (fungi and bacteria), extracts of animal origin, and plant extracts. Their advantage is usually low toxicity and low prices for their production.

The first part of the paper concerns fungicidal effects of plant extracts in *in vitro* conditions. In the second part some effective plant extracts were selected and used on intact plants. Fungicidal effects of plant extracts against powdery mildew caused by *Blumeria graminis* f.sp. *hordei* in barley were studied under glasshouse conditions. Extracts were tested in different concentrations and combinations with other extracts or with some additional compounds. The field trial aimed at plant extract effectivity against fungal pathogens in winter wheat was established under strong epidemy of leaf rust caused by *Puccinia recondita* and Fusarium head blight (FHB) caused by *Fusarium culmorum.*

Materials and Methods

1. Fungicidal effect of plant extracts in *in vitro* cultures

Extraction

50 g of fresh or frozen plant material was cut into small pieces (equal to 25 g of dry plants in powder form) and then homogenized by laboratory blender in 100 ml of 96 % ethanol.

The mixture was then placed in Erlenmeyer flasks and placed on a laboratory shaker for 8 hours. The final extract was filtered after 24 hours of extraction process and stored in a refrigerator at 5 °C.

Testing of the fungicidal effectivity of plant extracts in vitro

PDA agar medium was used in two replications for one experimental variant. 19 ml of agar were mixed with 1 ml of a particular plant extract. A small segment of *M. nivale* mycelium was placed in the middle of each Petri dish containing growing medium. The reduction of myceliar growth was measured after one week of incubation at room temperature and compared with non-treated control. Extracts from 95 plant species made from frozen plants and 8 extracts from dry plants originated in two regions of Asia (Hebei–China and Marmaris–Turkey) were assessed.

Phytopathogenic fungi use for this experiment

All plant extracts were tested in fungicidal effectivity against snow mold caused by *Microdochium nivale Fr. Samuels, I. C. Hallet.* The isolate used was originated from winter wheat plant.

2. Glasshouse experiment

Establishment of experiment

Spring barley variety Tolar, extremely susceptible to powdery mildew was used. For each treatment there were 4 replications with five plants per container. Barley plants were grown until they reached the two-leaf stage.

Plant treatments

In the first step, following plant extracts were tested: balsam fir *Abies balsamea* L. Mill., lesser stitchwort *Stellaria graminea* L., hoptree *Ptelea trifoliata* L., garlic *Allium sativum* L., common walnut *Juglans regia* L., lavender *Lavandula officinalis* Chaix et Kitt., northern white cedar *Thuja occidentalis* L., tasmanian blue gum *Eucalyptus globulus* Labill, thyme *Thymus serpyllum* L., mugwort *Artemisia vulgaris* L., austrian pine *Pinus nigra* Arnold, Szechuan lovage root *Ligusticum chanxiong* Hort, schisandra *Schisandra chinensis* (Turcz) Bail, and chinese sumac *Rhus chinensis* Mill. The extracts were named based on abbreviations of the Latin name of the original plant species (Table 1).

The extracts were tested in the following concentrations: 2.5%, 10%, 20%, mixtures of extract 20% + Greemax (G), and extract 20% + olive oil (O). Colloidal concentrate Greemax (Stallen Company, Switzerland) was used to improve penetration, translocation and utilization of the extracts at the recommended rate of 40 ml/ha.

The second part of the experiment was to continue the use of those extracts with high fungicidal activity against powdery mildew discovered in part one. In addition, two new plant extracts were prepared from henna *Lawsonia innermis* L. originated in North Africa and wild species of *Mentha* spp. collected in Zakynthos Island (Greece).

Wild garlic *Alllium ursinum* L., balsam fir *Abies balsamea* (L.) Mill., austrian pine *(Pinus nigra* Arnold*)*, Szechuan lovage root (*Ligusticum chanxiong* Hort) and schisandra (*Schisandra chinensis* (Turcz) Bail) were tested in monocomponent extracts in concentrations 2,5%, 10%, 20%, 20% + Greemax, and 20% + olive oil, in two-component and in four-component mixtures of extracts in final concentration 20% (10%+10%, and 5% + 5% + 5% + 5%, respectively). 10 ml of extract solution was sprayed per treatment and replication with a hand sprayer. After treatment, containers were left until plant surfaces were dry and then inoculated with *B. graminis* f. sp. *hordei* by shaking from above severely infected plants with developed conidia of powdery mildew. The temperature was maintained between 15—20 °C for following 10 days. In both parts of the experiment the plants treated with water were used as controls.

Table 1

List of the common and scientific names of plant species and the names of plant extracts

Common name	Scientific name	Extract name
balsam fir	*Abies balsamea* (L.) Mill.	ABBA
stitchwort	*Stellaria graminea* L.	STEGR
hoptree	*Ptelea trifoliata* L.	PTETRIO
garlic	*Allium sativum* L.	ALSAT
common walnut	*Juglans regia* L.	JUREG
lavender	*Lavandula officinalis* CHAIX et KITT.	LAVAO
Northern white cedar	*Thuja ocidentalis* L.	TOCIDENT
tasmanian blue gum	*Eucalyptus globulus* Labill.	EUCA
thym	*Thymus serpyllum* L.	THYM
mugwort	*Artemisia vulgaris* L.	MISIA
Austrian pine	*Pinus nigra* Arnold	PINIG
Szechuan lovage root	*Ligusticum chanxiong* Hort.	LUX
schisandra	*Schisandra chinensis* (Turcz)	SANDRA
Chinese sumac	*Rhus chinensis* Mill.	RUS
wild garlic	*Allium ursinum* L.	AURS
henna	*Lawsonia innermis* L.	HENNA
mentha	*Mentha* spp.	ZAKYNTH

Assessment

A standardized scale was used to estimate the infected leaf area: 0%, 1%, 5%, 10%, 25%, and 50% (Anonymous, 1997). Each treatment was assessed in 4 replications with 5 plants per replication. Results were compared using ANOVA.

3. Field experiment with winter wheat under strong FHB infection

Winter wheat cultivar Ebi was sown in randomized blocks of 10 m^2 in 4 replicates of each. The treatment with plant extracts *(Allium ursinum* L. — AURS, *Rhus chinensis* Mill — RUS, and *Thuja ocidentalis* L. — Tocident) and fungicides used as standards (Horizon 250 EC, (a.i. tebuconazole, 250 g/l) (dose 1.0 l/ha) and Juwel (a.i. epoxiconazole 125 g/l + kresoxym-methyl 125 g/ha) (dose 0,8 l/ha) was made immediately before inoculation.

The whole experiment was then inoculated with 6.10^6 conidia/ml of *F. culmorum* isolate with a high level of aggressiveness at the start of flowering (DC 61).

FHB and leaf rust development were assessed in the field, and DON (mg/kg) content in harvested grains was measured immunologically by ELISA. The results were compared statistically by ANOVA.

Results and Discussion

Experiment 1:

There were found 12 plant species among tested plant extracts that showed a significantly high fungicidal effect (Fig.1). The absolute suppression of mycelium growth was caused by the extract made from leaves of *Allium ursinum* L.

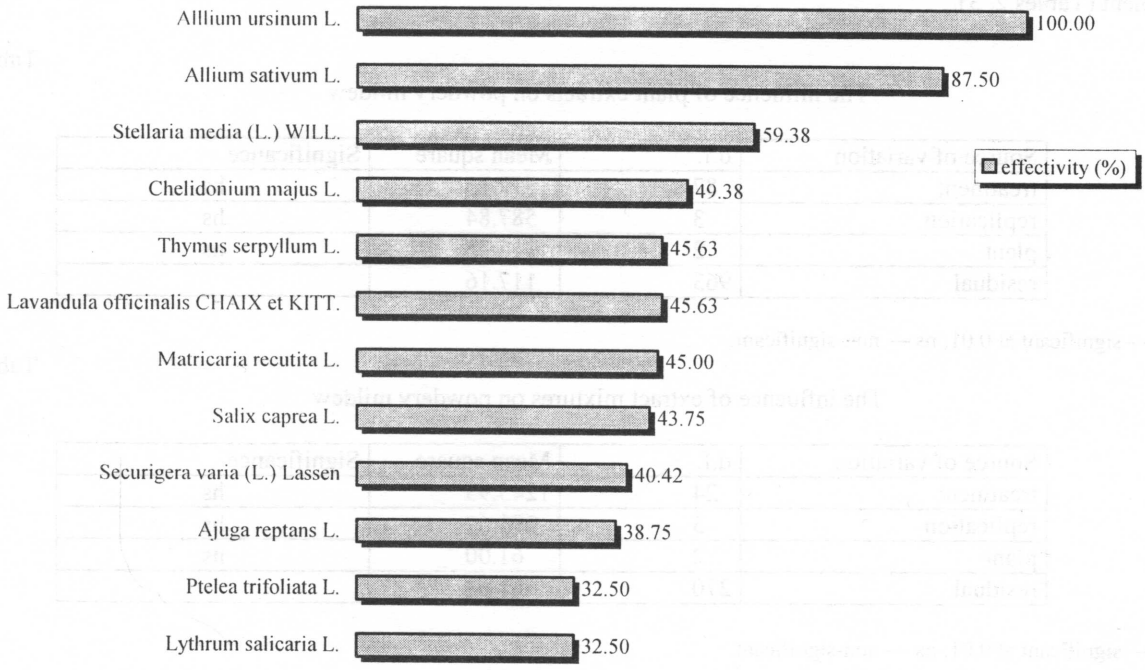

Fig. 1. Plant extracts with the highest fungicidal effectivity (%) against *M. nivale*

Three plant species originated in Asia were also highly effective. The best performance was found for *R. chinensis* Mill — 72.5 % (Fig. 2).

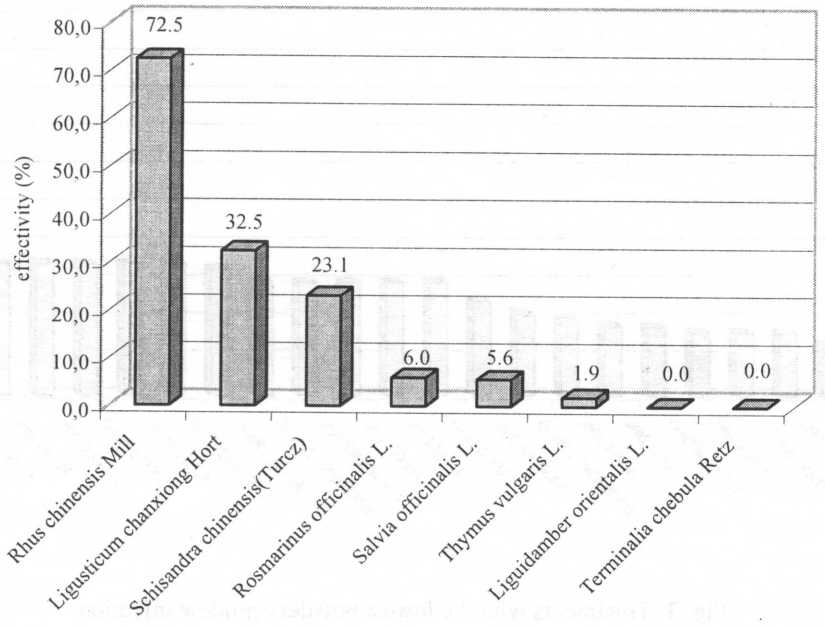

Fig. 2. Fungicidal effectivity of plant extracts, originated in Hebei (China) and Marmaris (Turkey), against *M. nivale*

Within the total number of 95 extracts prepared from frozen plant materials, 12 extracts were with significant fungicidal activity. The level of their activity was higher than 32.0%. Among them, plant species with known antiseptic and antibiotic activities can be found, as well as others — with unknown influence on fungal pathogens.

In the first group, garlic could be mentioned (*A. sativum* L.). By Müler (1936), the extract made from this plant can be used against bacterial diseases in human medicine. Rotting processes of intestines can be braked down by products from garlic. An important effective factor of garlic plant is sulfur essence, which is the basic element of above-mentioned antibiotic activity.

Experiment 2:

Practically all extracts and their combinations showed statistically significant suppression of powdery mildew development (Tables 2, 3).

Table 2

The influence of plant extracts on powdery mildew

Source of variation	d.f.	Mean square	Significance
treatment	87	1572.15	hs
replication	3	587.84	hs
plant	2	117.75	ns
residual	963	117.16	

Note: hs — significant at 0.01; ns — non-significant.

Table 3

The influence of extract mixtures on powdery mildew

Source of variation	d.f.	Mean square	Significance
treatment	24	1243.93	hs
replication	3	291.67	hs
plant	2	61.00	ns
residual	270	61.63	

Note: hs — significant at 0.01; ns — non-significant.

The lowest infection rate was found in the first part of the experiment after treatments with ABBA 20%+O (0.67%) and STEGR 20%+O (1.0%). Between 5 and 10% of infection level were LAVAO 20%+O, ABBA 2,5%, PTETRIO 20%+O, ABBA 10%, ALSAT 20%+O, LUX 20%, and ABBA 20%. On the contrary, the highest infection over 40 % was found after LAVAO 20%+G, THYM 20%, PINIG 2.5%, AURS 2.5%, PTETRIO 2.5%, EUCA 20%, and JUREG 20%+G (50.0%). The results of most effective treatments are summarized in Figure 3.

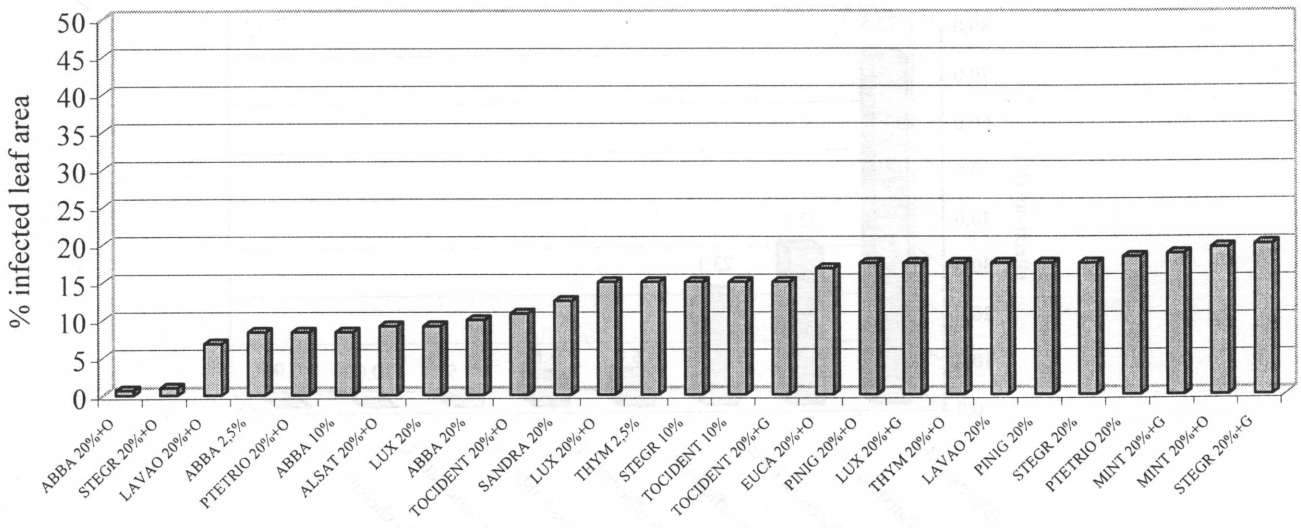

Fig. 3. Treatments with the lowest powdery mildew infection

The lowest powdery mildew infection in the second part of the experiment was found after the following treatments: AURS+PINIG+LUX+SANDRA+G (4.17%), AURS+PINIG +LUX +SANDRA+O (6.08%), AURS+ABBA+LUX+SANDRA (6.33%), AURS+ABBA +PINIG+LUX (6.67%), AUARS+HENNA+MINT+SANDRA (6.92%), LUX 20% (7.50%), AURS+PINIG+LUX+SANDRA (7.58%), AURS+HENNA +MINT+PINIG (9.17%), ABBA 20% (10.00%) (Fig. 4). The infection rate of non-treated checked plants reached 47.92%.

The plant extract originating from balsam fir needles showed the highest fungicidal activity against powdery mildew on barley in glasshouse experiments. The treatment was highly effective across all concentrations used, but the best result was achieved when the extract was mixed with oil. The combination with Greemax was only moderately effective. The extract from balsam fir is known as Canada turpentine, which is composed of 23—24% of volatile oils, 48—50% of alpha and beta canadinolic acids, and 11—12% of resin (Kaufman et al., 1999). The fungicidal active compounds from balsam fir may explain the suppression of *B. graminis* f.sp. *hordei* when sprayed on barley leaf surface. This effect was increased with addition of oil, which helped adherence of fungicidal compounds on the leaf surface. On the contrary, Greemax, which helps increase penetration of compounds into the leaf tissue, may have removed them from the place of maximal utilization.

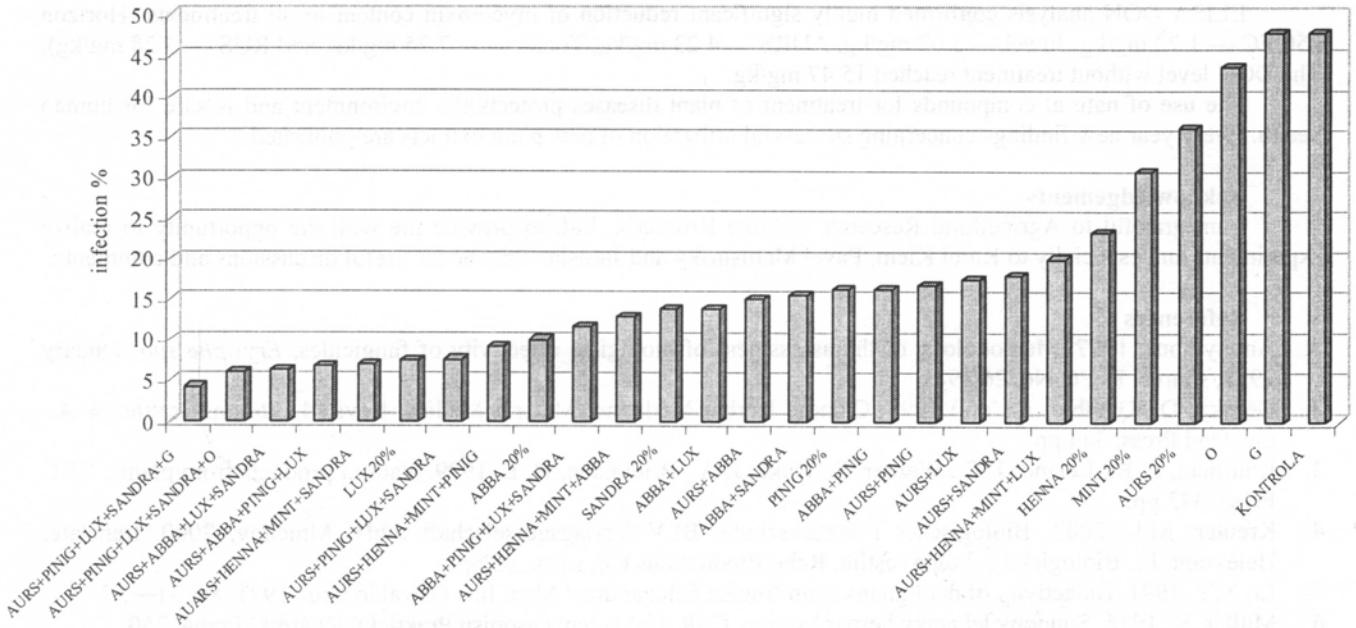

Fig. 4. The effectivity of plant extract mixtures against powdery mildew infection

Thirteen plant extracts from the total number of seventeen tested showed increase in fungicidal effectivity against powdery mildew in combination with plant oil. The effect of oil as an additive to fungicidal products is commonly known and currently used in production of chemical products (EC formulation). Quarles (2000) describes enhanced fungicidal effects of treatments containing different kinds of oils. Kreuter (2000) described biological products. Effective against fungal diseases of plants, which are based on combinations with natural oils: "Mehltau-Kombipack" used against mildew or "Neudo-Vital" which increases resistance of strawbery against *Botrytis cinerea* and rose plants against mildew, rust and black spot disease.

A mixture of 4 extracts — AURS+PINIG+LUX+SANDRA — was the most effective experimental treatment. *Schisandra chinensis* (Turcz) (SANDRA) powder prepared from dry fruits contains lignins and essential oils (Li, 1991). The fungicidal effect of extract from *Alium ursinum* L. (AURS) is based on sulphur essence. The active compounds of *Ligusticum chanxiong* Hort. (LUX) are alkaloids, tetramethylpyrazine and phenols (Shao et al., 1994). The composition of *Pinus nigra* (PINIG) extract is based on fixed or volatile oils, and solution of potassium or sodium hydrate. There are many compounds in this mixture, which have various biochemical and physiological properties. Classic Chinese herbal prescriptions also include between five and ten herbs per formula and contain hundreds of potentially active ingredients (Bensky, Gamble, 1993). They become difficult to evaluate using the Western pharmacological model of analyzing a solitary agent for a specific effect. It is therefore necessary to perform wide screening of possible extract combinations and to increase the probability of creating a combination with optimal influence on plant diseases.

Experiment 3:

The estimation of FHB infection rate was made on 17th day after inoculation. The lowest consistent infection rate was found after treatment with fungicides Horizon 250 EC and Juwel and plant extract AURS (1.1%). Tocident reduced infection of spikes to 2.8% and RUS — to level of 4.4%. All effects were statistically significant (Table 4).

Table 4

The influence of treatment with plant extracts and fungicides on leaf rust and FHB development in the field and on mycotoxin content in harvested grains

		Leaf rust (%)		FHB (%)		DON (mg/kg)	
Source of variation	d.f.	Mean square	Significance	Mean square	Significance	Mean square	Significance
treatment	5	18582.70	hs	104.80	hs	104.80	hs
replication	3	6.06	ns	0.02	ns	0.02	ns
residual	222	94.83		36.06		0.01	

Note: hs — significant at 0.01; ns — non-significant.

There were 55.6% of flag leaf area infected with leaf rust on 17th day without treatment with fungicides. Both the RUS and Tocident plant extracts reduced pathogen development to 20%. Only small infection was found after treatment with fungicide Horizon 250 EC (0.4%), and plants treated with Juwel and AURS were free of infection.

ELISA DON analysis confirmed highly significant reduction of mycotoxin content in all treatments (Horizon 250 EC — 1.72 mg/kg, Juwel — 3.02 mg/kg, AURS — 4.23 mg/kg, Tocident — 7.75 mg/kg, and RUS — 9.28 mg/kg). The DON level without treatment reached 15.47 mg/kg.

The use of natural compounds for treatment of plant diseases protects the environment and is safe for human health. Every year new findings concerning successful utilization of new plant extracts are published.

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References

1. Anonymous. 1997. Methodology of the assessment of biological effectivity of fungicides, *Erysiphe spp.* January 1997, source: EPPO No. 26/1981.
2. Bensky, D., Gamble, A. (ed.) 1993. Chinese Herbal Medicine: Materia Medica, Revised Edition. Seattle, W.A.: Eastland Press, 344 pp.
3. Kaufman, P. B., Leland, J. C., Warber, S., Duke, J. A., Brielmann, H. L. 1999. Natural products from plants. CRC Press, 343 pp.
4. Kreuter, M.L. 2002. Biologischer Pflanzenschutz. BLV Verlagsgesselschaft mbH, Mnichov, 2000. translate: Helebrant, L.: Biologická ochrana rostlin, Rebo Productions CZ, s.r.o., 95 pp.
5. Li, X.Y. 1991. Bioactivity of neolignans from fructus Schizandrae. Mem Inst Oswaldo Cruz 1991, 86, 31—37.
6. Müller, K. 1936. Soudoby lekarsky herbar kveteny CSR. Nákladem casopisu Praktický lékárník, Praha, 250.
7. Quarles, W. 2000. Least-Toxic Controls of Plant Diseases. Available at: http://www.bbg.org/, gar2/topics/sustainable/ handbooks/ natural disease/ least-toxic.html.bio
8. Shao, C.R., Chen, F.M., Tang, Y.X. 1994. Clinical and experimental study on Ligusticum wallichi mixture in preventing and treating bronchial asthma. Chung Kuo Chung Hsi I Chieh Ho Tsa Chih, 14, 465—468.
9. Sinclair, W.M.D., Pressinger, R.M. (ed.) 2002. Chemical pesticides health effects research. Available at: http://www.chem-tox.com/pesticides/.

EFFECT OF TREATMENT WITH CHITOSOL 1 ON FUSARIOSIS OF TOMATO

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Abstract

The effect of four concentrations of chitosol 1 on health of tomato plants grown in substratum, infested by *Fusarium oxysporum* f. sp. *radicis-lycopersic*, was tested. The seedlings were obtained from sterilized seeds sown into sterilized substratum. Before replanting in substratum infested with pathogen, the seedlings were soaked in four different concentrations of chitosol 1. After 14 days, the weight and disease index were determined. The results showed that chitosol 1 in all concentrations reduced the disease level but the concentration of 0.3% proved to be the most effective. In this combination the plant weight was higher by 37% than that of control, and disease level reduced by 45%.

Key words: chitosol, chitin, *Fusarium oxysporum* f. sp. *radicis-lycopersici.*

Introduction

Earlier investigations of tomato health status proved that *Fusarium oxysporum* f. sp. *radicis-lycopersici* was a cause of root and steam rot of tomato grown in plastic tunnels. As customers become more concerned about pesticide residues, alternate methods of disease controls are introduced in vegetable production, among them the compounds of chitin. Additionally, attempts to control this disease by use of chemicals or soil disinfestations have failed. Chitin stimulates defence responses to the pathogen infection by invoking activity of chitinases, phytoalexins, protease inhibitors and structural compounds such as callose and lignin (Benhamou, 1996). It also inhibits mycelial growth of numerous fungal pathogens (Stössel, Leuba, 1984). Our aim was to test the effect of root treatment of one of chitin compounds on the control of fusariosis of tomato.

Materials and Methods

Fusarium oxysporum f. sp. *radicis-lycopersici* isolate of tested pathogenicity to tomato seedlings of cultivar Raissa F[1], susceptible to the pathogen, were used in the experiment. The seedlings were obtained from sterilized seeds sown into sterilized substratum. Chitosol 1 — liquid preparation, pH = 6.7; Mv = 193.0 kD; DD = 99.4% with polymer content of 1.44% was diluted to concentrations of 0.05%, 0.1%, 0.2%, and 0.3%. The roots were washed with sterile, distilled water and treated by dipping them in certain concentration. Control plants were not treated.

Substrate inoculation was made using method described by Werner (1991—1992). Pathogen inoculum grown in rice was added to each pot (about 10 g of inoculum per each pot) before planting, and mixed with substratum. Five replications were made, four plants in each. All treated plants were maintained in the room at 22 °C and for 12 h under fluorescent light.

After 14 days, observations and measurements were made. The weight of replications for each chitosol 1 concentration was estimated. Disease index (DI) was determined according to the scale with five classes:

0° — no visible symptoms;
1° — light-brown discoloration or very few spots on hypocotyl or roots;
2° — several necrotic spots on tap root and hypocotyl;
3° — numerous necrotic spots on tap root and hypocotyl, covering whole roots;
4° — root rot, seedlings dead.

The survey was established in three replications. The data were analyzed using ANOVA test ($\alpha = 0.05$).

Results and Discussion

All tested tomato plants showed symptoms characteristic to those caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici.* Diseased plants stunted, their lower leaves turned yellow, wilted and decayed. On the stems, above the ground level, brown lesions were visible. Finally, whole plants wilted and died. Roots exhibited symptoms most clearly from numerous necrotic spots to root rot. Many plants without pronounced foliar symptoms showed necrosis on roots and cortex, as well as vascular discolorations.

Differences in disease development between plants treated with chitosol 1 and control were observed. However, no differences were stated between the tested concentrations, disease index was highest for the control and differed significantly from other concentrations. The best effect in disease inhibition was observed at concentration of 0.3%. Disease index was lower by 10% in comparison with other concentrations, and plants produced more green mass. In this combination, plant weight was higher by 37% than that of control, and disease level reduced by 45% (Fig. 1, Fig. 2).

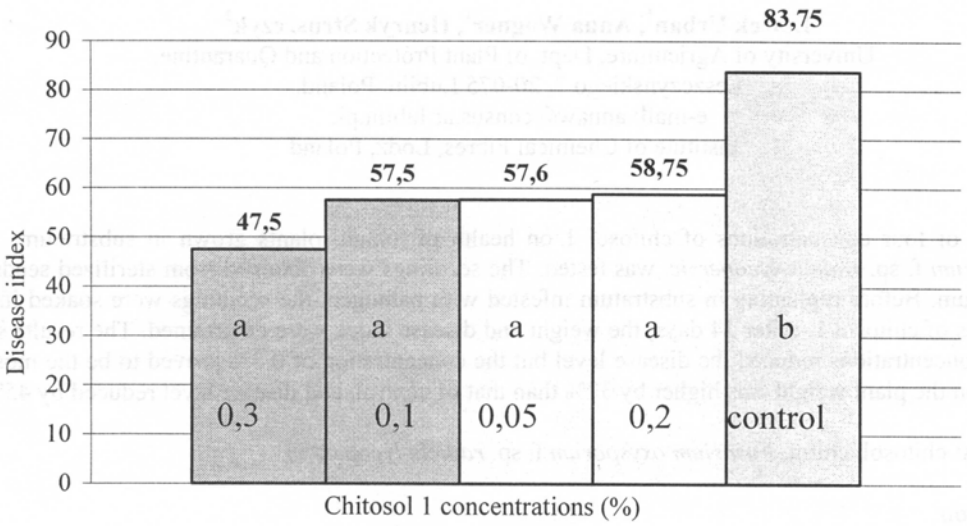

Fig. 1. The effect of chitosol 1 on severity of crown and root rot of tomato

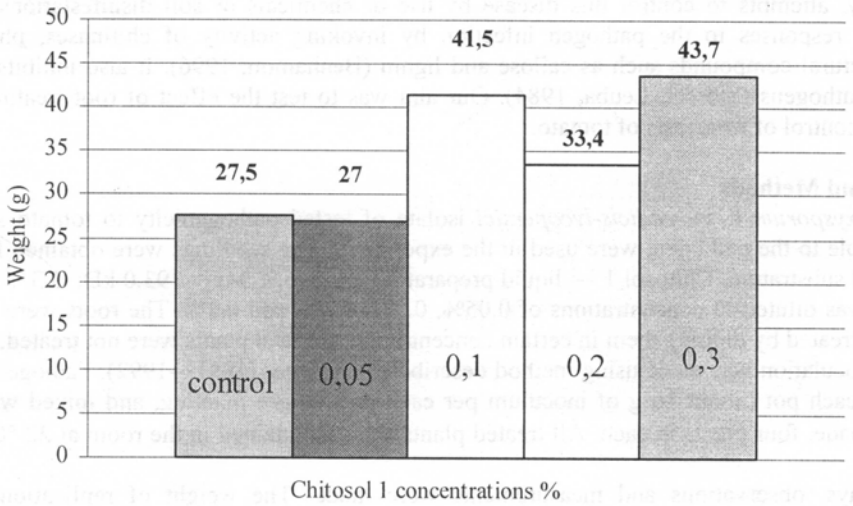

Fig. 2. Total weight of tomato plants inoculated with Fusarium oxysporum f. sp. radicis-lycopersici and treated with chitosol 1

The survey showed that chitosol 1 was effective against *Fusarium oxysporum* f. sp. *radicis-lycopersici.* Although root coating did not prevent plants from infection, disease development was significantly inhibited. It can be used as a biocontrol agent in integrated pest management systems. It not only halts growth of the pathogen, but also invokes morphological and biochemical changes in plant cells (Benhamou, Theriault, 1992).

Chitin stimulates growth of some antagonists as *Trichoderma* spp. and induces chitinases production. Induced by chitin, defence prevents plants from other pathogen infection, including viruses and bacteria (Pospieszny, 1997).

References

1. Benhamou, N. 1996. Elicitor-induced plant defence pathways. Trends in Plant Science, 1, 7: 233—240.
2. Benhamou, N., Thériault, G. 1992. Treatment with chitosan enhances resistance of tomato plants to the crown and root rot pathogen Fusarium oxysporum f. sp. radicis-lycopersici. Physiol. and Mol. Plant Path., 41, 33—52.
3. Pospieszny, H. 1997. Niektóre aspekty stosowania chitozanu w ochronie roślin. Post. w Ochr. Roś., 37, 1, 306—310.
4. Stössel, P., Leuba, J. L. 1984. Effect of chitosan, chitin and some aminosugars on growth of various soilborne phytopathogenic fungi. Phytopath. Z., 111, 82—90.
5. Werner, M. 1991/1992. Patogeniczność ośmiu form specjalnych Fusarium oxysporum Schlecht. względem wybranych gatunków roślin. Acta Mycol., 27, 127—136.

THE BIOTIC EFFECT OF PHYLLOSPHAERE MICROORGANISMS ON SOME FUNGI PATHOGENIC TO PLANTS

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Abstract

The biotic effect of saprophytic fungi and epiphytic bacteria on pathogenic fungi *Botrytis cinerea, Phomopsis viticola* and *Monilia coryli* was studied. It was shown that *Trichoderma* species were the most effective antagonists towards the above mentioned pathogenes. They strongly inhibited the growth of pathogenic fungi colonies and totally destroyed their mycelium and conidia. The antagonistic effect of bacteria from the genera of *Bacillus* spp. and *Pseudomonas fluorescens* was weaker as compared to *Trichoderma.*

Key words: antagonistic fungi and bacteria, *Botrytis cinerea, Phomopsis viticola, Monilia coryli.*

Introduction

The above ground parts of plants are colonized both by pathogenic and antagonistic microorganisms. The latter ones include saprophytic fungi and epiphytic bacteria (Papavizas, 1985; Łacicowa, 1989; Fokkema, 1993). The antagonistic effect of these microorganisms is based on competition, antibiosis, and mycoparasitism (Papavizas, 1985; Łacicowa, 1989). Owing to these properties they contribute to the natural limitation of population size of microorganisms pathogenic to plants. Recently, it has been shown that *Botrytis cinerea* and *Phomopsis viticola* were the main pathogenes occuring on grapevine shoots (Król, 1998; Machowicz-Stefaniak, 1998), and *Monilia coryli* — on fruitlets and hazelnut fruit (Zalewska, 1999). Saprophytic fungi species and epiphytic bacteria were also found on the studied parts of plant together with the above mentioned pathogenes (Król, 1998; Machowicz-Stefaniak, 1998; Zalewska, 1999).

The present studies were carried out to determine the interaction between the tested microorganisms.

Materials and Methods

The studied material consisted of saprophytic fungi species, the most frequently isolated from various parts of plants: *Trichoderma koningii, T. viride, T. harzianum, Gliocladium catenulatum, G. penicilloides, G. fimbriatum, G. roseum, Alternaria alternata, Epicoccum purpurascens, Penicillium funiculosum* and epiphytic species of bacteria from the genera of *Bacillus* spp. and *Pseudomonas fluorescens. Botrytis cinerea, Phomopsis viticola* and *Monilia coryli* were included among the pathogenic fungi. The above mentioned microorganisms were isolated in recent years from the above ground parts of grapevine and hazel (Król, 1998, 2004; Machowicz-Stefaniak, 1998; Zalewska, 1999). Isolates of *Bacillus* spp. and *Pseudomonas fluorescens* marked with letter orginated from grapevine and were tested towards the pathogenes of these plant, i.e. *B. cinerea, Ph. viticola.* Isolates with letter were obtained from hazel and were tested on *M. coryli.* Tests of the biotic effect among the fungi were carried out on PDA medium. The interaction among the fungi was studied on the basis of individual biotic effect (IBE) according to Mańka K. (1974) on PDA medium. The biotic effect was estimated in dual growth cultures of the studied fungi, using an 8-degree scale. When sparophytic fungi limited the growth of pathogenes, they were marked with a positive value (+) and in opposite situations with a negative one (-). The biotic effect between the fungi was estimated after 10 days of dual growth, between bacteria *B. cinerea* and *P. viticola* after 4 and 8 days of dual growth, and between bacteria and *M. coryli* after 8 and 14 days. In the case of *Trichoderma* and *Gliocladium* species, their influence on the vitality of the hyphae and conidia of the pathogens was also studied as long as there were colonies of the pathogen covered with a mycoparasite.

The biotic effect of bacteria was evaluated on the ground of the width of the growth inhibition zone of pathogenes, caused by bacteria on PDA medium, according to Sobiczewski et al. (1996).

Results and Discussion

Among the fungi tested in the present paper, *Trichoderma* spp. most effectively limited the growth of pathogene colonies, as can be seen from the high positive biotic effects (Table 1). *Trichoderma* spp. completely inhibited the growth of 10-day-old colonies of *P. viticola* and *M. coryli* and to a high degree limited the growth of quickly-growing fungus *B. cinerea.* After this time a loss of vitality of pathogenic fungi conidia and hyphae was observed, which was manifested by their shrinking and the cytoplasm getting detached from the cell walls. Such antagonistic activity of *Trichoderma* species results from their high capacity for competition, antibiosis and mycoparasitism (Papavizas, 1985; Łacicowa, 1989).

Gliocladium spp. studied in the present paper limited the growth of 10-day-old pathogen colonies in a small degree (Table 1), which indicates poor capacities of these antagonistic fungi for competition (Łacicowa, 1989; Machowicz-Stefaniak, 1998). However, after 16—22 days of dual growth the disintegration and lysis of hyphae and conidia *B. cinerea, P. viticola* and *M. coryli* was observed. Probably it resulted from exellent capacities of these fungi species for mycoparasitism and antibiosis; these antagonistic abilities of *Gliocladium* spp. manifested only after several days' contact with the pathogen mycelium (Łacicowa, 1989; Machowicz-Stefaniak, 1998; Zalewska, 1999).

Table 1

The biotic effect of fungi on some pathogenes, examined after 10 days of dual growth

Pathogenic fungi species / Saprophitic fungi species	*Botrytis cinerea* IBE*	*Phomopsis viticola* IBE	*Monilia coryli* IBE
Trichoderma koningii Oud.	+7	+8	+8
Trichoderma viride Pers. ex S.F.	+5	+8	+8
Trichoderma harzianum Rifai	+7	+8	+8
Gliocladium catenulatum Gilman et Abbott	+1	+2	+2
Gliocladium penicilloides Corda	+1	+2	+2
Gliocladium fimbriatum Gilman et Abbott	+1	+2	+2
Gliocladium roseum (Link.) Bainier	+2	+2	+3
Alternaria alternata Keissler	–7	+2	+1
Cladopsorium cladosporioides (Fres.) de Vries	–8	–5	–1
Epicoccum purpurascens Ehrenberg	+3	+4	+6
Penicillium funiculosum Thom.	–3	+3	+2

* IBE — individual biotic effect.

Among the other species of saprophytic fungi only *Epicoccum purpurascens* limited the growth of three studied pathogenic species effectively enough, and *Penicillium funiculosum* inhibited the growth of *M. coryli* and *P. viticola* (Table 1). The studied genera of bacteria showed high antagonistic activity in inhibiting the pathogenic fungi development, especially after 4 days of dual growth with *B. cinerea* and *P. viticola,* and after 8 days of dual growth with *M. coryli* (Table 2). After this time, the bacteria formed a clear zone of inhibition of pathogene colonies' growth, ranging from 7 to 31 mm in width, depending on the isolate and pathogen. The growth of *Monilia coryli* was inhibited most effectively and the development of *B. cinerea* was limited in the weakest degree (Table 2). The abilities of the studied bacteria to form a wide inhibition zone probably resulted from the capacity of *Bacillus* spp. and *Pseudomonas fluorescens* to produce numerous antibiotics and to affect pathogens on the ground of antibiosis (Fokkema, 1993). The antagonistic activity of the majority of the tested bacteria isolates decreased after 8 and 14 days of dual growth, because the width of the pathogen growth inhibition zone became considerably narrower (Table 2). The decrease of antagonistic activity of bacteria with time under in-vitro conditions was also observed by Fokkema (1993), Sobiczewski et al. (1996), Zalewska (1999), and Król (2004).

Table 2

Isolates of bacteria most effectively limiting the growth of pathogenic fungi species

Isolates of bacteria	The width of the growth inhibition zone in mm					
	Botrytis cinerea		*Phomopsis viticola*		*Monilia coryli*	
	a	b	a	b	a	b
Bacillus sp. W 1	10,0	2,7	24,0	7,7		
Bacillus sp. W 35	18,7	13,1	25,0	19,2		
Bacillus sp. W 54	13,3	6,2	24,0	20,7		
Bacillus sp. LKO 25					21,0	19,0
Bacillus sp. *LMO 58*					27,7	16,0
Pseudomonas fluorescens W 21 a	18,7	16,1	23,5	19,2		
Pseudomonas W 4 a	18,0	8,5	22,4	20,7		
Pseudomonas W 28 a	7,0	2,0	21,4	8,6		
Pseudomonas LMO 10					31,0	30,0
Pseudomonas LFO 65					30,3	21,0
Pseudomonas LKK 21					29,0	28,7
Pseudomonas sp. LKK 20					28,0	23,5
Pseudomonas LKO 75					27,3	21,5

a — after 4 days of dual growth with *B. cinerea, P. Viticola,* and after 8 days with *M. coryli;*
b — after 8 days of dual growth with *B. cinerea, P. Viticola,* and after 14 days with *M. coryli.*

Isolates of bacteria most effectively limited the pathogen growth, represented the genera known for high antagonistic abilities and common occurrence in the phyllosphaere of the cultivated plants (Fokkema, 1993; Sobiczewski et al., 1996).

It seems that sparophytic fungi and epiphytic bacteria with high antagonistic activity can create unfavourable conditions for the growth of the pathogenes on the above ground parts of grapevine and hazel.

References

1. Fokkema, N.J. 1993. Opportunities and Problems of Control of Foliar Pathogenes with Micro-organisms. Pestic. Sie., 411—416.
2. Król, E. 1998. Epiphytic bacteria isolated from grape leaves and its effect on *Botrytis cinerea* Pers. Phytopathol. Pol., 16, 53—61.
3. Król, E. 2004. Oddziaływanie epifitycznych bakterii z liści winorośli na *Phomopsis viticola* Sacc. Acta Agrobotanica, vol. 57, 1.
4. Łacicowa, B. 1989. Niektóre aspekty wykorzystania grzybów z rodzaju *Trichoderma* i *Gliocladium* w biologicznej ochronie roślin. Ochrona Roślin, 3, 8—10.
5. Machowicz-Stefaniak, Z. 1998. Antagonistic activity of epiphytic fungi from grape-vine against *Botrytis cinerea* Pers. Phytopathol. Pol., 16, 45—52.
6. Mańka K., 1974. Zbiorowiska grzybów jako kryterium oceny wpływu środowiska na choroby roslin. Zesz. Probl. Post. Nauk Roln., 160, 9—23.
7. Papavizas, G.G. 1985. *Trichoderma* and *Gliocladium*: biology, ecology and potential for biocontrol. Ann. Rev. Phytopathol., 23, 23—54.
8. Sobiczewski, P., Bryk, H., Berczyński, S. 1996. Evaluation of epiphytic bacteria isolated from apple leaves in the control of postharvest apple diseases. J. Fruit Orn. Plant Res., 4, 1, 35—45.
9. Zalewska, E. 1999. Effect of phyllosphere microorganisms on the growth of *Monilia coryli.* Phytopathol. Pol., 18, 57—67.

PATHOGENICITY OF *FUSARIUM* SPP. TO CHRYSANTHEMUM (*DENDRANTHEMA GRANDIFLORA* TZVELEV)

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Abstract

The isolates of *Fusarium oxysporum* Schlecht. and *Fusarium. avenaceum* (Cda ex Fr.) Sacc. were tested for their pathogenicity to chrysanthemum plants. The differences in pathogenic abilities between isolates were observed. All isolates of *F. avenaceum* were pathogenic, while only one isolate of *F. oxysporum* showed some pathogenic ability. However, even if disease symptoms did not occur on plants inoculated with *F. oxysporum*, the differences in plants growth and development were noticed.

Key words: chrysanthemum, *Fusarium* spp., pathogenicity.

Introduction

Fusarium spp. are known for their pathogenicity to many ornamentals grown under covers (Booth 1971). Chrysanthemum is one of the most important ornamental plants in Poland. The main production is aimed at the end of October as chrysanthemums are used for decorating tombs on All Saints Day. Recently, these beautiful ornamentals cultivated in pots are getting very popular all year round. They embellish our houses, terraces and balconies. Among factors affecting the market value of chrysanthemums, fungal diseases are probably the most important. Soil-borne pathogens have the most dramatic impact on economics of chrysanthemum production as they can cause the death of plants.

Earlier investigations showed that *Fusarium* spp. colonized roots and stem base of chrysanthemums (Kopacki and Wagner 2003). However, *Fusarium* populations are diversified in their pathogenicity to host plants (Alabouvette et al. 1992). Also, the cultivars differ in their susceptibility to soil-borne pathogens (Kopacki and Wagner 2003). Our aim was to test the susceptibility of cv. Snowdon, one of the most popular cultivars in Poland, to *F. oxysporum* and *F. avenaceum*, as well as to determine the pathogenicity of individual isolates.

