SEARCHING FOR THE CAUSE OF CLOVER FATIGUE

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Abstract. *There is little knowledge about the situation concerning clover fatigue in Norway. Clover is important in organic farming systems and problems with growing legumes will influence organic farming negatively. Both nematodes and fungi can cause clover fatigue. Plant parasitic nematodes were present in samples taken on organic farms in Norway 2011-2012. In 2014 clover plants and soil from 11 farms in Mid-Norway were analysed for both nematodes and fungi. All samples were infected with root rot Fusarium spp., mostly F. avenaceum. Plant parasitic nematodes were found in soil from all samples. Eight nematode groups were present. Spiral nematodes were the most common and abundant group; present on all the farms. No strong correlations were found between numbers of nematodes in the soil and the degree of Fusarium root rot or percentage of clover. However, the least severe root rot was found in the youngest leys.*

Key words: *Organic farming, clover fatigue, nematodes, Fusarium spp.*

INTRODUCTION

Before chemical fertilisers were widely used, different clover species were common in leys. However, early disappearance of the clover, now called clover fatigue, was a serious problem. In addition to abiotic winter damages, various diseases and pests, such as the clover rot fungus, *Sclerotinia sclerotiorum* and the stem nematode (*Ditylenchus dipsaci)*, were known to cause low persistence of clover in the leys in Norway [1],[2]. Sundheim reported that severe injuries in red clover roots during the summer were mainly caused by *Fusarium* species [3]. Nowadays, there is little awareness about clover fatigue. Lately, no systematic surveys have been done regarding the situation in Norway [4].

Legumes, such as clover, provide a major source of nitrogen in organic farming systems, and help to maintain soil fertility. The soil structure is also improved by the roots and biomass of clovers. Organic farming systems have been intensified in the past ten years. On a number of farms, clovers are frequently included in the crop rotation. Recently, some Norwegian organic farmers have noticed a decrease in the amount of clover in the ley and even a reduction of the total harvest. In Sweden, severe injuries caused by *Fusarium* root rot were found to be widely distributed on clover plants in organic leys [5]. In Finland, as part of a project to improve the profitability of organic milk production by increasing the efficiency of red clover cultivation, the fungal species composition and prevalence, and genetic variation and pathogenicity of clover rot (*S. trifoliorum*) and *Fusarium* spp. were studied [6]. The most common *Fusarium* species associated with red clover root rot was *Fusarium avenaceum*. Intensive use of clover in organic farming systems and a warmer climate are factors that indicate a need for attention to increased occurrence of diseases and pests in clover [7]. Problems with growing legumes will influence the nitrogen supply and economy in organic farming negatively.

Different species of fungi and nematodes can cause clover fatigue. Few cultivars are resistant to these organisms, so knowledge on the occurrence of fungi and nematodes is essential for effective crop rotations. In a preliminary study, samples from fields with clover/grass ley were taken on organic farms in different parts of Norway. The samples, taken 2011-2012, demonstrated the presence of different plant parasitic nematodes. In soil from 6 farms in different parts of Norway the following groups and species of plant parasitic nematodes were detected: Stunt nematodes (*Tylenchorhynchus dubius, T. maximus* and *Merlinius* sp.), spiral nematodes (*Helicotylenchus canadensis, H. pseudorobustus* and *Rotylenchus* sp.), root lesion nematodes (*Pratylenchus crenatus* and *P. fallax*), ring nematodes (fam. *Criconematidae*), pin nematodes (*Paratylenchus* sp. and *P. bukowinensis*), needle nematodes (*Longidorus elongatus*), stubby root nematodes (*Paratrichodorus pachydermus*) and cyst nematodes (*Heterodera trifolii*) [8].

In the present study, we examined the presence of fungi on red clover plants and plant parasitic nematodes in the soil in the root zone of the clover plants.

MATERIALS AND METHODS

Soil, roots and root necks of red clover plants were collected from 11 organic farms in Trøndelag and Nordmøre, in Mid-Norway. On six of the farms the crop rotation consists of grass/clover leys, on the other farms the crop rotation consists of both grass/clover leys and cereals. The samples were taken from grass/clover leys on both sandy soils, clay and moraine soil. The leys were from one to five years old.

The samples were collected on 1st-3rd September, week 36 in 2014. The samples for fungal analyses were rinsed in running water and transported to Bioforsk Plant Health where they, after splitting lengthwise with a scalpel, were incubated on moist filter paper in a humid atmosphere at 20°C. Symptoms of root rot, assessed as lesions on root surface and internal root necrosis and decay (0 = no attack, 3 = severe attack), and fungal growth, were observed in week 37 and 38. Pieces from roots with symptoms of root rot were surface disinfected and placed on agar (PDA) September 19th and assessed for fungal growth three and five days later.

The samples for plant parasitic nematode analyses included clover plants with roots and soil. The soil samples were extracted by the Seinhorst elutriator at Bioforsk Plant Health [9]. Plant parasitic nematodes were identified to family, subfamily and generic level in Leica M10 stereo microscope.