Materials and Methods

The cuttings of cv. Snowdon after roots sterilizing in 50% ethanol and rinsing in sterile water were planted in 11 cm pots filled with the substrate with or without the fungi. As inoculum, the isolates 166, 22, 10, 250 and 471 of *F. oxysporum* and 25, 221, 498, 31 and 401 of *F. avenaceum* were selected for the tests. The isolates were obtained from diseased chrysanthemum plants. Compost substrate with 5% amendment of rice was sterilized in the autoclave in 1000 ml Erlenmayer flasks and inoculated with 14-days cultures of individual isolates of *F. oxysporum* and *F. avenaceum*. The same substrate without pathogens was used as controls. Inoculated substrate was maintained at 20 °C for 3 weeks and after that period it was added (as 1:3) to sterilized standard substrate (soil + sand, 1:1) in pots. Chrysanthemum plants were grown in a growth chamber at 22—23 °C with 12 hours photoperiod for 9 weeks. Every week the health status of plants was evaluated according to 5-grade scale: 0 — no symptoms, 1 — yellowing of bottom leaves, 2 — yellow or necrotic spots on all leaves, 3 — wilting, 5 — death. The disease index was determined using McKinney formula (Łacicowa 1969). Also the height of plants was measured every week. Results were analyzed statistically with Duncan test. At the end of experiment the mycological analysis was conducted to check if the symptoms were caused by tested fungi.

Results and Discussion

Among tested isolates of *F. oxysporum* only Fo22 caused disease symptoms but the disease index did not differ significantly from the control. First symptoms of yellowing appeared after 4 weeks. No wilting or dead plants were observed. Mean disease index was 4.8% after 28 days and amounted only to 13.4% after 63 days. Other isolates of *F. oxysporum* did not cause any disease symptoms (Tab. 1).

The isolates of *F. avenaceum* differed in their pathogenicity to cv. Snowdon. After one week, yellow and necrotic leaves were observed on the plant inoculated with Fa401, Fa31, Fa498 and Fa221. Disease index ranged from 9.8% to 26.6%. No symptoms were observed on the plants inoculated with Fa25. The most harmful proved to be the isolates Fa401 and Fa31. The disease index from Fa401 ranged from 26.6% at the beginning of experiment to 98.4% at the end. For Fa31 the disease index amounted to 26.6% and 61.8%, respectively. The plants inoculated with Fa25 showed first symptoms after 7 weeks. Even at the end of the experiment the disease index was low (26.6%) and did not differ significantly from the control (Tab. 1).

Table 1

Mean disease index for chrysanthemum plants of cv. Snowdon inoculated with *Fusarium oxysporum* and *F. avenaceum*

Isolate	Dates of observations								
	3.05	10.05	17.05	24.05	31.05	7.06	14.06	21.06	28.06
Control	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a
Fo160	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a
Fo250	0a	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a
Fo10	0a	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a
Fa471	0a	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a
Fo25	0a	0a	0 a	0 a	0a	0 a	10.0 a	13.2 a	21.6 ab
Fo22	0a	0 a	0 a	4.8 a	10.0 a	11.8 a	13.4 a	13.4 a	13.4 ab
Fa498	9.8 a	10.0 a	11.6 a	11.6 a	11.6 a	15.2 a	21.8 ab	21.8 a	26.6 ab
Fa221	9.8 a	14.8 ab	16.6 ab	18.4 ab	20.2 ab	23.4 ab	28.4 ab	28.4 ab	30 b
Fa31	26.6 a	40.2 bc	43.6 bc	41.8 bc	43.4 bc	46.6 bc	46.6 b	56.6 bc	61.8 c
Fa401	26.6 a	86.8 d	90.0 d	96.6 d	96.6 d	96.6 d	96.6 d	96.6 d	98.4 d

Note: values marked with the same letter do not differ significantly at $P < 0.05$.

F. avenaceum affected also the height of plants. The strongest effect was observed for the isolates Fa401 and Fa31. The height of plants inoculated with the isolates Fo471, Fo160, Fo22 and Fa498 did not differ significantly from that of control plants. The isolates Fo10 and Fo250 which disease index was 0, caused a significant reduction of plants height (by 40% and 32%, respectively). Also Fa25 of low disease index decreased the height of plants by 41.6% at the end of the experiment (Tab. 2).

Table 2

Mean height of chrysanthemum plants of cv. Snowdon inoculated with *Fusarium oxysporum* and *F.avenaceum*

Isolate	3.05	10.05	17.05	24.05	31.05	7.06	14.06	21.06	28.06
Control	4.1 a	7.1 a	13.5 a	17.5 a	18.6 a	19.3 a	19.7 a	22.5 a	25.5 a
Fo471	4.4 a	5.4 bcd	10.3 ab	9.9 bcd	14.5 ab	15.2 ab	15.7 ab	18.4 ab	21.5 ab
Fa498	4.6 a	5.6 bd	9.0 bc	11.9 b	13.2 bc	13.8 bc	14.4 ab	16.3 abc	19.5 abc
Fo160	4.0 a	6.5 ab	9.5 bc	11.9 b	12.7 bcd	13.2 bc	14.2 b	16.0 abc	18.7 abcd
Fo22	4.6 a	6.4 ab	9.2 bc	10.9 bc	11.3 bcde	11.7 bcd	12.4 cd	14.9 bc	17.8 abcd
Fo250	4.5 a	6.4 ab	9.2 bc	10.5 bc	11.8 bcde	12.1 bcd	12.9 cd	14.8 bc	17.1 bcd
Fa221	4.6 a	5.4 bcd	6.7 cde	9.1 bcd	10.4 bcde	10.8 bcde	11.3 cde	14.2 bcd	16.8 bcd
Fo10	4.2 a	5.4 bcd	8.3 bc	9.9 bcd	10.2 bcde	10.6 bcde	11.3 cde	12.9 bcd	15.5 bcde
Fa25	4.9 a	5.8 abc	6.8 cde	8.8 bcde	9.5 bcdef	9.7 cde	10.4 cdef	12.7 bcd	14.6 bcde
Fa31	4.0 a	4.8 cde	5.2 de	6.6 cdef	7.7 defg	7.5 efg	8.1 cdefg	9.7 cde	12.1 def
Fa401	4.3 a	5.4 bcde	4.8 e	4.9 ef	5.0 gh	5.1 gh	5.1 fg	5.4 e	5.7 fg

Note: values marked with the same letter do not differ significantly at $P < 0.05$.

Results indicated the differentiation in pathogenicity between analyzed pathogens and between isolates. All isolates of *F. avenaceum* caused some disease symptoms, which confirms other reports (Horita and Kodama, 1996). The fungus is known for its pathogenicity to many ornamentals (Wojdyla 1993; Wagner et al., 2003). *F.avenaceum* can cause stem and root rot and sometimes it affects also buds, causing their rot (Horita and Kodama, 1996). The tested cv. Snowdon appeared to be susceptible to this pathogen.

On the contrary, the analyzed isolates of *F. oxysporum* did not cause serious damage to plants. However, some isolates reduced significantly their height. That might be the result of *F. oxysporum* effect on plant physiology. Plants defending themselves against pathogens use some of their energy sources for defense mechanisms (Pospieszny and Struszczyk, 2003) that can hinder a normal development of plants. Also, a negative effect of *Fusarium* spp. on photosynthetic capacity of plant can result in plants stunting (Lorenzini et al., 1997).

Regarding the effect on health status and plant development, some isolates seemed to be non-pathogenic. Especially the isolates Fo471, Fo160 and Fo22 seem to be interesting. If their non-pathogenicity is proved in the tests with other chrysanthemum cultivars, they can be regarded as biocontrol agents (Alabouvette et al., 1992).

References

1. Alabouvette, C., Eparvier, A., Couteaudier, Y., Steinberg, C. 1992. Methods to be used to study competitive interactions between pathogenic and nonpathogenic *Fusarium* spp. in the rhizosphere and at the root surface. IOBC/OILB Bulletin, XV, 1—7.
2. Booth, C. 1971. The Genus *Fusarium*. Kew, UK.
3. Horita, H., Kodama, F. 1996. Bud Rot of Chrysanthemum Caused by *Fusarium avenaceum*. Ann. Rep. of Soc. Pl. Prot. of North Japan, 47, 75—77.
4. Kopacki, M., Wagner, A. 2003. Health status of garden mums (*Dendranthema grandiflora* Tzvelev) in Lublin region. Sodininkystė ir Daržininkystė, 22 (3), 83—90.
5. Lorenzini, G., Guidi, L., Nali, C., Ciompi, S., Soldatini, G. F. 1997. Photosynthetic response of tomato to vascular wilt diseases. Pl. Science, 124, 143—152.
6. Łacicowa, B. 1969. [Laboratory method of quick evaluating the resistance of barley to *Helminthosporium sativum* P. K. et B.] Biul. IHAiR, 3—4, 61—62.
7. Pospieszny, H., Struszczyk, H., 2003. Factors Determining an Efficacy of Chitosan in the Control of Plant Pathogens. Bull. Pol. Acad. Sciences, Biol. Sciences, 51 (3), 251—258.
8. Wagner, A., Hetman, B., Kwiatkowski, S. 2003. Fungi colonizing different *Silphium* spp. Phyt. Polonica, 28, 69—73.
9. Wojdyła, A. T. 1993. Chemical control of *Fusarium avenaceum* (Cda ex Fr) Sacc. On carnations. II. Evaluation of fungicide effectiveness for substratum disinfection before rooting of carnation cuttings. Roczn. N. Roln. S. E, 23, 41—45.

PATHOGENICITY TEST OF *FUSARIUM OXYSPORUM* ISOLATES ORIGINATED FROM TOMATO

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Abstract

There are known two formae speciales associated with tomato: *Fusarium oxysporum* f. sp. *lycopersici* (FOL) and *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL). The aim of this investigation was to estimate pathogenicity of some *F. oxysporum* isolates obtained from diseased tomato plants. A rapid laboratory technique was used to differentiate isolates of *F. oxysporum* that cause crown- and root-rot or wilt from those that are saprophytic. Large variation among tested isolates was observed. Some of them did not cause infection of tomato. Others caused discrete, light tan discoloration of the entire primary root, which is characteristic for FOL. Sometimes symptoms of crown rot appeared on the root-stem transition and it often led to seedlings death that suggests FORL to be the causative organism.

Key words: tomato, *Fusarium oxysporum*, formae speciales, pathogenicity, seedlings.

Introduction

Diseases are still limiting factors in the production of tomato. *Fusarium oxysporum* Schlecht. is one of most important pathogens. Populations of *Fusarium oxysporum* are differentiated in formae speciales and races based on differences in pathogenicity to various hosts and specificity to cultivars of the host. There are known two formae speciales associated with tomato: *Fusarium oxysporum* Schlecht. f. sp. *lycopersici* (Sacc.) Snyder & Hansen (FOL) and *Fusarium oxysporum* f. sp. *radicis-lycopersici* Schlecht. Jarvis & Shoemaker (FORL) (Booth, 1971; Jarvis, Shoemaker, 1978). Both forms can become a significant problem in greenhouse production. FOL, the vascular pathogen of tomato, commonly exists as two races and many commercial varieties of tomato carry the resistance genes, which protect plants against both races 1 and 2. Resistance to FORL isn't so common (Sutherland, Pegg, 1995). Therefore FORL, which causes crown and root rot, appears to be more serious problem now and in the future.

All development stages of tomato are prone to FORL. This soil-borne disease when infects seedlings leads to their death or poor quality. Early symptoms on seedlings include stunting, yellowing, and loss of cotyledons and lower leaves. More advanced visible signs of disease are a pronounced brown lesion that girdles the hypocotyl, root rot, wilting and death. Symptoms of the later infection remain vascular wilt. During ripening of the first fruits sudden or slow wilt symptoms are observed. Diseased plants show a dark chocolate-brown discoloration in the vascular region but not higher than 25 cm above the root-stem transition zone. Root system often rots entirely, and chocolate-brown cancers appear at the soil line. Above the infection region an abnormal proliferation of adventitious roots may occur (Forsberg, 1989).

FORL infects other solanaceous species, and members of the *Leguminaceae*, *Cucurbitaceae* and *Chenopodiaceae*. Madhosingh (1995) suggests that FORL may cause a significant problem in other crops. Therefore, *Fusarium oxysporum* isolates differentiation considering their pathogenicity is so important.

The aim of this investigation was to estimate pathogenicity of some *Fusarium oxysporum* isolates obtained from diseased tomato plants.

Materials and Methods

50 isolates of *Fusarium oxysporum*, obtained from diseased tomato plants grown in plastic tunnels, with typical cultural and morphological characteristic to the genus, were chosen. Each isolate was obtained from a separate plant. Diseased plants were of varieties: Celzus F_1, Delfine F_1, Elena Groot F_1, Graziella F_1, Marissa F_1, and Raissa F_1.

Tomato seeds of cultivar Remiz F_1, known as susceptible to both forms of *Fusarium oxysporum*, were surface sterilized and placed on 2,5% water agar in Petri-dishes. Each agar plate was inoculated with three plugs of certain isolate, and then incubated for 12 h under fluorescent light in a room where temperatures were 20—22 °C. Disease development was visually rated after 10 and 14 days and finally expressed as disease index (DI) according to the scale with five classes:

0° — no visible symptoms;
1° — light-brown discoloration or very few spots on hypocotyl or roots;
2° — several necrotic spots on tap root and hypocotyl;
3° — numerous necrotic spots on tap root and hypocotyl, covering whole roots;
4° — root rot, seedlings dead.

The survey was established in three replications. The data were analyzed using ANOVA test ($\alpha = 0.05$).

Table 1

Results of pathogenicity test, observation after 10 days

Isolate number	Number of plants			Disease index	
	healthy	infected	died		
control	40	0	0	0,00	a
A67	35	5	0	3,12	a
A84	21	19	0	11,87	ab
A336	12	25	0	15,62	abc
A87	17	23	0	18,12	abcd
A323	9	31	0	20,62	abcde
A420	11	29	0	21,25	abcde
A257	9	31	0	21,87	abcdef
A417	9	31	0	25,00	abcdefg
A286	6	32	2	28,75	abcdefgh
A192	9	28	1	29,37	abcdefghi
A154	6	33	0	34,37	bcdefghij
A166	7	28	4	37,50	bcdefghijk
A388	5	35	0	37,50	bcdefghijk
A19	6	32	2	40,62	bcdefghijkl
A152	3	34	3	41,87	bcdefghijkl
A117	5	27	5	42,50	cdefghijkl
A306	0	39	1	42,50	cdefghijkl
A75	6	26	8	42,50	cdefghijkl
A406	5	34	1	43,12	cdefghijkl
A142	4	33	3	45,00	cdefghijkl
A157	0	39	0	45,62	cdefghijkl
A10	5	30	5	46,87	defghijkl
A3	5	28	7	47,50	defghijkl
A338	0	37	3	47,50	defghijkl
A405	6	31	2	47,50	defghijkl
A76	5	30	5	47,50	defghijkl
A308	2	36	4	48,12	defghijkl
A132	1	34	5	48,75	efghijkl
A307	5	29	6	49,37	efghijkl
A419	0	40	0	51,87	fghijklm
A403	0	40	0	52,50	ghijklm
A96	2	32	6	54,37	ghijklm
A325	0	38	2	55,00	ghijklm
A362	0	39	1	55,00	ghijklm
A366	0	37	3	56,25	hijklm
A324	0	36	4	57,50	hijklm
A337	0	38	2	59,37	ijklm
A294	0	40	0	60,62	jklm
A155	0	36	4	61,25	jklm
A58	0	31	9	62,50	jklm
A180	0	34	5	63,12	jklm
A23	1	31	8	63,75	jklm
A159	0	40	0	65,00	klm
A50	0	30	10	65,00	klm
A89	0	31	9	66,25	klm
A43	0	30	11	66,87	klm
A384	0	37	3	67,50	klm
A170	0	33	7	68,75	lm
A57	0	31	9	68,75	lm
A92	0	25	15	81,25	m

Note: values followed by the same letter do not differ significantly $LSD_{0,05} = 30,4481$.

Table 2

Results of pathogenicity test, observation after 14 days

Isolate number	Number of plants			Disease index	
	healthy	infected	died		
control	40	0	0	0,00	a
A67	31	9	0	6,25	a
A323	10	30	0	25,62	ab
A87	10	27	3	28,75	bc
A84	10	24	6	31,25	bc
A420	0	40	0	37,50	bcd
A286	0	37	3	41,87	bcde
A19	4	32	4	42,50	bcdef
A417	0	38	2	42,50	bcdef
A192	0	36	3	44,37	bcdefg
A117	4	30	6	45,62	bcdefgh
A3	5	25	9	46,25	bcdefgh
A154	1	30	6	47,50	bcdefghi
A306	0	33	6	47,50	bcdefghi
A75	0	32	8	48,12	bcdefghij
A142	1	33	6	50,00	bcdefghij
A152	0	36	4	50,00	bcdefghij
A166	0	32	6	50,00	bcdefghij
A405	0	37	3	51,87	bcdefghijk
A257	0	34	6	53,12	cdefghijk
A336	0	33	7	53,12	cdefghijk
A10	1	33	7	55,00	cdefghijkl
A76	0	30	8	58,12	defghijklm
A308	0	33	7	60,00	defghijklm
A419	0	37	3	60,00	defghijklm
A96	0	29	11	60,00	defghijklm
A132	0	32	8	61,25	defghijklmn
A307	0	31	9	65,00	efghijklmno
A23	0	27	13	67,50	efghijklmnop
A155	0	31	9	68,75	fghijklmnopr
A157	0	33	7	68,75	fghijklmnopr
A58	0	27	13	69,37	ghijklmnopr
A180	0	30	10	71,87	hijklmnopr
A406	0	25	15	73,12	ijklmnopr
A324	0	30	10	74,37	jklmnopr
A366	0	29	11	76,87	klmnopr
A388	0	25	15	80,00	lmnopr
A57	0	26	14	80,62	lmnopr
A89	0	24	16	80,62	lmnopr
A43	0	22	18	81,25	lmnopr
A50	0	28	12	82,50	mnopr
A362	0	23	17	84,37	mnopr
A325	0	16	24	87,50	nopr
A159	0	19	21	88,12	opr
A92	0	16	24	88,12	opr
A170	0	18	22	88,75	opr
A337	0	15	25	90,62	opr
A384	0	14	26	91,25	opr
A403	0	13	27	91,87	pr
A338	0	11	29	93,12	pr
A294	0	9	31	94,37	r

Note: values followed by the same letter do not differ significantly $LSD_{0,05} = 26,8001$.

Results and Discussions

The results showed significant diversity in the pathogenicity of chosen isolates of *Fusarium oxysporum* to tomato seedlings (Tables 1 and 2). Statistical analysis enables differentiation of isolates into groups, from those non-pathogenic to those which led to the seedlings death. Some isolates caused sudden effect, and after seven days some seedlings had light necrosis. After ten days symptoms were well noticeable.

According to Sanches et al. (1975), this survey can be a useful tool to distinguish isolates of *Fusarium oxysporum* that cause wilt from those that cause crown and root rot from those that are saprophytic. FORL is supposed to give first symptoms after 7—10 days from inoculation. Fungus caused dark brown lesions girdled the entire crown and the portion of primary root. Sometimes, fungus caused discrete lesions on the cotyledons and hypocotyl. Later, most of seedlings infected by FORL were killed (Malathrakis, 1985; Forsberg, 1989). However, FOL caused light tan discoloration of the entire primary root in 10—14 days. Occasionally developed no external symptoms except a brown speck on the tip of the main root. Saprophytic isolates did not infect the seedlings (Sanches et al., 1975; Malathrakis, 1985).

Similar observations were done during investigations. After 10 days 10 isolates (A67, A84, A336, A87, A323, A420, A257, A417, A286, and A192) and just two (A67 and A323) after 14 days, had no significant differences in comparison to control. Isolates A419, A403, A96, A325, A362, A366, A324, A337, A294, A155, A58, A180, A23, A159, A50, A89, A43, A384, A170, A57, and A92 were the most pathogenic ones. They might be FORL, but it is supposed to confirm that in the further experiments. Summarizing, this method is fast and easy technique which gives full answer about pathogenicity and is very helpful in dividing population of *Fusarium oxysporum,* associated with tomato, into saprophytes and formae speciales.

References

1. Booth, C. 1971. The genus Fusarium. CMI, Kew, Surrey.
2. Forsberg, A. S. 1989. Occurrence and identification of Fusarium root rot — *Fusarium oxysporum* f. sp. *radicis-lycopersici* — in greenhouse grown tomatoes in Sweden. Växtskyddsnotiser, 53, 4, 94—99.
3. Jarvis, W. R., Shoemaker, R.A., 1978. Taxonomic status of *Fusarium oxysporum* causing foot and root rot of tomato. Phytopathology, 68, 1679—1680.
4. Madhosingh, C. 1995. Rapid tomato seedling assay for virulent isolates of *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL), the tomato crown and root rot pathogen. J. Phytopathology, 143, 435—437.
5. Malathrakis, N. E. 1985. Tomato crown and root rot caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* in Greece. Plant Pathology, 34, 438—439.
6. Sanchez, L. E., Endo, R. M., Leary, J. V. 1975. A rapid method for identifying the clones of *Fusarium oxysporum* f.sp.*lycopersici* causing crown and root rot of tomato. Phytopathology, 65, 726—727.
7. Sutherland, M. L., Pegg, G. F. 1995. Purification of a toxin from *Fusarium oxysporum* f. sp. *lycopersici* race 1. Physiol. Mol. Plant Pathol., 46, 243—254.

REACTION OF OAT GENOTYPES TO *FUSARIUM AVENACEUM* (FR.) SACC., *FUSARIUM CULMORUM* (W.G. SM.) SACC. AND *FUSARIUM GRAMINEARUM* (SCHWABE.) INFECTION

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Abstract

Panicles of 12 oats genotypes were inoculated with conidial suspension of the isolates: *Fusarium avenaceum* № 122, *Fusarium culmorum* № 45 and *Fusarium graminearum* № 181. The number of kernels per panicle (NK), the weight of 1000 kernels (TKW) and the kernels yield (KY) were calculated for each genotype of the inoculated group and compared to the control — non inoculated group. Reduction of KY after inoculation with *F. avenaceum* ranged from 10 to 68,5%, with *F. culmorum* from 24 to 68%, and with *F. graminearum* from 0 to 52%. Reduction of TKW after inoculation with *F. avenaceum, F. culmorum* and *F. graminearum* was from 0 to 51,6%, from 10,7 to 44%, and from 3 to 34,6%, respectively, while NK from 0 to 61,6%, from 2 to 59,5, and from 0 to 49,9% respectively.

Key words: oat, fusarium panicles blight, inoculation.

Introduction

Fusarium panicles blight of oat is caused by several species. The most important are: *F. avenaceum, F. culmorum, F. graminearum* and *F. poae* (Langseth et al., 1995; Moschini, Fortugno, 1996; Veisz et al., 1997; Mielniczuk, 2001; Kiecana et al., 2002). Infection heads and panicles of plants by *Fusarium* spp. has a direct negative effect both on the yield size, causing its decrease, and on worse quality of the kernels. Losses caused by *Fusarium* spp. are also associated with accumulation of toxic secondary metabolites in infected kernels (Kiecana, 1994; Langseth et al., 1995; Parry et al., 1995; Bottalico, 1998; Mielniczuk, 2001; Kiecana et al., 2002). The disease symptoms are similar in all small grain cereal crops. Infected kernels are smaller than non-infected, shrivelled and discoloured (Chełkowski, 1989; Bai, Shaner, 1994; Kiecana, 1994). In the case of more resistant grnotypes of cereals, the fewer heads per plot and spikelets per head are infected. In extremaly resistant genotypes only some individual spikelets are affected (McMullen et al., 1997). *Fusarium* panicles blight of oat in Poland can reduce yield by 12—48% (Perkowski, Kiecana, 1997; Kiecana, Mielniczuk, 2000, 2002; Mielniczuk et al., 2000).

The aim of this paper was to determine the damage of *F. avenaceu, F culmorum* and *F. graminearum* to 12 oats genotypes.

Materials and Methods

Twelve oats genotypes (CHD 894, CHD 1095, CHD 1296, CHD 1607, CHD 1653, CHD 1694, STH 2293, STH 2393, STH 2494, STH 2694, Farys, and Sławko) were inoculated under field conditions of the experimental plots in Zamość region (South-Eastern Poland), with *F. avenaceum, F. culmorum* and *F. graminearum*. These isolates used for inoculation were obtained from the cultures collection of the Phytopathology Department at the Agricultural University of Lublin, Poland and were isolated from oat's kernels. The isolates *F. avenaceum* № 122, *F. culmorum* № 45 and *F. graminearum* № 181 were selected for the method of Mishra and Behr (1976). These strains reduced Ducat cv. kernels germination ability by up to 3, 2 and 8%, respectively.

The inoculum was prepared according to the modified method described by Mesterhazy (1978). The growing medium (1L) was composed as follows: water extract of 0.5 kg oat leaves with selective medium — SNA, was autoclaved (1 h, 121 °C and 1 atm). Subsequently, when cold, the cultures of *F. avenaceum, F.culmorum* and *F. graminearum* isolates were incubated for two weeks at 18—20 °C with a 12 h period of natural light (Kiecana, 1988). After incubation, the inoculum — stired for 10 min. — was filtred through a cheesecloth, and the conidial suspension (5×10^5 spores per ml) was used for the inoculation.

All oat cultivars and lines were studied in one location (Zamość region) in 2001. The experiment was carried out in a randomized complete blocks design with four replications. Eighty panicles of oat (20 panicles per replicate) were inoculated with three examined *Fusarium* spp., 4 days after the anthesis of minimum 50% of plants (21.—26.06.2001). The inoculum (2 ml per panicle), prepared as described above, was applied with a laboratory sprayer. The same cultivars and lines were sprayed with 2 ml. Distilled water instead of a conidial suspension, was used as the control (non–inoculated) group. After inoculation or water spraying, the panicles were protected with plastic bags for 24 h to avoid water evaporation and the spread of the inoculum.

Mature panicles were collected on 9th August 2001 and were threshed manually. Yield (KY), kernels number (NK) and the weight of 1000 kernels (TKW) of the experimental groups were measured and compared with controls. The results were calculated for each genotype using statistical analyses (multiple confidence interval T-Tukey, Oktaba, 1972).

Symptoms of the disease were evaluated according to earlier publications (Kiecana, 1994; Parry et al., 1995).

The mean values of kernels yield, 1000 kernels weight and kernels number after panicles of oat inoculation with *Fusarium* spp. tested and the control (non-inoculated group)

Genotypes	kernels yield [g]				1000 kernels weight [g]				kernels number per panicle			
	F. a.	*F. c.*	*F. g.*	control	*F. a.*	*F. c.*	*F. g.*	control	*F. a.*	*F. c.*	*F. g.*	control
CHD 894	6,70	4,95*	7,26	11,75	19,81*	24,25*	26,76*	40,93	32,62	20,52	27,25	29,47
CHD 1095	5,15*	6,22*	8,28	14,00	16,25*	17,39*	22,68	30,94	31,62	27,52	36,75	45,25
CHD 1296	6,67*	11,10	7,97*	14,57	20,00*	25,75	23,17*	33,50	33,22	43,00	34,50	43,80
CHD 1607	6,55*	7,17*	8,13*	15,20	20,62	19,94	23,85	30,25	31,82	36,95	34,50	50,45
CHD 1653	6,22	6,57	7,95	11,72	20,81*	22,06*	21,62*	32,81	30,57	29,92	36,75	35,60
CHD 1694	4,17*	6,26*	7,34	12,73	19,87	18,37*	20,65	28,93	21,25*	34,12	35,25	55,42
STH 2293	9,23	5,80	9,74	10,25	26,75	20,37	23,33	24,75	34,15	27,90	41,50	43,27
STH 2393	3,85*	4,07*	10,36	12,23	17,50	20,27	24,64	24,63	21,65*	19,87*	42,00	49,13
STH 2494	6,62*	4,27*	7,54	13,20	19,87	16,87*	24,96	28,16	33,60	24,65	29,50	46,75
STH 2694	8,27	6,62	11,14	9,30	31,25	22,50	25,35	26,13	26,00	29,55	44,00	34,35
Farys	5,10*	5,00*	8,06	11,57	23,87	19,75	22,23	30,00	21,52	25,92	35,00	38,82
Sławko	8,40*	10,90*	11,09*	23,12	25,62	30,12	30,93	33,75	32,52*	35,67*	36,00*	71,82

Means marked * differed significantly when compared with the control ($P < 0.05$).
F. a. — Fusarium avenaceum, F. c. — Fusarium culmorum, F. g. — Fusarium graminearum.

Results and Discussion

The inoculation, described in the Methods, was succesful in our experiments and the panicles of experimental groups exhibited scab symptoms typical for natural infection with a higher percentage of diseased spikelets. Scab symptoms in case of oat were similar to observations typical for fusarium head blight of other cereals (Mesterhazy, 1978; Kiecana, 1988, 1994; Parry et al., 1995; Kiecana et al., 1997; Goliński et al., 2002; Inch, Gilbert, 2003). Branes in a place of contact with spikelet axels along the nervure exhibited etiological symptoms of salmon pink or orange sporodochia with conidia of examined *Fusarium* spp. Sporodochia were also visible on internal and external glumes of all kernels in infected spikelet. The infected kernels were shriveled, discoloured and often outgrown with mycelium.

Different methods of inoculation have been described in literature (Takeda, Heta, 1989). In our experiment, according to the methods described by Mesterhazy (1978), Stack, McMullen (1985), or Kiecana (1994), panicles were sprayed with a water suspension of *F. avenaceum, F. culmorum* and *F. graminearum* macroconidia and this procedure, in our opinion, resembles natural infection.

The inoculation of oat's panicles by *F. avenaceum, F. culmorum* and *F. graminearum* during the flovering has the influence on the reduction of kernels number per panicle and worse kernels development. During the experiment, differences between all the tested genotypes of oat were observed. Significant differences in the kernels yield were found in eight oat genotypes after inoculation with *F. avenaceum* and *F. culmorum*, and in three genotypes after inoculation with *F. graminearum* (table). The reduction of yield after infection with *F. avenaceum, F. culmorum* and *F. graminearum* ranged from 10 to 68,5%, from 24 to 68%, and from 0 to 52%, respectively (Fig.1).

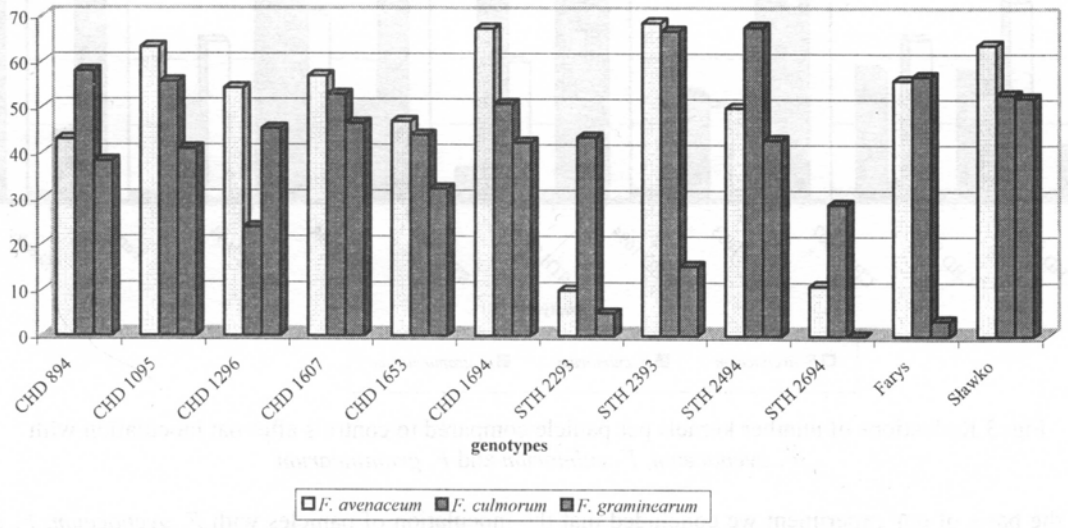

Fig. 1 Reductions of kernels yield compared to controls after oat panicles inoculation with F. *avenaceum, F. culmorum* and *F. graminearum*

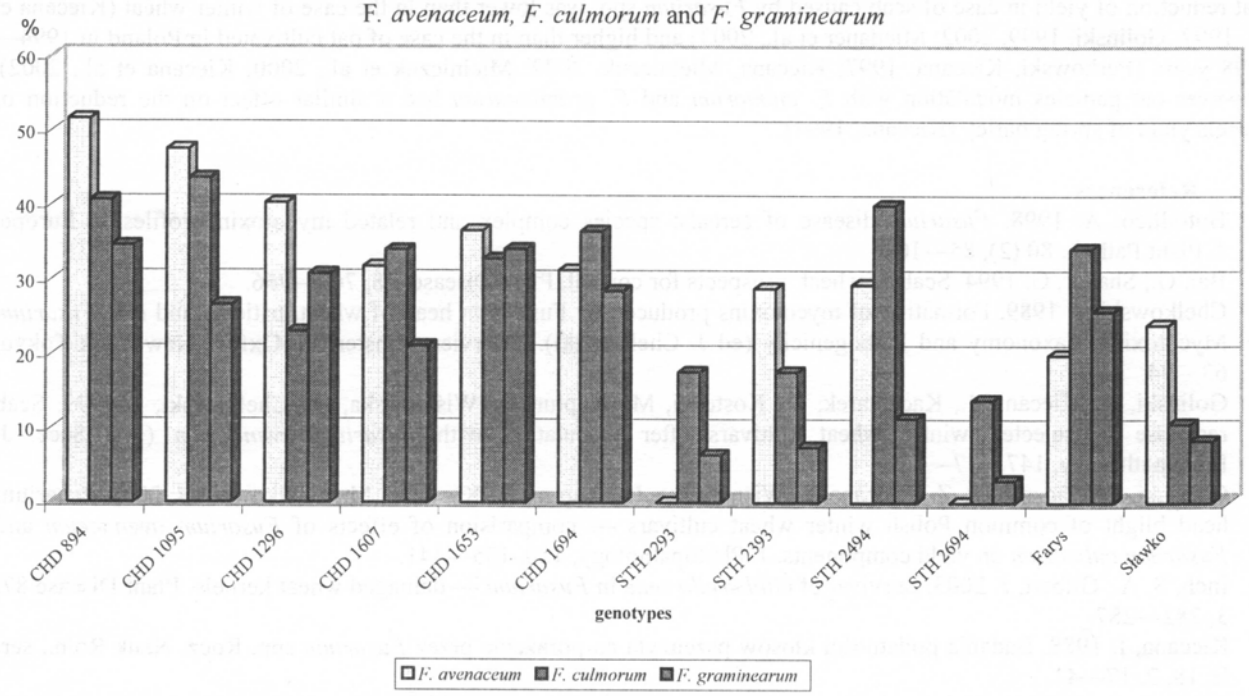

Fig. 2 Reductions of TKW compared to controls after oat panicles inoculation with *F. avenaceum, F. culmorum* and *F. graminearum*

Significant differences concerning TKW were observed in lines CHD 894, CHD 1095, CHD 1296 and CHD 1653 in case of panicles infected with *F. avenaceum* and CHD 894, CHD 1095, CHD 1653, CHD 1694 and STH 2494 in case of infection with *F. culmorum*. *Fusarium graminearum* significantly reduced TKW in three genotypes of oat (CHD 894, CHD 1296, CHD 1653) (table). The lowest TKW reduction was detected in lines STH 2293 and STH 2694 (0%) and the highest in CHD 894 — 51,6% in case of *F. avenaceum* (Fig. 2). *Fusarium culmorum* reduced TKW of oat's genotypes tested from 10,7 to 44% and *F. graminearum* from 3 to 34,6% (Fig. 2). Significant differences in kernels number caused by three pathogens tested (*F. avenaceum, F. culmorum* and *F. graminearum*) were presented in the table. The decrease of the number of kernels in a panicle for infection by *F. avenaceum, F. culmorum* and *F. graminearum* ranged from 0 to 61,6%, from 2 to 59,5%, and from 0 to 49,9%, respectively (Fig. 3).

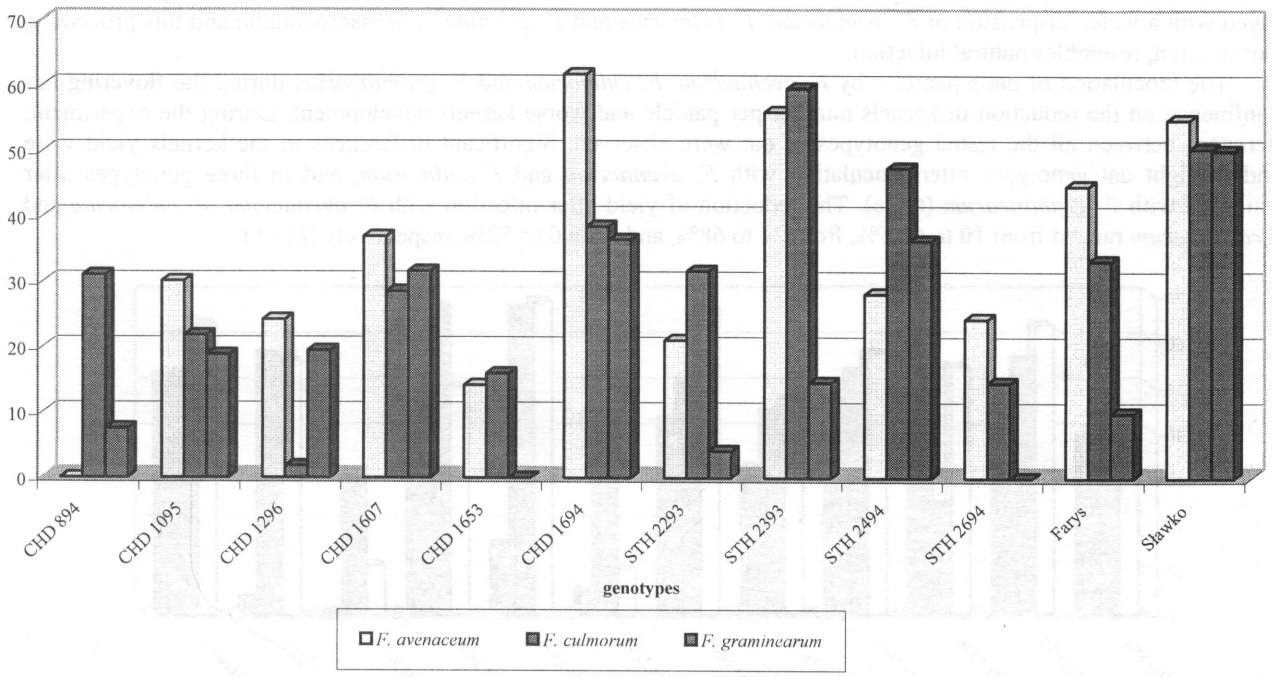

Fig. 3 Reductions of number kernels per panicle compared to controls after oat inoculation with *F. avenaceum, F. culmorum* and *F. graminearum*

On the basis of our experiment we concluded that the inoculation of panicles with *F. avenaceum, F. culmorum* and *F. graminearum* reduced the kernels yield on average by 49, 50 and 34%, respectively. The obtained results showed that reduction of yield in case of scab caused by *Fusarium* spp. was lower than in the case of winter wheat (Kiecana et al., 1997; Goliński, 1999, 2002; Miedaner et al., 2003) and higher than in the case of oat cultivated in Poland in 1994—1998 years (Perkowski, Kiecana, 1997; Kiecana, Mielniczuk, 2000; Mielniczuk et al., 2000; Kiecana et al., 2002). However oat panicles inoculation with *F. culmorum* and *F. graminearum* had a similar effect on the reduction of kernels yield of spring barley (Kiecana, 1994).

References

1. Bottallico, A. 1998. *Fusarium* disease of cereals: species complex and related mycotoxin profiles in Europe. J. Plant Pathol., 80 (2), 85—103.
2. Bai, G., Shaner, G. 1994. Scab of wheat: prospects for control. Plant Disease, 78, 760—766.
3. Chełkowski, J. 1989. Formation of mycotoxins produced by Fusaria in head of wheat, triticale and rye, *Fusarium* Mycotoxins, Taxonomy and Pathogenicity (ed J. Chełkowski). Elservier, Amsterdam-Oxford-New York-Tokyo, 63—84.
4. Goliński, P., Kiecana, I., Kaczmarek, Z., Kostecki, M., Kaptur, P., Wiśniewska, H., Chełkowski, J. 1999. Scab response of selected winter wheat cultivars after inoculation with *Fusarium avenaceum* (Fr.) Sacc. J. Phytopathology, 147, 717—123.
5. Goliński, P., Kaczmarek, Z., Kiecana, I., Wiśniewska, H., Kaptur, P., Kostecki, M., Chełkowski, J. 2002. Fusarium head blight of common Polish winter wheat cultivars — comparision of effects of *Fusarium avenaceum* and *Fusarium culmorum* on yield components. J. Phytopathology, 150, 135—141.
6. Inch, S. A., Gilbert, J. 2003. Survival of *Gibberella zeae* in *Fusarium* — damaged wheat kernels. Plant Disease 87, 3, 282—287.
7. Kiecana, I. 1988. Badania podatności kłosów pszenżyta na porażenie przez *Fusarium* spp. Rocz. Nauk Roln., ser. E, 18, 2, 17—41.
8. Kiecana, I. 1994. Badania nad fuzariozą kłosów jęczmienia jarego (*Hordeum vulgare* L.) z uwzględnieniem podatności odmian i zawartości mikotoksyn w ziarnie. Seria Wydaw., Rozprawy Naukowe, 161, 1—49.

9. Kiecana, I., Wojciechowski, S., Chełkowski, J. 1997. Reaction of winter wheat cultivars to *Fusarium avenaceum* (Fr.) Sacc. and *F. culmorum* (W.G.Sm.) Sacc. under different localities. Polish Agric. Ann., ser. E, 26, ½, 61—65.
10. Kiecana, I., Mielniczuk, E. 2000. The occurrence of *Fusarium avenaceum* (Fr.) Sacc. and *Fusarium culmorum* (W. G. Sm.) Sacc. in oats (*Avena sativa* L.). 6th European Fusarium Seminar and Third COST 835 Workshop (Agriculturally Important Toxigenic Fungi) at the BBA and FU Berlin, Germany, 11—16 September 2000, 67—68.
11. Kiecana, I., Mielniczuk, E., Kaczmarek, Z., Kostecki, M., Goliński, P. 2002. Scab Response and Moniliformin Accumulation in Kernels of Oat Genotypes Inoculated with *Fusarium avenaceum* in Poland. Europ. J. Plant Pathol., 108, 245—251.
12. Langseth, W., Hoie, R., Gullord, M. 1995. The influence of cultivars, location and climate on deoxynivalenol contamination in Norwegian oats 1985—1990. Acta Agric., Scand. sect. B, Soil and Plant Sci., 45, 63—67.
13. McMullen, M., Jones, R., Gallenberg, D. 1997. Scab of wheat and barley: Pre-emerging disease of devastating impact. Plant Disease, 81, 1340—1348.
14. Mesterhazy, A. 1978. Comparative analysis of artificial inoculation methods with *Fusarium* spp. on winter wheat varieties. Phytopathol. Z. 93, 1, 12—25.
15. Miedaner, T., Moldovan, M., Ittu, M. 2003. Comparison of spray and point inoculation to assess resistance to fusarium head blight in a multienvironment wheat trial. Phytopahology 93, 9, 1068—1072.
16. Mielniczuk, E., Kiecana, I., Perkowski, J. 2000. Reduction of yield and mycotoxins accumulation in oat cultivars and lines after *Fusarium culmorum* (W.G.Sm.) Sacc. and *F. sporotrichioides* Sherb. inoculation. 6th European Fusarium Seminar and Third COST 835 Workshop (Agriculturally Important Toxigenic Fungi) at the BBA and FU Berlin, Germany, 11—16 September 2000, 67.
17. Mielniczuk, E., 2001. The occurrence of *Fusarium* spp. on panicles of oat (*Avena sativa* L.). Journal of Plant Protection Research, 41, 2, 173—180.
18. Mishra, C.B.P., Behr, L. 1976. Der Einfle von Kulturfiltraten von *Fusarium culmorum* (W.G.Sm.) Sacc., *Fusarium avenaceum* (Fr.) Sacc. und *Fusarium nivale* (Fr.) Ces., *Griphosphaeria nivalis* Müller et v Arx auf die Keimung des Weizeus. Arch. Phytopathol. Pflanzenschutz 12, 6, 373—377.
19. Moschini, R. C., Fortugno, C. 1996. Predicting wheat head blight incidence using models based on meteorological factors in Pergamino Argentina. European Journal of Plant Pathology, 102, 211—218.
20. Oktaba, W. 1972. Metody statystyki matematycznej w doświadczalnictwie. PWN, Warszawa.
21. Parry, D. W., Jenkinson, P., McLoad, L. 1995. *Fusarium* ear blight (scab) in small grain cereals - a review. Plant Pathol., 44, 207—238.
22. Perkowski, J., Kecana, I. 1997. Reduction of yield and mycotoxins accumulation in oats cultivars after *Fusarium culmorum* inoculation. Cer. Res. Comm. Proceedings of the 5th European *Fusarium* Seminar, Szeged, Hungary 25, 3/2, 801—803.
23. Stack, R.W., McMullen, M.P. 1985. Head blighting potention of *Fusrium* species associated with spring wheat heads. Can. J. Plant Pathol., 7, 79—82.
24. Takeda, K., Heta, H. 1989. Establishing the testing method and a search for the resistant varieties to *Fusarium* head blight in barley. Jap. J. Breed., 39, 203—216.
25. Veisz, O., Szunics, Lu., Szunics, L. 1997. *Fusarium* infection of oat varieties. Cer. Res. Comm. Proceedings of the 5th European *Fusarium* Seminar, Szeged, Hungary 25, 3/2, 829—831.

Ecological approach of modern weed control systems

WEED CONTROL IN SPRING BARLEY BY LOWER DOSES OF HERBICIDES IN ESTONIA

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Abstract

As herbicides are expensive and detrimental to the environment, it is necessary to restrict their use. One option is to reduce the used herbicide concentration. To study the efficiency of reduced herbicide concentrations, field experiments have been performed in Estonia since 2000. All the herbicides were applied at their standard, half and quarter doses. The experiments were conducted in spring barley in three replications. During treatment with herbicides, the weeds were in stage of 3—4 true leaves. Prior to spraying, the dominant weed species in the field were determined, as well their density per 1 m^2 and growth stage. The unsprayed control variant was used for comparison. Using the databases compiled by the Danish Institute of Agricultural Sciences, two prototypes of different weed control capacity were created in Estonia for spring wheat and barley. The local prototype was tested in the field experiments in 2002 and 2003. The efficiency of the used herbicide depends on the species composition of the prevalent weeds in the field. If only weeds sensitive to the herbicide used are present, adequate efficiency is attained already with a half dose or even a quarter dose. The preliminary results from the medium- and high-efficiency weed control models used in the experiment are positive. In the experiment with barley, it was possible to reduce the herbicide rate to 45% of the recommended full concentration. The program-calculated herbicide doses put the weed control efficiency at 62—86%.

Key words: herbicide concentration, herbicide efficiency, growth stage.

Introduction

In today's farming, abundant use is made of chemical plant agents, primarily herbicides. As herbicides are expensive and detrimental to the environment, it is necessary to restrict their use. One option is to reduce herbicide concentration.

In Denmark, Sweden, Norway, Great Britain and the Netherlands, herbicide applications at doses reduced by 10—70% have already become a norm and full doses are used sporadically and only on distinct recommendations of agricultural advisers (Domaradzki et al., 2003).

The sensitivity of different weed species to herbicide is diverse. For some weed species, 10% of the normal dose is enough to get good control, for some — much higher doses are needed (Auskalnis, 2003).

Most selective herbicides will control only a limited spectrum of the weeds dominating in the region. With increasing complexity in local weed infestations, the relevance of using tank-mixtures increases simultaneously. Economic and other interests can also motivate the use of tank-mixtures (Rydahl, 1999).

Herbicide activity is influenced by many complex interactions involving weed flora, growth stage of weeds, environmental conditions, and competitive ability of the crop. Under favourable conditions, satisfactory weed control can be obtained with doses much lower than the recommended dose while under unfavourable conditions not even the highest dose recommended on the label may provide satisfactory weed control (Kudsk, 2001). Climatic conditions before, at and after herbicide application can change the efficacy of herbicides.

Materials and Methods

Field trials testing herbicide efficacy were conducted at Tartu, Saku, Jõgeva and Jäneda (different regions of Estonia) in the period 2000—2003. The trials were a randomized bloc design with three replicates. All herbicides were used at full, ½ and ¼ doses. Tested herbicides are listed in Table 1. Herbicides were applied when weeds were at 2—6 true leaf stage and cereals at growth stage BBCH 20–22. Weed assessment was made on individual weed species in 3 × 0.25 m^2 per plot 4 weeks after herbicide application. All weed specimens in all replicates were collected, counted and weighted by species. The unsprayed control variant was used for comparison.

Efficacy of the herbicides by number and by mass of weeds was calculated by formula:

$E = (M_1–M_2)/M_1*100$,

where M_1 — weeds number or mass per m^2 on untreated plots;

M_2 — weeds number or mass per m^2 on plots treated with herbicides.

Table 1

The tested herbicides

Trade name	Active ingredients	Full dosage
Banvel 4S *	Dicamba 480 g/l	220 g/ha
Sekator*	Amidosulfuron 5 % + jodosulforon-metyl-natrium 1.25 %	300 g/ha
Mustang*	Florasulam 6.25 g/l + 2,4 D 2EHE 452.5 g/l	0.6 l/ha
Granstar 75DF	Tribenuron-methyl 75 g/kg	15 g/ha
Lintur 70 WG	Triasulfuron+dicamba 4.1+659 g/kg	150 g/ha
MCPA	MCPA 750 g/l	2.0 l/ha
Duplosan Super 600 SL	Dichlorprop-P+MCPA+mechlorprop-P 310+160+130 g/l	2.0 l/ha
Primus	Florasulam 50 g/l	0.1 l/ha

* included in the experiments in 2003.

This article is built on data from years 2002 and 2003. The year 2002 was extremely dry whereas 2003 was suitable for plant growth and development. The weather data are given in Figure 1.

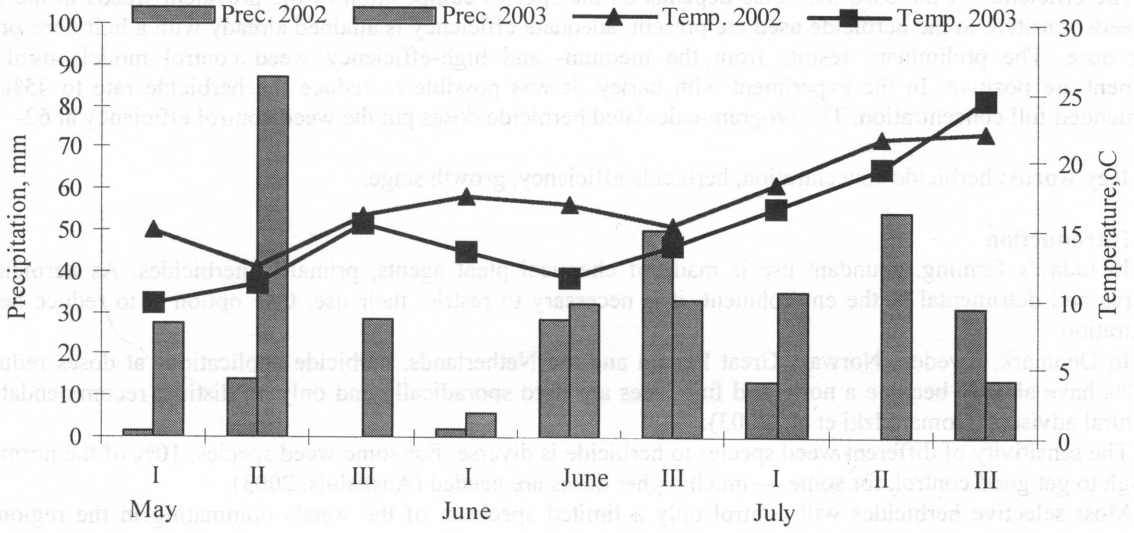

Fig. 1. Climatic conditions in Estonia in 2002—2003

Results

The most widespread weeds in the trial area were *Viola arvensis, Chenopodium album, Stellaria media, Galeopsis speciosa, Fumaria officinalis, Polygonum convolvulus, Capsella bursa-pastoris, Veronica sp., Thlapsi arvense* and *Galium aparine.*

By skillful use of herbicides or their mixes it should be possible to control all the most widespread weeds on a cereal field.

The shorter the weed growth stage the greater the herbicide efficacy. Other important parameters are the crop growth stage, the expected yield, and the soil. Herbicide efficacy is substantially influenced by the weather. If following a prolonged drought plants have run short of water, herbicide efficacy is low.

Due to the drought in the spring of 2002 and the extraordinary deficiency of soil moisture, both cereals and weeds sprouted unevenly, the latter also relatively late. For that reason the trial variants were sprayed with herbicides later than the optimal time for barley growth (BBCH – 23–31). Nevertheless, the density of weeds was small at the time of spraying, with many of them still in the cotyledonal stage. Weeds sprouted in large numbers also after spraying. The plants were in a state of stress. The low soil moisture activated defensive reactions in some weed species, as a result of which herbicide-treated weeds were stunted but not completely destroyed. Consequently, the herbicide efficacies were lower with regard to weed density compared to weed weight (Table 2).

Table 2

The efficacy of herbicides used in the trial at different doses for controlling the most widespread weeds in 2002

Weed species	Dosage	Efficacy 2002									
		Granstar		Lintur		Primus		MCPA		Dupl. S	
		W	N	W	N	W	N	W	N	W	N
Chenopodium album CHEAL	1	74	56	61	45	46	22	87	81	78	69
	½	64	53	53	41	40	26	51	41	62	35
	1/4	49	39	44	50	46	14	51	38	58	27
Viola arvensis VIOAR	1	72	68	68	66	24	40	43	45	40	65
	½	45	50	41	74	24	35	23	40	33	57
	1/4	36	61	32	59	53	26	24	41	36	53
Veronica sp VERSP	1	85	46	43	93	46	68	10	28	59	53
	½	26	26	72	89	8	14	46	53	26	44
	1/4	55	50	43	36	22	17	30	31	33	33
Thlapsi arvense THLAR	1	100	86	92	96	70	56	96	93	97	99
	½	85	94	75	71	48	64	92	83	86	77
	1/4	53	44	59	70	44	59	66	59	58	50
Stellaria media STEME	1	100	X	67	X	100	X	66	X	100	X
	½	83	X	100	X	100	X	55	X	55	X
	1/4	98	X	84	X	67	X	61	X	63	X
Polygonum convovulvus POLCO	1	90	74	94	70	68	75	90	77	60	59
	½	84	79	62	62	72	65	57	58	63	51
	1/4	0	0	65	67	82	81	62	71	56	55
Fumaria officinalis FUMOF	1	60	51	37	53	30	24	37	54	41	53
	½	60	36	38	45	41	33	45	43	26	47
	1/4	37	23	12	15	33	43	15	15	25	34

Efficacy of weed control: 90—100% — well, 65—89% — sufficient, 50—64% — satisfactory, <49 — poor.
N — number of weeds, W — weight of weeds.

The weather conditions of 2003 were more advantageous; accordingly, efficacy of herbicides was higher.

The hardest-to-control weed in the trial area was *Galium aparine*. This weed was impossible to control with either Granstar or MCPA. No herbicide can be used at reduced doses. The efficacy of Granstar was low in controlling *Viola arvensis* and *Polygonum convovulvus*. *Fumaria officinalis* should be controlled at full herbicide doses (Table 3).

In 2003, three new herbicides — Mustang, Sekator and Banvel 4S — were included in the experiment, whereas Duplosan Super and Primus were excluded. As Sekator contains two agents (Amidosulfuron 5% + iodosulfuron-methyl-natrium 1.25%) its efficacy was higher compared to the other herbicides tested for all the weeds included in the trial. Sekator proved effective in controlling all the weeds at both half and full doses. Quarter doses proved incapable of controlling *Viola arvense, Galium aparine*, and *Fumaria officinalis*. In controlling other most widespread species, the efficacy of reduced herbicide doses was 53—98% (Fig. 2).

The efficacy of Mustang in controlling *Viola arvensis*, *Galium aparine* and *Polygonum convovulvus* was low at half and quarter doses. Full doses were unable to control *Lamium purpureum* and *Fumaria officinalis*, the efficacy being 37% and 48%, respectively.

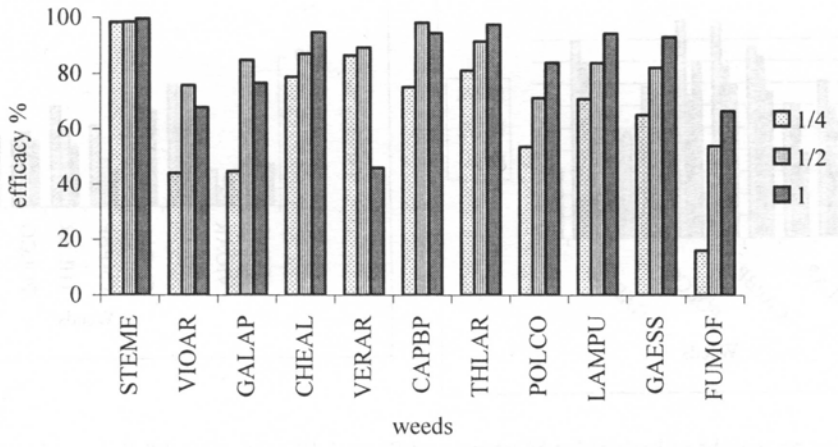

Fig. 2. The efficacy of herbicide Sekator at ¼, ½ and full doses in controlling the most widespread weeds

Table 3

The efficacy of herbicides used in the trial at different doses for controlling the most widespread weeds in 2003

Weed species	Dosage	Efficacy 2003					
		Granstar		Lintur		MCPA	
		W	N	W	N	W	N
Stellaria media STEME	1	100	X	84	X	51	X
	½	98	X	81	X	57	X
	1/4	99	X	84	X	39	X
Viola arvensis VIOAR	1	39	37	81	65	82	79
	½	37	30	71	64	62	65
	1/4	26	20	56	44	49	48
Galium aparine GALAP	1	52	42	77	55	26	24
	½	41	28	49	38	29	24
	1/4	24	8	65	18	23	21
Chenopodium album CHEAL	1	100	99	89	71	85	90
	½	94	92	50	52	92	89
	1/4	81	69	46	42	74	70
Capsella-bursa pastoris CAPBP	1	60	64	91	88	98	98
	½	60	63	89	84	84	89
	1/4	58	59	54	53	45	58
Thlaspi arvense THLAR	1	88	80	90	77	93	90
	½	87	88	76	65	82	85
	1/4	70	73	72	53	61	68
Polygonum convovulvus POLCO	1	61	45	75	80	58	51
	½	53	48	73	75	23	9
	1/4	48	32	47	45	35	26
Lamium purpureum LAMPU	1	89	54	79	51	62	39
	½	64	54	71	38	53	41
	1/4	76	46	59	21	57	22
Fumaria officinalis FUMOF	1	86	72	89	78	87	62
	½	58	43	76	37	42	21
	1/4	81	80	31	11	20	12

N — number of weeds, W — weight of weeds.

The lowest efficacy was evidenced by the narrow-spectrum Banvel 4S, whose efficacy in controlling most of the weeds remained low even at full doses. Quarter doses can be used to control *Chenopodium album* (efficacy on weight 63%, efficacy on number 66%) and *Galium sp.* (weight 74%, number 60%). At full doses the efficacy proved low in controlling *Stellaria media*, *Viola arvensis*, *Polygonum convolvulus*, *Lamium purpureum* and *Galeopsis speciosa* (Fig. 3).

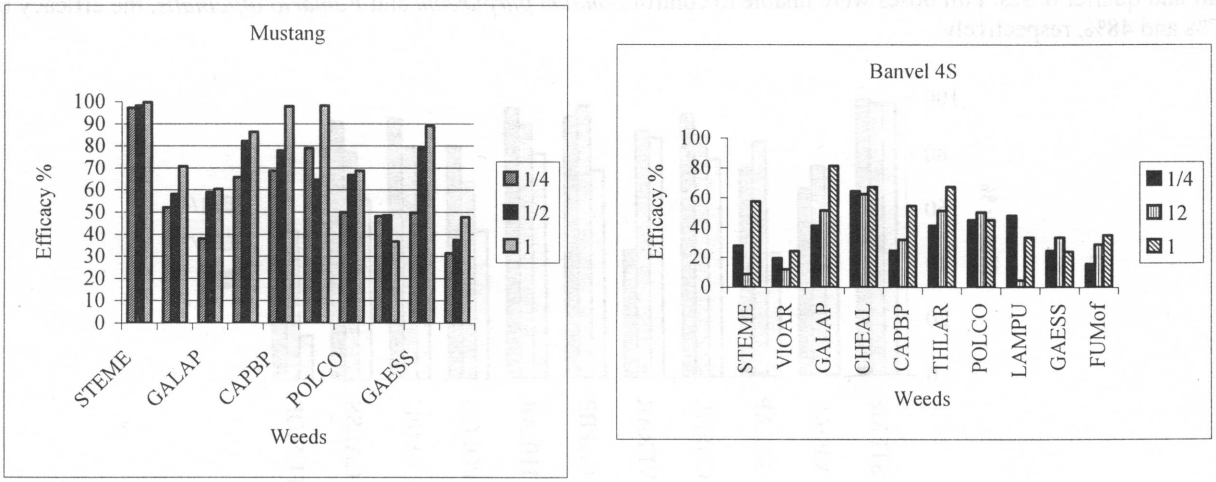

Fig. 3. The efficacy on weight of herbicides Mustang and Banvel 4S at ¼, ½ and full doses in controlling the most widespread weeds

Conclusion

Regular doses of herbicides can be reduced if the species composition of the weeds growing in the field corresponds to the spectrum of action of the herbicide to be used.

If the weeds growing in a field are vulnerable to herbicide it can be used at a ¼ dose.

The low soil moisture and the high air temperature in 2002 activated defensive reactions in some weed species, resulting in low efficacy of herbicide treatment.

The selection of herbicides and their doses should be based on the species composition and growth stage of the weeds and on the weather conditions.

References

1. Auskalnis, A. 2003. Experience with "Plant Protection On line" for weed control in Lithuania. Proceedings of the Crop Protection Conference for the Baltic Sea Region, 166—175.
2. Domaradzki, K., Praczyk, T., Matysaik, K. 2003. Prototype of Polish version of decision support system for weeds. Proceedings of the Crop Protection Conference for the Baltic Sea Region, 175—180.
3. Kudsk, P. 2001. How to investigate the influence of environmental factors on herbicide performance. The BCPC Conference — Weeds, 495—503.
4. Rydahl, P. 1999. Optimising mixtures of herbicide within a decision support system. The 1999 Brighton Crop Protection Conference, Weeds, Vol. 3, 761—766.

DYNAMICS OF THE FLORA OF ARABLE FIELDS IN CENTRAL LATVIA

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Abstract

For nine years, starting from 1994, annual surveys of the flora of five arable fields in five regions of central Latvia have been made to assess the effects of crop rotation, crop husbandry practices and other factors on the dynamics of non-crop plant species. This work is part of a programme to provide information for agronomists and farmers to help determine the need for weed control and plan future control measures. The observation fields were chosen to be typical of arable fields in respective regions. Assessments were made early in July when plants were well developed and species could be determined easily. Sixty-four non-crop species were recorded: 20 species occurred in all five fields; 14 species occurred only in one field; no species occurred in all five fields in all nine years. The lowest number of species, recorded in any field in any year, was 2; the highest was 32. The numbers of plants of non-crop species ranged from 5 m^{-2} to 305 m^{-2}, with large annual fluctuations in all fields. The numbers in all fields were lowest in 1996. There were significant differences among fields and among years for numbers of species and numbers of plants of all weed groups. The numbers of annual dicot plants were highest in crops of spring wheat, winter wheat and spring oats, and lowest in a grass crop. The numbers of perennial dicots and monocots were lowest in crops of sugar beet and spring barley, and highest in a crop of timothy and red clover and in fields that were fallowed or uncropped. Numbers of perennial dicot and monocot plants were significantly associated with previous crop, but numbers of annual dicots were not. The numbers of perennial dicot plants were lower where a herbicide had been used, but there were no differences in the numbers of annual dicot and monocot plants. Regression analysis showed that no one of the five factors (field, year, crop, previous crop, herbicide) best accounted for the observed variation in numbers of species and numbers of plants of all weed groups, but, overall, crop accounted for the most variation (16% to 60% of variance accounted for). Adding previous crop and herbicide to the regression model accounted for more variation in only four of the eight comparisons. The optimal models for species numbers and plant numbers of the weed groups were all different but accounted for 47% to 86% of the variance.

Key words: weeds, surveys, dynamics, crop husbandry.

Introduction

Periodic surveys of the weed flora of agricultural fields in Latvia have been undertaken since the 1940s, when the first surveys were made by Alfreds Rasiņš (Rasiņš et al., 1985; Рубенис, 1995; Lapiņš, 1999; Lejiņš, Āboliņš, 2000; Lapiņš et al., 2002). The results from these surveys have shown great changes in the relative and absolute frequencies of some species during this period: some species that were rare are now common, some that were common are now rare or absent, while the frequency of others has increased in recent years (Vanaga, 2002). These changes in the flora can be related to several major changes in Latvian agriculture during the past 60 years (Vanaga, 2002).

The present study was undertaken to investigate the dynamics of the weed flora within individual fields in relation to cropping and crop husbandry practices. Assessments were made in the same five fields each year for nine successive years from 1994. From 1997, soil samples for weed seed assessment were taken at the same time as the plant counts, but these results will be reported elsewhere. This study was part of a joint programme by the Latvian State Centre of Plant Protection, the Department of Soil Management of Latvia University of Agriculture, and Skriveri Research Centre. The larger programme included investigations on weed populations, abundance of weed species, changes in composition, and species dynamics and harmfulness, as well as research for optimisation of weed control measures.

Materials and Methods

Five observation fields were selected to be typical of farming systems and conditions in central Latvia. The crop rotations and crop husbandry in each field were determined by the respective farmers (Table 1). The soil type in the observation fields was sod podzolic. The soil textures in the observation fields were loam (Cēsis, Rīga, Valka) or loamy sand (Limbaži, Valmiera). Herbicides for broad-leaved weed control were used frequently in the Cēsis and Rīga observation fields, but only once in the nine years in the Limbaži, Valka and Valmiera observation fields.

Table 1

Crop rotation of observation fields and herbicide use 1994—2002

Region		1994	1995	1996	1997	1998	1999	2000	2001	2002
Rīga	Previous crop	WR	SB	P	SB	SB	SB	SB	SW	UN
	Crop	SB	P	SB	SB	SB	SB	SW	UN	UN
	Herbicide	No	No	Yes	Yes	Yes	Yes	Yes	No	No
Cēsis	Previous crop	WR	SBT	SB	G	G	SB	SB	P	WT
	Crop	SBT	SB	G	G	SB	SB	P	WT	RC
	Herbicide	No	Yes	No	No	No	Yes	Yes	No	Yes
Valka	Previous crop	WR	SBT	SB	WR	WR	SB	F	WR	P
	Crop	SBT	SB	WR	WR	SB	F	WR	P	SB
	Herbicide	No	No	No	No	No	No	No	No	Yes
Valmiera	Previous crop	P	WW	SW	P	SW	SO	SO	SW	TRC
	Crop	WW	SW	P	SW	SO	SO	SW	TRC	TRC
	Herbicide	No	No	No	No	No	Yes	No	No	No
Limbaži	Previous crop	WW	P	SO	SB	SO	WW	WR	SO	WW
	Crop	P	SO	SB	SO	WW	WR	SO	WW	SO
	Herbicide	No	Yes	No	No	No	No	No	No	No

Crop Codes: SB — spring barley; SO — spring oats; SW — spring wheat; WW — winter wheat; WR — winter rye; WT — winter triticale; P — potato; SBT — sugar beet; G — grass; RC — red clover; TRC — timothy & red clover; F — fallow; UN — uncropped.

From 1994 to 2002, growing weeds were assessed in observation fields at the beginning of July, when cereal crops were at the inflorescence stage. The occurrence of each non-crop (weed) species within a 200 cm^2 frame was recorded at 50 points in each field. Plant density (plants m^{-2}) were calculated from the percentage frequencies for each species by the method of A. Rasiņš and M. Tauriņa (1982). Density correction factors of x1.5 for *Spergula arvensis* and *Stellaria media* and of x3 for *Elymus repens* were applied as recommended by A. Rasiņš and M. Tauriņa. Weed species (Lejiņš et al., 1997) were considered in five groups (Bāliņš et al., 1988): annual dicots, perennial dicots, non-grass monocots, annual grasses, perennial grasses. For some of the analyses, the three monocot groups were taken together because of the small number of species within this group (Tables 4—9). The numbers of plants and species were transformed to log10 (number + 1) for statistical analysis. The data were analysed with the aid of Microsoft Excel and GenStat for Windows.

Results and Discussion

Sixty-four species of weeds were recorded in this survey. Twenty species occurred in all five fields: 14 species occurred in only one field. No species was recorded in all five fields in all nine years. The highest number of species recorded in any field in any year was 32 (Valmiera 2000) and the lowest was 2 (Riga 1996) (Table 2). The highest plant density recorded was 305 plants m^{-2}, of which two-thirds were *Elymus repens* (Limbaži 1998); the lowest plant density was 5 m^{-2} (Riga 1996) (Table 3).

Thirty-six species of annual dicots were recorded: 13 occurred in all five fields; seven occurred in only one field. The most frequent species of annual dicot (as determined by frequency of occurrence in the 45 field-year observations) were: *Tripleurospermum inodorum* (36), *Chenopodium album* (34), *Polygonum convolvulus* (34), *Capsella bursa-pastoris* (27) and *Stellaria media* (26). These five species were also the most abundant overall, but *Galium aparine* (frequency 12/45) was the most abundant, with an average density of 11.2 plants m^{-2} where it occurred, compared to 10.5 plants m^{-2} for *Ch. album* and *S. media*. No annual dicots were found in the Cēsis field in 1997.

Twenty-three species of perennial dicots were recorded: five occurred in all five fields; five occurred in only one field. The most frequent species of perennial dicot (as determined by frequency of occurrence in the 45 field-year observations) were: *Cirsium arvense* (27), *Sonchus arvensis* (23), *Taraxacum officinale* (19), *Artemisia vulgaris* (18) and *Mentha arvensis* (18). *Linaria vulgaris* was, however, the most abundant perennial dicot overall because of the high density of plants it produced where it occurred, 20.2 m^{-2}, as compared to 8.8 m^{-2} for *S. arvensis* which was the next most abundant species in this group. No perennial dicots were found in the Rīga field in 1994 and 1996.

Table 2

The number of species of weeds in five observation fields, annually during 1994—2002

Region	Weed group	1994	1995	1996	1997	1998	1999	2000	2001	2002
	Annual dicot	10	6	2	13	12	15	14	19	10
	Perennial dicot	0	1	0	2	4	3	3	8	9
Rīga	Non grass	0	0	0	0	0	0	0	0	0
	Annual grass	0	0	0	0	1	1	1	1	2
	Perennial grass	1	1	0	1	1	1	1	1	1
	Annual dicot	8	11	1	0	8	8	6	11	3
	Perennial dicot	1	1	1	3	1	2	4	5	7
Cēsis	Non grass	0	0	0	0	0	0	0	1	0
	Annual grass	0	1	0	0	0	1	0	1	1
	Perennial grass	1	0	1	1	1	1	1	1	1
	Annual dicot	12	11	4	8	10	15	11	4	10
	Perennial dicot	2	1	2	9	8	10	6	4	7
Valka	Non grass	0	0	0	0	0	0	0	0	0
	Annual grass	0	0	0	0	0	0	1	0	0
	Perennial grass	0	0	0	0	1	1	1	1	0
	Annual dicot	15	12	8	18	19	17	20	3	6
	Perennial dicot	6	6	3	8	7	9	11	10	12
Valmiera	Non grass	1	0	0	0	0	0	0	0	0
	Annual grass	1	0	0	0	0	1	0	0	0
	Perennial grass	1	1	1	1	1	1	1	1	1
	Annual dicot	13	5	5	17	14	17	17	19	17
	Perennial dicot	3	3	2	7	6	6	5	8	11
Limbaži	Non grass	0	0	0	0	0	0	0	0	0
	Annual grass	0	0	0	0	0	0	0	1	1
	Perennial grass	1	1	0	1	1	1	1	1	1

Table 3

Weed density in five observation fields (plants m^{-2}), annually during 1994—2002

Region	Weed group	1994	1995	1996	1997	1998	1999	2000	2001	2002
	Annual dicot	94	23	5	83	47	60	61	90	34
	Perennial dicot	0	3	0	4	8	3	21	38	48
Rīga	Non grass	0	0	0	0	0	0	0	0	0
	Annual grass	0	0	0	0	7	4	2	3	3
	Perennial grass	0	24	0	21	3	24	72	84	159
	Annual dicot	20	41	6	0	12	13	34	58	11
	Perennial dicot	3	2	3	26	1	2	7	10	18
Cēsis	Non grass	0	0	0	0	0	0	0	8	0
	Annual grass	0	2	0	0	0	1	0	1	8
	Perennial grass	9	0	3	21	18	15	42	27	36
	Annual dicot	60	66	8	39	34	62	62	8	60
	Perennial dicot	2	1	7	28	21	57	42	8	9
Valka	Non grass	0	0	0	0	0	0	0	0	0
	Annual grass	0	0	0	0	0	0	1	0	0
	Perennial grass	0	0	0	0	6	33	21	6	0
	Annual dicot	87	52	18	127	97	120	177	4	28
	Perennial dicot	19	27	8	33	15	50	57	69	94
Valmiera	Non grass	1	0	0	0	0	0	0	0	0
	Annual grass	1	0	0	0	0	1	0	0	0
	Perennial grass	3	27	9	9	60	27	33	90	99
	Annual dicot	54	49	20	76	62	79	100	116	102
	Perennial dicot	59	5	19	47	49	31	70	70	43
Limbaži	Non grass	0	0	0	0	0	0	0	0	0
	Annual grass	0	0	0	0	0	0	0	3	3
	Perennial grass	132	6	0	27	99	195	30	51	9

Only five species of monocots were recorded. Two of these — *Elymus repens* (perennial grass) and *Poa annua* (annual grass) — occurred in all five fields, with frequencies of 36/45 and 11/45, respectively. *E. repens* was much the most abundant species of all the weeds recorded in the survey, producing an average of 42.5 plants m^{-2} where it occurred. For *P. annua* the average plant density was 2.4 m^{-2}. The non-grass monocot *Juncus bufonius* was recorded

only twice: Cēsis 2001 (8 plants m^{-2}), Valmiera 1994 (1 plant m^{-2}). The annual grasses *Apera spica-venti* and *Echinochloa crus-gali* were found only in the Rīga field, in 2002 and in 1998, 1999, and 2000, respectively.

To examine the main effects of fields and years, the total numbers of species and plants within each weed group were analysed by analysis of variance using the field x year interaction as an estimate of residual variance. There were statistically significant differences in the mean numbers of species among fields for all three weed groups analysed and for all species combined (Table 4). For annual dicots, the Cēsis field had significantly fewer species than the other four fields. For perennial dicots, the Rīga and Cēsis fields were significantly lower and the Valmiera field significantly higher than the average. The mean number of monocot species in the Valka field was significantly lower than in the other four fields. For all species together, the significant difference was due to the higher numbers in the Valmiera and Limbaži fields. There were significant differences among years in the mean numbers of species of all weed groups, mainly due to the lower numbers recorded in 1996 (Table 5). The numbers of species of annual dicots in other eight years were very similar, but low numbers of perennial dicots were recorded in 1994 and 1995 as well as in 1996. The mean numbers of monocot species recorded in the first four years were lower than in the subsequent five years.

Table 4

The mean number of species recorded in five observation fields, 1994—2002 (data transformed to log10 (number + 1))

Weed group	Rīga	Cēsis	Valka	Valmiera	Limbaži	LSD 5%
Annual dicot	1.06	0.78	0.95	1.10	1.15	0.226
Perennial dicot	0.52	0.52	0.74	0.93	0.79	0.140
Monocot	0.35	0.37	0.15	0.35	0.31	0.132
All species	1.18	1.04	1.16	1.35	1.31	0.144

Table 5

The mean number of species recorded in nine years in five observation fields (data transformed to log10 (number + 1))

Weed group	1994	1995	1996	1997	1998	1999	2000	2001	2002	LSD 5%
Annual dicot	1.11	1.02	0.65	0.92	1.13	1.18	1.15	0.98	0.94	0.304
Perennial dicot	0.45	0.47	0.37	0.79	0.74	0.79	0.80	0.89	1.00	0.188
Monocot	0.24	0.24	0.12	0.24	0.34	0.41	0.37	0.43	0.37	0.177
All species	1.20	1.13	0.79	1.21	1.29	1.35	1.32	1.28	1.30	0.193

There were statistically significant differences in the mean numbers of plants among fields for all three weed groups and for all species combined, but the patterns were different for each weed group (Table 6). For annual dicots, the mean numbers in the Cēsis field were significantly lower than in the other four fields. For perennial dicots, a significant difference was the higher mean numbers in the Valmiera and Limbaži fields. The mean numbers of monocots in the Valka field were significantly lower than in other fields. These patterns combined to give significantly higher mean numbers for all species in the Valmiera and Limbaži fields. The lower mean numbers of plants of all weed groups in 1996 made large contributions to the significant differences among years (Table 7). There were no other significant differences for annual dicots. For perennial dicots, the mean numbers of plants in the first three years were generally lower than in subsequent six years; the mean numbers in 2000, 2001 and 2002 were particularly high. For monocots, the mean numbers in the first three years were significantly lower than those in all of the last four years.

Table 6

The mean number of plants m^{-2} in five observation fields, 1994—2002 (data transformed to log10 (number + 1))

Weed group	Rīga	Cēsis	Valka	Valmiera	Limbaži	LSD 5%
Annual dicot	1.67	1.30	1.54	1.70	1.86	0.380
Perennial dicot	0.83	0.79	1.09	1.53	1.57	0.337
Monocot	1.25	1.20	0.51	1.44	1.45	0.457
All species	1.93	1.72	1.76	2.16	2.20	0.188

Table 7

The mean number of plants m^{-2} in nine years in five observation fields (data transformed to log10 (number + 1))

Weed group	1994	1995	1996	1997	1998	1999	2000	2001	2002	LSD 5%
Annual dicot	1.80	1.67	1.04	1.50	1.67	1.83	1.97	1.52	1.53	0.509
Perennial dicot	0.83	0.72	0.75	1.36	1.10	1.21	1.50	1.46	1.52	0.452
Monocot	0.78	0.83	0.32	1.03	1.39	1.60	1.58	1.61	1.40	0.613
All species	1.98	1.82	1.25	1.99	1.96	2.14	2.24	2.08	2.13	0.252

The overall patterns for mean numbers of species and mean numbers of plants show low values in 1996 and generally increasing values in later years of this survey. Preliminary analysis of data from the meteorological stations nearest to the five observation fields has indicated that the lower numbers of species and plants in 1996 may be associated with a period of cold, dry weather during the spring of that year. The generally higher numbers of species and plants in later years have been contributed by different weed groups in different fields. This increase cannot be simply related to any of the crop sequences or non-use of herbicides (Table 1). The progressive increase of *E. repens* (perennial grass weed group) in the Rīga field during later years of the survey may be related to a change from deep ploughing (25 cm) to shallow ploughing (15 cm). For the last two years of the survey, this field was not cultivated or cropped.

Because the numbers of contributing observations varied, the effects crop, previous crop and herbicide were examined by the method of residual maximum likelihood (REML), using a linear model. This technique allows the analysis of unbalanced datasets and produces output equivalent to the analysis of variance. The results in Table 8 must be interpreted with care because the extracted effects of crop are confounded with the effects of specific fields and specific years. The numbers of species of annual dicots were below the average in the crops of red clover (Cēsis 2002) and timothy and red clover (Valmiera 2001, 2002), but the numbers of annual dicot plants were lower only in the grass crops (Cēsis 1996, 1997). The numbers of annual dicot plants were highest in the crops of spring wheat (Rīga, 2000; Valmiera 1995, 1997, 2000), winter wheat (Valmiera 1994; Limbaži 1998, 2001), and spring oats (Valmiera 1998, 1999; Limbaži 1995, 1997, 2000, 2002). The lowest numbers of perennial dicots were in the crops of sugar beet (Cēsis 1994; Valka 1994) and spring barley (all fields except Valmiera, and all years except 2000 and 2001). Above average numbers of perennial dicot plants occurred in the timothy and red clover crops and in the fields that were fallowed or left uncropped and uncultivated. The lowest numbers of monocots were also found in the sugar beet and spring barley crops. The highest numbers of monocot plants were in the field that was uncropped (Rīga 2001, 2002) and the timothy and red clover crops (Valmiera 2001, 2002).

Table 8

Predicted mean number of species and plants m^{-2} in five observation fields in nine years classified by crop (REML analysis; data transformed to log10 (number + 1))

Crop	Number of observations	Mean number of species				Mean number of plants m^{-2}			
		Annual dicot	Perennial dicot	Monocot	All species	Annual dicot	Perennial dicot	Monocot	All species
SB	12	1.01	0.46	0.22	1.12	1.61	0.62	0.64	1.74
SO	6	1.20	0.88	0.36	1.37	1.96	1.48	1.36	2.18
SW	4	1.24	0.87	0.35	1.40	2.00	1.52	1.46	2.24
WW	3	1.23	0.88	0.46	1.41	1.98	1.62	1.51	2.27
WR	4	0.94	0.79	0.20	1.17	1.54	1.38	0.91	1.90
WT	1	1.11	0.78	0.60	1.32	1.86	1.04	1.57	2.06
P	5	0.91	0.58	0.30	1.09	1.44	1.04	1.40	1.82
SBT	2	1.06	0.39	0.15	1.13	1.62	0.54	0.50	1.71
G	2	1.05	0.45	0.30	0.65	0.42	1.02	0.97	1.40
RC	1	0.60	0.90	0.48	1.11	1.08	1.28	1.65	1.87
TRC	2	0.69	1.08	0.30	1.23	0.95	1.91	1.98	2.27
F	1	1.18	1.04	0.30	1.42	1.76	1.76	1.53	2.17
UN	2	1.17	0.98	0.54	1.42	1.75	1.64	2.08	2.36
average SED		0.175	0.215	0.151	0.174	0.312	0.371	0.577	0.311

Note: crop codes as in Table 1.

The numbers of perennial dicot and monocot plants were significantly associated with previous crop, but the numbers of annual dicot plants were not. The numbers of plants of perennial dicots were significantly lower where a herbicide had been applied: herbicide used 0.85, no herbicide used 1.27 (SED 0.175). There were no differences in the numbers of annual dicot plants: herbicide used 1.65, no herbicide 1.60 (SED 0.157); or in the numbers of monocot plants: herbicide used 1.09, no herbicide 1.20 (SED 0.234). The lack of effect on monocots was not unexpected because the herbicides applied by the farmers were active only against broad-leaved weeds. The lack of effect on the annual dicots was mainly due to the presence of large numbers of *G. aparine* plants in five of the 12 crops sprayed with herbicide (average abundance in infested crops — 16.2 plants m^{-2}) and to smaller numbers in seven of the 33 crops not sprayed with herbicide (average abundance in infested crops — 7.6 plants m^{2}). The herbicides chosen by the farmers for broad-leaved weed control were not effective against *G.aparine*. Adding previous crop to the crop model fitted to plant numbers had no significant effect on either groups of dicots, but gave a significant improvement for the monocots. Adding herbicide to the crop model gave no improvement for any weed group.

The relative importance of field, year, crop, previous crop and herbicide as factors to explain the observed variation in numbers of species and numbers of plants was determined by fitting simple linear regressions and multiple linear regressions to the data for each of the weed groups. The goodness of fit of the various models was assessed by

reference to the 'percentage variance accounted for' statistic (adjusted R^2, expressed as a percentage) produced with the analysis of variance for each fitted regression.

No one of the five factors (field, year, crop, previous crop, herbicide) best accounted for the observed variation in numbers of species and numbers of plants of all weed groups, but crop provided the best fit in five of the eight comparisons (Table 9). For species numbers, the percentage variance accounted for by the best fitting single factor ranged from 16.4% (year, monocots) to 59.7% (crop, annual dicots). For plant numbers, the percentage variance accounted for by the best fitting single factor ranged from 30.5% (previous crop, monocots) to 50.5% (crop, annual dicots). Adding previous crop and herbicide to the regression model for crop accounted for more variation in only four of the eight comparisons.

Table 9

Percentage variance accounted by linear models of crop and crop husbandry factors (data transformed to log10 (number + 1))

Factors in model	Mean number of species				Mean number of plants, m^{-2}			
	Annual dicot	Perennial dicot	Monocot	All species	Annual dicot	Perennial dicot	Monocot	All species
Simple linear models								
Field	13.1%	24.5%	14.1%	15.5%	8.4%	31.4%	18.3%	20.1%
Year	14.5%	39.5%	16.4%	35.9%	14.6%	17.2%	26.1%	42.8%
Crop	59.7%	40.8%	16.1%	40.6%	50.5%	49.6%	23.5%	26.5%
Previous crop	5.3%	13.0%	1.8%	<0	12.5%	26.1%	30.5%	7.1%
Herbicide	<0	1.6%	<0	<0	<0	9.9%	<0	0.5%
Multiple linear models								
Crop + Previous Crop	63.3%	23.9%	28.0%	35.3%	48.4%	52.2%	43.6%	22.2%
Crop + Previous crop + Herbicide	61.6%	21.7%	38.3%	33.1%	46.0%	49.9%	49.7%	20.5%
Optimal model *	78.1%	81.9%	47.2%	83.8%	65.9%	72.8%	80.8%	86.2%

* see text for details of factors in individual optimal models.

The optimal models of combinations of the main effects of the five factors were determined by stepwise multiple linear regression. The optimal models for species numbers and plant numbers of the weed groups were all different and accounted for 47% to 86% of the variance (Table 9). The eight optimal models are given below, with the factors listed in the order in which they were added to individual regressions:

Optimal models for numbers of species:

annual dicots: crop + previous crop + year + field
perennial dicots: crop + year + field + herbicide
monocots: year + field + previous crop
all species: crop + year + field + previous crop + herbicide

Optimal models for numbers of plants:

annual dicots: crop + year + previous crop + herbicide
perennial dicots: crop + field + year
monocots: previous crop + year + field + herbicide
all species year + previous crop + herbicide + field

As with the REML analysis, these optimal regression models must be interpreted with care because the effects of crop and previous crop are confounded with specific fields and specific years. For annual dicots and perennial dicots, crop was the principal factor for both numbers of species and numbers of plants. Year and field predominated among the second and third order factors, indicating the importance of season and location respectively. The numbers of species of monocots were very small, but were related primarily to the year and field. The numbers of monocot plants, which were much higher, were related primarily to previous crop. The perennial grass *E. repens* was the most abundant monocot and its numbers were greatly affected by the changes from winter cereals to spring cereals that occurred twice in the Limbaži field and by the Riga field being left uncropped for two consecutive years.

Conclusions

Sixty-four non-crop species were recorded: 20 species occurred in all five fields; 14 species occurred in only one field; no species occurred in all five fields in all nine years. The lowest number of species recorded in any field in any year was 2; the highest was 32. The numbers of plants of non-crop species ranged from 5 $m^{"2}$ to 305 $m^{"2}$, with large annual fluctuations in all fields. The numbers in all fields were lowest in 1996.

There were significant differences among fields and among years for numbers of species and numbers of plants of all weed groups. The numbers of annual dicot plants were highest in crops of spring wheat, winter wheat and spring oats, and lowest in a grass crop. The numbers of perennial dicots and monocots vvere lowest in crops of sugar beet and spring barlev, and highest in a crop of timothy and red clover and in fields that were fallowed or uncropped.

Numbers of perennial dicot and monocot plants vvere significantly associated vvith previous crop, būt numbers of annual dicots vvere not. The numbers of perennial dicot plants were lovver where a herbicide had been used, būt there vvere no differences in the numbers of annual dicot and monocot plants.

Regression analysis shovved that no one of the five factors (field, year, crop, previous crop, herbicide) best accounted for the observed variation in numbers of species and numbers of plants of all weed groups, būt, overall, crop accounted for the most variation (16% to 60% of variance accounted for). Adding previous crop and herbicide to the regression modei accounted for more variation in only four of the eight comparisons. The optimal models for species numbers and plant numbers of the weed groups were all different būt accounted for 47% to 86% of the variance.