RESULTS AND DISCUSSION

All samples were infected with root rot *(Fusarium* spp.*),* mostly *F. avenaceum.* On plants from three of the farms the infection was severe, with dark rot in the root neck and inside the roots. Two of these farms had no crop rotation with cereals, while on the third farm the samples were taken from a five year old grass/clover ley, where also the clover proportion was the lowest recorded among the fields sampled in this study. Plants from two fields with low root rot occurrence (farm no 1 and farm no 7) were among the farms with the highest clover proportion and the youngest leys. This is in agreement with a previous Norwegian survey showing more necrotic roots of older plants than roots from one year old plants [3]. No signs of clover rot *(S. trifoliorum)* were detected in the observation period. The reason for this might be that damage from clover rot is most visible in the spring, when damaged plants can be seen and black sclerotia can be found around the root necks of infected plants.

Plant parasitic nematodes were found in soil samples from all farms. The material contained eight nematode groups: *Tylenchus sensu lato*, stunt nematodes (*Tylenchorhynchus* spp.*), root lesion nematodes (Pratylenchus* spp.*), spiral nematodes (*fam. Hoplolaimidae), pin nematodes (*Paratylenchus*), ring nematodes (subfam. Criconematinae), stubby root nematodes (fam. Trichodoridae) and cyst nematodes (*Heterodera* sp).

Spiral nematodes were the most common group and occurred in samples from all farms. The groups *Tylenchus sensu lato* and the root lesion nematodes were observed on 10 farms. Stunt nematodes were present on eight farms, pine nematodes on five farms, while stubby root nematodes and ring nematodes were found on four and three farms respectively. In the soil from one farm a cyst nematode juvenile was detected.

The spiral nematodes were the most abundant group, occurring in high densities in samples from eight farms. In one farm with 5 year old ley, 1030 ind./250ml soil were recorded and the clover cover in this case was only 5-10%. In a young 1 year old ley, which was established after a long cereal cultivation, the numbers of spiral nematodes were 620 ind./250 ml soil with a clover cover of 50-60%. *Tylenchus sensu lato*, root lesion nematodes and stunt nematodes were less common. This was also the case of pin nematodes and ring nematodes. Stubby root nematodes occurred infrequently, but the very high level of 190 ind./250 ml soil was recorded in a 3 year old ley with 20% cover of clover.

In the present material, no strong correlations were found between numbers of nematodes in the soil and the degree of *Fusarium* root rot or percentage of clover.

Like in an earlier study, spiral-, root lesion and stunt nematodes were abundant groups of nematodes in the present material [8]. The numbers of spiral nematodes are similar to the levels observed earlier, while the numbers of root lesion- and stunt nematodes are lower. In the earlier study, samples were collected from more southern locations, which may be a possible explanation for the higher abundance of root lesion and stunt nematodes reported in 2013. It appears that spiral nematodes are abundant and well distributed in most areas of Norway. Population densities of spiral nematodes exceeding 500 ind./250 ml soil may be highly damaging, so the loss of clover cover in the 5-year-old ley may be related to the high nematode numbers. The high population density in the 1-year-old ley may be a pre-crop effect from the prolonged cereal cultivation. These nematodes may, over time, reduce the productivity of the ley, since spiral nematodes have been reported to be associated with growth reductions in red clover in Poland [10].

Spiral nematodes in high densities were also associated with a low percentage of clover in a previous study in Norway [8].

Table 1

Numbers of different nematode groups in samples of 250 ml soil and degree of attack (0: no attack, 3: severe attack) of root rot *(Fusarium* spp.*)* on red clover plants from eleven organic farms in Mid-Norway in 2014.

Farm no.	Age of ley	Tylenchus sensu lato	Stunt nematodes	Root lesion nematodes	Spiral nematodes	Pin nematodes	Ring nematodes	Stubby root nematodes	Cyst nematodes	Fusarium
1	**0**	0	15	13	4	18	0	0	0	**0,5**
2	**5**	28	10	34	1030	0	0	1	0	**3**
3	**2**	78	0	4	315	31	7	0	0	**1**
4	**2**	22	2	6	460	0	0	0	0	**1,5**
5	**5+**	30	0	1	132	0	5	0	0	**2**
6	**2**	10	36	75	25	20	2	190	1	**2**
7	**1**	80	10	2	620	0	0	0	0	**1**
8	**2**	5	0	0	279	0	0	0	0	**3**
9	**2**	55	34	23	215	4	0	2	0	**2**
10	**2**	25	93	29	190	25	0	8	0	**2**
11	**2**	6	10	35	14	0	0	0	0	**3**

Plant parasitic nematodes and fungi are involved in a multitude of interactions related to root damage [11]. For a long time soil-borne fungi have been suspected to interact with nematodes in promoting root decay [12]. There have been no specific studies on the potential complex of clover, involving *Fusarium* root rot and nematodes. Our results did not reveal correlations between nematode densities and degrees of root decay by *Fusarium* spp. It is plausible that the wounds and cavities caused by root-lesion nematodes could enhance a subsequent root infection by *Fusarium* spp., but in this case the restricted material and the high variability in nematode numbers might have obscured such a relationship. Due to the complex dynamics of sequential etiology, where nematode predisposition of plants to pathogen infection may vary in time and space, organism interactions in disease complexes may be difficult to demonstrate in the field [13].

CONCLUSIONS

The results show that damages caused by root rot occurred in plants from all sampled farms and the main *Fusarium* species detected was *F. avenaceum*.

Spiral nematodes were the most prevalent and abundant nematode group, occasionally reaching potentially damaging densities.

In this study, strong correlations between nematode numbers and *Fusarium* root rot could not be detected.

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