References

1. Bāliņš, M., Āboliņš, J., Lapiņš, D., Resnais, A., Bērziņš, A., Lejiņš, A. 1988. Latvijas PSR izplatītākās nezāles, graudaugu un kartupeļu slimības. Latvijas PSR Valsts Agrorūpnieciskā komiteja. Republikāniskā augu aizsardzības stacija, Rīga, 176 lpp.
2. Lapiņš, D. 1999. Dynamics of weediness in Latvia during last fifty years. Proceedings of the Scientific Conference of Baltic States Agricultural Universities — Agroecological optimization of husbandry technologies. Jelgava, Latvia University of Agriculture, 211—218.
3. Lapiņš, D., Bērziņš, A., Koroļova, J., Sprincina, A. 2002. Dynamics of weed level in spring grain sowings in Kurzeme and Zemgale. Proceedings in Agronomy, No. 4, Jelgava, Latvia University of Agriculture, 97—101.
4. Lejiņš, A., Āboliņš, J. 2000. The weediness and its changes in fields of Eastern regions of Latvia. Proceedings of the International Conference — Development of environmentally friendly plant protection in the Baltic region, Tartu, Estonia, 103—106.
5. Lejiņš, A., Rasiņš, A., Āboliņš, J., Gavrilova, G., Lapiņš, D., Ozols, J., Vimba, E. 1997. Nezāļu, to grupu un augu aizsardzības tehnikas terminoloģijas vārdnīca. LRZM, LVZZPI "Agra", Skrīveri, 302 lpp.
6. Rasiņš, A., Tauriņa, M. 1982. Nezāļu kvantitatīvās uzskaites metodika Latvijas PSR apstākļos. Ieteikumi. LM ZTIP, Rīga, 24 lpp.
7. Rasiņš, A., Tauriņa, M., Lejiņš, A., Rimicāne, D. 1985. Nezāļu racionālas uzskaites un apkarošanas problēmas Latvijas PSR apstākļos. ZTI Padomju Latvijas Lauksaimniecība, Nr. 6, 20—25.
8. Vanaga, I. 2002. Changes of weed flora in arable fields in central Latvia and prospects for control with herbicides. Proceedings Crop Protection in Northern Britain 2002, 33—40.
9. Рубенис, Е., Лапиньш, Д., Берзиньш, А., Вадоне, Д., Леиньш, А., Леиня, Б., Страмкале, В., Туманс, В. 1995. Засоренность посевов крестьянских хозяйств в разных регионах Латвии. Труды межд. конф. — Проблема зосоренности в Балтийском регионе в современных условиях сельского хозяйства, Каунас, Литовская сельскохозяйственная академия, 226—232.

APPLICATION OF SYNTHETIC AUXINS HERBICIDES IN SPRING BARLEY AT REDUCED DOSAGES

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Abstract

Field trials were carried out at the Research and Study Farm "Vecauce" of the Latvia University of Agriculture (LLU) during 2001—2003. Spring barley cv 'Ansis' was grown under different soil properties, weed infestation levels, and weather conditions. Herbicides MCPA (MCPA 750 SL, BASF, full dosage 2 L ha^{-1}) and dichlorprop-P + mecoprop-P + MCPA (Duplosan Super 600 SL, BASF, dichlorprop-P 310 g L^{-1}, mecoprop-P 130 g L^{-1}, MCPA 160 g L^{-1}, full dosage 2 L ha^{-1}) were applied at three dosages: a full recommended dosage, one half, and one quarter of a full recommended dosage. Data show that there are no significant ($P < 95\%$) decreases in control of common lambsquarter (*Chenopodium album* L.) and field pennycress (*Thlaspi arvense* L.) by decreasing the dosages of applied herbicides. Applied dosages could be reduced twice using MCPA against field violet (*Viola arvensis* (L.) Murr.) and dichlorprop-P + mecoprop-P + MCPA against speedwell (*Veronica arvensis* L.). Reduction of applied dosages of these synthetic auxins made no significant ($P < 95\%$) changes in spring barley grain yield. There were no significant differences in grain yield between untreated plots and treatments with herbicide application in three years' average, which shows high impact of other factors on spring barley grain yield.

Key words: synthetic auxins, herbicides, spring barley, reduced dosages, weeds, grain yield.

Introduction

Organic herbicides began to be produced in earnest with dinitrophenol compounds in 1932. A breakthrough occurred in the 1940s with 2,4-D (2,4-dichlorophenoxyacetic acid), a compound similar to plant hormones, which is a highly selective systemic herbicide when used in very small quantities. 2,4-D was quickly adopted to control broad-leaved weeds in corn, sorghum, small grains, and grass pastures, as well as in lawns and other ornamental turf. The phenoxyaliphatic acids and their derivatives, another major group of organic herbicides, succeeded because of their selectivity and ease of translocation. MCPA ((4-chloro-2-metylfenoxy) acetyl acid) first appeared on the market in 1946 under trade name 'Agrokson' (Cobb, 1992). In the 1960s and 1970s, a combination of 2,4-D and 2,4,5-T was widely used in Vietnam as a defoliant under the name Agent Orange. As a result of questions concerning the possible health effects of the use of Agent Orange, heightened awareness of possible ecological and health dangers attributable to herbicides has resulted in reevaluation of many compounds and has called indiscriminate use into question. Use of the dioxin-containing 2,4,5-T was prohibited in the United States in 1984[1].

However, despite years of intense study, the details of the mechanism of action of these herbicides remain elusive. It is known that they bind to auxin receptors in plant cells, triggering a series of spontaneous effects that quickly alter gene regulation and disrupt normal plant growth (Sterling, Hall, 1997). These herbicides change nutrient transport in phloem and its active ingredients accumulate in the main growing points (Cobb, 1992). There are results from the trial with dichlorprop-P + mecoprop-P + MCPA application at dosage 2.5 L ha^{-1} in spring barley that two hours after spraying, dichlorprop-P concentration in spring barley leaves was 8.0 mg kg^{-1}. 29 days after application, dichlorprop-P was found in ears in concentration 1.75 mg kg^{-1}. Before harvesting (68 days after spraying), dichlorprop-P was not found in grains but its concentration in soil was 2.0 mg kg^{-1}. Whereas mecoprop-P and MCPA were not found neither two hours after spraying nor 29 or 68 days after it (Zikova el al., 1997).

Resistance to these herbicides is uncommon, considering their history of intensive use in cereal cropping systems. First occasion of resistance was observed already in 1952 in Canada, Ontario. Till nowadays there are discovered 24 against synthetic auxins herbicides resistant weed biotypes[2]. Ironically, the availability of these resistant plants and other auxins-resistant mutants provides useful research tools to study both the mechanism of action of the herbicides and the resistance mechanisms (Devine, Shukla, 2000).

Synthetic auxins herbicides MCPA and dichlorprop-P + mecoprop-P + MCPA are one of the most popular herbicides in Latvia mainly because of its low price. Reducing applying dosages will decrease inputs and allows farmers to produce cheaper production. The question is how such practice effects weed control and cereals yield. Many investigations show that applied doses of synthetic auxins herbicides could be reduced at appropriate conditions — suitable weather conditions, susceptible weed species, weeds mainly in cotyledon stage, crop stands with strong competitiveness (Bostrom, Fogelfors, 2002; Lundkvist, 1997; Salonen, 1993a).

Materials and Methods

Field trials were carried out at the Research and Study Farm "Vecauce" of the Latvia University of Agriculture (LLU) from 2001 to 2003. Soil properties were different in all trial years: sandy loam podzolic soil, humus content

[1] http://bartleby.com/65/he/herbicid.html (06. 02. 2004)
[2] http://www.weedscience.org/summary/MOASummary.asp (07. 02. 2004.)

31 g kg^{-1}, pH_{KCl} 7.1, content of phosphorus 253 mg kg^{-1}, content of potassium 198 mg kg^{-1} (2001); loam podzolic soil, humus 20 g kg^{-1}, pH_{KCl} 6.8, content of phosphorus 98 mg kg^{-1}, content of potassium 186 mg kg^{-1} (2002); sandy loam sod carbonate leached soil, humus content 21 g kg^{-1}, pH_{KCl} 6.8, content of phosphorus 85 mg kg^{-1}, content of potassium 77 mg kg^{-1} (2003). The trial was arranged in 4 replications, plot size 25 m^2. Spring barley cv 'Ansis' was grown after caraway (2001), maize (2002), and potatoes (2003). Soil tillage was traditional: autumn ploughing and presowing tillage with rotortiller "Amazone KG – 452". Spring barley was sown on April 30, 2001, April 18, 2002 and April 29, 2003 with trial sowing machine "Hege 80". Sowing rate — 400 fertile seeds per m^2. Mineral fertilizers were used before sowing for 6 t ha^{-1} high yields according to calculation using soil agrochemical properties. Herbicide application was done at the spring barley tillering stage (GS 21-29 by Zadoks). Two synthetic auxins herbicides — MCPA (MCPA 750 SL, BASF, full dosage 2 L ha^{-1}) and dichlorprop-P + mecoprop-P + MCPA (Duplosan Super 600 SL, BASF, dichlorprop-P 310 g L^{-1}, mecoprop-P 130 g L^{-1}, MCPA 160 g L^{-1}, full dosage 2 L ha^{-1}) were applied at three dosages: a full recommended dosage, one half, and one quarter of a full recommended dosage (hereafter 1/1, 1/2 and 1/4). Weed assessments were done three times: first time — before spraying, second — on the 6th week after spraying, third — before harvesting. Assaying was done using a 0.25 m^2 big circle in three places per plot identifying weed species and counting the number of weeds per species and in the second assay measuring weeds fresh weight. First two assessments were done in fixed places. The yield was harvested on 16 August 2001, 9 August 2002 and 7 August 2003 by trial harvester "Hege 140" and was adjusted to 86% dry matter content and 100% purity. Data analysis for significance was done by ANOVA. Interactions between factors were calculated using correlation-regression analyses.

Meteorological conditions were different in all trial years. Spring 2001 was late but weather conditions at spraying time were optimal. After spraying, the beginning of June was cool and wet but from the end of June air temperature was very high. It was accompanied with a lot of precipitation, which called serious lodging of spring barley. Spring of 2002 was early, which allows to sow barley quite early but suitable weather conditions caused also massive weed germination. At the time of spraying, weather conditions were not favorable — high air humidity and small rain 2 hours after spraying — what could effect herbicide efficacy. Spring 2003, like in year 2001, was late. Plants suffered from water deficit, which was caused by extremely dry autumn in 2002 and lack of precipitation in spring 2003. From the end of July very hot and dry weather started what forced barley maturation. Barley was yielded in due time, and strong rainfalls in the middle of August did not affect the barley yield.

Results and Discussion

Weed infestation in spring barley in 2001 was medium to low — on average 57.5 weeds per square meter. The most widespread species were common lambsquarter (*Chenopodium album* L.) (37.7% of total population), small nettle (*Urtica urens* L.) (25.2%), field pennycress (*Thlaspi arvense* L.) (14.6%), and speedwell (*Veronica arvensis* L.) (6.1%). Almost 60% of all weeds were in 2—4 true leave stage at the time of spraying. In year 2002, weed infestation was very big — on average 364.3 weeds per square meter. The dominant weed was field violet (*Viola arvensis* (L.) Murr.) — on average 54% of total population. Widespread were also dead-nettle (*Lamium purpureum* L.) (11.5%), common chickweed (*Stellaria media* (L.) Vill.) (10.8 %), common lambsquarter (7.3%), and field pennycress (4.4%). More than 50% from weeds were in 2 true leave stage before spraying. Weed infestation was considerable also in year 2003 — 187.3 weeds per square meter and the dominant weed again was field violet — 68.1% of total population. Approximately 70% of weeds were in cotyledon stage before herbicide application, so spraying was done in this year in the most suitable time.

Herbicide efficacy was estimated by the years and by herbicides and weed species separately. Calculations included weed species with distribution of at least one plant per square meter. Only common lambsquarter (hereafter CHEAL — codes according to Bayer (1992)), dead-nettle (LAMPU) un field pennycress (THLAR) were distributed on average more than one plant per square meter before spraying in all trial years.

Results from first weed accounting show no significant differences (at probability level 95%) in weed number among treatments for all the most common weed species. That allows to conclude that trials were settled in places with homogenous weed infestation in all trial area.

Data from second weed accounting show that application of MCPA has significant ($p < 0.05$) effect on the control of CHEAL, THLAR, common chickweed (STEME), and field violet (VIOAR). No differences were observed among applied dosages of MCPA in the number and fresh weight of CHEAL and THLAR in all trial years. Significant ($p < 0.05$) differences among applied dosages of MCPA we can find in the number and fresh weight of VIOAR (Table 1). In both years, application of full recommended dosage has significant better effect on the reduction of the number and fresh weight of VIOAR compared to 1/4 dosage (except fresh weight of VIOAR in year 2003). Whereas 1/2 dosage has no statistically provable differences from the effect of full dosage but has given significant reduction in the number and fresh weight of VIOAR compared to untreated. We can find no significant differences in control of STEME in year 2003, which can be explained with a very small amount of this weed compared to 2002.

Table 1

The number and fresh weight of weeds six weeks after application of herbicide MCPA

Year	Weed		Untreated	MCPA 1/1	MCPA 1/2	MCPA 1/4	$\gamma_{0.05}$
2002	STEME	number per m^2	0*	0	0	0	–
		weight, g m^{-2}	83.0	11.1	22.4	29.9	44.71
	VIOAR	number per m^2	286.3	62.7	184.3	207.3	109.74
		weight, g m^{-2}	116.2	13.0	44.9	66.1	43.15
2003	STEME	number per m^2	7.3	2.0	1.7	6.7	4.53
		weight, g m^{-2}	2.5	0.3	0.2	1.3	3.00
	VIOAR	number per m^2	152.0	22.0	59.0	105.7	67.52
		weight, g m^{-2}	31.3	1.4	5.5	12.0	19.20

* the number of STEME per m^2 was not possible to determine in year 2002.

Application of MCPA has also shown a statistically significant reduction in the number and fresh weight of shepherd's purse (*Capsella bursa-pastoris* (L.) Med.; CAPBP) in 2002 but this was the only year when this weed was observed in a notable amount. The same effect has been shown by herbicide dichlorprop-P + mecoprop-P + MCPA.

Reduction in weeds fresh weight better characterizes efficacy of the herbicide. Data of weeds fresh weight control are presented in Fig. 1.

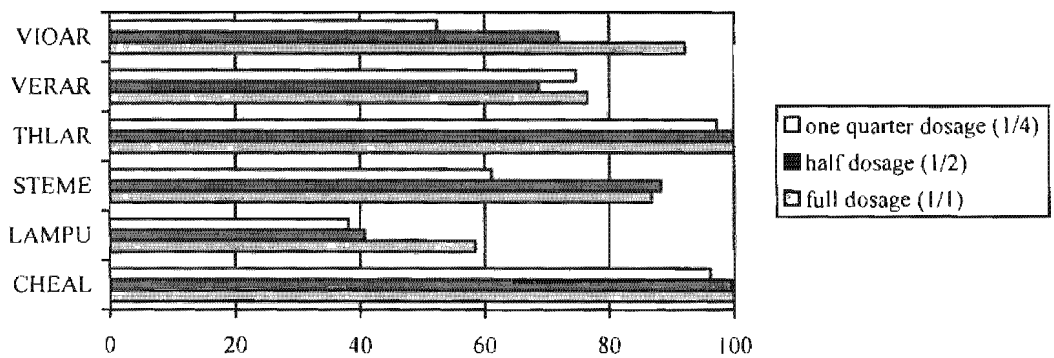

Fig. 1. Weed control by MCPA, average in three years (except VIOAR — average in two years), %

CHEAL was the only weed the number of which per m^2 differed significantly among treatments before harvesting — in all treatments it was smaller than in untreated plots. Such coherence can be observed in all trial years and for both tested herbicides.

Table 2

The number and fresh weight of weeds six weeks after application of herbicide dichlorprop-P + mecoprop-P + MCPA

Year	Weed		Untreated	Dichlorprop-P + mecoprop-P + MCPA 1/1	Dichlorprop-P + mecoprop-P + MCPA 1/2	Dichlorprop-P + mecoprop-P + MCPA 1/4	$\gamma_{0.05}$
2001	VERAR	number per m^2	9.3	0.7	2.3	6.3	6.12
		weight, g m^{-2}	1.9	0.0	0.2	0.4	1.22
2002	VIOAR	number per m^2	286.3	187.0	262.3	270.7	149.84
		weight, g m^{-2}	116.2	70.6	93.8	108.8	78.98
2003	VERAR	number per m^2	9.3	1.0	2.0	4.0	6.01
		weight, g m^{-2}	1.9	0.1	0.1	0.5	1.57
	VIOAR	number per m^2	152.0	21.7	55.0	89.0	61.17
		weight, g m^{-2}	31.3	1.3	5.3	11.7	19.52

A similar situation is with efficacy of herbicide dichlorprop-P + mecoprop-P + MCPA (Fig. 2). Also this herbicide shows good control of CHEAL, THLAR and STEME, but not so good control of VIOAR. Reduction in the number and fresh weight of VIOAR in 2002 was insignificant ($p>0.05$). It had better effect on speedwell (*Veronica arvensis* L.; VERAR) than MCPA (Table 2).

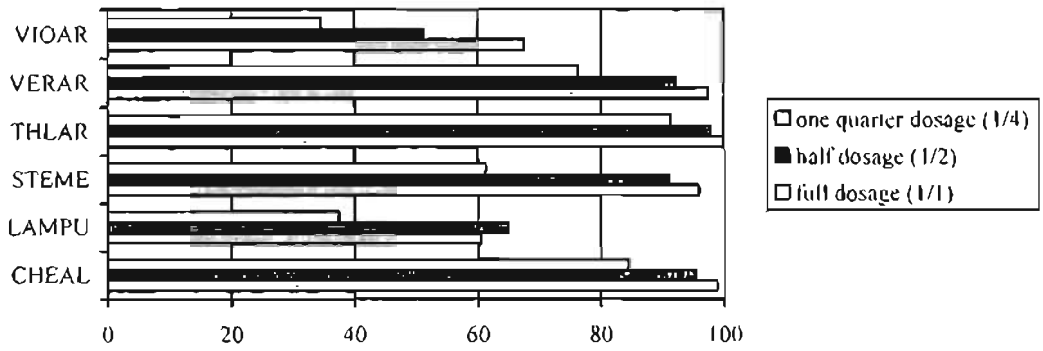

Fig. 2. Weed control by dichlorprop-P + mecoprop-P + MCPA, average in three years (except STEME, VERAR and VIOAR — average in two years), %

The yield of spring barley was within the predicted level — on average more than 6 t ha^{-1}. Also untreated plots gave high yield, which shows the great role of good quality seed material, precise sowing, an adequate amount of mineral fertilizers, and high capacity of competitiveness of spring barley.

Table 3

The yield of spring barley grain in treatments with MCPA and dichlorprop-P + mecoprop-P + MCPA, t ha^{-1}

Treatment	Year			
	2001	2002	2003	Average
Untreated	6.05	5.22	7.41	6.22
MCPA 1/1	6.09	5.74	7.84	6.56
MCPA 1/2	6.02	5.71	7.69	6.47
MCPA 1/4	5.98	5.49	7.76	6.41
Dichlorprop-P + mecoprop-P + MCPA 1/1	6.08	5.74	7.50	6.44
Dichlorprop-P + mecoprop-P + MCPA 1/2	6.05	5.53	7.57	6.38
Dichlorprop-P + mecoprop-P + MCPA 1/4	6.18	5.42	7.71	6.44

There were no significant differences in yields among treatments in years 2001 and 2002 ($p > 0.05$), whereas yield differences were significant ($p < 0.036$) in 2003 for herbicide MCPA — $\gamma_{0.05} = 0.287$. This means that all treatments except MCPA 1/2 dosage gave higher yield than untreated plots but they have no significant differences among them (Table 3). Such results coincide with results presented by Salonen (1993a) that spring barley grain yields differ within 5% among treatments with herbicides and untreated plots. In this case, differences among yields are within 5.3% on average in three years. It is obvious that herbicide treatments have low influence on spring barley grain yield. According to results from other trials in Latvia (Malecka, Lapins, 1997), usage of herbicides has just 10.2% big density of impact on barley grain yield.

Courtney and Johnson (1986) have stated that herbicide application increases yields when the average weed infestation reaches 150 plants per square meter. Also in trials carried out by Vanaga (2001), statistically significant yield differences were observed when weed infestation reached 142 plants of dicotyledonous weeds per square meter and grain yield was 6 t ha^{-1}. These trials show that this threshold could be even higher — at least 200 plants per square meter, although in year 2002 when weed infestation exceeded 350 plants per square meter no significant differences were observed in grain yield. For sure, we have to take into mind the spectrum of weed species that may differ and therefore change such a hypothetical threshold.

Salonen (1993b) has found that the total biomass production of annual dicotyledonous weeds correlated only weakly ($r = 0.48$) with the total weed density. On the contrary, in this trial we can find strong and statistically significant correlation between the total weeds fresh weight and total weed density six weeks after spraying, except in treatments with herbicide dichlorprop-P + mecoprop-P + MCPA in year 2002 (Table 4). The relationship in treatments with MCPA (average for three years) is described by equation $y = 0.88917x + 80.50321$, but in treatments with dichlorprop-P + mecoprop-P + MCPA – $y = 0.90683x + 76.05141$.

Table 4

Linear relationships between the total number of weeds and total weeds fresh weight six weeks after spraying

Variables	MCPA				Dichlorprop-P + mecoprop-P + MCPA			
	2001	2002	2003	Average	2001	2002	2003	Average
r_{yx}	0.71	0.71	0.96	0.80	0.69	0.32	0.97	0.79
R^2, %	50.0	51.0	91.5	64.3	46.9	10.3	94.2	63.2
P, %	99.8	99.8	99.9	99.9	99.7	77.6	99.9	99.9

Statistically significant negative relationships between spring barley grain yield and weed infestation were observed just in some particular cases (Table 5).

Table 5

Linear relationships between spring barley grain yield and weed infestation, on average during 2001—2003, r_{yx}

Variables	MCPA	Dichlorprop-P + mecoprop-P + MCPA
Total number of weeds before spraying	–0.26	–0.46
Total number of weeds six weeks after spraying	–0.25	–0.47
Total weeds fresh weight six weeks after spraying	–0.41	–0.57
Total number of weeds before harvesting	–0.62	–0.64
Number of CHEAL six weeks after spraying	0.09	0.07
Fresh weight of CHEAL six weeks after spraying	–0.21	–0.26
Number of VIOAR six weeks after spraying	–0.25	–0.48
Fresh weight of VIOAR six weeks after spraying	–0.38	–0.59
	$r_{0.05} = 0.285$	

Such results about the effect of CHEAL on barley grain yield differ from those obtained by Vanaga (2001). In her trials, statistically provable negative correlation existed only at a high level of grain yield. In this trial, on the contrary, in all three years the grain yield was higher than 6 t ha^{-1}, but linear relationships between the grain yield and the number and fresh weight of CHEAL were inessential at probability level 95% for both applied herbicides.

Conclusions

The results allow to conclude that reduced dosages of synthetic auxins herbicides can be applied in spring barley if sowings are infested with target weeds of these herbicides. Such practice surely in most cases would not change the barley grain yield significantly because herbicide application has a comparatively low effect on it.

References

1. BAYER. 1992. Important crops of the world and their weeds. 2nd ed., Bayer AG, Leverkusen, 1681 pp.
2. Bostrom, U., Fogelfors, H. 2002. Long-term effects of herbicide-application strategies on weeds and yield in spring-sown cereals. Weed Science, 50: 2, 196—203.
3. Cobb, A. 1992. Herbicides and plant physiology. Chapman & Hall, London, 176 pp.
4. Courtney, A. D., Johnson, R. T. 1986. An assessment of weed population and yield response in spring barley subjected to a programme of reduced herbicide usage. In: Proceedings, EWRS symposium on economic weed control, Wageningen, Netherlands, 301—308.
5. Devine, M. D., Shukla, A. 2000. Altered target sites as a mechanism of herbicide resistance. Crop protection, 19, 881—889.
6. Lundkvist, A. 1997. Influence of weather on the efficacy of dichlorprop-P/MCPA and tribenuron-methyl. Weed research, 37, 361—371.
7. Małecka, S., Lapiņš, D. 1997. Influence of herbicides for the weediness of spring barley. In: Weed control in Baltic region. Proceedings of international conference, Jelgava, LUA, 232—235.
8. Salonen, J. 1993a. Performance of reduced herbicide doses in spring cereals. Agricultural Science in Finland, 2:6, 537—548.
9. Salonen, J. 1993b. Reducing herbicide use in spring cereal production. Agric. sci. Finl. 2: Supplement No. 2, 42 pp.
10. Sterling, T. M., Hall, J.C. 1997. Mechanism of action of natural auxins and the auxinic herbicides. In: Herbicide Activity: Toxicology, Biochemistry and Molecular Biology (eds. R. M. Roe, J. D. Burton and R. J. Kuhr), IOS Press, Amsterdam, 111—141.
11. Vanaga, I. 2001. Spring cereal sowings and soil infestation with *Chenopodium album* and its influence on the spring barley yield in distinctive weather conditions. In: Brighton crop protection conference, weeds. Proceedings of an international conference, Brighton, UK, Vol. 1, 313—316.
12. Zikova, S., Ipatova, T., Luta, I. 1997. Dynamics of the decomposition of the herbicide Duplosan super and its residues in the cereal crops and in soils in Latvia. In: Weed control in Baltic region. Proceedings of international conference, LUA, Jelgava, 24—28.

THE INFLUENCE OF ADJUVANTS ON THE EFFICACY OF NICOSULFURON IN MAIZE

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Abstract

The improvement of chemical method involves use of modern application techniques, synthesis of new herbicides and adjuvants. Adding adjuvants to tank mixtures of pesticides is common practice in many countries of the world. For Ukrainian agriculture, which is characterized by limited financial resources and high degree of dependence upon imported pesticides, the search for cheap and ecologically friendly adjuvants is of great importance. Use of tank mixtures of herbicides with a different mode of action is a possibility of optimization of chemical method of weed control. The efficacy of nicosulfuron was the highest when applied at early stages of weed plant development, when adequate level of weed control was achieved by applying nicosulfuron at the dose of 37.5 g a.i. ha^{-1}. Inclusion of ammonium nitrate to the spray solution of nicosulfuron allows reducing application rate by 25% without any loss of efficacy.

Key words: nicosulfuron, adjuvants, weeds, maize.

Introduction

Herbicides are an important element in modern systems of the protection agricultural crops against weeds. However, agricultural practices in the countries of EC tend to reduce the dependence of weed management upon herbicides (Lotz et al., 2002). First of all it is achieved not by the refusal of herbicides, but by means of improved techniques application, synthesis of new herbicides and adjuvants and accurately defined strategies of their application (Kudsk, Streibig, 2002; Jensen, 2004), as growth of the number of crops including maize is not possible without effective weed control (Barkaszi, 2004; Задорожний, 2001). In many countries application of adjuvants to tank mixtures of herbicides including oils and nitrogen fertilizers becomes more and more wide-spread (Dogan et al., 2002; Швартау, 2002).

For Ukrainian agriculture, which is characterized by limited financial resources and high degree of dependence upon imported pesticides, the search for cheap and ecologically friendly adjuvants is of great importance. These substances include biodegradable exopolysaccharides of microbial nature, which are capable of enhancing "sticking" ability of herbicides to weed plants (Воцелко et al., 2001).

The objective of this research was to investigate the influence of polysaccharide enposan and nitrogen fertilizers on the efficacy of nicosulfuron for possibility of optimization of weed control in maize under conditions of forest-steppe zone of Ukraine.

Materials and Methods

Field trials were conducted during 1998—2003 at the Feed Research Institute of UAAS. Soil was of grey wooded type with 2.2—2.4% o.m. content and pH 5.2—5.5.

The trials were carried out on plots with a size of 25.2 m^2 (4.2 × 6 m) and in four random replications. In the trials, nicosulfuron was used (Milagro, 40 g a.i. L^{-1}, Syngenta). Herbicides were applied when the crop and grass weeds were at 3—5 leaf-stage and broad-leaved weeds at the first true leaf-stage. Herbicides were applied with a knapsack sprayer. The spray volume in experiments was 250 l ha^{-1}. Efficacy of herbicides was assessed 30 days after treatment (DAT) by counting weeds in a 0.25 m^2 frame in four different randomly selected spots for each plot and at crop harvest by measuring the above-ground weed fresh weight. Data of the maize yield were subjected to the analysis of variance. Means were compared using Least Significant Difference test (LSD) at the 5% level. The influence of adjuvants — enposan and ammonium nitrate — on the efficacy of nicosulfuron in maize was studied in field trials in the conditions of forest-steppe zone of Ukraine.

Higher air temperatures characterized weather conditions in May throughout the trial years, compared to average long-term indices. The level of precipitation was lower than the average in this period except for May 2002 and June 2000 and 2002 (Table1).

Table 1

Mean daily temperature and monthly rainfall from April to June in 1998—2003

Year	Temperature (°C)		Precipitation (mm)	
	May	June	May	June
1998	13,4	18,9	42	41
1999	11,9	20,8	37	16
2000	14,8	17,2	49	92
2002	15,9	17,1	73	144
2003	18,5	17,0	30	28
Long-term mean	10.6	17.7	60	74

Results and Discussion

In 1998—2000, the efficacy of different application rates and possibility of application of reduced doses of nicosulfuron in maize was studied. Results of the experiment show that at the time of nicosulfuron application at T_1, plants *E.crus-galli* and *S. glauca* had 1—3 leaves and height — 1,5—2,5 cm, plants *C. album* and *P. scabrum* — 2,0—5,0 cm, *T.arvense* — height up to 1,5 cm. The overall weed infestation of maize, 30 DAT after application of 37.5 and 50 g a.i. ha^{-1} of nicosulfuron, was reduced by 78—84%. The efficiency of nicosulfuron at the first date of application (T_1) was 5—7% higher than at the second date of application (T_2). At later terms of application, sensibility to nicosulfuron of broad-leaved species, especially *C. album*, reduced. Under such conditions, at T_2 the best herbicide effect could be obtained only with the highest rate of herbicide (Table 2).

Table 2

The influence of time application on the efficacy of nicosulfuron in 1998—2000

Treatment	Rate, g a.i. ha^{-1}	Application time	Weed control, %					
			30 DAT			before harvest		
			1998	1999	2000	1998	1999	2000
Nicosulfuron	50	T_1	87	86	79	84	84	72
Nicosulfuron	37.5	T_1	82	76	67	81	71	61
Nicosulfuron	25	T_1	76	57	63	78	61	58
Nicosulfuron	50	T_2	73	82	78	76	82	61
Nicosulfuron	37.5	T_2	73	74	39	74	69	51
Nicosulfuron	25	T_2	62	51	18	63	60	25

The results of researches conducted during 2002—2003 showed that 30 DAT nicosulfuron 40 g a.i. ha^{-1} controlled 78—87% of weeds depending on the year of research. Application of 50 g a.i. ha^{-1} increased the level of weed control by 2—12%. Before maize harvesting, the weed mass was lower than 89—90% compared to untreated plots.

Differences in the efficacy of herbicide activity, to a great extent, can be explained by weather conditions during the experiment. Under conditions of high soil infestation with weed seeds during some of the years, the second wave of weeds appeared. As a result, number of weeds in of untreated plots increased from 49 to 460 plants m^2. These years before harvesting, weed mass in the plots treated with herbicides reduced by 62—94%. To some extent it can be said that the seasons showed effect on nicosulfuron application. Nicosulfuron has a favorable effect on maize yield (Table 3).

Table 3

The efficacy of nicosulfuron and the maize yield in 2002—2003

Treatment	Rate, g a.i. ha^{-1}	Weed control, %		Maize yield, t ha^{-1}
		30 DAT	before harvest	
		2002		
Untreated		0	0	3.52
Nicosulfuron	40	78	85	6,14
Nicosulfuron	50	90	94	6,52
		2003		
Untreated		0	0	4.78
Nicosulfuron	40	87	62	7,55
Nicosulfuron	50	89	67	7,28

The possibility of increasing effect of nicosulfuron by using ammonium nitrate and enposan as additive has been studied in 2002 and 2003. Observations 30 DAT showed that nicosulfuron alone controlled 46—82% of weeds. The 1.0% ammonium nitrate addition to nicosulfuron at 20—40 g a.i. ha^{-1} increased activity by 8—22% of the herbicide against weeds.

Thanks to enposan sticking properties, the herbicide activity of milagro against annual grass and broad leaf weeds in maize was enhanced. At the rate of application of 30 g a.i. ha^{-1}, the mixture of nicosulfuron + enposan ensured weed control at 74%. At harvest, the biomass of weeds in maize was 71% lower compared to control plots (Table 4). The obtained results show that it is possible to reduce the rate of application of nicosulfuron by 25% provided it is used with enposan without decrease in its herbicidal activity. No phytotoxic effect of this mixture on maize was observed.

Table 4

The influence of ammonium nitrate (AN) and enposan on the efficacy of nicosulfuron in maize for grain in 2002 and 2003

Treatment	Rate, g a.i. ha^{-1}	Weed control, %			Yield, t ha^{-1}
		30 DAT	before harvest	reduction biomass	
Untreated*		0	0	0	4,16
Nicosulfuron	40	82	59	74	6,71
Nicosulfuron	30	69	66	67	6,20
Nicosulfuron	20	46	44	56	5,16
Nicosulfuron + AN	40+ 1.0 %	90	79	91	7,19
Nicosulfuron + AN	30+ 1.0 %	77	67	78	6,86
Nicosulfuron + AN	20+ 1.0 %	68	49	68	6,13
Nicosulfuron + enposan	30+ 1.0 %	73	64	71	6,36

Use of ammonium nitrate and *enposan* as an additive contributes to improved and enhanced phytotoxicity of nicosulfuron by 4—14% on *Setaria glauca* and by 13—18% on *Chenopodium album* (Table 5).

Table 5

Efficacy of nicosulfuron against major species of weeds in 2002 and 2003

Treatment	Rate, g a.i. ha^{-1}	Weed control 30 DAT, %	
		SETPF	CHEAL
Untreated		0	0
Nicosulfuron	40	89	60
Nicosulfuron	30	65	55
Nicosulfuron	20	46	28
Nicosulfuron + AN	40+ 1.0 %	93	73
Nicosulfuron + AN	30+ 1.0 %	78	73
Nicosulfuron + AN	20+ 1.0 %	60	37
Nicosulfuron + enposan	30+ 1.0 %	74	68

Conclusions

The efficiency of nicosulfuron was the highest when it was applied at early stages of weed plant development. In this case, an adequate level of weed control can be achieved with the dose of nicosulfuron at 37.5 g a.i. ha^{-1}. Inclusion of ammonium nitrate and enposan to the spray solution of nicosulfuron allows reducing application rate by 25% without any loss of efficacy.

References

1. Barkaszi, L. 2004. Theoretical analysis of the relationship between harvest-time weed density and corn production profitability at different intensity levels. Herbologia, Vol. 5, No. 1, 103—111.
2. Dogan, M.N., Boz, O., Albay, F. 2002. Influence of some additives on the efficacy of nicosulfuron in maize and fenoxaprop-P-ethyl in wheat. In: Proceedings of 12th EWRS Symposium, Wageningen, The Netherlands, 174—175.
3. Jensen, J.E. 2004. Weed control: presence and future – the Danish view. Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz. Sonderheft XIX, 19—26.
4. Kudsk, P., Streibig, J.C. 2002. Herbicides — a double-edged sword? In: Proceedings of 12th EWRS Symposium, Wageningen, The Netherlands, 94—95.
5. Lotz, L.A.P., Van der Weide, R.Y., Horeman, G.H., Joosten, L.T.A. 2002. Weed management and policies: From prevention and precision technology to certification of individual farms. In: Proceedings of 12th EWRS Symposium, Wageningen, The Netherlands, 2—3.
6. Воцелко, С.К., Литвинчук, О.О., Токарчук, Л.В. 2001. Липко генні носії для системного захисту сільськогосподарських культур на основі мікробних полісахаридів. Наук. Вісник УжНУ. Серія: Біологія, 9, 147—149.
7. Задорожний, В.С. 2001. Регулювання бур'янів в посівах кукурудзи на силос. Корми і кормовиробництво, 47, 138—140.
8. Швартау, В.В. 2002. Напрями регулювання фітотоксичності гербіцидів за допомогою хімічних сполук. In: Мат.3-ї наук.-теор. конф. "Забур'яненість посівів та засоби і методи їх знищення", Київ, 147—157.

EFFECT OF REDUCED TILLAGE AND COVER CROPS ON WEED INFESTATION OF VEGETABLES

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Abstract

The effect of no-tillage cultivation system and soil mulching with cover crops (white mustard, spring vetch, tansy phacelia, oats) on weed infestation, yielding of onion and carrot was studied. Species composition and number of weeds on onion and carrot plantations were estimated. Annual weed species: *Matricaria chamomilla* L., *Senecio vulgaris* L., *Galinsoga parviflora* Cav., *Urtica urens* L., and *Chenopodium album* L. dominated in primary weed infestation on plots with onion and carrot. Application of plant mulches and conventional tillage limited weed infestation of vegetables. A significant increase in weeds number was noticed in objects without plant mulches on onion and carrot plantations. The total and marketable yield of onion was highest under conditions created by no-tillage cultivation system. A higher yield of carrot roots was harvested from objects with conventional tillage.

Key words: reduced tillage, plant mulches, weed infestation, yield of vegetables.

Introduction

During wintertime, the surface of soil, not covered by plants, is particularly susceptible to destructive activity of atmospheric factors. Inter-crop plants left on the field for the winter period can play main role in soil protection (Dzienia, 1990). Cover crop mulches retard erosion process, hinder weed emergence, add organic matter into the soil, prevent nitrogen leaching, reduce evaporation, and retain warmth (Hembry, Davies, 1994; Abdul-Baki et al., 1996). Some of inter-crop plants effectively limit weed infestation during the vegetation season. Rye is a valuable winter-hard cover crop efficient at reducing weed infestation (Liebl et al., 1992; Smeda, Weller, 1996). An intensive system of vegetable production causes changes in soil physical properties (Radecki, 1986). Employment of heavy machines and implements, which pass the field several times, increases soil compaction and decreases soil porosity, and thus can influence the growth of vegetable roots (Kesik, Konopinski, 1993). Application of a simplified soil cultivation system and plant mulches reduced labour requirements and greater nutrient recycling. In conservation tillage practices, inter-crop plants are important because they modify microclimate of the crop which influences pest populations and crop yield (Masiunas, 1998). A reduced soil cultivation system using cover crop mulches has also many disadvantages, which may include higher chemical input (desiccants and herbicides), potential pest carryover in residues, and enhancement of some diseases (Hoyt et al., 1994). The last studies (Kesik et al., 2000; Borowy et al., 2000) showed that simplified soil tillage and use of plant mulches is an effective sustainable production system which can be used for vegetable production.

The purpose of this experiment was to compare the effect of differentiated cultivation systems and cover crop mulches on the yield and weed infestation of onion and carrot.

Materials and Methods

A field experiment was conducted during 1998—2000 at the Agricultural Research Station Felin near Lublin, on lessive soil derived from silty medium loam. The cultivated plants were onion c.v. 'Wolska' and carrot c.v. 'Perfekcja'. In the experiment the following cultivation variants were compared: I. Pre-sowing soil cultivation system (no-tillage and conventional soil cultivation with spring ploughing); II. Plant mulches — cover crops (control — without plant mulch, white mustard, spring vetch, tansy phacelia, oats). Cover crop seeds were sown in the third decade of August. The plants were left in the field for the winter period. In spring, conventional soil cultivation system was applied to half of each cover plot. The second part of each plot was left without ploughing. Before seeding, 150 kg P_2O_5 and 200 kg K_2O per hectare were incorporated into the soil. Nitrogen was delivered in two doses, 75 kg N before seeding and second dose as top-dressing. Cultivation and herbicide applications were standard practices in conventional onion and carrot production. The statistical design was split-plots with four replications. The results were subject to analysis of variance at $\alpha = 0,05$.

Results and Discussion

In primary weed infestation of onion plantation, four short lasting weed species dominated: *Matricaria chamomilla* (16,0 plants·m^{-2}), *Senecio vulgaris* (11,4 plants·m^{-2}), *Urtica urens* (10,8 plants·m^{-2}), and *Galinsoga parviflora* (9,9 plants·m^{-2}). Among long lasting weed species, three of them — *Agropyron repens* (2,8 plants·m^{-2}), *Equisetum arvense* (0,7 plants·m^{-2}) and *Taraxacum officinale* (0,3 plants·m^{-2}) — dominated (Table 1). In the field experiment, pre-sowing soil cultivation system had a considerable influence on weed infestation of onion plantation. Irrespective of the soil cultivation methods, the number of weeds growing on 1 m^2 amounted to 71,7. Increase of primary weed infestation in objects with no-tillage cultivation (mean 90,7 plants·m^{-2}), compared to objects with conventional soil tillage (52,7 plants·m^{-2}), was observed. Increase of weed infestation on the field with no-tillage cultivation was also confirmed by Szymankiewicz (1995).

Table 1

The effect of soil cultivation method and plant mulches on primary weed infestation of an onion plantation, mean during 1998—2000 (in plants·m^2)

Weed species	Conventional soil tillage						No-tillage						Mean
	control	white mustard	spring vetch	tansy phacelia	oats	mean	control	white mustard	spring vetch	tansy phacelia	oats	mean	
Short lasting weeds:													
Matricaria chamomilla	23,9	18,2	9,4	4,4	5,3	12,2	62,3	3,8	3,3	15,8	14,0	19,8	16,0
Senecio vulgaris	12,0	4,0	7,1	2,2	9,3	6,9	20,9	5,8	15,7	32,7	4,2	15,9	11,4
Urtica urens	6,2	10,3	2,2	6,9	5,5	10,2	4,2	14,9	12,9	6,0	18,9	11,4	10,8
Galinsoga parviflora	11,8	9,8	8,2	1,3	7,8	7,8	20,7	24,3	3,5	8,0	4,0	12,1	9,9
Capsella bursa-pastoris	1,8	0,6	2,0	1,3	2,5	1,6	23,7	0,7	2,0	16,2	5,3	9,6	5,6
Chenopodium album	6,0	1,7	2,4	4,9	4,7	3,9	7,8	4,0	3,2	3,8	5,3	4,8	4,4
Galinsoga hispida	6,6	2,9	1,5	2,0	4,2	3,4	7,1	2,2	2,7	3,8	2,5	3,7	3,6
Sinapis alba	0,0	7,1	0,0	0,0	0,0	1,4	0,0	19,8	0,0	0,0	0,0	4,0	2,7
Tripleurospermum inodorum	3,9	0,7	1,1	0,7	1,1	1,5	13,4	0,4	3,1	0,2	1,6	3,7	2,6
Echinochloa crus-galli	2,2	0,7	0,9	2,0	1,3	1,4	0,7	2,7	4,9	4,2	1,8	2,9	2,1
Poa annua	2,4	2,9	0,7	0,2	0,9	1,4	3,2	0,4	1,9	0,0	0,5	1,2	1,3
Stellaria media	1,0	0,7	0,4	0,9	0,9	0,8	2,2	0,9	0,9	1,1	3,3	1,7	1,2
Total	77,8	59,6	55,9	26,8	43,5	52,7	166,2	79,9	54,1	91,8	61,4	90,7	71,7
Long lasting weeds:													
Agropyron repens	1,8	1,8	3,1	2,0	3,8	2,5	6,8	4,2	0,5	1,8	2,4	3,1	2,8
Equisetum arvense	1,1	2,0	0,2	1,1	1,3	1,1	0,0	0,0	0,0	1,6	0,0	0,3	0,7
Taraxacum officinale	0,2	0,0	0,0	0,0	0,5	0,1	0,7	0,4	0,0	0,7	0,5	0,5	0,3
Cirsium arvense	0,0	0,3	0,0	0,0	0,0	0,1	0,2	0,0	0,0	0,0	0,0	0,0	0,1
Plantago maior	0,2	0,0	0,0	0,2	0,0	0,1	0,2	0,3	0,0	0,0	0,0	0,1	0,1
Total	3,3	4,1	3,3	3,3	5,6	3,9	7,9	4,9	0,5	4,1	2,9	4,1	4,0
Total number of weeds	81,1	63,7	59,2	30,1	49,1	56,6	174,1	84,8	54,6	95,9	64,3	94,7	75,7

Table 2

The effect of soil cultivation method and plant mulches on primary weed infestation of a carrot plantation, mean during 1998—2000 (in plants·m^2)

Weed species	Conventional soil tillage						No-tillage						Mean
	control	white mustard	spring vetch	tansy phacelia	oats	mean	control	white mustard	spring vetch	tansy phacelia	oats	mean	
Short lasting weeds:													
Matricaria chamomilla	10,7	0,0	0,0	0,0	5,3	3,2	13,4	0,4	0,0	0,0	7,6	4,3	3,7
Senecio vulgaris	10,1	1,1	3,7	9,1	9,3	6,7	20,0	2,5	42,6	18,9	13,1	23,4	15,0
Urtica urens	4,1	2,2	2,3	1,1	1,8	2,3	5,4	0,0	3,8	0,2	0,0	1,9	2,1
Galinsoga parviflora	6,2	0,7	1,1	0,0	0,7	1,7	10,4	3,1	4,9	0,2	1,6	4,0	2,9
Capsella bursa-pastoris	1,3	0,7	0,4	1,3	1,3	1,0	4,5	1,4	0,4	0,0	2,4	1,7	1,4
Chenopodium album	2,7	0,2	1,3	0,7	2,9	1,6	11,7	1,0	6,2	0,7	0,9	4,1	2,8
Galinsoga hispida	1,8	0,7	0,0	0,9	0,4	0,8	4,4	0,0	1,3	0,9	1,1	1,5	1,2
Sinapis alba	0,0	3,6	0,0	0,0	0,0	0,7	0,0	11,1	0,0	0,0	0,0	2,2	1,5
Tripleurospermum inodorum	1,7	1,3	0,0	2,9	1,1	1,4	5,0	1,1	1,6	1,1	2,2	2,2	1,8
Echinochloa crus-galli	0,3	0,4	0,4	1,3	0,0	0,5	0,6	2,2	3,3	4,5	0,0	2,1	1,3
Poa annua	1,0	0,0	0,0	0,0	0,0	0,2	2,3	0,9	0,0	0,0	0,0	0,6	0,4
Polygonum persicaria	0,1	0,0	0,6	0,2	0,4	0,3	1,5	0,4	0,0	0,0	0,7	0,5	0,4
Total	40,0	10,9	9,8	17,5	23,2	20,3	79,2	44,1	64,1	26,5	29,6	48,7	34,5
Long lasting weeds:													
Agropyron repens	2,9	4,5	1,3	1,5	0,0	2,0	7,0	4,7	2,0	0,5	4,0	3,6	2,8
Equisetum arvense	0,4	0,0	0,0	0,0	0,0	0,1	0,0	0,0	0,0	0,0	0,4	0,1	0,1
Sonchus arvensis	0,3	0,0	0,0	0,0	0,0	0,1	2,4	1,1	0,0	0,0	0,0	0,7	0,4
Convolvulus arvensis	0,0	0,0	0,0	0,0	0,0	0,0	0,2	0,2	0,2	0,0	0,0	0,1	0,1
Cirsium arvense	1,0	0,0	0,0	0,7	0,0	0,3	2,1	1,1	0,0	0,4	2,2	1,2	0,8
Taraxacum officinale	0,0	0,0	0,0	0,0	0,0	0,0	1,4	0,2	0,0	0,2	0,0	0,4	0,2
Plantago maior	0,0	0,0	0,0	0,0	0,0	0,0	1,1	0,0	0,0	0,0	0,7	0,4	0,2
Total	4,6	4,5	1,3	2,2	0,0	2,5	14,2	7,3	2,2	1,1	7,3	6,4	4,5
Total number of weeds	44,6	15,4	11,1	19,7	23,2	22,8	93,4	51,4	66,3	27,6	36,9	55,1	39,0

Application of plant mulches received from cover crops limited the number of weeds on the onion plantation. Decrease in the number of weeds was found on plots with tansy phacelia mulch and conventional tillage (26,8 plants·m^{-2} compared to control — 77,8 plants·m^{-2}), and also on plots with spring vetch mulch and no-tillage cultivation system (54,1 plants·m^{-2} compared to control — 166,2 plants·m^{-2}). Profitable effect of cover crop use on the decrease of weed infestation in vegetable cultivation was observed also by Borowy et al. (1999). Differentiated soil cultivation systems and plant mulches did not have a significant influence on the occurrence of long lasting weed species. But the lowest number of long lasting weeds in the objects with conventional and reduced soil tillage was observed under spring vetch mulch (3,3 and 0,5 plants·m^{-2}, respectively).

In primary weed infestation of carrot plantation, irrespective of the cultivation system and plant mulches, the following short lasting weed species: *Senecio vulgaris* (15,0 plants·m^{-2}), *Matricaria chamomilla* (3,7 plants·m^{-2}), *Galinsoga parviflora* (2,9 plants·m^{-2}) and *Chenopodium album* (2,8 plants·m^{-2}) dominated (Table 2). Mean number of weeds in objects with conventional tillage amounted to 20,3 plants·m^{-2}, while in objects with reduced soil tillage the number of weed species amounted to 48,7 plants·m^{-2}. Application of spring vetch mulch in combination with conventional soil tillage decreased the number of weeds (9,8 plants·m^{-2}) compared to control (40,0). In objects with reduced soil tillage, tansy phacelia mulch most of all limited weed infestation (26,5 plants·m^{-2}).

Among long lasting weed species, *Agropyron repens* (mean 2,8 plants·m^{-2}), *Cirsium arvense* (0,8 plants·m^{-2}) and *Sonchus arvensis* (0,4 plants·m^{-2}) dominated. Irrespective of differentiated plant mulches, a higher number of long lasting weeds (mean 6,4 plants·m^{-2}) was noticed in objects with a reduced soil cultivation system, compared to conventional soil tillage (2,5 plants·m^{-2}). Application of cover crops in cultivation of carrot limited occurrence of long lasting weed species. Oats and spring vetch mulches, in combination with conventional soil tillage and tansy phacelia mulch, together with reduced soil tillage had the biggest influence on the decrease of the number of long lasting weeds.

The cultivation method of plants significantly influenced the total and marketable yield of onion and carrot. Onion produced higher yields on plots with conventional soil tillage. Total yield of onion on average amounted to 48,6 t·ha^{-1}, whereas the yield in objects with no-tillage was lower by 5,6 t·ha^{-1} (Table 3). Also marketable yield of onion was significantly higher on plots with conventional tillage (41,7 t·ha^{-1}) than on plots with no-tillage cultivation system (37,0 t·ha^{-1}). Irrespective of soil cultivation methods, the plant mulches had a significant influence on the yield of onion. The highest total and marketable yield was harvested from plots with spring vetch mulch (49,1 and 43,7 t·ha^{-1}, respectively). The lowest total and marketable yield was noticed on plots with white mustard mulch (39,4 and 32,7 t·ha^{-1}, respectively).

Table 3

The effect of soil cultivation method and plant mulches on total and marketable yield of onion, mean during 1998—2000 (in t·ha^{-1})

Plant mulches	Total yield			Marketable yield		
	conventional soil tillage	no tillage	mean	conventional soil tillage	no tillage	Mean
Control	47,2	44,6	45,9	40,8	38,6	39,7
White mustard	40,7	38,0	39,4	34,2	31,1	32,7
Spring vetch	51,7	46,5	49,1	44,6	42,8	43,7
Tansy phacelia	50,5	46,8	48,7	42,8	39,9	41,4
Oats	52,7	38,9	45,8	45,9	32,8	39,4
Mean	48,6	43,0	45,8	41,7	37,0	39,4
$LSD_{(0,05)}$ for:	soil tillage		2,0			2,1
	plant mulches		4,5			4,7

The yield of carrot roots was significantly higher in objects with conventional soil tillage. Total yield of carrot roots on average amounted to 76,5 t·ha^{-1}, whereas in no-tillage cultivation system — to 69,4 t·ha^{-1} (Table 4). Similarly, the marketable yield was bigger on plots with conventional tillage (45,0 t·ha^{-1}) compared to reduced soil tillage (37,6 t·ha^{-1}). Irrespective of soil cultivation methods, the highest total and marketable yield of carrot roots was harvested from plots with tansy phacelia mulch (76,4 and 44 t·ha^{-1}, respectively). White mustard mulch limited the yielding of carrots. Another result with carrot cultivation by obtained Borowy et al. (2000); in a field experiment a higher yield of carrot roots was obtained from objects with no-tillage cultivation system. It is necessary to mark that the weather conditions during the vegetation season considerably influenced the onion and carrot yields.

Table 4

The effect of soil cultivation method and plant mulches on total and marketable yield of carrot roots, mean during 1998—2000 (in $t \cdot ha^{-1}$)

Plant mulches	Total yield			Marketable yield		
	conventional soil tillage	no tillage	mean	conventional soil tillage	no tillage	mean
Control	78,0	69,8	73,9	46,0	33,9	40,0
White mustard	68,5	68,2	68,4	40,2	37,1	38,7
Spring vetch	75,5	70,8	73,2	43,0	43,0	43,0
Tansy phacelia	79,6	73,2	76,4	45,6	43,2	44,4
Oats	80,9	65,2	73,1	50,3	30,7	40,5
Mean	76,5	69,4	73,0	45,0	37,6	41,3
$LSD_{(0,05)}$ for:	soil tillage		2,5			2,3
	plant mulches		5,7			5,1

Conclusions

1. In primary weed infestation of onion and carrot plantations, five weed species *Matricaria chamomilla* L., *Senecio vulgaris* L., *Urtica urens* L., *Galinsoga parviflora* Cav. and *Agropyron repens* Beauv. dominated.
2. Conventional soil tillage with spring ploughing considerably limited the weed infestation on onion and carrot plantations compared to no-tillage cultivation system.
3. Application of plant mulches received from cover crops limited the number of weeds on onion and carrot plantations. Among investigated mulches, tansy phacelia and spring vetch had profitable influence on the decrease of the number of weeds.
4. The yield of onion and carrot roots was significantly higher in objects with conventional soil tillage. Irrespective of soil cultivation methods, the highest total and marketable yield of onion was harvested from plots with spring vetch mulch, and of carrot roots – from objects with tansy phacelia mulch.

References

1. Abdul-Baki, A., Teasdale, J.R., Korcak, R., Chitwood, D.J., Huettel, R.N. 1996. Fresh-market tomato production in a low-input alternative system using cover crop mulch. HortScience, 31, 65—69.
2. Borowy, A., Jelonkiewicz, M. 1999. Zachwaszczenie oraz plonowanie osmiu gatunkow warzyw uprawianych metoda siewu bezposredniego w mulcz żytni. Zesz. Prob. Post. Nauk Rol., 466, 291—300.
3. Borowy, A., Konopinski, M., Jelonkiewicz, M. 2000. Effect of no-tillage and rye mulch on soil properties, weed infestation and yield of carrot and red beet. Annales AFPP, Dijon — France, 339—345.
4. Dzienia, S., Sosnowski, A. 1990. Uproszczenia w podstawowej uprawie roli, a wysokosc nakładow energii. Fragm. Agron., 3 (27) IV, 71—79.
5. Hembry, J.K., Davies, J.S. 1994. Using mulches for weed control and preventing leaching of nitrogen fertilizer. Acta Hort., 371, 311—316.
6. Hoyt, G.D., Monks, D.W., Monaco, T.J. 1994. Reviews. Conservation tillage for vegetable production. HortTechnology, 4 (2), 129—135.
7. Kesik, T., Konopinski, M. 1993. Effect of some agrotechnic factors on soil properties, yield and some physical features of carrot. Zesz. Prob. PNR, 399, 113—118.
8. Kesik, T., Konopinski, M., Błazewicz-Wozniak, M. 2000. Weed infestation and yield of onion and carrot under no-tillage cultivation using four cover crops. Annales AFPP, Dijon — France, 437—444.
9. Liebl, R., Simmons, F.W., Wax, L.M., Stoller, E.W. 1992. Effect of Rye (Secale cereale) Mulch on Weed Control and Soil Moisture in Soybean (Glycine max). Weed Technology, 6, 838—846.
10. Masiunas, J.B. 1998. Production of vegetables using cover crop and living mulches — a review. Journal of Vegetable Crop Production, 4 (1), 11—31.
11. Radecki, A. 1986. Studia nad mozliwoscia zastosowania siewu bezposredniego na czarnych ziemiach właściwych. Rozprawy naukowe i monografie. SGGW AR Warszawa, 1—86.
12. Smeda, R.J., Weller, S.C. 1996. Potential of rye (Secale cereale L.) for weed management in transplanted tomatoes (Lycopersicon esculentum). Weed Sci., 44, 596—602.
13. Szymankiewicz, K. 1995. Sposoby uprawy roli a zachwaszczenie kukurydzy uprawianej na ziarno w monokulturze. Mat. Konf. Nauk. „Siew bezposredni w teorii i praktyce", Szczecin-Barzkowice, 81—88.

WEED INFESTATION IN WHEAT SOWINGS IN CENTRAL AND WESTERN PART OF LATVIA

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Abstract

A weed infestation monitoring was carried out annually at the end of July in stationary observed areas in the Jelgava, Dobele, Saldus, Talsi, Tukums and Kuldiga districts of Latvia during 1994—2002. The weed infestation of sowings was determined using quantitative currency method developed by A. Rasiņš and M. Tauriņa (1989). The occurrence index was calculated for characterizing separate weed species and groups of weeds. Shannon biological diversity index was used as a complex indicator for weed infestation of sowings. Evaluation of weed infestation monitoring results was done using data ranging and grouping. To characterize dynamics of separate weed species, the index of changes of the number of weed species was used. Fisher criterion was applied for evaluating the influence of herbicides and crop rotation. Dominant weeds (> 5 p. m^{-2}) in winter wheat sowings were *Elytrigia repens*, *Viola* spp., *Stellaria media*, *Matricaria perforata*, *Polygonum* spp., and in spring wheat sowings — *Elytrigia repens*, *Lamium purpureum*, *Stellaria media* and *Fallopia convolvulus*. The number of weed species varied from 13 to 18 during the 5 investigation years. The number of weed species with occurrence over 50% decreased in winter wheat sowings, but the number of species with occurrence up to 10—20% increased. Shannon index for annual and perennial weeds tended to increase in winter wheat sowings. Biological diversity index in spring wheat sowings was higher for annual weed species than for perennial weeds — in annual weed species it increased from 0.69 in 1998 to 0.80 in 2002. Periodicity of dominant of weed species was established for *Polygonum* spp., which reproduces with seeds, and for winter weeds *Matricaria perforata* and *Viola* spp. The amplitude of cyclical changes in winter weeds was greater when initial level of weed infestation was higher. Changes in the number of perennial weeds *Elytrigia repens* and *Cirsium* spp. were not cyclical. The weed infestation in repeated spring wheat sowings was significantly higher than in sowings with crop rotation, especially for perennial weeds *Circium* spp. and *Elytrigia repens*. Differences in the weed infestation in repeated winter wheat sowings were insignificant, compared to sowings with crop rotation. The effect of years as a factor was higher than that of the pre-crop. The influence of herbicides was significant for occurrence of *Cirsium* spp. in winter wheat and for the number of *Sonchus arvensis* and *Myosotis* spp. in spring wheat.

Key words. wheat, weed infestation, monitoring of weeds.

Introduction

The importance of monitoring of weed infestation in sowings has already been discussed in previous publications of D. Lapinsh, J. Korolova, A. Berzinsh (2000), D. Lapiņš, A. Bērziņš, J. Koroļova, A. Sprincina (2002), I. Vanaga, D. Lapiņš, A. Bērziņš, J. Koroļova, A. Sprincina (2002). This has also been confirmed by research results in Finland (Salonen et al., 2001), Lithuania (Kavoliunaite et al., 2000), Estonia (Тойво, 1997), Belarus (Проpacов, 1995; Сорока, Романюк, 1997) and Russia (Ульянова, 1997; Кравченко, 1997). The analysis of long-term and annual observations of weed infestation of sowings weediness allows to determine the effect of crop rotation and chemical weed control compared to years as a factor of influence. Indexes are being more and more used for evaluating dynamics of weeds' biological diversity. The aim of the research was to analyze dynamics of weeds basing on the monitoring data in sowings in the Kurzeme and Zemgale regions of Latvia during 1998—2002.

Materials and Methods

The registration of weed infestation and data analysis methods

The weed infestation monitoring was carried out annually at the end of July in stationary observed areas in the Jelgava, Dobele, Saldus, Talsi, Tukums and Kuldiga districts starting from 1998. The observations were made on traditional and biological farms. Crop rotation and field areas were determined by the respective farmer. The weed infestation of sowings was determined using quantitative currency method developed by A. Rasiņš and M. Tauriņa (1989). The method is based on correlation between the occurrence of weed species in the field and the number of this weed species in 1 m^2 of field area. The invariability of the method allows to compare changes in weed infestation over a longer period of time (Лапиньш, 1999).

The observations were made using 200 cm^{-2} frames at particular field points. Determination of weed species was done in 100 places and, if the field area was less than 20 ha, in 50 places. From these data, the occurrence of weed species (%) was calculated, and then the number of weed species in 1 m^2 was determined.

Table 1

Areas of crop sowings observed in 1998—2002

Crops	Area, ha				
	1998	1999	2000	2001	2002
Spring wheat	83	176	166	109	60
Winter wheat	205	191.5	190	329.5	144

Simultaneously with evaluation of weed infestation, data about herbicide use were collected. A precise list of herbicides was not obtained, because farmers quite often don't have their own plant protection technique.

The index (%) of occurrence of weed species or a group of weeds was calculated as weighted mean and was weighted against the total field area, where this species or group of weeds was observed:

$$Is_i'' = \frac{\sum_{m}^{1}(Pl \times x_i)}{\sum_{m}^{1} Pl}, \qquad (1)$$

where: Is_i'' — occurrence of weed species i in year n by A. Rasiņš's method;

i — symbol of weed species;

m — number of stationary observed areas for a particular crop in n year;

$\sum_{m}^{1} Pl$ — total field area for a particular crop, ha.

Shannon biological diversity index was used as a complex indicator of weed infestation of sowings, which gives evaluation of total weed infestation of sowings according to all observed characteristics — number of weed species, number of weeds, and total number of weeds. A greater mathematical value of the index corresponds to greater total weed infestation of sowings (Magurran, 1988).

One of weed infestation's indicators is the index of change for the number of weeds, which shows the dynamics of weediness in one year compared to the previous year:

$$I'_{izm} = \frac{X_i'' - X_i^{n-1}}{X_i^{n-1}}, \qquad (2)$$

I'_{izm} — index of change for the number of weeds of a particular weed species i,

X_i'' — number of weeds of a particular weed species i in n year,

X_i^{n-1} — number of weeds of a particular weed species i in $n-1$ year.

Analysis of the weed infestation was carried out using data ranging and grouping. The index of change for the number of weeds of a particular weed species was used to characterize the dynamics of dominating weed species. Fisher criterion was applied to evaluate the influence of herbicides and crop rotation.

The number of determined weed species, changes in weed structure and weed occurrence were evaluated to analyze the dynamics of weeds in stationary observed areas. Species of weeds were grouped in clusters where the number of weeds is less than 1 p. m^{-2}, 1—5 p.m^{-2}, and > 5 p.m^{-2}. Analysis of occurrence of weed species during 5 inspection years was carried out by grouping the weed species into five clusters: 1) occurrence > 50; 2) 40—50; 3) 30—40; 4) 20—30, and 5) occurrence of 10—20% of the field area.

Meteorological conditions

The growth of weeds was dependent on meteorological conditions. Good meteorological conditions for weed growth were from April to June in all investigation years when daily temperature was higher than average long term norm.

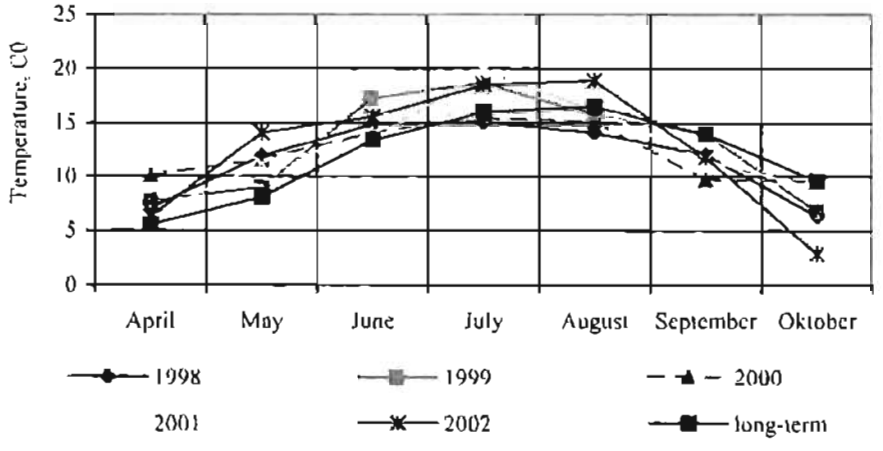

Fig. 1. The average daily temperature compared to long-term norm during 1998—2002

The precipitation was lower than norm in May and September in these years.

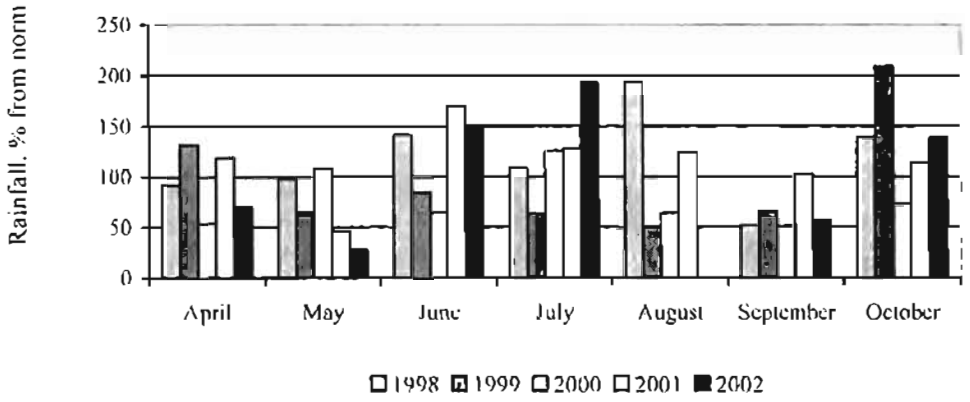

Fig. 2. The amount of precipitation, % from climatic norm, during the vegetation

Greatest deficit of precipitation was observed in August 2002 when average monthly rainfall was almost 0 mm. Greatest amounts of precipitation were observed in October 1999 — 209% above the norm. Much rainfall was also in July 2002, whereas April 2000 was the driest period. On the whole, the vegetation period of 1999 favored weed growth and development, as well as the effect of herbicides. The weather was cool with much precipitation throughout the second part of vegetation in 2000, which facilitated development of new weed shoots. Herbicides, sprayed at the end of May, had no effect on these weeds.

In 2001, the vegetation recovered on 3—5 April. Heavy rainfall in the second part of April delayed the field work, wheat was sown overdue, which might have effected occurrence of weeds. In May and June, the average air temperature was equal to average long-term indices. The amount of rainfall in these months: 72 mm in May and 99 mm in June, which makes 146% and 223% of the norm. Plants produced great vegetation mass that limited the growth of weeds. On the whole, the vegetation period in 2001 and 2002 favored weed growth and development.

Results and Discussion

The number and structure of weed species in wheat sowings

The number of weed species in winter wheat sowings varied within the range of 42—56 species in stationary observed areas during 1998—2002. Each second observation year demonstrated higher field pollution with different weed species (Table 2). The structure of weed species also varied – perennial weeds appeared to be more widespread. Higher variability of weed species was in 2001 — 56 weed species in winter wheat sowings. The lowest variability of weed species was observed in 2000 — in total 42 weed species. In 1998, 45 weed species were established in winter wheat sowings, three of which — *Elytrigia repens*, *Stellaria media* and *Viola* spp. — were of greatest occurrence > 15 p. m^{-2}. The structure of dominant weed species changed in 1999. The most widespread was *Elytrigia repens* — 107 pieces per 1 m^2. Then follow *Matricaria perforata* and *Apera spica-venti* – more than 15 p. m^2. The greatest quantity of weed species had the average number of weeds less than 1 piece per 1 m^2. Table 2 shows that the most significant was cluster with weed species > 5 p. m^2. The number of weed species decreased almost three times (from 8 to 3 weed species) during 1998—2002.

Table 2

The number of weed species in clusters by pieces in 1 m^2 in winter wheat sowings, 1998—2002

Clusters by pieces in m^2	Number of weeds				
	1998	1999	2000	2001	2002
< 1	27	39	29	38	32
> 1	18	15	13	18	15
of which: 1—5	10	7	9	12	12
> 5	8	8	4	6	3

Table 3

The number of weed species in clusters by pieces in m^2 in spring wheat sowings, 1998—2002

Clusters by pieces in m^2	Number of weed species				
	1998	1999	2000	2001	2002
<1	33	30	22	32	19
1—5	9	10	9	11	10
>5	4	3	6	4	2
>1	13	13	15	15	12
Total	46	43	37	47	31

In spring wheat sowings in Kurzeme—Zemgale stationary observed areas, species with average number of weeds per 1 m^2 less than one had the greatest variety (Table 3). The number of weed species in the cluster >1 piece per 1 m^2 was 12 in 2002 and 15 — in previous years. The number of weed species in the cluster >1 piece per 1 m^2 was relatively constant — 9—11during 1998—2002. Highest variety of weed species was in 2001.

In all observation years in winter wheat sowings, dominating perennial weeds were *Elytrigia repens* and *Cirsium* spp., dominant annual weeds — *Galium aparine, Lamium purpureum, Veronica* spp., *Chenopodium* spp., *Matricaria perforata, Euphorbia helioscopia*, and *Polygonum covolvulus. Lamium purpureum* and *Galium aparine* dominated in the cluster >5 p. m^{-2} during 1998—2001. Whereas *Polygonum convolvulus* and *Viola* spp. dominated during 2000—2002. During the last two years, *Solanum nigrum, Galeopsis* spp., *Taraxacum officinale* have been observed among dominating weed species. On the whole, four weed species had the highest occurrence in winter wheat sowings during five inspection years: *Elytrigia repens* — 47 p.m^2, *Galium aparine* — 7,7 p.m^{-2}, *Laminum purpureum* — 7,4 p.m^2, and *Polygonum convolvulus* — 6,5 p.m^{-2}.

In spring wheat sowings, thirteen species of weeds with occurrence >1 p. m^{-2} were established. *Elytrigia repens* dominated also in spring wheat sowings during all five years. The number of *Polygonum convolvulus* increased from 4,9 p.m^{-2} in 1998 to 8,2 p.m^{-2} in 2002. *Galium aparine* decreased from 10,6 p.m^{-2} in 1998 to 1,8 p.m^{-2} in 2002. The number of fourth dominant weed species *Laminum purpureum* varied from 12 in 1998 to 7,7 p.m^{-2} in 2000 spring wheat sowings, and then decreased to 3 p.m^{-2} in 2001–2002.

The occurrence of weed species in wheat sowings

The greatest number of weed species in winter wheat sowings was established in the cluster with occurrence up to 10% of field area during 1998—2002. The number of these species was very variable by years — 15—26 species. The number of weed species in the cluster 10—20% was 8—13, which tended to grow. A similar number of weed species was in the cluster with occurrence more than 50%, which tended to decrease — from 13 in 1998 to 6 species in 2002.

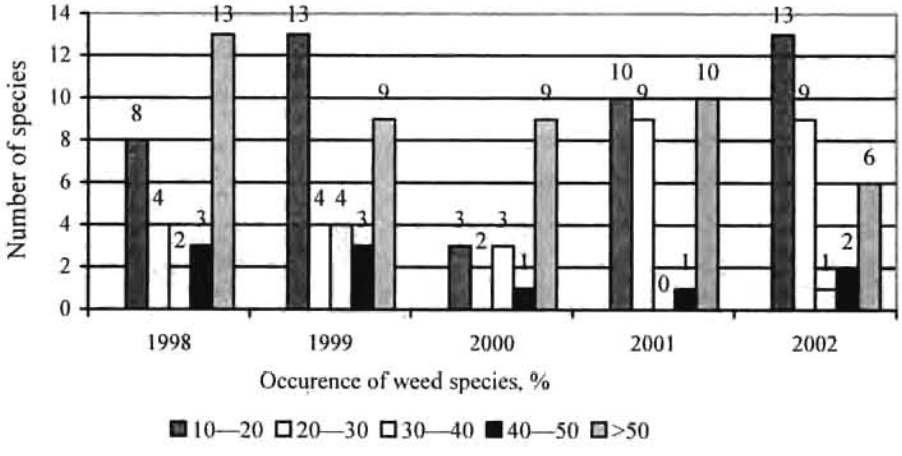

Fig. 3. The number of weed species compared to their occurrence in winter wheat sowings

The number of weed species in clusters with occurrence 20—30%, 30—40% and 40—50%, was not high. In winter wheat sowings, the number of weed species with occurrence greater than 50% decreased, while that of weeds with occurrence 10—20% — increased (Fig. 3).

Annual dominating weed species with occurrence >50% were *Viola* spp., *Matricaria perforata, Galium aparine, Stellaria media, Veronica* spp., *Polygonum covolvulus*, and *Polygonum* spp., perennial dominant weed species was *Elytrigia repens* and in some years also *Cirsium* spp. (Table 4).

The most common annual weeds with occurrence 20—30% in winter wheat sowings were *Consolida regalis, Capsella bursa- pastoris* and *Centaurea cyanus*, but the most common perennial weed – *Stachys palustris*. Other weed species had high variability of occurrence — within 10—50% during all five investigation years. In the cluster with

occurrence 10—20%, typical species were *Thlaspi arvense*, *Melandryum album*, and *Achillea millefolium*. The occurrence of *Consolida regalis*, *Myosotis* spp. and *Equisetum arvense* significantly decreased during 1999—2002.

Table 4

Occurrence of weed species in wheat sowings

Species of weeds	Occurrence, % from field area					On average, %
	1998	1999	2000	2001	2002	
Winter wheat						
Viola spp.	95.1	57.1	96	70	68.8	77.4
Polygonum spp.	66.3	86.3	54	90.7	73.6	74.1
Polygonum convulvolos	67.8	57	65	87.7	78.5	71.2
Elytrigia repens	75.1	82	82	72.5	39.1	70.1
Matricaria perforata	87.3	78	42	51.1	79.2	67.5
Galium aparine	82.9	57.6	67	69.9	42.4	64
Veronica spp.	75.6	52.1	78	54.6	51.4	62.3
Stellaria media	82.8	48.3	63	65.4	29.2	57.6
Cirsium spp.	82.4	87.8	31	53.3	28.5	56.6
Spring wheat						
Polygonum convulvolus	96.4	82.5	71	96.3	76.7	84.6
Galium aparine	80.7	75.8	74	98	45	74.6
Lamium purpureum	98.8	86	61	58.7	50	71
Veronica spp.	92.8	41.1	50	27.5	73.3	56.9
Euphorbia heloscopia	77.1	29.6	55	58.7	55	55
Polygonum spp.	78.3	88.2	50	28.4	25	54

Weed species with occurrence above 50% in spring wheat sowings decreased from 11 species in 1998 to 5 in 2002 (Fig. 4).

Annual weeds *Lamium purpureum*, *Polygonum convolvulus*, *Veronica* spp., *Galium aparine*, *Euphorbia helioscopi* were the most frequent species with occurrence > 50% in the observed spring wheat sowings. Perennial weed *Elytrigia repens* showed invariable occurrence (more than 50%) throughout all the investigation years.

Chenopodium spp. and *Polygonum* spp. were most dominating weed species in 1998, 1999 and 2000, with occurrence > 50% that decreased to 30—40% during the last investigation years. *Avena fatua*, *Capsella bursa — pastoris* and *Taraxacum officinale* exhibited high occurrence only in 1998.

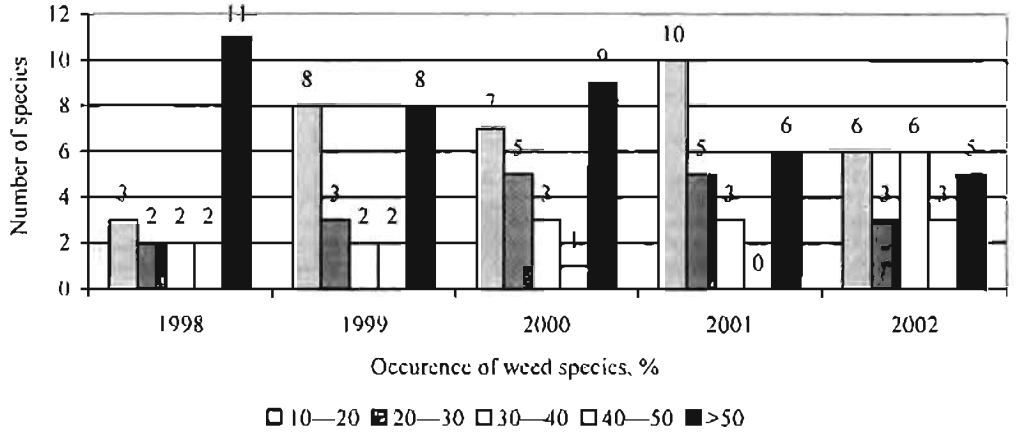

Fig. 4. The number of weed species compared to their occurrence in spring wheat sowings

The occurrence of *Stellaria media* and *Equisetum arvense* varied within the range of 20—40%, occurrence of *Galeopsis* spp., *Sonchus arvense* and *Convolvulus arvensis* within 10—30%, *Stachys palustris* and *Plantago* spp. — 10—20%. In 1998, annual weeds *Lamium purpureum*, *Polygonum convolvulus*, *Veronica* spp., *Galium aparine*, *Polygonum* spp., *Euphorbia helioscopia*, *Avena fatua*, *Capsella bursa — pastoris* and *Chenopodium* spp. demonstrated high occurrence (more than 50% of field area).

Complex evaluation of weed infestation variety

The highest Shannon index (0.72—0.79 on average) was established for annual weed species in winter wheat sowings during 1998–2002. The lowest Shannon index (0.68) was in 1999, but the highest (0.79) was in 2002, which shows that the index of annual weeds in winter wheat sowings tends to grow (Fig. 5).

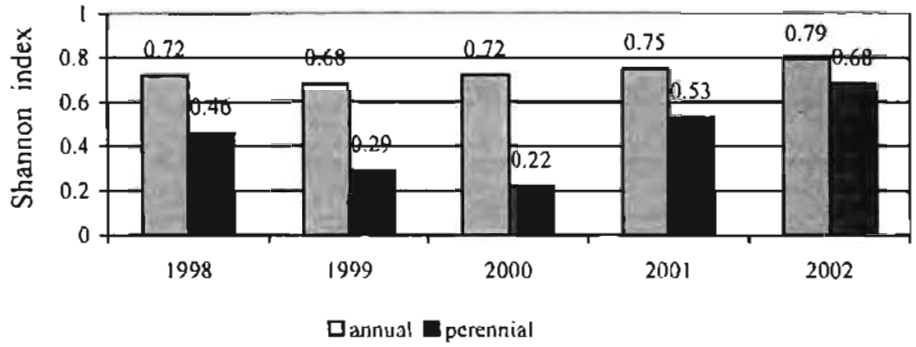

Fig. 5. Evaluation of winter wheat sowings with Shannon index

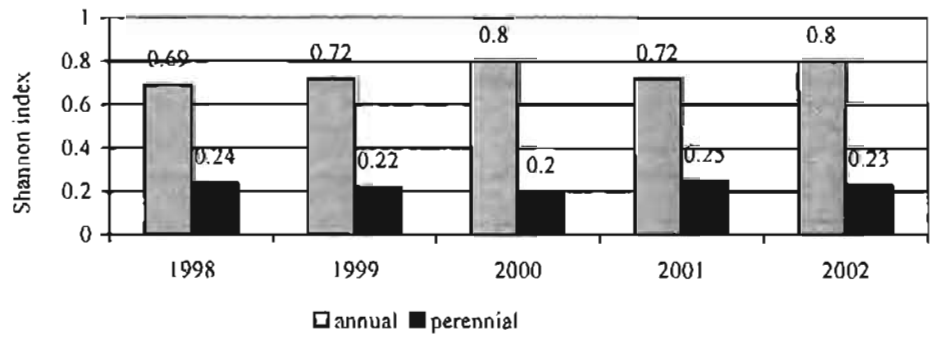

Fig. 6. Evaluation of spring wheat sowings with Shannon index

Shannon index for perennial weeds in winter wheat sowings ranged from 0.22 to 0.68 — it was very low in 1999 and 2000 (0.29 and 0.22, respectively), but the highest (0.68) in 2002.

Biological diversity index for annual weeds in spring wheat sowings was higher than for perennial weeds. Annual weed index increased from 0.69 in 1998 to 0.80 in 2002. Variance of Shannon index for annual weeds was 0.8 each year, but for perennial weeds it varied within 0.2—0.25.

Indexes of changes for dominant weeds in wheat sowings

It is possible to analyze indexes of changes in weed infestation more precisely in a longer time period, therefore additionally investigation results of the Department of Soil Management from earlier years (1994—1997) were used. The periodicity of change for the number of weeds was established for dominant weeds that reproduce with seeds — *Polygonum* spp., and for winter weeds *Matricaria perforate* and *Viola* spp. (Fig. 7). The amplitude of cyclic changes was higher when higher was the initial weed infestation in 1994, which is best demonstrated by the change in the number of two dominant weeds *Matricaria perforata* and *Viola* spp.

Changes in the indexes of perennial dominants *Elytrigia repens* and *Cirsium* spp. were not cyclic. The increase in these indexes during some years was dependent not only on soil tillage, but also on other meteorological and biological conditions.

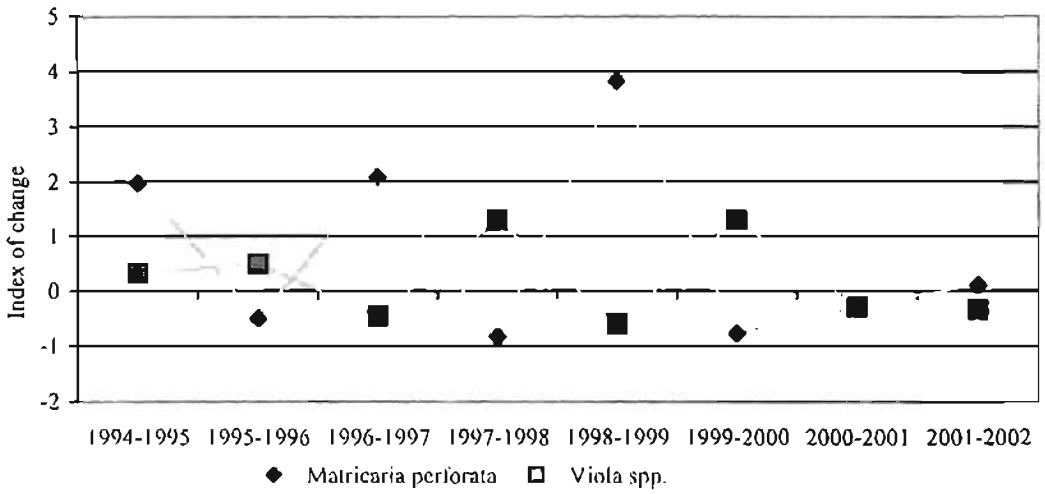

Fig. 7. Indexes of change for dominant winter weeds in winter wheat sowings

The periodicity of change for the number of weeds that reproduce with seeds in spring wheat sowings was not pronounced compared to winter wheat sowings. Dominant weed species were *Galium aparine* and *Lamium purpureum*, and also winter weeds *Matricaria perforata* and *Viola* spp. (Fig. 8). Low cyclic changes were established for *Galium aparine* and *Lamium purpureum* during 1996—2002. Changes in the number of perennial weeds *Elytrigia repens* and *Cirsium* spp. in spring wheat sowings don't have any agronomical explanation.

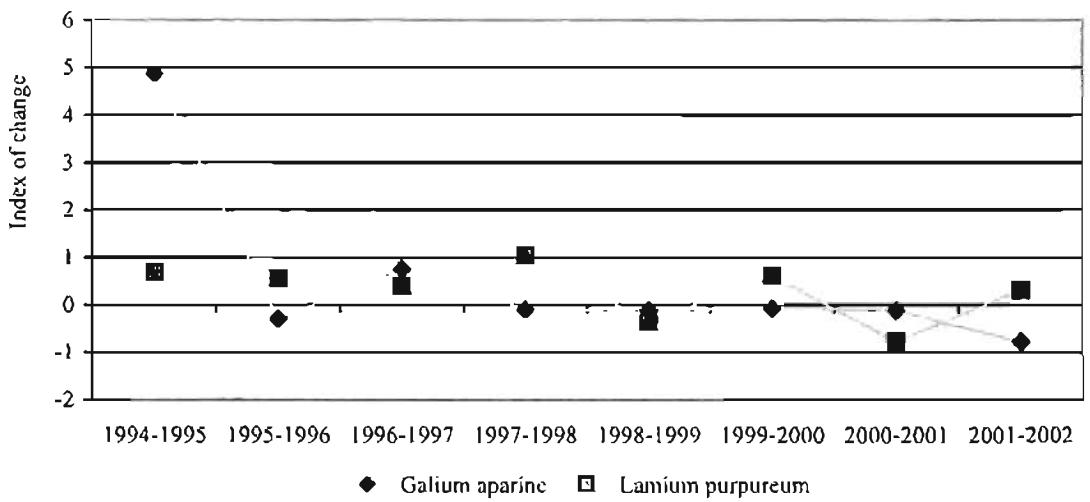

Fig. 8. Indexes of change for dominant weeds in spring wheat sowings

The effect of pre-crop on weed infestation in of wheat

The investigation results show that not always repeated sowings were the reason for a high level of weed infestation, especially in 2001 and 2002. The difference between the number of weeds in repeated winter wheat sowings and sowings with crop rotation was insignificant for all weeds.

Table 5

Weed infestation in of spring wheat in 2001 compared to crop rotation, $p.m^{-2}$

Species of weeds	Number of weeds, $p.m^{-2}$			Confidence, P%
	repeated sowings	crop rotation	±	
Cirsium spp.	9.33	0.83	8.5	96.4
Chenopodium spp.	8.33	0.33	8	91.8
Elytrigia repens	128	14	114	98.1
Polygonum convolvulus	2.33	10.17	-7.8	87.9
Matricaria perforata	3.00	1.00	2	70.6
Veronica spp.	2.67	1.5	1.2	39.7
Galium aparine	8.67	6.83	1.8	33.0
Polygonum spp.	2.33	1.33	1	19.1

In spring wheat sowings, the negative effect of repeated sowings was typical, also the number of weeds per 1 m^2 was greater than in sowings with crop rotation. A significant difference was established for *Cirsium* spp. and *Elytrigia repens*. The preplant as a factor of influence on weed infestation had low confidence probability (P%>90). Higher but insignificant levels of probability were established for *Galium aparine* and *Lamium purpureum*, but the lowest for *Veronica* spp.

The effect of herbicides

The weed infestation of wheat sowings was analyzed by comparing use of herbicides. High confidence probability (> 90%) was established to *Cirsium* spp., *Matricaria perforata*, and *Chenopodium* spp. Use of herbicides might be one of factors influencing occurrence of these weeds (Table 6). The confidence of difference for other dominant weeds *Veronica* spp., *Polygonum* spp., *Fallopia convolvulus*, *Viola* spp., *Stellaria media* was low.

Table 6

Weed infestation of winter wheat compared to use of herbicides, on average during 1995—2002

Species of weeds	Number of weeds, p. m^{-2}		±difference	Confidence, P%
	without herbicides	with herbicides		
Cirsium spp.	8	2.5	–5.5	97.1
Matricaria perforata	26.3	6.6	–19.7	90.5
Chenopodium spp.	4.5	1.2	–3.5	93.0
Sonchus arvensis	5.9	0.8	–5.2	83.4
Galium aparine	10.1	5.0	–5.1	84.2
Polygonum spp.	6.6	3.8	–2.8	84.1

Table 7

Weed infestation of spring wheat compared to use of herbicides, on average during 1995—2002

Species of weeds	Number of weeds, p. m^{-2}		±difference	Confidence, P%
	without herbicides	with herbicides		
Sonchus arvensis	9.6	0.4	–9.2	99.7
Myosotis spp.	3.4	0.3	–3.10	96.0
Stellaria media	23.1	9	–14.0	93.4
Galeopsis spp.	0.4	2.2	1.8	90.6
Cirsium spp.	14.1	3	–11.0	91.5
Chenopodium spp.	9.5	3.5	–6.0	89.0
Fallopia convolvulus	3.4	5.2	1.8	74.9

Differences in the number of *Myosotis* spp. and *Sonchus arvensis* were significant in spring wheat sowings. On the whole, the effect of herbicides on the number of weed species was low. A significant effect was established for *Cirsium* spp., *Sonchus arvensis* and *Myosotis* spp. The lowest probability was for weeds *Veronica* spp., *Viola* spp. and *Galium aparine*. Insufficient effect of herbicides could be explained only by wrong choice and inadequate use of herbicides.

Conclusions

In winter wheat sowings, dominant weed species with more than 5 pieces per 1 m^2 were *Elytrigia repens*, *Viola* spp., *Stellaria media*, *Matricaria perforata*, and *Polygonum* spp., in spring wheat sowings — *Elytrigia repens*, *Lamium purpureum*, *Stellaria media*, *Fallopia convolvulus*. The number of dominant weed species varied from 13 to 18 during the five investigation years. The number of weed species with occurrence over 50% decreased in winter wheat sowings, but the number of species with occurrence up to 10—20% increased during the investigations. Shannon index for annual and perennial weeds increased in winter wheat sowings. In spring wheat sowings, biological diversity index for annual species was higher (an increase from 0.69 in 1998 to 0.80 in 2002) than for perennial weeds. The periodicity of dominant weed species was established for *Polygonum* spp., which reproduces with seeds, and for winter weeds *Matricaria perforata* and *Viola* spp.

The amplitude of cyclic changes for winter weeds was greater when initial level of weed infestation was higher. The changes in the number of perennial weeds *Elytrigia repens* and *Cirsium* spp. were not cyclical.

The weed infestation in repeated spring wheat sowings was significantly higher than in sowings with crop rotation, especially for perennial weeds *Circium* spp. and *Elytrigia repens*. Differences in the weed infestation in repeated winter wheat sowings were insignificant, compared to sowings with crop rotation. The effect of years as a factor was higher than that of the pre-crop. The influence of herbicides was significant for occurrence of *Cirsium* spp. in winter wheat and for the number of *Sonchus arvensis* and *Myosotis* spp. in spring wheat.

References

1. Kavoliunaite, I., Monstvilaite, J., Šakaliene, O. 2000. Changes in weed flora and trends of herbology science under the present Lithuanian conditions. Proceedings of the International Conference, Tartu, Estonia, Sept. 28—29, 60—63.
2. Lapiņš, D., Bērziņš, A., Koroļova, J., Sprincina, A. 2002. Nezāļu skaita un sugu sastāva dinamika vasarāju labību sējumos Kurzemē un Zemgalē. Agronomijas Vēstis, Nr. 4, 97—106.
3. Lapinsh, D., Korolova, J., Berzinsh, A. 2000. The weediness of spring barley and wheat sowings in the districts of western Latvia. Development of enviromentally friendly plant protection in the Baltic Region: Proceedings of the International Conference, Tartu, Estonia, September 28—29, 94—97.
4. Magurran, A. E. 1988. Ecological Diversity and Its Measurement. New Jersey, Princeton University Press, 179 pp.
5. Rasiņš, A., Tauriņa, M. 1982. Nezāļu kvantitatīvās uzskaites metodika Latvijas PSR apstākļos. Ieteikumi. Rīga: LM ZTIP, 24 lpp.
6. Salonen, J., Hyvonen, T., Jalli, H. 2001. Weeds in spring cereal fields in Finland — a third survey. Agricultural and food science in Finland, N° 10, 347—364.
7. Vanaga, I., Lapiņš, D., Bērziņš, A., Koroļova, J., Sprincina, A. 2002. Dynamics of weed infestation in spring cereals in Latvia. Proceedings of 12th Symposium of European Weed research Society. Netherlands, Waageningen, 2002, 24—27. June, 316—317.
8. Кравченко, О. 1997. Заметки к современному состоянию сегетального элемента флори Ленинградской области. Труды международной конференции гербологов. Jelgava: LLU, 54—57.
9. Лапиньш, Д. 1999. Динамика количества и видовово состава сорных растений в Латвии за последние пятьдесять лет. Agroecological optimization of husbandry technologies: Scientific Conference of Baltic states. Jelgava: LLU, 8—10 July, 211—218.
10. Протасов, Н. 1995. Проблемы засоренности посевов в Балтийском регионе в современных условыях сельсково хозяйства. Труды международной конфоренции. Каунас-Академия, 354—358.
11. Сорока, С. Романнок, И. 1997. Увеличение засоренности посевов основных сельскохозяйственных культур в Беларуси. Труды международной конференции гербологов. Jelgava: LLU, 140—144.
12. Тоиво, К. 1997. Засоренность оставленных под залежь полей. Труды международной конференции гербологов. Jelgava: LLU, 183—185.
13. Ульянова, Т. 1997. Сорные растения Северозапода России. Труды международной конференции гербологов. Jelgava: LLU, 47—53.

POSSIBILITIES FOR USING TANK MIXTURES OF THE HERBICIDES GOLTIX AND BETANAL ON BEET

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Abstract

Pesticides, including herbicides, are expensive and their use inevitably causes problems in the area of environment protection. Therefore, the use of herbicides should be restricted. One of the possibilities to diminish the load of herbicides on the environment is to apply smaller doses than recommended. The aim of this research was to elucidate the effectiveness of small doses of herbicides Goltix WP 70 and Betanal, used as tank mixtures, on annual weeds and yields of beet roots. The field experiment was established in Estonia near Tartu. The results of the field experiment revealed that in the dry and hot vegetation period of 2002, the influence of Goltix WP 70, through the soil, was slight both on weeds and yields. Betanal affects weeds through their overground parts, and already a quarter and a half of the recommended Betanal dose proved effective. By using tank mixtures of Betanal and Goltix WP 70, the doses of both the herbicides can be reduced by a half. Thus there is no need to treat fields twice, whereas, in addition, the load of herbicides on the environment diminishes.

Key words: garden beet, herbicides, Goltix WP 70, Betanal.

Introduction

Most of the pesticides used in Estonia are herbicides, particularly in grain cultivation, where 80% of the used pesticides are herbicides. Herbicides are also relatively widely exploited in vegetable cultivation to reduce the times of cultivation between rows and manual hoeing.

Pesticides, including herbicides, are expensive, and their use inevitably entails problems in the area of environment protection. Therefore their use should be restricted. One option for reducing the load of pesticides on the environment is to apply smaller doses. Such an experiment was carried out in 2000 when it became evident that, to control weeds around beet plants, both Betanal and Goltix can be applied in quantities diminished by at least ¼ (Lauk, Kallion, 2001; Lauk, 2002). However, it is possible to reduce herbicide doses even more, by using two different herbicides in tank mixtures to strengthen their effect on weeds. To establish whether the named herbicides can be mixed and what doses are the most suitable was the aim of this research. The use of smaller doses in tank mixtures would reduce the load of pesticides on the environment, and it would also prove more economical as there would be no multiple treatments with different necessary preparations.

Material, methods and experimental conditions

In 2002, Betanal and Goltix 70 WP — two herbicides most used on beet — were tested. Both preparations had a systemic and, to some extent, contact effect, affecting sprouting of weeds effectively. Thus, the herbicides can be used simultaneously to control weeds.

Betanal contains active substance agent phenmediphan, 160 g/l. Preparative form — emulsion concentration. A herbicide with mainly a systemic selective effect to control short-lived dicotyledonous weeds. The effect on weeds can be noticed 4—8 days after spraying. Betanal enters plants through leaves and inhibits photosynthesis, as a result of which plants turn yellow and wither. Recommended doses on sugar beet, fodder beet and beet are 5.0—6.0 l/ha before or after the sprouting of beet (Taimekaitsevahendid ..., 2002).

Goltix 70 WP contains an active substance agent metamitron, 700 g/kg. Preparative form — wetting powder. A preparation with a systemic selective effect, entering plants both through roots and leaves. Therefore it is recommended to use it before or after the sprouting of beet. The recommended dose on sugar beet, fodder beet and beet — 2 kg/ha (Taimekaitsevahendid ..., 2002).

On the farm of Kalju Saar, two varieties of red beet were grown — `Rocket` and `Pablo F1`. Since part of the field with `Pablo` was more even, it was chosen for the experiment.

`Pablo F1` is a recognised standard variety that can be marketed when it is fresh, used as a raw material in industry, and preserved. It is highly tolerant towards bolting and, consequently, suitable for early sowing under conditions of Estonia. Its roots are totally red and ideally round, smooth and with no beet rings and additional roots. Its leaf spoke is small.

The field experiment was established in the beet field of the vegetable farm belonging to Kalju Saar and situated in Luunja rural municipality in Tartu County. The pre-crop in the field was potato. After potato harvesting, autumn ploughing was carried out. In spring the field was re-ploughed and cultivated. Under cultivation, a chlorine-free complex fertiliser (16:16:16) produced by the firm Regle, was applied at 600 kg/ha. The beet variety `Pablo` was sown on 2 May. The experiment was planned (marked down) on 20—24 May. Due to the dry weather of the period, beet started sprouting only on 17 June. Spraying with two herbicides — Betanal and Goltix WP 70 — and their mixtures was performed on 20 June.

As in earlier experiments it was established that spraying of full doses is not reasonable, the biggest herbicide norms were excluded from the experiment. Thus, the test variants were as follows:

1. 0;
2. Betanal, 1.5 l/ha;
3. Betanal, 3.0 l/ha;
4. Betanal, 4.5 l/ha;
5. Goltix 70WP, 0.5 kg/ha;
6. Goltix 70WP, 1.0 kg/ha;
7. Goltix 70WP, 1.5 kg/ha;
8. Betanal, 1.5 l/ha+Goltix 70WP, 0.5 kg/ha;
9. Betanal, 3.0 l/ha+Goltix 70WP, 1.0 kg/ha;
10. Betanal, 4.5 l/ha+Goltix 70WP, 1.5 kg/ha.

The experiment was carried out in three replications, enabling to process test results by dispersion analysis method. The total size of an experimental plot was 10 m^2. There were 30 experimental plots in total, used for observation and measurements.

Counting of weeds was started on 28 June, i.e. 8 days after spraying with herbicides. Weeds were at the cotyledon stage. The next counting was performed on 5 July — 15 days after spraying. Weeds were counted by the frame method, i.e. sprouting annual weeds were counted in two random plots; the size of a frame was 0.5 × 0.5 m.

The experiment was harvested on 3—4 October. On each plot the yield was harvested from 4-metre long parts of two parallel furrows. Thus, the size of the actual field plot was 4.96 m^2. As due to the dry and warm summer beet plants sprouted unevenly, there were empty spaces inside the actual plot. Root yield from each plot was weighed. A structure analysis was also performed — roots of up to 4 cm and over 4 cm were weighed separately.

Experimental results

Due to the long and droughty spring, when precipitation in April and May was very small, beet sown at the beginning of May sprouted only after a month and a half — around 17 June. Weed control with the two herbicides and their mixture was performed on 20 June, which was a month later than usual. The weather was not favourable for spraying the soil herbicide Goltix either. The whole period from sowing to sprouting was very dry and warm. Therefore the spraying solution was applied to dry soil, and the effect of the soil herbicide was indignificant, although a bigger liquid amount than usual was used. In the case of later spraying, weeds would have grown too big, and the effectiveness of Betanal would have been very small.

The first counting of weeds was carried out on 28 June, i.e. 8 days after spraying. By that time Betanal had considerably affected the number of weeds ($LSD_{95\%}$ — 115.8 and $LSD_{99\%}$ — 158.3) at all doses (Fig. 1). The number of weeds decreased most in the case of the biggest dose of Betanal, 4.5 l/ha, however, the decrease was not remarkable compared to that of smaller doses. The soil herbicide Goltix did not substantially affect the number of weeds, and the small decrease in the number of weeds was within the limits of the standard error.

In the case of herbicide mixtures, the number of weeds was even lower than in variants with Betanal, showing that Goltix, to some extent, had also decreased the number of weeds.

The next counting of weeds was carried out on 5 July, i.e. on the 15th day after spraying. On field plots sprayed only with Betanal, the number of weeds was nearly on the same level as during the previous counting (Fig. 2) when already a smaller herbicide dose reduced the number of weeds considerably ($LSD_{99\%}$= 64.4). Two weeks after spraying, the number of weeds also diminished significantly on plots treated with Goltix 70 WP. However, differences between different doses were not significant here.

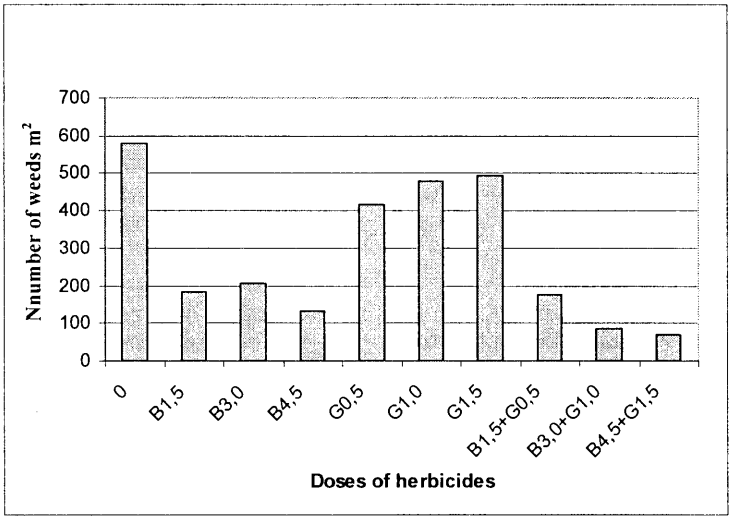

Fig. 1. The number of weeds depending on the doses of herbicides on 8th day after treatment

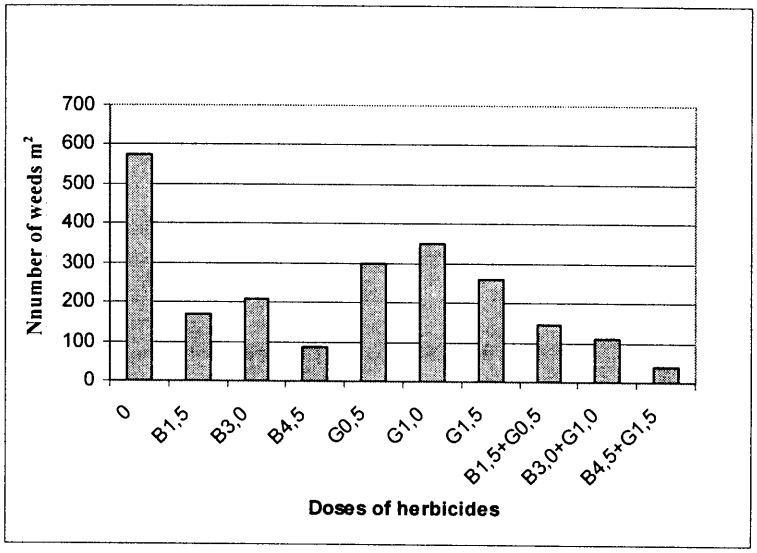

Fig. 2. The number of weeds depending on the doses of herbicides on 15th day after treatment

In the case of mixtures of Betanal and Goltix, the number of weeds was the smallest. The reduced number of weeds can be explained by the use of Goltix, the effect of which is not as quick as that of Betanal.

Thus, Betanal destroys weeds faster — already during a week, and it is rather effective at smaller doses, 1.5 and 3.0 l/ha. The effect of Goltix diminished due to the dry summer of 2002 — both separately and in tank mixtures. Its effect reveals itself also later than that of Betanal, i.e. ca in 2 weeks. In the case of such an extreme year, it is difficult to provide any recommendations about herbicide doses in tank mixtures. However, on the basis of the results of the experiment, it can be said that both preparations can be used in tank mixtures, in quantities smaller by a half than recommended, i.e. Betanal — 3.0 l/ha, and Goltix 70 WP — 1.0 kg/ha.

In the year of the experiment, the forming of **beet yield** to a large extent depended on the weather. Due to the dry and warm April, seeds sown on the first days of May had to endure dry soil. As there was no considerable precipitation in May and June, beet sprouted only after a month and a half — on 17 June, and the sprouting was uneven. Precipitation during the second and third decades of June favoured growth of beet, however, the yields were still small, primarily due to the short growth period. Moreover, the proportion of undersized roots (with diameters under 4 cm) was large in the yield. The beet yield from the experimental area in 2002 was only 9—23 tons per hectare. As a comparison, the yield per hectare was 45—55 tons in the experiment of 2000 (Lauk, 2001).

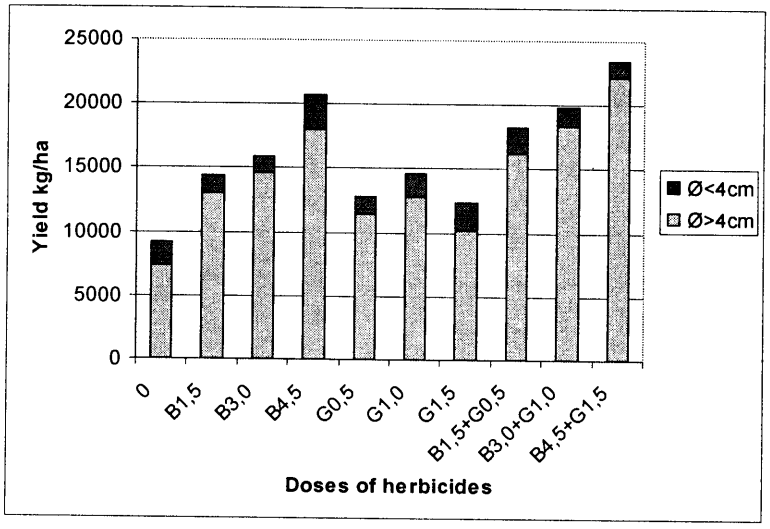

Fig. 3. Beet yield depending on the doses of herbicides

The biggest yields were gained from experimental plots where Betanal was used for weed control (Fig. 3). Mostly, already at a dose of 3.0 l/ha, the yield was bigger by 6,672 kg/ha, compared to unsprayed plots. The largest proportion of the yield, over 14,201 kg/ha, was obtained by applying larger doses of Betanal and Goltix 70 WP as tank mixtures with credibility 99.9% ($LSD_{99.9\%}$ on 11,827.33). Consequently, Goltix 70 WP had exerted an effect on weeds. However, as a rule, the effect of Goltix on the yield was small due to the dry and hot year and was not within the limits of credibility ($LSD_{96\%}$ — 6,375.12).

To determine the structure of the yield, roots with the diameter under and over 4 cm were weighed separately. The diameters of the largest roots did not exceed 10 cm. Depending on the variant, the proportion of undersized roots was 5—20% and it was bigger on unsprayed patches and patches sprayed with Goltix 70 WP.

Figure 3 presents the yield of roots with the diameter over 4 cm, depending on the doses of herbicide. The tendency here is the same as in the case of the total yield. With bigger doses of Betanal the yield of commercial roots was significantly bigger than that of unsprayed plots, however, already small doses of herbicides had increased the yield. The effect of Goltix was small also here.

There were more undersized roots on unsprayed plots and in the case of small Betanal doses. With Goltix, the total yield was comparatively smaller with a larger proportion of undersized roots.

Summary and conclusions

Testing of two herbicides, Betanal and Goltix 70 WP, at different doses and in different tank mixtures for control of weeds gave the following results:

- in the dry and hot summer of 2002, the effect of Goltix 70 WP, affecting weeds through soil, was small despite the use of bigger doses of herbicide solution (400 l/ha). A week after spraying there were no significant differences between plots sprayed with Goltix 70 WP. After two weeks, the number of weeds on plots sprayed with Goltix 70 WP had diminished, but there were no substantial differences between different doses. Consequently, in droughty years it is not reasonable to use herbicides exerting their effect through soil;
- Betanal affects weeds mainly through their overground parts, and this experiment revealed that already small doses of herbicides reduced the number of weeds considerably. The effect of Betanal on weeds was also faster — weeds can be destroyed in a week;
- it is reasonable to use tank mixtures enabling to reduce doses of herbicides and avoid multiple treating of the field. On the basis of the experiment, a tank mixture of Betanal and Goltix 70 WP, 1.5 l/ha and 0.5 kg/ha, respectively, can be recommended;
- in 2002, the biggest yields were gained from experimental plots treated with Betanal. Already at a dose of 3.0 l/ha the beet yield was by over 6.6 t/ha bigger than that of untreated experimental plots. The largest proportion of the yield — over 14.2 t/ha — was obtained by using tank mixtures of larger doses. The effect of Goltix 70 WP on the yield proved to be small in the dry and hot year 2002.

Acknowledgements

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References

1. Lauk, Ü., Kallion, H. 2001. Vähendatud herbitsiidiannuste kasutamise võimalustest peedi umbrohutõrjel. Efektiivne keskkonda säästev põllumajandus. EPMÜ Teadustööde kogumik, Nr. 212, 145—148.
2. Lauk, Ü. 2001. Vähendatud herbitsiidiannuste mõju söögipeedi saagile. EPMÜ Teadustööde kogumik, Nr. 213, 96—100.
3. Lauk, Ü. 2002. Influence of reduced herbicide doses on the yield and weedness of beet. International scientific conference "Plant protection in the Baltic region in the context of integration to EU", Kaunas, 61—63.
4. Taimekaitsevahendid ja kasvuregulaatorid kasutamiseks Eesti Vabariigis 2002. Taimetoodangu Inspektsioon, Saku, 2002, 177 pp.

THE EFFECT OF SOIL MULCHING ON WEED INFESTATION IN A STRAWBERRY PLANTATION

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Abstract

In climatic and soil conditions of the Vilnius Region, a four years' research on the effectivity of mulching of a strawberry 'Senga Sengana' plantation was carried out. The strawberry plantation was covered with mulches with compost (peat and organic farm materials), rye straw, sawdust from needle trees, wood bark (from needle trees), and black plastic foil. The investigations were conducted in four replications, on brown soil formed from light clay sand. Estimation of weed infestation was conducted twice during each year: after strawberry blooming (June — primary weeds) and during full summer (August — secondary weeds). The number and species composition of weeds were estimated. Independently of the years of study and its objects, average number of weeds amounted to 39,3 units per 1 m^2 in primary weed infestation and 21,0 units per 1 m^2 in secondary weed infestation. Annual weeds dominated in primary as well as in secondary weed infestation; the total number of perennial weeds was much smaller. During the four years of investigations, 23 diverse weed species were observed (15 annual and 8 perennial). Among annual weeds, *Galinsoga parviflora* Cav., *Chenopodium album* L. and *Capsella bursa-pastoris* (L.) Med. were found in the greatest number. In conditions of field experiment, the best method of limiting weed infestation was soil mulching with black plastic foil. Organic mulches like pine bark, sawdust and straw also limited appearance of weeds. Compost mulch caused higher weed infestation compared to not mulched soil.

Key words: weed infestation, mulching, strawberry.

Introduction

One of most useful forms of soil cultivation in strawberry plantations is mulching with organic as well as synthetic materials. Many authors have found that mulching hinders water from infiltrating into the soil thus restraining mineral components from rinsing, especially nitrogen (Elmar, Terrandino, 1991; Locoscio et al., 1985; Truax, Gargon, 1993; Stojanowska, 1996). The majority of surveyed cases show that mulches favorably influence the plants' supply with both water and nutritive elements. Organic mulches decay thus enriching soil with humus, which supplies minerals to plants (Lipecki, 1992). The impact of mulching on the content of minerals in soil first of all depends on the used material. Some materials like bark or sawdust decay slowly, others — quickly, so they play their role only for one season and they must be continuously supplemented (Smolarz, 1982). The ecological aspect of mulching is also very important, i.e. soil protection from weeds and reduction of herbicide usage (Kulesza, 1994). Struggling against weeds is especially important in berry shrub plantations from early spring to summer because weeds have to compete to gain enough water and nutritive elements. Weeds absorb many mineral components and their aboveground portions contain much more nitrogen, potassium, calcium, phosphorus or magnesium compared to leaves of cultivated plants (Lipecki, 1991).

Within 1994—1997, complex surveys have been carried out regarding the impact of strawberry plantation mulching with different materials on the plants' growth, blooming, fructification as well as on the content of mineral components in soil and strawberry leaves. Irrespective of research years, the mean number of inflorescences was the greatest on plants from the control object and on plants mulched with black foil. The smallest number of inflorescences was formed on plants from the object with straw and wood bark mulches. Among the studied mulches, black foil had the most profitable influence on the number of fruit settings. In this combination, in each research year the percentage of fruit settings was the highest (Kesik, Maskalaniec, 2003). The mulch with compost had profitable influence on the P and Mg content in soil, and mulch with straw — on K content. The experiment demonstrated that use of sawdust and wood bark decreased the K content in soil, but use of straw decreased the content of Mg. The applied mulches modified the N, P, K and Mg contents in strawberry leaves (Kesik, Maskalaniec, 2004).

The survey concerns assessment of the plantation's weeding state — the number of weeds on area unit and the sorts of weeds.

Materials and Methods

In climatic and soil conditions of the Vilnus region, a four years' research (1994—1997) on the effectiveness of mulching of a strawberry 'Senga Sengana' plantation was carried out. The investigation was conducted in 4 replications on brown soil composed of light clay sands. The period of vegetation on the surveyed area is 180—190 days. The last spring ground frost is usually recorded at the end of May, but the first — in autumn, in mid-September. The years 1994—1997 were generally cool, and in 1997 the last ground frosts were on 26 May. The most advantageous thermal conditions for strawberry growth were in 1995, when average monthly air temperatures were higher than in other years. Annual rainfalls are on average from 600 to 700 mm, but in the vegetation period — from 450 to 470 mm. The heaviest rainfalls were in 1994 (751 mm), but the lightest — in 1996 (525 mm). The information about climatic conditions comes from the Meteorological Station of the Soil Cultivation Institute Department in Troku Wokie near Vilnius.

The strawberry plantation was covered with mulches with compost (peat and organic farm materials), rye straw, sawdust from needle trees, wood bark (from needle trees), and black plastic foil. Organic mulch (compost, sawdust, bark), which created a 10 cm layer, was supplemented after two years of the study; straw mulch was complemented every year; black plastic foil was exchanged after 2 vegetation seasons. The estimation of weed infestation was conducted using the frame method twice each year: after blooming (June — primary weeds) and during full summer (August — secondary weeds). On each frame of a 1 m^2 area, at which the fields were divided, the number and species composition of weeds (in 4 replications) were estimated. Each time after this operation all weeds were manually removed from the fields. Other cultivation operations were not applied.

Results and Discussion

The survey showed that the number of weeds was very differentiated irrespective of mulching combinations. The greatest number of weeds was in 1994. In primary weeding (estimated in June), there were on average 75,2 units per 1 m^2 of the experimental plot, and 16,9 units per 1 m^2 in secondary weeding (estimated in August). Also in 1996 the level of weeding was very high — 52,2 units per 1 m^2 in primary weeding, and 43,5 weed units in secondary weeding. Within 1995—1997, weed infestation was lower — on average a dozen or so units per 1 m^2. Making independent analysis of objects with mulching, annual weeds dominated both in primary and secondary weeding — 35,2 units per 1 m^2 in early vegetation period and 17,6 units per 1 m^2 in full summer. The number of perennial weeds was considerably lower: 4,5 weeds per 1 m^2 in primary weeding and 3,4 weeds per 1 m^2 in secondary weeding, on average.

The 4-year surveys carried on experimental plots resulted in stating 23 weed species: 15 annual and 8 perennial weed species. A full weeds representation was not performed every year (Table 1), i.e., in 1994 — the spectrum of weed species included 17 species (11 annual and 6 perennial), in 1995 — 16 species (9 annual and 7 perennial), in 1996 — 17 species (10 annual and 7 perennial), and in 1997 — 16 species (10 annual and 6 perennial).

Among annual weeds, *Chenopodium album* L. was found in the greatest number, especially in combination with leaf-mould. Making analysis of primary weeding, 184 weed units per 1 m^2 were stated in 1994, and 64,7 units per 1 m^2 — in 1996. *Galinsoga parviflora* Cav. was the most frequently observed weed species in object mulching with leaf-mould in 1996 — about 127 units per 1 m^2 in primary weeding and 134 units in secondary weeding. *Fumaria officinalis* L. occurred more frequently only in 1994 in primary weeding on control plots (35,7 units per 1 m^2), and in the next years it disappeared.

The flora of perennial weeds was not too numerous — during 4 years only 8 sorts of weeds were stated, among them *Agropyron repens* L. appeared every year, but *Taraxacum officinale* Web. — only in 1995. The plots mulched with bark and sawdust were a favorite place for *Equisetum arvense* L.

The number of weeds in particular experiment combinations was very differentiated (Table 3). Weeds appeared mostly on plots mulched with compost (136,9 units per 1 m^2 in primary weeding and 59,7 units per 1 m^2 in secondary weeding), and on control plots which were not mulched (82,6 units per 1 m^2 in primary weeding and 32,2 units per 1 m^2 in secondary weeding). The least number of weeds both in primary and secondary weeding appeared on plots mulched with black foil (1,2 and 1,7 units per 1 m^2), with bark (6,1 and 7,6 units per 1 m^2), with sawdust (4,2 and 9,0 units per 1 m^2), and straw (4,0 and 16,5 units per 1 m^2).

Table 1

Species composition and number of weeds per 1 m^2 in a strawberry plantation during 1994—1997

Weed species	1994		1995		1996		1997		Mean	
	A	B	A	B	A	B	A	B	A	B
Annual weeds										
1. *Chenopodium album* L.	39,0	6,9	1,9	1,8	12,1	5,4	2,9	1,3	14,0	3,9
2. *Galinsoga parviflora* Cav.	16,3	5,6	5,3	5,8	25,1	26,8	5,2	3,8	13,0	10,5
3. *Capsella bursa-pastoris* (L.) Med.	5,0	0,5	1,1	0,3	2,7	3,6	2,1	0,4	2,7	1,2
4. *Echinochloa crus-galli* (L.) P.B.	2,1	1,4	1,0	1,4	1,0	0,6	1,6	0,4	1,4	1,0
5. *Stellaria media* L. Vill.	0,8	–	0,2	0,1	0,7	0,3	0,5	0,3	0,6	0,2
6. *Fumaria officinalis* L.	6,7	–	–	–	–	–	–	–	1,7	–
7. *Galium aparine* L.	1,3	–	0,1	–	0,6	–	–	–	0,5	–
8. *Polygonum persicaria* L.	0,5	0,2	0,1	–	0,3	–	0,2	–	0,3	0,1
9. *Amaranthus retroflexus* L.	0,1	–	–	–	0,8	0,6	–	–	0,4	0,2
10. *Galeopsis tetrahit* L.	0,1	0,1	0,1	–	–	–	–	–	0,1	–
11. *Lamium purpureum* L.	–	0,1	0,1	–	0,2	0,1	–	–	0,1	0,1
12. *Erigeron canadensis* L.	–	–	–	–	2,3	2,1	0,3	0,2	0,7	0,6
13. *Poa annua* L.	–	–	–	–	–	–	0,1	0,3	–	0,1
14. *Veronica arvense* L.	–	–	–	–	–	–	0,3	–	0,1	–
15. *Erodium arvensis* L. L'Her.	–	–	–	–	–	–	0,1	0,1	–	0,1

Table 1 (continuation)

Weed species	1994		1995		1996		1997		Mean	
	A	B	A	B	A	B	A	B	A	B
Total annual weeds	71,9	14,8	9,9	9,4	45,8	39,5	13,3	6,8	35,2	17,6
Perennial weeds										
1. *Mentha arvensis* L.	0,2	–	0,1	–	–	–	0,1	–	0,1	–
2. *Agropyron repens* (L.) P.B.	0,5	0,5	1,4	1,4	2,0	1,9	0,9	2,1	1,3	1,3
3. *Convulvulus arvensis* L.	0,1	–	0,1	–	–	–	–	–	0,1	–
4. *Equisetum arvense* L.	0,9	1,0	1,0	1,0	0,6	0,2	0,2	0,2	0,9	0,8
5. *Urtica dioica* L.	1,6	0,5	0,4	0,2	2,8	0,7	0,3	0,3	1,3	0,4
6. *Artemisia vulgaris* L.	–	0,1	0,1	–	–	0,1	0,1	–	0,1	0,1
7. *Taraxacum officinale* Web.	–	–	0,8	0,8	0,9	1,0	1,1	1,2	0,6	0,7
8. *Cirsium arvense* (L.) Scop.	–	–	–	–	0,1	0,1	–	–	0,1	0,1
Total perennial weeds	3,3	2,1	3,9	3,4	6,4	4,0	2,7	3,8	4,5	3,4
Total	75,2	16,9	13,8	12,8	52,2	43,5	16,0	10,6	39,3	21,0

A — primary weed infestation — after blooming (beginning of June);
B — secondary weed infestation — full summer (August).

The results of the survey show that black foil used as mulch reduced weed infestation successfully. According to the opinion of many authors, use of black foil allows eliminating herbicides entirely (Dobromilska et al., 1995; Kunicka et al., 1997; Rechnio, 1988; Szewczuk, 1995; Zmuda, 1986). But use of organic mulches as a weed infestation reducer was not so successful and reduced weeds only to some extent. Similar effects of mulching were experienced using organic materials like bark, straw, and sawdust (Kulesza et al., 1996; Mika, Krzewinska, 1996; Rechnio, 1988; Lipecki, 1992; Szewczuk, 1995). It was observed that organic mulches hampered growth of weeds, especially annual weeds; these mulches were overgrown by perennial weeds, which demand use of leaf herbicides to liquidate them.

Table 2

Species composition and number of weed units per 1 m^2, average during 1994—1997

Weed species	Objects											
	Control		Compost		Straw		Sawdust		Bark		Foil	
	A	B	A	B	A	B	A	B	A	B	A	B
Annual weeds												
1. *Chenopodium album* L.	17,7	5,1	64,3	7,7	0,9	5,9	0,2	3,2	0,4	1,2	–	0,1
2. *Galinsoga parviflora* Cav.	29,6	11,9	46,7	42,0	0,6	5,9	0,1	1,7	0,6	1,3	0,1	0,4
3. *Capsella bursa-pastoris* (L.) Med.	12,6	5,0	3,6	1,4	–	0,2	–	0,4	0,1	0,3	–	0,05
4. *Echinochloa crus-gali* (L.) P.B.	4,2	2,2	4,0	2,7	0,2	0,5	0,1	0,1	0,1	0,2	–	0,05
5. *Stellaria media* L. Vill.	1,8	0,6	1,6	0,3	–	0,1	–	–	–	–	–	–
6. *Fumaria officinalis* L.	8,9	–	0,1	–	–	–	–	–	–	–	–	–
7. *Galium aparine* L.	0,05	–	2,9	–	–	–	–	–	–	–	–	–
8. *Polygonum persicaria* L.	–	–	1,4	0,05	–	0,2	–	0,1	0,1	-	–	–
9. *Amaranthus retroflexus* L.	–	–	1,4	0,9	–	–	–	–	-	-	–	–
10. *Galeopsis tetrahit* L.	–	–	0,1	–	0,1	0,1	–	–	-	-	–	–
11. *Lamium purpureum* L.	0,05	0,05	0,2	0,05	–	0,1	0,1	–	-	0,1	0,1	–
12. *Erigeron canadensis* L.	1,2	2,6	3,1	0,6	0,1	0,1	0,3	–	0,7	–	0,1	–
13. *Poa annua* L.	–	0,3	–	–	0,2	–	–	–	–	–	0,1	0,2
14. *Veronica arvense* L.	–	–	0,4	–	–	–	–	–	–	–	–	–
15. *Erodium arvensis* L. L'Her.	–	–	–	–	–	0,1	–	–	–	–	–	–
Total annual weeds	76,1	27,8	129,8	55,7	2,1	13,2	0,8	5,5	2,0	3,1	0,3	0,8

Table 2 (continuation)

Weed species	Objects											
	Control		Compost		Straw		Sawdust		Bark		Foil	
	A	B	A	B	A	B	A	B	A	B	A	B
Perennial weeds												
1. *Mentha arvensis* L.	0,4	–	–	–	0,05	–	–	–	–	–	–	–
2. *Agropyron repens* (L.) P.B.	3,3	2,9	1,4	2,2	0,7	1,7	0,9	1,3	0,9	0,6	0,1	0,3
3. *Convulvulus arvensis* L.	0,1	–	–	–	–	–	0,1	–	–	–	–	–
4. *Equisetum arvense* L.	0,5	0,1	0,1	–	0,2	0,1	1,0	1,0	2,3	2,4	–	–
5. *Urtica dioica* L.	2,0	1,0	5,4	0,9	–	0,2	–	0,1	–	0,2	–	–
6. *Artemisia vulgaris* L.	–	–	0,1	–	–	–	–	–	0,1	0,2	–	–
7. *Taraxacum officinale* Web.	0,2	0,4	0,1	0,8	1,0	1,3	1,4	1,1	0,8	1,1	0,8	0,6
8. *Cirsium arvense* (L.) Scop.	–	–	0,1	0,05	–	–	–	–	–	–	–	–
Total perennial weeds	6,5	4,4	7,1	4,0	1,9	3,3	3,4	3,5	4,1	4,5	0,9	0,9
Total annual and perennial weeds	82,6	32,2	136,9	59,7	4,0	16,5	4,2	9,0	6,1	7,6	1,2	1,7

A — primary weed infestation (beginning of June);
B — secondary weed infestation (August).

The survey on competitiveness of some weed sorts in strawberry cultivation shows that at proper weed density they do not compete with strawberry, and some sorts like *Capsella bursa pastoris* L. can be living mulch for strawberry plants (Zmuda, 1992).

Table 3

The influence of mulching on primary and secondary weeding during 1994—1997

Objects	Weeding	1994	1995	1996	1997	Mean
Control	primary	202,0	31,0	55,8	41,0	82,6
	secondary	10,2	28,0	73,0	17,2	32,2
Compost	primary	237,0	33,0	240,0	34,2	136,9
	secondary	8,2	30,0	177,0	23,0	59,7
Straw	primary	3,4	3,6	3,3	5,4	4,0
	secondary	46,6	5,8	3,0	7,6	16,5
Sawdust	primary	2,6	4,8	6,0	3,0	4,2
	secondary	20,3	5,2	1,7	8,1	9,0
Bark	primary	3,2	8,0	6,0	7,2	6,1
	secondary	13,8	6,7	2,6	6,2	7,6
Foil	primary	0,0	0,5	1,0	3,0	1,2
	secondary	1,8	0,2	2,5	2,0	1,7
Mean	primary	75,2	13,8	52,2	16,0	39,1
	secondary	16,9	12,8	43,5	10,6	21,0

Conclusions

1. During four-year experiments in mulching a strawberry plantation, it was observed that the source of the highest weed infestation was leaf-mould made from peat and organic materials from farm and soil without mulch protection.
2. Mulching with straw, sawdust, bark and, first of all, with black foil were most successful to reduce the number of weeds.
3. *Chenopodium album* L. and *Galinsoga parviflora* Cav. were observed as the most noxious weeds in strawberry plantations.
4. A larger number of weeds was observed in primary weeding at the beginning of June after strawberry blooming, compared to secondary weeding in full summer.

References

1. Dobromilska, R., Orlowski, M., Rekowska, E., Slodkowski, P. 1995. Sciolkowanie gleby w uprawie warzyw cieplolubnych. Mat. Ogolnopol. Konf. Nauk. "Nauka praktyce ogrodniczej", Lublin, 761—764.
2. Elmer, W. H., Ferrandino, F. J. 1991. Early and late — season blossom — and rot of tomato following mulching. Hort. Sci., 26, 9, 1154—1155.
3. Kesik, T., Maskalaniec, T. 2003. Wplyw sciolkowania plantacji na wzrost, kwitnienie i owocowanie truskawki (*Fragaria ananassa* D.). Annales UMCS, sectio EEE, vol. XIII. 243—248.
4. Kesik, T., Maskalaniec, T. 2004. Wplyw sciolkowania plantacji na zawartosc skladnikow mineralnych w glebie i w lisciach truskawki. Roczniki Akademii Rolniczej w Poznaniu (in press).
5. Kulesza, W. 1994. Wplyw siedliska oraz mulczowania gleby kora na plonowanie truskawek w drugim roku uzytkowania plantacji. XXXIII Ogolnopol. Konf. Nauk. Sad., Olsztyn, 330—332.
6. Kulesza, W., Szafranek, R. C., Zielenkiewicz, J. 1996. Produkcyjne i ekologiczne efekty umiarkowanego nawozenia azotem i sciolkowania gleby w sadzie jabloniowym. Symp. Miedzynarod: "Ekologia w ogrodnictwie", Olsztyn, 76—79.
7. Kunicka, E., Siwek, P., Capecka, E. 1997. Wplyw oslon i sciolek z tworzyw sztucznych na plonowanie brokulu w uprawie wiosennej. Ogolnopol. Konf. Nauk. "Doskonalenie technologii roslin warzywniczych", Krakow, 154–156.
8. Lipecki, J. 1991. Co dalej z chwastami i herbicydami? Sad Nowoczesny, 3—14.
9. Lipecki, J. 1992. Susza i co dalej? Sad Nowoczesny, 10—16.
10. Lipecki, J., Wieniarska, J. 1990. Czy mozna juz zrezygnowac z herbicydow w sadach? Ogrodnictwo, 1, 12—14.
11. Locascio, S.J., Fiskell, J.G.A., Graetz, D.A. 1985. Nitrogen accumulation by pepper as influenced by mulch and time fertilizer application. J. Amer. Soc. Hort. Sci., 110, 3, 325—28.
12. Mika, A., Krzewinska, D. 1996. Rezultaty sciolkowania gleby w rzedach drzew w polkarlowym sadzie jabloniowym. XXXIV Ogolnopol. Konf. Sad., Skierniewice, 158—161.
13. Rechnio, H. 1988. Wplyw sciolkowania na plonowanie czarnej maliny. Sad Nowoczesny, 8, 6—7.
14. Smolarz, K. 1982. Sadownictwo, Praca zbiorowa, PWRiL Warszawa, 132—134.
15. Stojanowska, J. 1996. Wplyw sciolkowania folia na wzrost i plonowanie jabloni. Ogrodnictwo, 5, 7—9.
16. Szewczuk, A., Licznar-Malanczuk, M. 1995: Wplyw sciolkowania roznymi materialami rzedow drzew na wlasciwosci gleby oraz plonowanie i wzrost odmiany Elstar. Mat. Ogolnopol. Konf. Nauk. „Nauka praktyce ogrodniczej, Lublin, 39—42.
17. Truax, B., Gagron, D. 1993. Effects of straw and black plastic mulching on the initial growth and nutrition of butternut, white ash and bur oak. For. Ecol. Manage, 57, 17—27.
18. Zmuda, E. 1989. Wplyw sposobow pielegnowania gleby na plonowanie roslin i jakosc owocow truskawek. Annales UMCS, sectio E, vol. XLIV, 21, 181—186.
19. Zmuda, E. 1994. Zachwaszczenie a wzrost i produktywnosc roslin truskawki. Annales UMCS, sectio EEE, vol. II, 17, 125—134.

WEEDS IN SPRING BARLEY AND RESULTS OF WEED LIMITING

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Abstract

Field trials were carried out on organic farming fields at the Skriveri Research center of the Latvia University of Agriculture (LLU). The influence of previous plants (red clover, bare fallow, winter rye for green manure, winter rye), use of stable manure (60 t ha^{-1} or without) and harrowing (without harrowing, before first leaf emergence, at the stage of tillering, before first leaf emergence and at the stage of tillering) on the yield and weediness of spring barley 'Sencis' were tested. Data show that previous plants and stable manure influenced barley grain yields. Harrowing increased the yields of barley only after winter rye for green manure and using stable manure, but the time of harrowing had no influence on the yield of barley. The highest weed infestation in barley was obtained after red clover. Use of stable manure increased the number of annual weeds.

Key words: organic farming, spring barley, weeds, harrowing, previous plants, yields.

Introduction

The main preconditions for organic farming are use of organic fertilizers and crop rotation that maintain soil fertility [1]. One of factors limiting barley grain yields is weeds. In Latvia, during the last 10 years, weeds have been freely propagating in large areas, especially perennial weeds. From 1946, every year in Latvia expeditions are regularly organized to record weeds in different plant sowings. In 1999, in the eastern part of Latvia, 47 species of weeds were ascertained in barley sowings. The most spread annual weeds were *Chenopodium* album L., *Stellaria media* (L.) Will., *Galium aparine* L., *Convolvulus arvensis* L., *Matricaria inodorum* L., and perennial weeds *Elytrigia repens* (L.) Nevski., *Sonchus arvense* L., *Cirsium arvense* L., *Artemisia vulgaris*, and *Tussilago farfara* L. The weed infestation in of barley sowings increased from 79.0 pieces m^{-2} in 1996 to 165.1 pieces m^{-2} in 1999 [2, 3].

The aim of the article is to present the research results on productivity of barley and weed infestation in sowings depending on different agrotechnical elements in organic farming trials at the Skriveri Research centre.

Materials and Methods

The object of research: spring barley 'Sencis'.

The field trials were carried out on turf podsolic soil: pH_{KCl} — 6,75, P_2O_5 — 162 mg kg^{-1}, K_2O — 15,8 mg kg^{-1}, organic matter content — 3,25%, N_{total} — 0,11%.

After bare fallow + winter rye for green manure, 17 t ha^{-1} of biomass of winter rye were cultivated. Winter rye was at the stage of stemelongation (GS 32 after Zadoks). The number of plants — 335 plants m^{-2}. In the trial, variants with stable manure were included (doses of 60 t ha^{-1}). Before sowing, grains were treated with 1,5 kg of ashes of foliage trees and 1,5 l of water per 100 kg of grain.

The variants in the trial:

factor A — previous plants with graduation:
- A_1 — red clover,
- A_2 — bare fallow,
- A_3 — winter rye,
- A_4 — winter rye for green manure;

factor B — time of harrowing with graduation:
- B_1 — without harrowing,
- B_2 — before first leaf emergence,
- B_3 — at the stage of tillering,
- B_4 — before first leaf emergence and at the stage of clustering;

factor C — use of stable manure with graduation:
- C_1 — without stable manure,
- C_2 — stable manure 60 t ha^{-1}.

Weed assessment was established by the method of number and weight of weeds using a 0.25 m^{-2} by frame.

Results and Discussion

The year 2003 was very favorable for growth and development of spring barley. April was rainy and cold, which hampered the time of sowing. In May, the air was getting warmer gradually, at nights the temperature was under 10 °C, and frosts were frequent on the soil surface. Barley germinated and clustered quickly. In June, the average air temperature was 0,7 degrees lower than the norm, but the amount of precipitation made 75% of the norm. July with average air temperature 19 °C was the second warmest middle-summer month during the last 80 years in Latvia. Barley grew and developed well and produced good and qualitative grain yields. Depending on the variants, the yields in the field trial varied from 2,18 to 3,56 t ha^{-1} (Table 1).

Table 1

The spring barley 'Sencis' grain yields depending on the previous plant, use of stable manure and time of harrowing, t ha^{-1}, 2003

Previous plant* Factor A	Time of harrowing (factor B)	Stable manure** (Factor C)		
		without	60 t ha^{-1}	on average
A_1	1. without	3,03	3,37	3,20
	2. at GS 7	2,72	3,26	2,99
	3. at GS 23	2,94	3,38	3,16
	4. at GS 7 and GS 23	3,00	3,56	3,28
	$\gamma_{0,05}$ = 0,46 t ha^{-1}	$\gamma_{0,05}B$ =0,33 t ha^{-1}	$\gamma_{0,05}C$ = 0,23 t ha^{-1}	
A_2	1. without	2,24	2,96	2,60
	2. at GS 7	2,23	2,73	2,48
	3. at GS 23	2,20	2,71	2,46
	4. at GS 7 and GS 23	2,46	2,79	2,62
	$\gamma_{0,05}$ = 0,52 t ha^{-1}	$\gamma_{0,05}B$ =0,37 t ha^{-1}	$\gamma_{0,05}C$ = 0,26 t ha^{-1}	
A_3	1. without	3,30	3,16	3,23
	2. at GS 7	3,16	3,58	3,37
	3. at GS 23	3,13	3,46	3,29
	4. at GS 7 and GS 23	3,08	3,51	3,30
	$\gamma_{0,05}$ = 0,52 t ha^{-1}	$\gamma_{0,05}B$ =0,36 t ha^{-1}	$\gamma_{0,05}C$ = 0,26 t ha^{-1}	
A_4	1. without	2,43	3,31	2,87
	2. at GS 7	2,23	3,30	2,76
	3. at GS 23	2,19	3,24	2,71
	4. at GS 7 and GS 23	2,31	3,28	2,80
	$\gamma_{0,05}$ = 0,34 t ha^{-1}	$\gamma_{0,05}B$ =0,24 t ha^{-1}	$\gamma_{0,05}C$ = 0,17 t ha^{-1}	

* A_1 — bare fallow; A_2 — red clover; A_3 — bare fallow and winter rye for green manure; A_4 — bare fallow and winter rye for grain.

The data in Table 1 demonstrate the influence of stable manure on spring barley — grain yields have increased by 0,47—0,99 t ha^{-1} on average. Use of stable manure for barley after winter rye provided the highest yield increase. It is known that winter rye impoverishes the soil; use of stable manure provided increase in yields by 0,99 t ha^{-1} on average.

After different previous plants the highest yields were obtained after winter rye for green manure and after bare fallow in both variants, with stable manure and without it. Increase in grain yields after winter rye for green manure can be explained by the activity of microorganisms. After decomposition of winter rye biomass, plants can use nitrogen and CO_2 for development.

1000 kernel weight was medium — 33,5—38,4 g, medium was also specific weight — 609,0—633,5 g. The content of total protein in grain after bare fallow and winter rye for green manure was good — 11,7—11,5%, but after red clover and winter rye for grain — unsatisfactory (9,7—9,5%).

Excess precipitation and warm weather in most part of the vegetation period favored fast development of barley and suppression of weeds. Harrowing increased the grain yields essentially only after winter rye for green manure with stable manure, whereas time of harrowing had no significant influence on the grain yield. The microorganisms, carried into the soil by stable manure, provided fast decomposition of green manure.

At the stage of heading (3 weeks after harrowing) 18 species of weeds were established in the sowings. The number and weight of annual and perennial weeds are presented in Table 2.

Table 2

Weed infestation in spring barley 'Sencis' sowings in 2003

Previous plant (factor A)*	Stable manure (factor C)**	Harrowing (factor B)	Number of weeds, pieces m^{-2}			Biomass of weeds, g m^{-2}		
			total	annual	perennial	total	annual	perennial
A_1	0	without	108	108	0	370,0	370,0	0,0
		GS 7	90	80	10	113,6	93,6	20,0
		GS 23	78	78	0	170,7	170,7	0,0
		GS 7, GS 23	62	62	0	55,1	55,1	0,0
	60	without	134	134	0	138,3	138,3	0,0
		GS 7	104	104	0	107,8	107,8	0,0
		GS 23	72	72	0	51,1	51,1	0,0
		GS 7, GS 23	50	48	2	47,4	46,1	0,0
A_2	0	without	148	64	50	50,0	50,0	0,0
		GS 7	84	54	16	16,0	16,0	0,0
		GS 23	88	46	42	42,0	42,0	0,0
		GS 7, GS 23	94	50	44	44,0	44,0	0,0
	60	without	215	163	52	52,0	52,0	0,0
		GS 7	147	105	42	42,0	42,0	0,0
		GS 23	165	117	48	48,0	48,0	0,0
		GS 7, GS 23	150	114	36	36,0	36,0	0,0
A_3	0	without	120	120	0	104,9	104,9	0,0
		GS 7	74	74	0	98,8	98,8	0,0
		GS 23	62	60	2	93,2	91,2	2,0
		GS 7, GS 23	46	42	4	134,2	126,1	8,2
	60	without	161	161	0	190,6	190,6	0,0
		GS 7	97	97	0	110,7	110,7	0,0
		GS 23	118	112	6	198,4	190,0	8,4
		GS 7, GS 23	82	82	0	139,4	139,4	0,0
A_4	0	without	120	120	0	86,0	86,0	0,0
		GS 7	46	44	2	58,8	46,1	12,7
		GS 23	90	82	8	114,5	97,3	17,3
		GS 7, GS 23	70	70	0	64,8	64,8	0,0
	60	without	108	106	2	137,6	87,6	50,0
		GS 7	102	102	0	108,8	108,8	0,0
		GS 23	80	78	2	131,3	129,3	2,0
		GS 7, GS 23	76	76	0	205,1	205,1	0,0

* A_1 — bare fallow; A_2 — red clover; A_3 — bare fallow and winter rye for green manure; A_4 — bare fallow and winter rye for grain;
** 0 — without stable manure; 60—60 t ha^{-1}.

In variants without stable manure, the previous plant did not influence the total number of weeds. In variants with stable manure, the number of weeds was higher after red clover. After use of stable manure the number of weeds increased essentially when barley was grown after red clover (+ 65 pieces m^{-2}) and after winter rye for green manure (+ 39 pieces m^{-2}).

The total number of weeds influenced barley grain yields without use of stable manure only after winter rye for green manure ($r = 0,81$), but in variants with stable manure — when barley was grown after bare fallow ($r = -0,91$) and after winter rye for green manure ($r = -0,80$). Total biomass of weeds influenced the grain yield significantly only after winter rye with stable manure ($r = 0,96$).

From annual weeds, *Chenopodium album* L, *Stellaria media* L., *Capsella bursa-pastoris* L., *Polygonum* spp. and *Matricaria inodorum* L. were ascertained in all variants. In some variants, *Spergula arvensis* L., *Viola* spp., *Galeopsis speciosa* Mill., *Raphanus raphanistrum* L. and *Thlaspi arvense* L. were established, though the number of these weeds was negligible.

The number of annual weeds influenced the yields of barley significantly only after red clover without stable manure ($r = 0,86$). Whereas biomass of these weeds influenced the grain yield only after winter rye without stable manure ($r = -0,93$).

In barley sowings, six perennial weed species were established, the most widespread of which were *Elytrigia repens* (L.) Nevski and *Sonchus arvense* L. After red clover a higher number of weeds were established compared to

other previous plants, though neither the number nor biomass of weeds influenced the yields of grain. Sowings of previous plant red clover were sparse, which favored fast spread of perennial weeds.

Table 3

The influence of harrowing time and use of stable manure, after different previous plants, on the number of weeds in spring barley 'Sencis' in 2003

Time of harrowing (factor B)	Stable manure (factor C)		On average
	0	60	
1. after bare fallow			
1. without	108	134	121
2. at GS 7	92	104	113
3. at GS 23	78	72	75
4. at GS 7 and GS 23	62	50	56
$\gamma_{0,05}$ = 58 pieces m^{-2}	$\gamma_{0,05}$ B = 41 pieces m^{-2}		$\gamma_{0,05}$ C = 29 pieces m^{-2}
2. after red clover			
1. without	148	215	182
2. at GS 7	84	147	116
3. at GS 23	88	165	127
4. at GS 7 and GS 23	94	150	122
$\gamma_{0,05}$ = 79 pieces m^{-2}	$\gamma_{0,05}$ B = 56 pieces m^{-2}		$\gamma_{0,05}$ C = 39 pieces m^{-2}
3. after winter rye for green manure			
1. without	120	161	141
2. at GS 7	74	97	86
3. at GS 23	62	88	90
4. at GS 7 and GS 23	46	82	64
$\gamma_{0,05}$ = 47 pieces m^{-2}	$\gamma_{0,05}$ B = 33 pieces m^{-2}		$\gamma_{0,05}$ C = 23 pieces m^{-2}
4. after winter rye for grain			
1. without	120	118	114
2. at GS 7	90	102	74
3. at GS 23	70	80	85
4. at GS 7 and GS 23	46	76	73
$\gamma_{0,05}$ = 42 pieces m^{-2}	$\gamma_{0,05}$ B = 30 pieces m^{-2}		$\gamma_{0,05}$ C = 21 pieces m^{-2}

Table 3 shows that harrowing significantly decreased the number of weeds after all previous plants.

After bare fallow without stable manure, double harrowing decreased the number of weeds essentially but only in variants without harrowing. In variants with stable manure, harrowing decreased the number of weeds significantly at growth stage 23 and, if barley was harrowed twice, at GS 7 and GS 23. After winter rye for green manure the time of harrowing had no influence on the number of weeds. After winter rye without stable manure harrowing at growth stage 23 and double harrowing decreased the number of weeds, but in variants with stable manure the time of harrowing did not influence the number of weeds.

Conclusions

1. In the field trial, grain yields varied within the range of 2,18—3,56 t ha^{-1}. Use of stable manure increased the grain yield by 0,47—0,99 t ha^{-1}. The highest yields after different previous plants were obtained after winter rye for green manure and after bare fallow in both variants, with stable manure and without. Harrowing increased grain yields essentially only after winter rye for green manure with stable manure, though the time of harrowing had no essential influence on the yield of grain.
2. At the stage of heading (3 weeks after harrowing), 18 species of weeds were ascertained in barley sowings. Use of stable manure increased the number of weeds significantly when barley was grown after red clover and after winter rye for green manure.
3. The number of annual weeds had a significant influence on barley grain yield only after red clover without stable manure. Whereas biomass of these weeds influenced grain yields only after winter rye without stable manure.
4. Harrowing essentially decreased the number of weeds after all previous plants.

References

1. Lejiņš, A., Rasiņš, A., Āboliņš, J., Gavrilova, G., Lapiņš, D., Ozols, J., Vimba, E. (sast.) 1997. Nezāļu, to grupu un augu aizsardzības tehnikas terminoloģijas vārdnīca. LVZZPI "Agra", Skrīveri, 300 lpp.
2. Lejins, A., Abolins, J. 2000. The weediness and its changes in fields of eastern regions of Latvia. Development of Environmentally Friendly Plant Protection in the Baltic Region: Proceedings of the International Conference, Tartu, Estonia, September 28—29, 2000, 103—106.
3. Vaivare, M. (sast.) 2002. Praktiskā bioloģiskā lauksaimniecība Latvijā. I—III. Rīga: McĀbols, 10—85.

THE EFFECT OF SOIL TILLAGE, SOWING TECHNOLOGY AND HERBICIDE APPLICATION ON WEED INFESTATION IN SPRING BARLEY

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Abstract

Field trials were carried out at the Research and Study farm (RSF) "Vecauce" of the Latvia University of Agriculture (LLU) during 2002—2003. The effect of soil deep loosening, sowing technology and weed control on the yield of spring barley was studied on sod podzolic (2002) and sod carbonate leached (2003) loam soils with humus content 14 g kg^{-1} (2002) and 20 g kg^{-1} (2003), soil reaction pH_{KCl} 6.0 and 6.6, content of phosphorus 204 and 207 mg kg^{-1}, content of potassium 96 and 105 mg kg^{-1}, respectively. Spring barley was grown in recurrent sowing. Following treatments were investigated in the trial: Factor A — usage of herbicide Glifoss in autumn in treatments with direct sowing (done before the trial year): A1 — without Glifoss (untreated); A2 — Glifoss 0.5 L ha^{-1}; A3 — Glifoss 2.0 L ha^{-1}; A4 — soil ploughing at the depth of 18—22 cm; Factor B — sowing technologies: B1 — using a disc driller and local deposition of mineral fertilizers (except ammonium fertilizers) ("Rapid 400 C", hereafter "Rapid"); B2 — using an anchor-type driller with a rotortiller at the depth of 5—7 cm and dispersion of mineral fertilizers before sowing ("Amazone AD-403 super", hereafter "Amazone h1"); B3 — using anchor-type driller with rotortiller in depth 7—10 cm and dispersion of mineral fertilizers before sowing ("Amazone AD-403 super", hereafter "Amazone h2").

Diverse meteorological conditions in the trial years had determinative importance to the effect of soil tillage, application of Glifoss and sowing technologies on weed infestation in sowings and growth and development of spring barley. Significant decrease in the number of annual weeds was observed by increasing the depth of the rotortiller from 5—7 cm to 7—10 cm using sowing machine "Amazone AD-403 super". A significant negative effect on the growth and development of spring barley was caused by increased number of *Elytrigia repens* (L.) Desv. ex Nevski. The negative effect of sowing infestation with annual weeds on the growth and development of spring barley was not significant.

Key words: spring barley, soil tillage, sowing, herbicide application.

Introduction

Direct sowing of spring barley or minimal soil tillage becomes a very popular method worldwide. Such sowing technology allows economizing resources hereto not decreasing yields of cereals. This fact has also been approved in earlier researches made by the scientists of the Department of Soil management of the Latvia University of Agriculture (LLU) (Lapins et al., 2000; 2001). Similar researches have been made in Lithuania (Maikstiene, 2000; Stancevicius et al., 2000) and Estonia (Lauringson et al., 2001). Earlier researches have shown that one of the yield's limiting factors is increased numbers of annual and perennial weeds, especially *Elytrigia repens* (L.) Desv. ex Nevski when direct sowing is used.

The aim of this work is to evaluate the effect of soil tillage, herbicide application and sowing technology on the yield of spring barley and weed infestation in sowings.

Materials and Methods

Field trials were carried out at the Research and Study farm (RSF) "Vecauce" of the Latvia University of Agriculture (LLU) during the years 2002 and 2003. The effect of soil deep loosening, sowing technology and weed control on the yield of spring barley was studied on sod podzolic (2002) and sod carbonate leached (2003) loam soils with humus content 14 g kg^{-1} (2002) and 20 g kg^{-1} (2003), soil reaction pH_{KCl} 6.0 and 6.6, content of phosphorus 204 and 207 mg kg^{-1}, content of potassium 96 and 105 mg kg^{-1}, respectively. Spring barley was grown in recurrent sowing.

Following treatments were investigated in the trial:

Factor A — usage of herbicide Glifoss in autumn in treatments with direct sowing (done before the trial year): A1 — without Glifoss (untreated); A2 — Glifoss 0.5 L ha^{-1}; A3 — Glifoss 2.0 L ha^{-1}; A4 — soil ploughing at the depth of 18—22 cm;

Factor B — sowing technologies: B1 — using a disc driller and local deposition of mineral fertilizers (except ammonium fertilizers) ("Rapid 400 C", hereafter "Rapid"); B2 — using an anchor-type driller with a rotortiller at the depth of 5—7 cm and dispersion of mineral fertilizers before sowing ("Amazone AD-403 super", hereafter "Amazone h1"); B3 — using an anchor-type driller with a rotortiller at the depth of 7—10 cm and dispersion of mineral fertilizers before sowing ("Amazone AD-403 super", hereafter "Amazone h2").

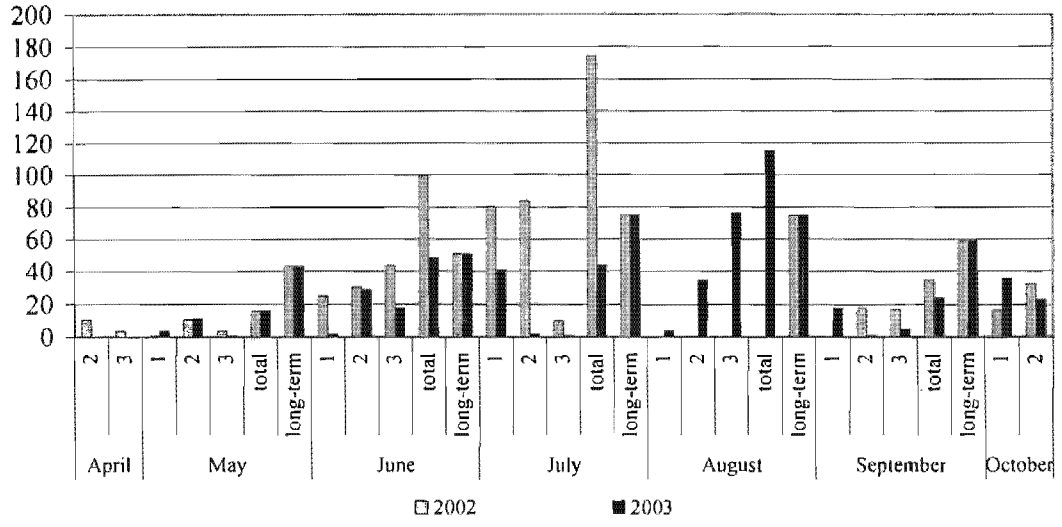

Fig. 1. Amount of precipitation, LLU RSF "Vecauce", 2002—2003, mm

Characterization of meteorological conditions

There was a lack of precipitation from the second decade of April till the second decade of May in both trial years (Fig. 1). Very dry weather was in 2002 when from the third decade of July till the end of October total amount of precipitation was less than 40 mm. Differences in the average air temperature were not so remarkable. Particularly dry weather in the autumn of 2002 was complemented by rapid decrease in temperature — from 12 °C in the second decade of September to 2 °C in the second decade of October (Fig. 2).

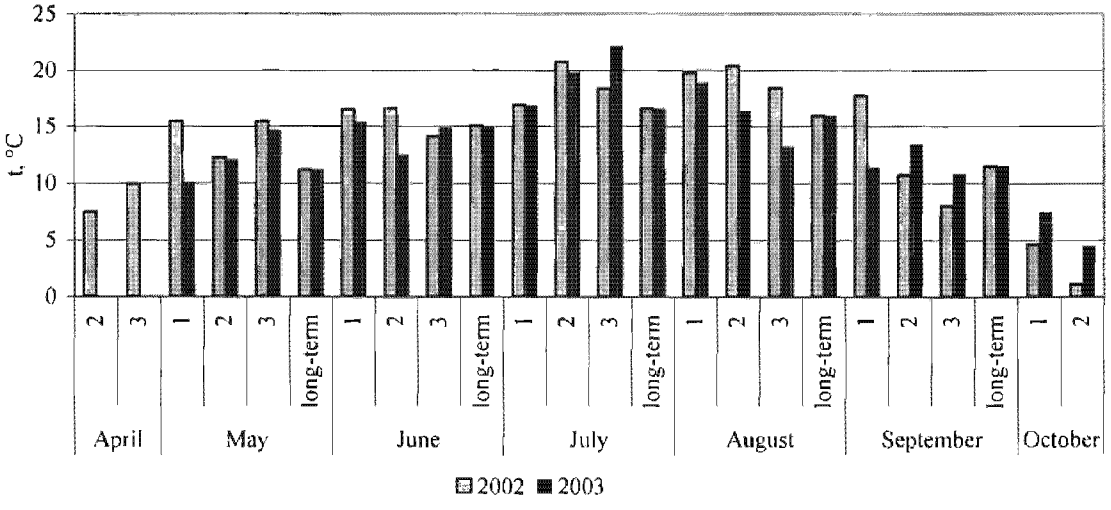

Fig. 2. Average air temperature, LLU RSF "Vecauce", 2002—2003, °C

Spring barley growing technologies

Soil was ploughed by Overum-6 DVL combined with Pakomat DK-205-335 CM. Herbicide Glifoss was applied on 06.09.2001. and 19.09.2002. Spring barley cv. Klinta was sown on 22.04.2002. and 02.05.2003. Sowing rate was 400 fertile seeds per m^2. Mineral fertilizers $N_6P_{26}K_{30}$ 300 kg ha^{-1} and NH_4NO_3 150 kg ha^{-1} (2002) were used, but in year 2003 — $N_4P_{20}K_{20}$ 300 kg ha^{-1} and NH_4NO_3 200 kg ha^{-1}. Pneumatic diffuser "Amazone" dispersed mineral fertilizers. Herbicide Duplozans super 2 L ha^{-1} was applied on 23.05.2002., but in 2003 mixture Granstars 10 g ha^{-1} + Primuss 60 ml ha^{-1} + Kontakts (adjuvant) 100 ml per 100 L water (28.05.2003.) was used. Insecticide Fastaks 0.15 L ha^{-1} was used in 2002 because of serious infestation of aphids.

Dry weight of shoot of spring barley was determined for 30 plants from each treatment. The number and length of lateral roots and coefficient of tillering were determined for 25 plants from each treatment. Weed assessment was done two times in the growing season using a 0.1 m^{-2} big frame.

The yield was harvested with trial harvester "Hege-140", adjusted to 14% moisture content and 100% purity. Data analysis was done using three factor analyses of variance.

Results and Discussion

Results in year 2002 show that a significant increase in the number of annual weeds was observed in the treatment with sowing after ploughing. Such results are obtained in both weed assessments — at the beginning and at the end of spring barley life cycle (Fig. 3). In the treatment where the disc-sowing machine was used, the number of annual weeds was smaller than in treatments with the anchor-type driller with a rotortiller after intensive soil tillage. Data show that in treatments with a greater number of annual weeds in early growth stages of spring barley before herbicide application, the number of annual weeds will be greater also in late stages. This coherence is verified by the coefficient of correlation between the numbers of annual weeds in both growth stages of spring barley ($r_{yx} = 0.677 > r_{0.05} = 0.231$).

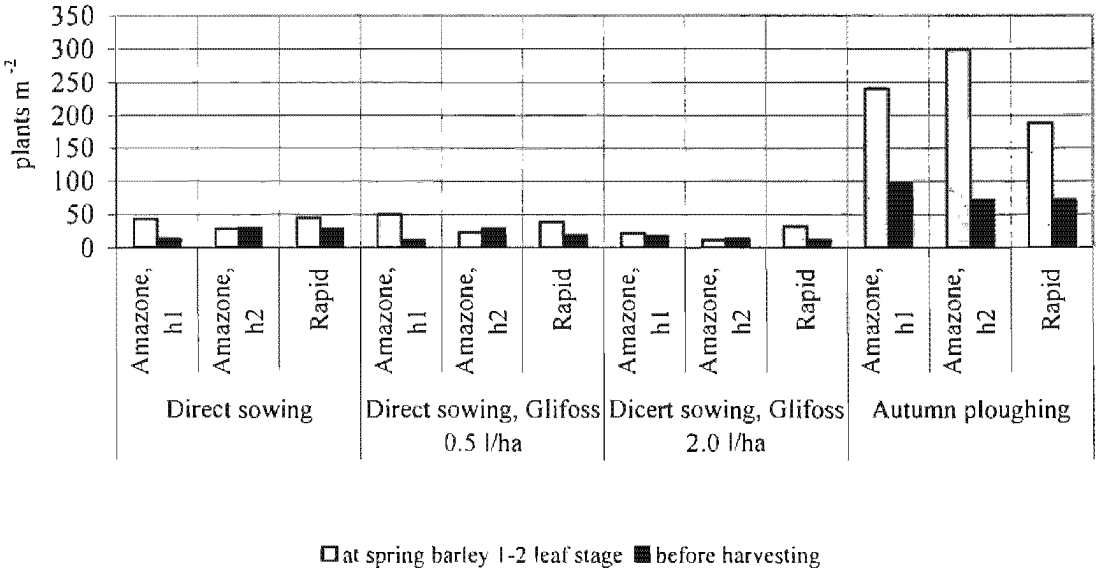

Fig. 3. The number of annual weeds in spring barley, 2002

Usage of gliphosate in the stubble-field provided a smaller number of annual weeds in 2002 at spring barley 1—2 leaf stage. There are no significant differences between the applied dosages of Glifoss — both provided similar efficiency. Increased depths of the rotortiller for sowing machine "Amazone" made significant decrease in the number of annual weeds in the treatment with direct sowing after applying Glifoss at the dosage of 0.5 L ha^{-1} (Fig. 4).

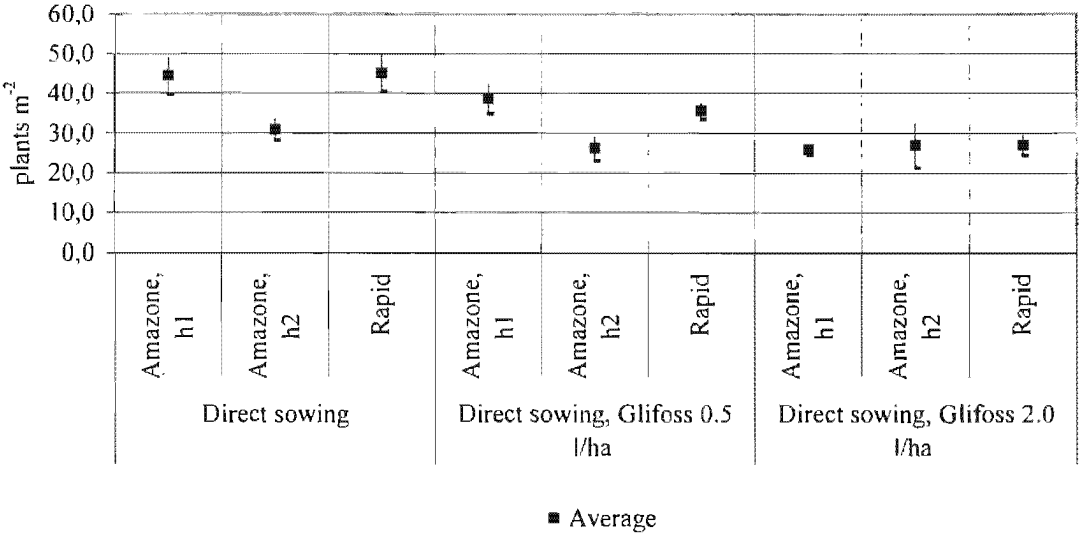

Fig. 4. The number of annual weeds at spring barley 1—2 leaf stage, 2002

There were no significant differences in the number of annual weeds among treatments with direct sowing before harvesting in 2002.

Usage of the disc sowing machine and anchor-type driller with the rotortiller at deeper working depths in direct sowing provided a significant decrease in the number of *Elytrigia repens* (L.) Desv. ex Nevski at spring barley

1—2 leaf stage. In treatments with autumn ploughing, the average number of *Elytrigia repens* (L.) Desv. ex Nevski was below one plant per square meter. Even a small number of *Elytrigia repens* (L.) Desv. ex Nevski in treatments with usage of gliphosate made a significant increase of this weed during the growing season. The same increase was observed in treatments with autumn ploughing (Fig. 5).

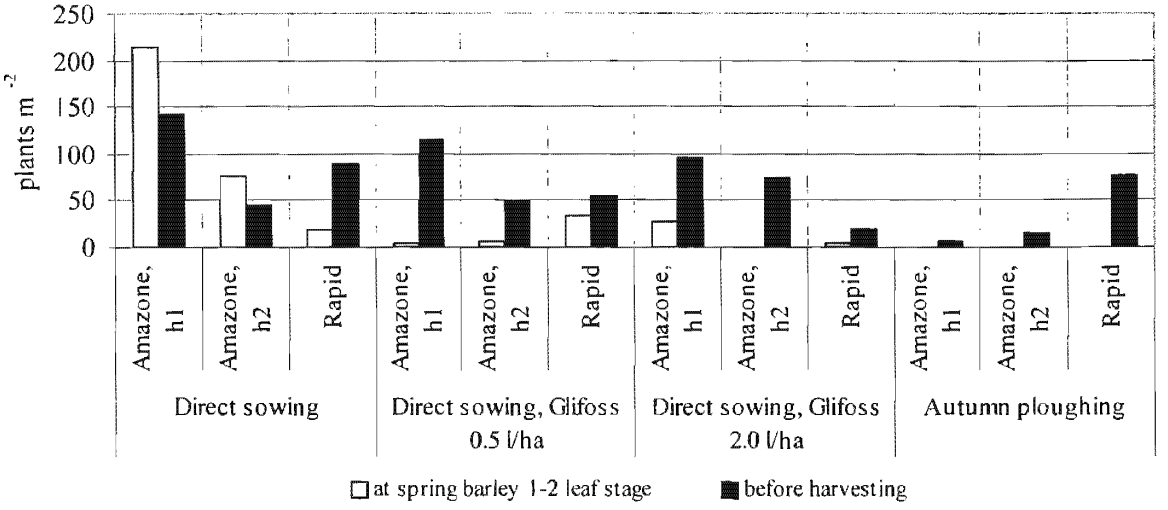

Fig. 5. The number of *Elytrigia repens* (L.) Desv. ex Nevski. in spring barley, 2002

Analyses of correlation allow concluding that only number of *Elytrigia repens* (L.) Desv. ex Nevski at spring barley 1—2 leaf stage made a significant negative impact on the development and yield of spring barley in 2002. The number of annual weeds at spring barley 1—2 leaf stage had positive correlation with indicators of spring barley development but relationship with the spring barley grain yield was insignificant (Table 1). There was a significant negative correlation between the numbers of *Elytrigia repens* (L.) Desv. ex Nevski at both barley growth stages and the grain yield.

Table 1

Correlation coefficients among weed infestation and indicators of the development of spring barley and grain yield

Resultant indications *(y)*	2002 r_{yx}^{2002}	2003 r_{yx}^{2003}
Number of annual weeds at spring barley 1-2 leaf stage *(x_1)*		
Length of spring barley roots at tillering stage	0.4834 *	0.2416 *
Weight of spring barley roots at tillering stage	0.3992 *	0.2596 *
Weight of spring barley plant at tillering stage	0.3992 *	0.2486 *
Spring barley grain yield	0.2679 *	–0.1657
Number of *Elytrigia repens* (L.) Desv. ex Nevski at spring barley 1-2 leaf stage *(x_2)*		
Length of spring barley roots at tillering stage	–0.4962 *	0.2046
Weight of spring barley roots at tillering stage	–0.3919 *	–0.0781
Weight of spring barley plant at tillering stage	–0.4000 *	–0.0073
Spring barley grain yield	–0.6440 *	–0.2344 *
Number of annual weeds before harvesting *(x_3)*		
Spring barley grain yield	0.05189	–0.3589 *
Number of *Elytrigia repens* (L.) Desv. ex Nevski before harvesting *(x_4)*		
Spring barley grail yield	–0.5250 *	–0.6482 *
$r_{0.05}$	0.231	0.231

* correlation coefficient significant at 95% probability.

The number of annual weeds before harvesting in 2002 was very small because of lack of precipitation, therefore correlation with spring barley grain yield was insignificant. Relationship between the number of *Elytrigia repens* (L.) Desv. ex Nevski at spring barley 1—2 leaf stage and before harvesting is also described by the results of analyses of regression (Figs. 6 and 7). In both cases, equations of linear regression are statistically significant.

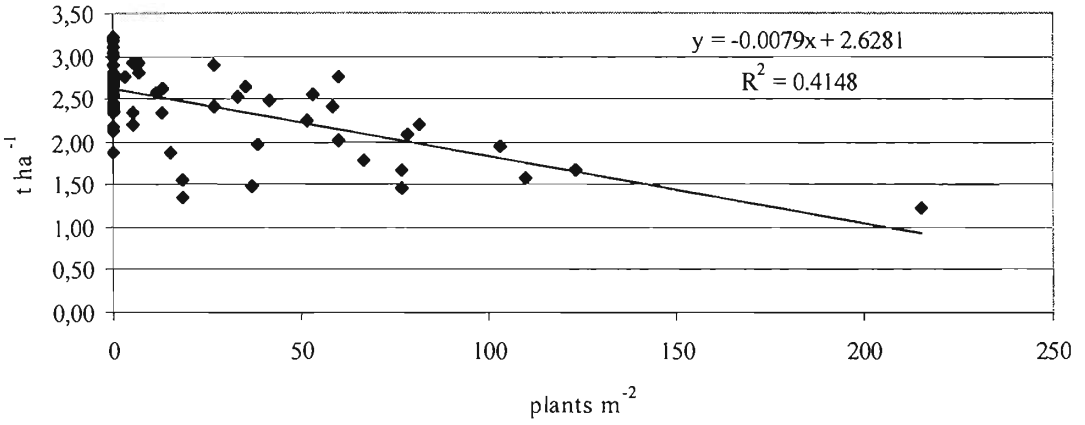

Fig. 6. Relationship between the number of *Elytrigia repens* (L.) Desv. ex Nevski. at spring barley 1—2 leaf stage and the grain yield, 2002

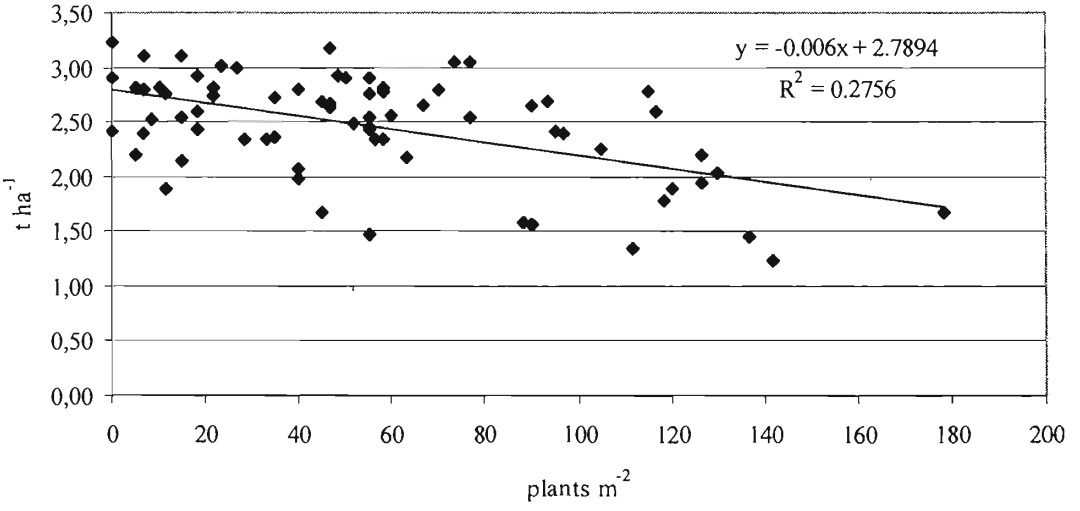

Fig. 7. Relationship between the number of *Elytrigia repens* (L.) Desv. ex Nevski. before harvesting and the spring barley grain yield, 2002

Comparison of the coefficients of linear regression from the relationships between the number of *Elytrigia repens* (L.) Desv. ex Nevski before harvesting and the grain yield in 2002 and 2003 shows that the noxious effect of *Elytrigia repens* (L.) Desv. ex Nevski was significantly higher in 2003 (Figs. 7 and 8). Linear relationship between the number of *Elytrigia repens* (L.) Desv. ex Nevski before harvesting and the grain yield in 2003 was also statistically significant.

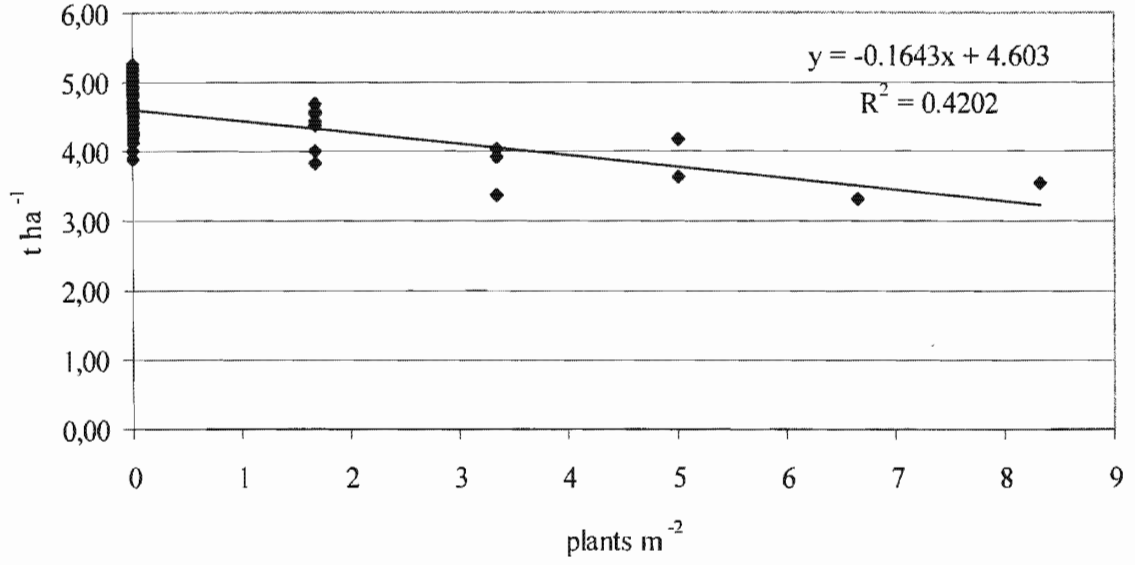

Fig. 8. Relatioship between the number of *Elytrigia repens* (L.) Desv. ex Nevski. before harvesting and the spring barley grain yield, 2003

A considerably lower but also a significant negative effect on spring barley grain yield was determined for the number of annual weeds before harvesting in year 2003. Comparison of the results of analyses of regression between both trial years shows that the noxious effect of weed infestation in sowings increases at higher levels of grain yield.

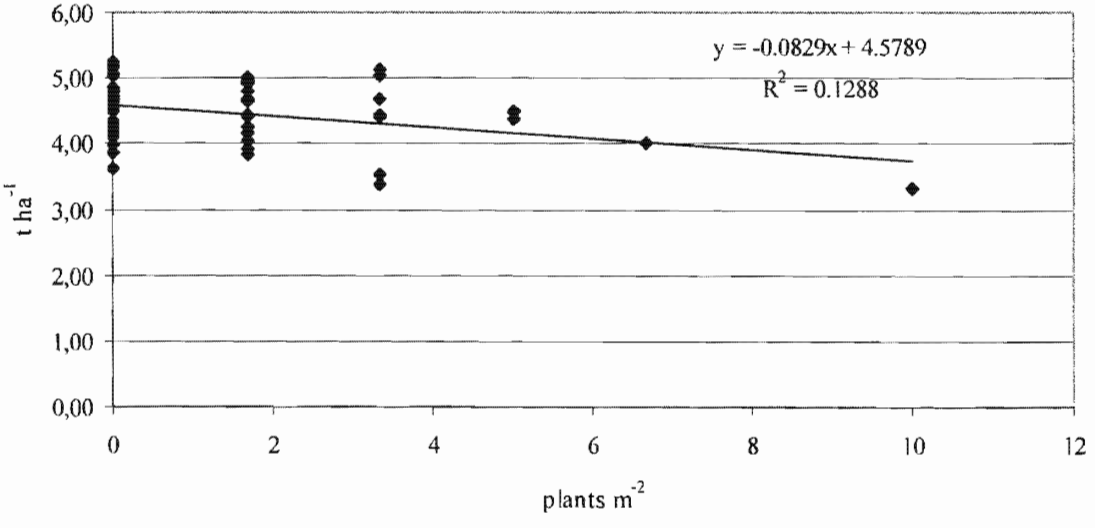

Fig. 9. Relatioship between the number of annual weeds before harvesting and the spring barley grain yield, 2003

A significant negative effect of annual weeds on the development and grain yield of spring barley was determined only before harvesting in 2003. Treatments with application of Glifoss at dosage of 2.0 L ha^{-1} and sowing after autumn ploughing show better results. A significantly higher number of annual weeds was observed in treatments where sowing was done with the anchor type driller ("Amazone") with a rotortiller at the lowest working depth (5—7 cm) (Fig. 10).

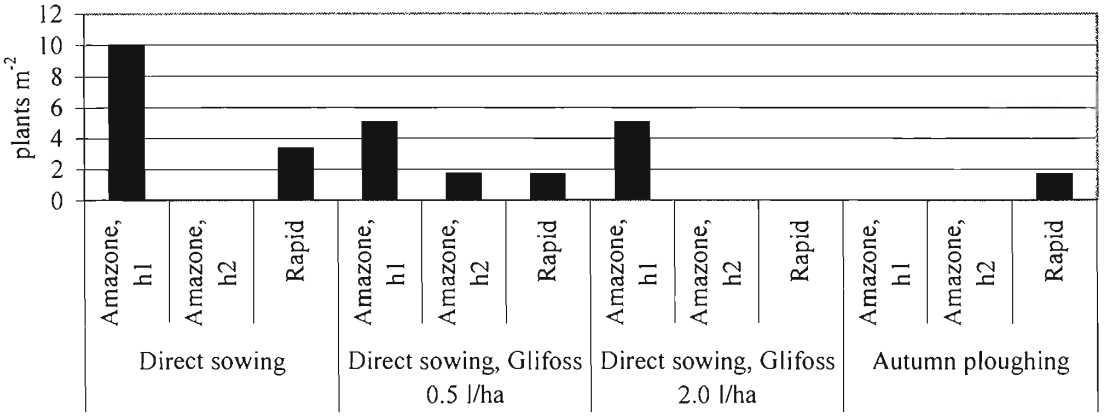

Fig. 10. The number of annual weeds before harvesting, 2003

Increasing the depth of the rotortiller for driller "Amazone" from 5—7 cm to 7—10 cm provided a significant decrease in the number of annual weeds in sowings of spring barley.

Conclusions

Diverse meteorological conditions in the trial years had determinative importance to the effect of soil tillage, application of Glifoss and sowing technologies on weed infestation in sowings and growth and development of spring barley.

Significant decrease in the number of annual weeds was observed by increasing the depth of the rotortiller from 5—7 cm to 7—10 cm using sowing machine "Amazone AD-403 super".

A significant negative effect on the growth and development of spring barley was caused by increased number of *Elytrigia repens* (L.) Desv. ex Nevski. This regularity was determined both at barley 1—2 leaf growth stage and before harvesting.

The negative effect of sowing infestation with annual weeds on the growth and development of spring barley was not significant at barley 1—2 leaf stage. The effect of weed infestation before harvesting differs between the trial years.

An increased number of annual weeds at barley 1—2 leaf stage also caused greater weed infestation before harvesting.

References

1. Lapiņš, D., Gaile, Z., Bērziņš, A., Liepiņš, J., Ausmane, M., Melngalvis, I., Gužāne, V., Sprincina, A., Freipiča, A., Kuplais, Ē., Kreišmane, B. 2000. Augsnes apstrādes — sējas tehnoloģiju efektivitāte graudaugiem LLU mācību un pētījumu saimniecībā "Vecauce". Agronomijas vēstis, Nr. 2, 26—39.
2. Lapiņš, D., Bērziņš, A., Gaile, Z., Koroļova, J. 2001. Soil Tillage and Sowing Tehnologies for Spring Barley and Winter Wheat. Baltic States Banch of Istro — 1st International Conference of BSB of Istro & Meeting of Working Group 3 of the INCO-COPERNICUS Concerted Action on Subsoil Compaction. Modern Ways of Soil Tillage and Assessment of Soil Compaction and Seedbed Quality, 21—24 August, EAU Tartu, Estonia, 150—160.
3. Lauringson, E., Vipper, H., Kuill, T., Talgre, L., Hirsnik, L. 2001. The Effect of the Minimisation of Autumn Tillage on Weediness and Yield. Baltic States Banch of Istro – 1st International Conference of BSB of Istro & Meeting of Working Group 3 of the INCO-COPERNICUS Concerted Action on Subsoil Compaction. Modern Ways of Soil Tillage and Assessment of Soil Compaction and Seedbed Quality, 21—24 August, EAU Tartu Estonia, 81—92.
4. Maiksteniene, S. 2000. Possibilities of primary tillage reduction on clay loam soil. The Results of Long-Term Field Experiments in Baltic States, Proceedings of the International Conference, Jelgava, Latvia, 22—23 November, 106—114.
5. Stancevicius, A., Spokiene, N., Raudonius, S., Treciokas, K., Jodaugiene, D., Kemesius, J. 2000. Reduced Primary soil Tillage of the Light Loamy Soils. The Results of Long-Term Field Experiments in Baltic States, Proceedings of the International Conference, Jelgava, Latvia, 22—23 November, 133—147.

DETERMINATION OF WEEDS IN AGROPHYTOCENOSIS IN SITE-SPECIFIC AGRICULTURE

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Abstract

A study has been conducted in cooperation with the University of Bonn. The weed determination and weed control system based on digital photo analysis, GPS and GIS, usable for various farms under the conditions of Latvia, is being worked out. Significant reduction in pesticide usage and environmental preservation have been reached via site specific, selective herbicide application.

Digital image analysis is used to identify weed seedlings of the most typical vegetation in cereal sowings in Latvia. The weed species identification is needed to select post emergence herbicides and to assess weed seedlings distribution in the fields. The reduction of herbicide treatments can be realized by selective herbicide application, when weed control is limited to field areas where specific weeds are present.

Images were captured in near-to-infrared light with a digital camera. The images were processed with the computer — binarized, weeds contours were extracted, and shapes — analyzed (including parameters of the external contour such as roundness, compactness, area, and 40 Fourier descriptors). The investigations were carried out in cereal sowings in Latvia.

Key words: site-specific agriculture, automated weed recognition.

Introduction

A study has been conducted to prevent nature pollution caused by unnecessary herbicide usage, to obtain economical gain for agriculture via developing and improving an automated weed recognition system designed at the Friedrich-Wilhelm University of Bonn, and to use it for weed detection and patch spraying under the conditions of Latvia.

In Latvia, weed patch spraying is actual in most of the fields, which are five hectares large or more. Of course, it depends — there might be some large fields, where uniform spraying can be applied without significant herbicide wasting (compared to site-specific application).

Most of the fields have non-homogenous weed density with weeds located in several patches where, as in other field areas, herbicides (for example, specific, expensive herbicides against CIRAR, AVEFA) might not be used at all. This threshold level of weeds depends on the possible yield loss and economical calculation as "not worth to spend, but worth to save".

This paper is mainly a theoretical literature review of methodology and techniques for creating and improving an automated weed detection system for use under the conditions of Latvia.

Materials and Methods

In vegetation hall conditions at the University of Bonn, Germany, germinating plant samples (60 species) were grown. Over 40 weed species and six crops (barley, wheat, oat, sugar beet, rape, maize) were successfully growing, and pictures were successfully taken.

The purpose of the investigation: for creating a plant-form parameters' knowledgebase, collect digital pictures of weeds, which later will be used for automated weed detection in field conditions. The pictures of weeds were taken once every two days until the weeds' six leaves stage.

For image recording, the nIR camera-framegrabber-computer system was used (a Kodak grayscale camera with resolution 1024*1024 pixels, sensitive in nIR). Very high image precision — approximately 5 pixel/mm — was used. For image processing, hardware and software equipment of the Institute of Plant Cultivation of the University of Bonn was used.

Results and Discussion

Possible economical and environmental gain from precise herbicide application in patch spraying

A lot of research have been done showing clearly that not all parts of fields require the same amounts of herbicides. For example, Von Lettner et al. summarize[1] that economic threshold is often reached only on 50% of arable land. The possible savings in herbicide usage vary between 50 and 70%. Significant savings in precise herbicide use are possible by implementing adequate thresholds.

In areas where weed density is below the weed control threshold, herbicides are not used. In a 4-year experiment with a GPS-equipped sprayer at the Dikhopshof research station of the University of Bonn in Germany, average calculated savings of herbicide usage made 54% (Timmermann, Gerhards, Kuehbauch, 2004). Mr. Gerhards points out that savings are strongly dependent on crop and year. For grass weed herbicides, the savings were 90% in winter cereals, 78% — in maize, and 36% — in sugar beet; for herbicides against broadleaf weeds, 60% savings were in winter cereals, 11% — in maize, and 41% — in sugar beet fields. The economical gain from reduction in herbicide use varied between the crops, it was dependent on the amount and price of herbicides used. In maize, savings made 42 €/ha, in winter wheat — 32 €/ha, and in sugar beet — 20 €/ha (Gerhard, Christensen, 2003). In barley sowings (Dicke, 2004)

[1] Von Lettner J., Hank K. und Wagner P. Ökonomische Potenziale der teilflächenspezifischen Unkrautbekämpfung. http://www.weihenstephan.de/ui/veroeff/tfu.htm

with site-specific weed control, 54% of herbicides against *Gallium aparine* (GALAP) and *Cirsium arvense* (CIRAR), 96% of herbicides against grass weeds, and 94% of herbicides against broadleaved weed species were reduced.

Techniques of image acquisition and processing

To provide a precise, environment-saving herbicide application system, a weeds patch spraying and automated weed detection-decision-making system with good computer vision capabilities is necessary. To detect weeds automatically, good quality images, as well as powerful computer equipment, are needed because image processing "costs" a lot of CPU usage.

Several types of cameras are used worldwide, and different approaches exist — either tractor-mounted and close-to-soil surface or airborne mounted (remote sensing). As J.V. Statfford says (Statfford, 1997), it is possible to see weed patches via airborne remote sensing, but data must be complemented by others such as assisted manual surveying to create application maps for patch spraying.

Recognition principles

Some researchers (e.g. R.H. Biller et al., 1997) do not use video-camera systems but optoelectronical sensors. They detect and analyze light reflected by soil and plants to distinguish green objects — plants (Biller et al., 1997). Such systems might be very useful to spray and save glyphosphate herbicides. Unfortunately, this system is not capable of distinguishing and detecting crop plants from weeds. D. Ehlert (2004), for detecting weeds, worked in empty space between crop rows. There detection principle was to count every small, green plant as a weed, but big objects — as crop plants. In early 1985, Woebbecke and Meyer (1995b) established an interesting fact that the Fast Hadamard and Fast Fourier frequency transform methods can be used to classify inter-row video images, and the Fast Hadamard transform was as effective as the Fast Fourier transformation, but over 20 times faster.

Kühbauch, Gerhard, Sökefeld et al. have worked for some ten years at the University of Bonn to create a complete system, which now is capable not only of distinguishing crop plants from weeds, but also of detecting several weed species. For the weed species detection, plant shape parameters are used (length, width of leaves, and form factors). M. Sökefeld (Sökefeld, 1997) describes in detail mathematics used to detect weeds and crop species in his PhD paper. This system for plants two-dimensional description uses not only the plant's size, area and span width, but also the Fast Fourier transformation description.

Image acquisition

There are two main approaches to capture images and extract plant shape contours for comparison with samples in the knowledgebase: first is to use RGB (red-green-blue) human visible light images, second is near-to-infrared (further in the text abbreviation nIR is used). Images in nIR are taken in a 700~1000 nm wavelength. This wavelength is very appropriate due to plants' higher reflectance of light than that of soil's. The final result is quite a good contrast image with brighter plants over darker soil. Woebbecke et al. (1995a) valued the near-infrared video system as superior to the color system for detecting weeds in inter-rows. R. Gerhard, W. Kühbauch et al. use nIR and bispectral (nIR+green) cameras in field conditions. Bispectral cameras with image preprocessing in chip level give excellent contrast images where plant contours are perfectly visible, but non-plant objects (stones) are not visible (Sökefeld, 2004[2]). There might be used RGB cameras, too, similarly as Petry (1998) and Peretz et al. (2000) do it. In this case, a specific plants image extraction program with specific plants color space coordinates calculation is needed (Woebbecke et al., 1995a).

Weed patch spraying can be done either online or offline. Offline processing, with spraying later, has a benefit – it allows calculating precisely the amount of each herbicide to be used. Online processing should work "as go", but Sökefeld (2004) says that requirement for a direct injection system for site-specific herbicide application is based on weed detection. The type of weed detection and the resulting detection time is the crucial factor for the whole weed detection system (on-line or off-line) and for its performance, especially for the acceptable delay time of the direct injection system.

Conclusions

From literature studies it can be concluded that the Precision Farming Weed Control technology system can give significant savings in herbicide use by automatic weed detection and precise patch spraying application.

Even nowadays image capturing for such needs has not been solved via remote sensing due to lack of the required very high image precision (more than 1 pixel/mm). For the best quality, very detailed pictures are needed; therefore use of close-to-surface, tractor-mounted cameras are preferable.

Generally, for weed recognition, there might be used all types of cameras — RGB and nIR, but the best results are possible by using some combination of IR and visible light spectrum. To find the best low-cost solution, it is worth to study possibilities of using ordinary industrial RGB cameras.

For weed detection, a knowledgebase of weeds form span-width, Fast Fourior transformation and other parameters can be successfully used.

Computer-based image analysis and weed detection consume a lot of CPU time requiring powerful computers. As more images per field and per every second we take, as more computing resources are needed.

Now, in the development stage of the project, even significant savings of herbicides might justify only some costs of developing the automatic weed detection and application of the Precision Farming Weed Control technology system.

Optimistically, the system might be started testing in practical farming in Latvia in 2006 at the very earliest.

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[2] Sökefeld M., Gerhards R. Unkrautkartierung mit digitaler Bildverarbeitung. http://www.landtechnik.uni-bonn.de/ifl_research/pp_5/unkrautkartierung_mit_digitaler_bildverarbeitung.pdf, 7 p.

References

1. Biller, R.H., Hollstein, A., Somer, R. C. 1997. Precision application of herbicides by use of optoelectronic sensors. Precision Agriculture. In: STAFFORD, J.V. (ed.) Precision Agriculture '97, First European Conference on Precision Agriculture, Warwick University, UK, 451—458.
2. Dicke, D., Fries, A., Gerhards, R. 2004. Ermittlung von Schadschwellen für die teilschlagspezifische Unkrautbekämpfung im Braugerstenanbau. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz (Journal of Plant Diseases and Protection), Sonderheft XIX, 413—421.
3. Ehlert, D., Langner, H.R. 2004. Sensoren für die Präzisionslandwirtschaft. Forschungsreport Verbraucherschutz, Ernährung, Landwirtschaft, ISSN 0931-2277, Deutschland, 8—11.
4. Franz, E., Gebhardt, M. R., Unklesba, K. B. 1991. The use of local spectral properties of leaves as an aid for identifying weed seedlings in digital images. Trans. ASEA 34(2), 682—687.
5. Gerhards, R., Christensen, S. 2003. Real time weed detection, decision making and patch spraying in maize, sugarbeet, winter wheat and winter barley. Weed research, 43, 358—392.
6. Krohmann, P. 2003. Effizienz teilschlagspezifischer Unkrautkontrolle und räumlich-zeitliche Dynamik der Unkrautpopulationen in einer Felderfolge und Monokultur. Dissertation der Hohen Landwirtschaftlichen Fakultät der Rheinischen Friedrich-Wilhelms-Universität zu Bonn, 135 pp.
7. Naboul, A.A. 1996. Einzelstruktureinheit zur Echtzeit-konturextractuion, naGIT Automatisierungstechnik & Qualitätsmanagement. Symposium "Aktuelle Entwicklungen und industieller Einsatz der BildVerarbeitung" 5. und 6. September 1996, Aachen.
8. Perez, A. J., Lopeza, F., Benlloch, J. V., Christensen, S. 2000. Colour and shape analysis techniques for weed detection in cereal fields. Computers and Electronics in Agriculture, 25, 197—212.
9. Petry, W. 1989. Dissertation. Unkrautkontrolle im landwirtschaftlichen Pflanzenbau mit Hilfe der quantitativen Bildanalyse, Universität Bonn, 1—74.
10. Shropshire, G. J., Von Bargen, K. 1989. Fourier and Hadamard transforms, for detecting weeds in video images. Paper No. 89—7522, An ASAE meeting presentation, New Orleans.
11. Sökefeld, M. 1997. Zugl. Dissertation. Automatische Erkennung von Unkrautarten im Keimblattstadium mit digitaler Bildverarbeitung. Bonn, 60 pp.
12. Sökefeld, M., Gerhards, R., Therburg, R.D., Nabout, A., Jacobi, J., Lock, R., Kühbauch, W. 2002. Multispektrale-Bildanalyse zur Erfassung von Unkraut und Blattkrankheiten. Z. PflKrankh. PflSchutz, Sonderheft XVIII, 437—442.
13. Sökefeld, M., Hloben, P., Schulze Lammers, P. 2004. Möglichkeiten und Grenzen der Direkteinspeisung von Pflanzenschutzmitteln zur teilschlagspezifischen Unkrautkontrolle. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz (Journal of Plant Diseases and Protection), Sonderheft XIX, ISSN 0938-9938, 431—437.
14. Statfford, J.V. 1997. Machine-assisted detection of weeds and weed patches. Precision Agriculture, Vol.2: Technology, IT and Management, Oxford, 511—518.
15. Timmermann, C., Gerhards, R., Kuehbauch, W. 2004. The Economic Impact of Site-Specific Weed Control. Precision Agriculture, 4, 249—260.
16. Woebbecke, D. M., Meyer, G. E., Von Bargen, K., Mortensen, D. A. 1995a. Color indices for weed identification under various soil, residue and lighting conditions. Transsactions of the ASAE, Vol. 38(1), 259—269.
17. Woebbecke, D. M., Meyer, G. E., Von Bargen, K., Mortensen, D. A. 1995b. Shape features for identifying young weeds using image analysis. Transactions of the ASAE, Vol. 38, No. 1, 271—281.

PRODUCTIVITY OF THE SECOND GENERATION WEEDS, RESISTANT TO HERBICIDE MCPA

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Summary

The experiments were carried out at the Experimental Station of the Lithuanian University of Agriculture in the period of 2002—2003. The seeds of some annual weeds were collected in 2001 from the crops applied with herbicide MCPA (1 l/ha) and from the organic field of the University's training farm, in which herbicides had not been used since 1996. In winter, seeds were kept in soil. In spring, seeds of weeds were sown in barley crop, which had been sprayed according to the following scheme: 1) control, 2) MCPA 1 l/ha, 3) MCPA 2 l/ha.

The impact of the herbicide on the progeny of the weeds, which survived the spraying, was determined depending on species characteristics. After spraying weeds of the first and second generation, resistance of *Galeopsis tetrahit* L. to the herbicide decreased in the second generation. After spraying 1 and 2 l/ha of the preparation, the productivity of the first generation *Galeopsis tetrahit* L. decreased by 48.0—64.6%, while repeated spraying of the second generation plants decreased the productivity by 80.0—99.0%, respectively. At the same time, herbicide resistance of the second generation plants of other weed species (*Persicaria lapathifolia* L., *Fallopia convolvulus* L., *Stellaria media* L., *Galium aparine* L.) increased. A lower dose of MCPA (1 l/ha) even stimulated propagation of these weeds. *Galium aparine* appeared to be particularly resistant to herbicide MCPA. After spraying progeny of sprayed plants with this herbicide (1 l/ha), the second generation of *Galium aparine* produced dry mass and matured seeds 3.4 times more compared to the control, where only the first generation was sprayed. The obtained difference was significant.

The influence of MCPA on weeds did not disappear in the second generation, although progeny of the applied plants was untreated with this herbicide. MCPA stimulated productivity of the second generation plants of *Chenopodium album*, *Polygonum lapathifolia*, and *Stellaria media*. Thus agrophytocenosis that was damaged by herbicide MCPA naturally recovered in the following year as the surviving weeds produced more productive progeny.

Key words: MCPA (4-chloro-2methylphenoxyacetic acid), weeds, generation, progeny, resistant.

Introduction

Herbicides of 75 names were registered in Lithuania in 2003 (Valioniene, 2003). Derivatives of phenoxyacids make 15% (11 names) of all herbicides. These are compounds of 2.4-dichlorphenoxy acetic acid: aminopielik, DMA 6 2.4-D "RETRO", 2.4-D Nufarm, BASF 2.4-D. Compounds of 4-chloro-2methylphenoxyacetic acid such as chvastox, danacetate, MCPA, MCPA Super, Kemira MCPA, Nufarm MCPA are of similar selectivity. These herbicides are 3—4 times cheaper compared to the new and efficient compounds of sulfonylurea, therefore they are still in demand.

Compounds of phenoxyacids have been used in Lithuania since 1957. It has been determined that after long-term application of herbicides of the same or similar effect the species that are sensitive to them disappear, while the number of resistant ones is increasing. It has already been observed that regular application of 2,4-D herbicides caused disappearance or decrease in the amount of *Chenopodium album* L., *Thlaspi arvense* L., *Capsella bursa-pastoris* (L.) Med., *Sinapis arvensis* L., *Raphanus raphanistrum* L., *Erysimum cheiranthoides* L. and increase in *Agrostis stalonifera* L., *Elytrigia repens* (L.) Nevski, *Equisetum arvense* L., *Poa annua* L., and *Polygonum aviculare* L. (Stancevicius, 1972).

2.4-D herbicides have been applied in the crops of gramineous cereals in many countries for a long time, therefore wide spread of resistant weeds is observed. In Latvia, the increase of *Galium aparine* L., *Veronica sp., Euphorbia helioscopia* L., *Lapsana communis* L., *Poa annua* L., *Lamium purpureum* L., *Stellaria media* (L.) Vill., *Artemisia vulgaris* L., *Mentha arvensis* L., *Elytrigia repens* (L.) Nevski in grain crops has been observed in recent years (Lapins, 1999).

In Finland, a survey of weeds in 80 fields in 1980 and in 90 fields in 1990 showed that before 1980, when phenoxyacids herbicides were mostly used in the crops of gramineous cereals, the most frequent weed species were as follows: *Viola arvensis* Murray, *Galeopsis sp., Stellaria media* (L.) Vill., *Fumaria officinalis* L., and *Elytrigia repens* (L.) Nevski. When application of sulfonylurea derivatives started, the most frequent weed species in the crops of gramineous cereals were *Viola arvensis* Murray, *Chanopodium album* L., *Stellaria media* (L.) Vill., *Elytrigia repens* (L.) Nevski, and *Galium spurium* L. In a 10–year period the spread of *Chenopodium album* L., *Galium spurium* L., *Elytrigia repens* (L.) Nevski, *Cirsium arvense* (L.) Scop. in Finland was the strongest (Hyvönen, Ketoja, Salonen, 2003). The spread of *Elytrigia repens* was also observed in Germany (Albrecht, 1995) and in Hungary (Toth et al., 1999).

The experiments carried out under different agroclimatic conditions in 1997—1998 in the Czech Republic showed the existence of few weed species in separate fields (from 8 to 23), while predominating species were as follows: *Viola arvensis* Murray, *Galium aparine* L., *Chenopodium album* L., *Fallopia convolvulus* L., and *Elytrigia repens* (L.) Nevski (Tyser, Holec, 2004).

The spread of some weeds can have different reasons. The increase can be caused by bigger space in agrophytocenoses and lower competition of weeds due to disappearance of sensitive species. Resistance of weeds to herbicides can be considered as the second reason. It is a phenomenon when after application of the same herbicides for a long time the sensitive weed species become the resistant ones. The third reason is as follows: herbicides as physiologically active materials can stimulate the growth and development of resistant weeds. The stimulating impact of 2.4-D on some annual weeds was observed already when this herbicide was applied in spring barley and winter rye crops at the Experimental Station of the Lithuanian University of Agriculture. 2.4-D stimulated growth and propagation of the second generation of *Chenopodium album* L., *Centaurea cyanus* L., *Tripleurospermum perforatum* (Merat) M.Lainz, and *Spergula arvensis* L. (Spokiene, 1971).

Thus, so far in the registration of new herbicides their biological efficacy is evaluated on the basis of the number of surviving weeds and mass of dry matter. After that the amount of killed weeds is calculated. No investigation of biological characteristics of herbicides-resistant weeds that survived in the treated crops has been carried out in Lithuania or in other countries. In recent years, foreign editions have been writing a lot about the physiological mechanism of weeds resistance.

The aim of this work — to analyze the impact of herbicide MCPA on the productivity of the progeny of some widely spread annual weeds.

Materials and Methods

The investigations were carried out at the Experimental Station of the Lithuanian University of Agriculture in 2002—2003. The soil of the experiment site was medium loam on sandy loam over the moraine clay *Endohypogleyic-Eutric Planasol — Ple-gln-w* (Jodaugiene, 2002).

Widely spread annual weeds *Chenopodium album* L., *Galeopsis tetrahit* L., *Stellaria media* (L.) Vill., *Persicaria lapathifolia* (L.) Gray, *Fallopia convolvulus* L., *Galium aparine* L., *Viola arvensis* Murray were chosen for the investigations. Seeds of these weeds were collected in 2001 in the crops of the Experimental Station of the Lithuanian University of Agriculture that had been sprayed with herbicide MCPA (1 l/ha) and in the organic field of the Training Farm, in which herbicides had not been applied since 1996. To discontinue the dormant state, the weed seeds were put into capron packages and buried in soil at the depth of 20 cm in autumn. There they were kept during winter.

In spring, the weed seeds were sown in small plots of spring barley crop. The barley was sown manually at 20 cm distances between the rows. Seed rate — 180 kg/ha. In the spaces between the rows, seeds of the weeds that had been untreated and sprayed with herbicide MCPA were sown in the segment of 50 cm (sites of 0.1 m^2). 100 seeds of each weed species were sown. Herbicide MCPA was sprayed in the phase of barley tillering. Thus the experiment was arranged according to the following scheme:

The first generation, grown in 2001 (Factor A):

1. untreated,
2. MCPA 1 l/ha;

The second generation, grown in 2002 (Factor B):

1. untreated,
2. MCPA 1 l/ha,
3. MCPA 2 l/ha.

Area of the plots: 6.8 m^2 in 2002; 4.5 m^2 in 2003; in 4 replications.

In 2003, the third generation of weeds was grown. However, their germination was uneven. *Galium aparine* seeds did not germinate at all, germination of *Fallopia convolvulus* and *Stellaria media* seeds was poor. Germination of *Chenopodium album* was abundant, therefore their sprouts were thinned by leaving not more than 20 sprouts in a line. The experiment of small plots was arranged in the same way as in 2002, but barley was untreated.

The influence of herbicide MCPA on germination of weed seeds in field conditions was determined. The weeds were counted 20 days after sowing and 20 days after spraying. The weeds were rooted out before barley harvesting. Their productivity was established in different ways taking into consideration morphological characteristics. Plants of weeds that had an upright stem — *Chenopodium album, Galeopsis tetrahit* and *Persicaria lapathifolia* — were counted and their height was measured. Every plant was threshed manually and the seeds were counted.

Other weed species have different morphological characteristics therefore the establishment of their productivity varied. Stems of *Stellaria media* branch and often form a hummock. Stems of *Galium aparine* cling and climb, while those of *Fallopia convolvulus* twist round other plants, therefore, at the end of vegetation, the exact number of these weeds can be hardly determined. Therefore, in the sites of each plot (0.1 m^2) where the analyzed weeds had been sown all plants were rooted out, wrapped into paper packages and dried in dry premises. Dry weed mass was weighed and manually threshed. When the remains after threshing did not exceed 2 g all seeds were separated and counted. When mass of the remains after threshing exceeded 2 g, two samples of 1 g each were made and the seeds were separated and counted. After obtaining average number of seeds in the mass of 1 g, the number of seeds in the total mass was calculated. The investigation data was processed with computer program SYSTAT 10 by transforming the data lg $10(x+1)$.

Results

The influence of herbicide MCPA on germination of weed seeds in field conditions

The accounting 20 days after sowing showed that after sowing, germination of weed seeds, which were collected in untreated crop and wintered in soil, was dependent on species characteristics and reached 20.9—55.0%. Herbicide MCPA significantly decreased germination of the seeds of two species. After sowing, germination of *Chenopodium album* seeds, collected in crop treated with herbicide MCPA (1 l/ha), was 2—3 times lower and that of *Galeopsis tetrahit* — 60% lower compared to germination of seeds collected from plants not treated with the herbicide. MCPA had no significant influence on seed germination of other weed species (Table 1).

Table 1

The influence of herbicide MCPA on germination of weed seeds in field conditions

No.	Weed species	Germination in field conditions, %	
		Untreated	MCPA, 1 l/ha
1.	*Chenopodium album* L.	38.0	16.4***
2.	*Galeopsis tetrahit* L.	37.3	14.9***
3.	*Fallopia convolvulus* L.	20.9	17.3
4.	*Persicaria lapathifolia* (L.) Gray	24.6	26.9
5.	*Stellaria media* (L.) Vill.	45.1	42.3
6.	*Galium aparine* L.	55.0	56.2

*** significant differences at 99.9% probability level.

Biological efficacy of herbicide MCPA

As to the number of weeds that survived a 20-day period after spraying, *Chenopodium album* proved to be the most sensitive to herbicide MCPA. After spraying progeny of untreated weeds with 1 l/ha of the preparation, 95.1% of progeny were killed (Table 2). *Viola arvensis* was in the second place — 48.2% of plants died. Other weed species were more tolerant, the percentage of killed plants was 5.1—13.2%. A bigger dose of the herbicide was more effective for all tested weed species except for *Galium aparine*. Both bigger and smaller doses of MCPA had similar effect on this weed.

The herbicide had different influence on progeny of treated weeds when sprayed two years in turn. *Chenopodium album* became more resistant — 1 l/ha of MCPA killed 69.3% of plants. Resistance of *Fallopia convolvulus* and *Stellaria media* decreased, while that of *Galeopsis tetrahit*, *Galium aparine* and *Viola arvensis* increased. A lower dose of the herbicide did not kill the weeds. After spraying 1 l/ha of the preparation, the number of *Galeopsis tetrahit* plants was by 8.6% bigger, *Galium aparine* — by 4.0%, *Viola arvensis* — by 30.9% bigger than that in the control. The increase of *Viola arvensis* was significant. It should be noted that a bigger dose of MCPA did not kill *Galium aparine*. A bigger dose of the preparation decreased the number of plants of other weed species.

Table 2

Biological efficacy of herbicide MCPA

No.	Weed species	The first generation, grown in 2001 (factor A)					
		Untreated			MCPA 1 l/ha		
		The second generation, grown in 2002 (factor B)					
		Untreated	MCPA 1 l/ha	MCPA 2 l/ha	Untreated	MCPA 1 l/ha	MCPA 2 l/ha
		Number of plants 20 days after spraying					
		Unit/m^2	%		Unit/m^2	%	
1.	*Chenopodium album*	26.7	4.9***	3.7***	15.3	54.2	8.5*
2.	*Galeopsis tetrahit*	39.0	93.1	43.6*	16.3	108.6	26.4*
3.	*Fallopia convolvulus*	19.7	91.4	44.2*	21.7	50.7	36.9*
4.	*Stellaria media*	65.3	94.9	70.9	98.0	39.1*	20.7*
5.	*Galium aparine*	50.7	86.8	87.4	43.0	104.0	109.3
6.	*Viola arvensis*	8.3	51.8	56.6	9.7	130.9	23.7

* significant differences at 95% probability level.
*** significant differences at 99.9% probability level.

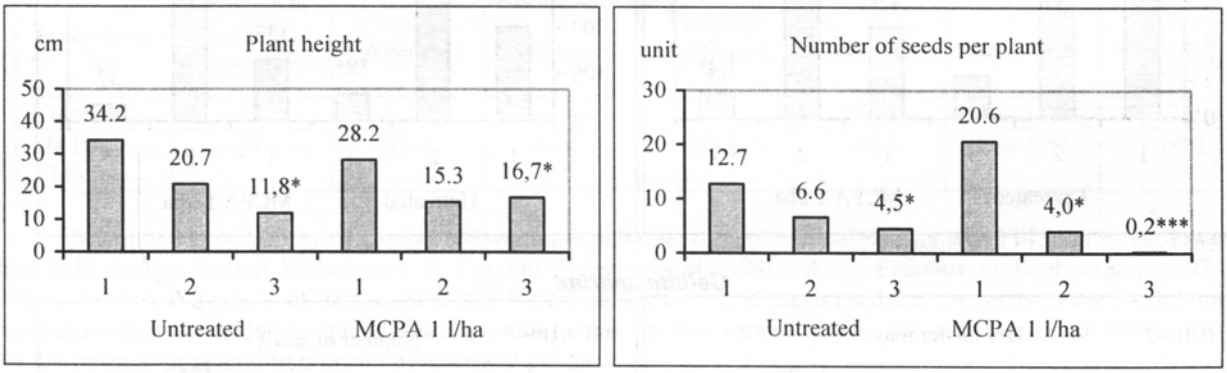
In order to evaluate the influence of herbicides on weeds it is not sufficient to determine the number of surviving weeds. The knowledge of the productivity of progeny, produced by weeds treated with herbicides, is very important.

Productivity of the second generation weeds, having survived in sprayed crop

Average height of weeds or their dry matter mass in the plot where they grew (g/0,1 m^2) and average productivity of one plant or number of matured seeds in the plot of 0,1 m^2 were considered the main indices of weed productivity. Competition of weeds depends on the mass of their aboveground part. The higher the plant and the bigger the mass of its stems and leaves, the stronger is the weed to compete with agricultural plants for space, light, water and nutrients. The amount of produced weed seeds is the most important condition for weeds to survive in agrophytocenosis. As influence of herbicide MCPA on productivity of second generation weeds depended on species characteristics, further investigation data are analyzed according to species.

Chenopodium album is very sensitive to herbicide MCPA. Only few plants remained in barley crop after spraying and they can not give an objective view of the whole analyzed object, therefore productivity data are not presented. Seeds of *Chenopodium album* plants, which survived after spraying with the herbicide two years in turn, were collected and left for further investigation the next year.

Galeopsis tetrahit. Herbicide MCPA had a significant effect impeding the growth and development of second generation plants. Spraying progeny of untreated plants with the herbicide irrespective of the dose resulted in a lower height of plants (by 39.5—65.5%) and lower amount of matured seeds (by 48.0—64.6), compared to the control (Fig. 1). After spraying MCPA, average height of the progeny of treated plants was lower by 45.3—40.8% and productivity — lower by 80.6—99%. The observed difference of productivity was significant. The data suggest that after treating with MCPA for two years in turn, *Galeopsis tetrahit* becomes less resistant to this herbicide. However, comparison of progeny of untreated and sprayed plants, which grow in crop without application of herbicides, shows that productivity of the second generation of sprayed plants is higher by 62%.



* significant differences at 95% probability level.
*** significant differences at 99.9% probability level.

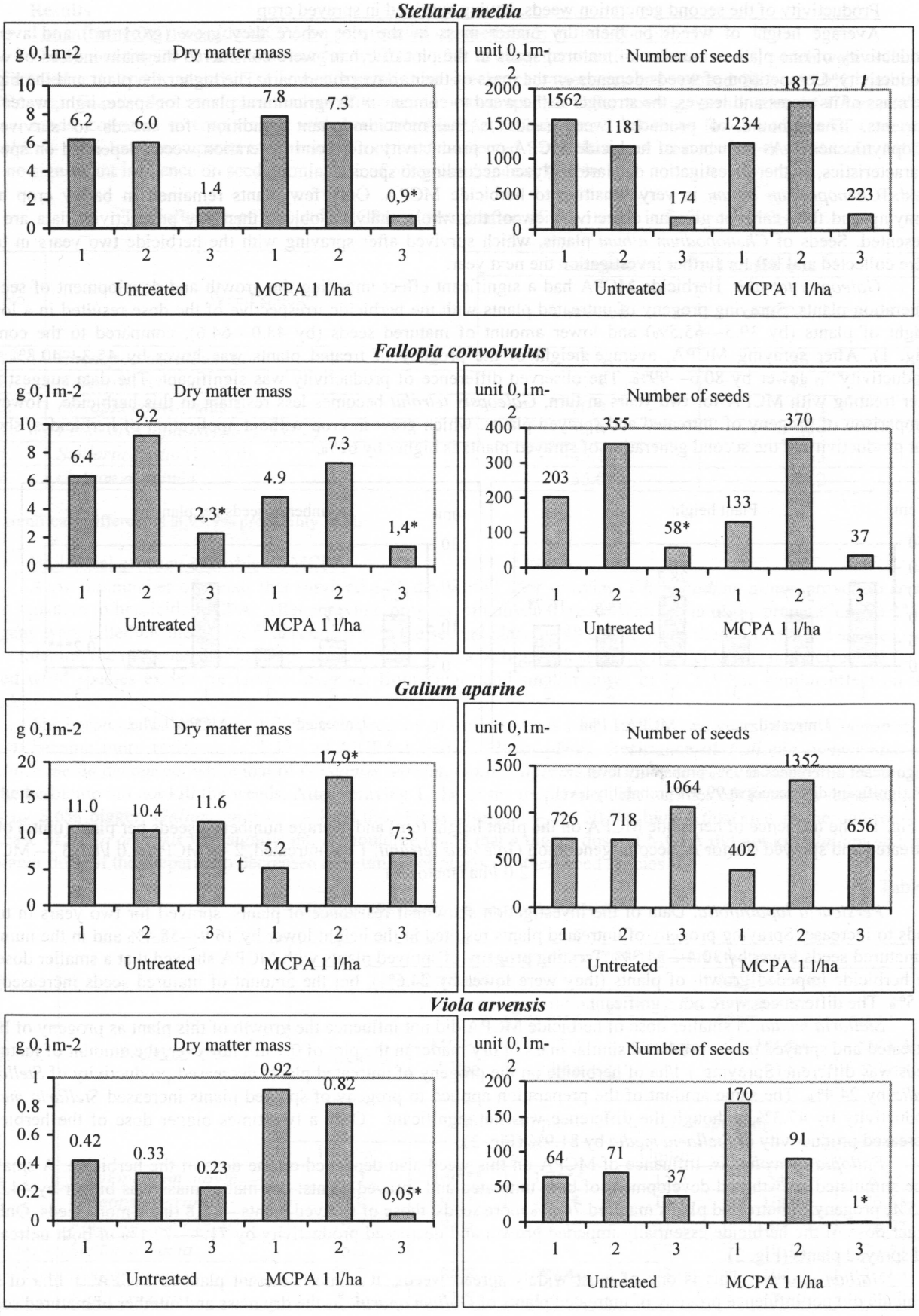
Fig. 1. The influence of herbicide MCPA on the plant height (cm) and average number of seeds per plant (units) of untreated and sprayed (factor A) second-generation *Galeopsis tetrahit*: 1 — untreated, 2 — MCPA 1.0 l/ha, 3 — MCPA 2.0 l/ha (factor B)

Persicaria lapathifolia. Data of the investigation show that resistance of plants, sprayed for two years in turn, tends to increase. Spraying progeny of untreated plants resulted in the height lower by 16.9—58.4% and in the number of matured seeds lower by 30.4—34.3%. Treating progeny of sprayed plants with MCPA showed that a smaller dose of the herbicide impeded growth of plants (they were lower by 24.6%), but the amount of matured seeds increased by 28.5%. The differences were not significant.

Stellaria media. A smaller dose of herbicide MCPA did not influence the growth of this plant as progeny of both untreated and sprayed plants produced similar mass of dry matter in the plot of 0.1 m^2, however, the amount of matured seeds was different. Spraying 1 l/ha of herbicide on the progeny of untreated plants decreased productivity of *Stellaria media* by 24.4%. The same amount of the preparation applied to progeny of sprayed plants increased *Stellaria media* productivity by 47.3%, although the difference was not significant. Only a two times bigger dose of the herbicide decreased productivity of *Stellaria media* by 81.9% (Fig. 2).

Fallopia convolvulus. Influence of MCPA on this weed also depended on the dose of the herbicide. A smaller dose stimulated growth and development of both untreated and sprayed plants: dry matter mass was bigger by 44.9—49.5%, progeny of untreated plants matured 74.6% more seeds, those of sprayed plants — 2.8 times more seeds. Only a bigger dose of the herbicide essentially impeded growth and decreased productivity by 71.4—72.1% of both untreated and sprayed plants (Fig. 2).

Galium aparine. This is one of most widely spread weeds. It is most tolerant plant to MCPA. 1 l/ha of the herbicide did not influence progeny of untreated plants of *Galium aparine* as the dry mass and number of matured seeds were similar to those in the control variant. Spraying 2 l/ha of MCPA stimulated productivity of the first time sprayed plants — they matured 46.5% more seeds compared to the control. MCPA stimulated the growth and development of this weed even more when applied to progeny of sprayed plants. After applying a smaller dose of the preparation for two years in turn, dry mass and productivity of *Galium aparine* were 3.4 times bigger compared to the control, which was sprayed only in 2001. The difference was significant. Spraying 2 l/ha of the preparation also resulted in more productive plants but with a slight difference: dry mass increased by 40.4%, number of seeds — by 63.1% (Fig. 2).



* significant differences at 95% probability level.

Fig. 2. The influence of herbicide MCPA on the productivity of untreated and sprayed second-generation weeds (dry matter mass g m^{-2} and number of seed units m^{-2}): 1 — untreated, 2 — MCPA 1.0 l/ha, 3 — MCPA 2.0 l/ha

Viola arvensis. This is a dwarfish weed, which is less harmful than *Galium aparine* or *Fallopia convolvulus*, but it is resistant to many herbicides and, therefore, widely spread. The investigations show that this weed is resistant to herbicide MCPA when 1 l/ha of the preparation is sprayed. Only a twice bigger dose of the herbicide significantly impeded the growth and development of *Viola arvensis*. Progeny of the plants, sprayed with 1 l/ha of MCPA, was 2.6 times more productive compared to progeny of untreated plants (Fig. 2).

Influence of herbicide MCPA on the third generation of weeds

Seeds of five weed species, matured in the experiment of 2002, were sown in barley crop in 2003. However, only progeny of *Chenopodium album*, *Persicaria lapathifolia* and *Stellaria media* grew. Seeds of other weed species either did not germinate at all or only few plants appeared. In 2003, barley was untreated. Thus, the influence of two years' application of herbicide MCPA on weed progeny, grown without herbicides, was determined.

Chenopodium album. 100 seeds were sown in each plot. Their germination in field conditions reached 36%. Such number of plants in 0.1 m^2 was too big, therefore, in all plots the sprouts were thinned by leaving 20 plants of approximately the same size. 80 plants were expected to grow in four repetitions. As not only interspecies competition for environmental conditions but also competition inside the species took place in the crop, some plants (9.6—12.3) died during the vegetation period. 73 plants grew in the plots of control variant and 71 plant in the plots of progeny of plants sprayed with MCPA (Table 3). Average height of progeny of untreated plants in barley crop reached 52.8 cm. One plant matured 255 seeds on average.

Table 3

The impact of herbicide MCPA on productivity of third generation plants of *Chenopodium album*

Treatments	Number of grown plants	Height of a plant, cm			Number of seeds per plant		
		Average	Min-max	Variation coefficient, %	Average	Min-max	Variation coefficient, %
Untreated	73	52.8	18—100	27.6	254.8	10—1145	82.2
MCPA 1 l/ha	71	47.2	27—71	33.8	343.6	12—977	100.1

Maximum productivity of *Chenopodium album* in barley crop of optimal density was 1145 seeds, i.e. lower than that indicated by famous herbologists E. Korsmo (Korsmo et al., 1986), A. V. Fisiunov (А.В. Фисюнов, 1984) and others. Statistical analysis of data showed that if herbicide MCPA had been used for two years in turn it did not have essential influence on the height of *Chenopodium album*. In this variant plants matured on average 344 seeds or by 34.8% more if compared to progeny of untreated plants.

Persicaria lapathifolia. Germination of seeds in field conditions was lower than that of *Chenopodium album*, therefore fewer plants of *Persicaria lapathifolia* grew: in plots of control variant — 20, progeny of plants sprayed with herbicide MCPA – 34—36. Progeny of untreated plants grew higher by 20—40%. A lower dose of the herbicide had no influence on productivity of progeny, while a bigger norm stimulated their propagation. After spraying 2 l/ha of herbicide, the second generation of survived plants matured 2 times more seeds compared to progeny of untreated plants (Table 4).

Table 4

The impact of herbicide MCPA on productivity of third generation plants of *Persicaria lapathifolia*

Treatments	Number of grown plants	Height of a plant, cm			Number of seeds per plant		
		Average	Min-max	Variation coefficient, %	Average	Min-max	Variation coefficient, %
Untreated	20	38.7	13.0—68.0	40.0	80.7	0.0—416	117.9
MCPA 1 l/ha	36	46.3	16.0—84.0	40.4	80.7	0.0—630.0	153.3
MCPA 2 l/ha	34	54.2	14.0—84.0	32.4	159.9	0.0—479.0	79.5

Variations of both plant height and productivity are very wide.

Stellaria media. Due to poor germination of the seeds sown in barley crop, only few plants were grown: progeny of untreated plants — only 3, of sprayed with MCPA 1 l/ha — 14, of sprayed with MCPA 2 l/ha — 6 (Table 5). No statistical analysis was done because of little selection of the observed plants. However, the presented data clearly show the tendency of MCPA impact. Seeds of the plants, sprayed with a smaller dose of the herbicide, had stronger germinating power, they produced more plants of the third generation, which were more productive — average productivity of one plant was 2.4 times bigger than that in the control. A bigger dose of the herbicide significantly impeded the growth and development of *Stellaria media* plants in the year of spraying (2002). However, plants grown from their seeds were more productive — the number of matured seeds was 2.8 times bigger than that in the control.

Table 5

The impact of MCPA on productivity of third generation plants of *Stellaria media*

Treatments	Number of grown plants	Average productivity per plant	
		Dry matter mass, g	Number of seeds
Untreated	3	1.38	169.0
MCPA 1 l/ha	14	1.54	416.6
MCPA 2 l/ha	6	1.41	485.8

The presented data of two years' investigations prove the characteristic feature of auxins — small doses of these materials stimulate, but bigger — impede the growth of plants. 2.4-dichlorfenoksyacetic acid and 4-chloro-2methylphenoxyacetic acid are numbered among auxins. These are 2.4-D and MCPA active materials. The influence of herbicides on the survived weeds lasts longer than one year. In the year of spraying they impede the growth of plants, but in the following year they stimulate growth and development of progeny. In this way the agrophytocenosis that has been disturbed by the herbicide recovers again.

Conclusions

1. The impact of herbicide MCPA on progeny of survived resistant weeds depends on species characteristics. After spraying this herbicide on the first and second generation weeds, the resistance of *Galeopsis tetrahit* to the herbicide decreases in the second generation. After spraying 1 and 2 l/ha of the preparation, productivity of the first generation *Galeopsis tetrahit* decreases by 48.0—64.6%, while repeated spraying of the second generation plants results in a corresponding decrease — by 80.6—99.0%.
2. Application of MCPA for two years in turn increases resistance of the second generation *Persicaria lapathifolia, Fallopia convolvulus, Stellaria media*, *Galium aparine*, and *Viola arvensis.* A lower dose of MCPA (1 l/ha) even stimulates propagation of these weeds.
3. Treating progeny of sprayed plants with 1 l/ha of the preparation stimulated maturation of more seeds compared to the control: *Persicaria lapathifolia* — by 28.5%, *Stellaria media* — by 47.3%, *Viola arvensis* — 2.6 times, *Fallopia convolvulus* — 2.8 times.
4. Competitive power of *Galium aparine* significantly increases — plants that have been sprayed with 1 l/ha of MCPA for two years produce dry matter mass and mature seeds by 3.4 times more compared to the control, in which only the first generation was sprayed.
5. The impact of MCPA on weeds does not disappear in the second generation although progeny of the sprayed plants is not treated with this herbicide. MCPA stimulates productivity of the second generation plants of *Chenopodium album, Persicaria lapathifolia*, and *Stellaria media.*
6. The agrophytocenosis that has been disturbed by herbicide MCPA naturally recovers in the following year as the survived weeds give more productive progeny.

References

1. Albrecht, H. 1995. Changes in arable weed flora of Germany during the last five decades. Proceedings 1995, 9th EWRS Symposium. Budapest, 41—48.
2. Hyvonen, T., Ketoja, E., Salonen, J. 2004. Changes in the abundance of weeds in spring cereal fields in Finland. Weed research, 43, 348—356.
3. Jodaugienė, D. 2002. Ilgamečio arimo ir purenimo įtaka dirvožemiui ir žemės ūkio augalų pasėliams supaprastinto žemės dirbimo sistemoje. Daktaro disertacijos santrauka. Akademija, 35 pp.
4. Korsmo, E., Vidme, T., Fykse, H. 1986. Korsmos ugrasplansjer. Oslo, 295 pp.
5. Lapinš, D. 1999. Динамика количества и видового состава сорных растений в Латвии за последние пятьдесять лет. Agroecological optimization of husbandry technologies. Proceedings of the Scientific Conference of Baltic States Agricultural Universities, Jelgava, Latvia University of Agriculture, 211—218.
6. Špokienė, N. 1971. 2,4-D natrio druskos įtaka miežių, žieminių rugių ir kai kurių piktžolių biologiniams pakitimams. Ž.ū.m.k. disertacija. Kaunas, 212 pp.
7. Stancevičius, A. 1972. Reguliaraus ir ilgalaikio 2,4-D (natrio druskos) naudojimo įtaka piktžolėtumo ir piktžolių rūšinės sudėties dinamikai. Žemės ūkio gamybos intensyvinimas. LŽŪA mokslo darbai, T .47, 5—14.
8. Toth, A., Benecs-Bardi, G., Balzas, G. 1999. Results of national weed surveys in arable land during the past 50 years in Hungary. Proceedings 1999 Brighton Conference — Weeds. Brighton, UK, 805—810.
9. Tyšer, L., Holec, J. 2004. Evaluation of the weed composition of agrophytocenoses in selected agricultural regions of the Czech Republic. Journal of Plant Diseases and Protection. Proceedings 22nd German Conference on Weed Biology and Weed Control 2—4 March, Stuttgart-Hohenheim, S.-H. 19, 209—214.
10. Valionienė, K. 2003. Profesionalaus naudojimo augalų apsaugos priemonių sąrašas. Vilnius, 64 pp.
11. Фисюнов, А.В. 1984. Сорные растения. Москва, 319 с.