EFFECT OF POST-HARVEST MOWING ON STRAWBERRY ‘DARSELECT’ GROWTH AND YIELD GROWN ON PLASTIC MulCH

Reelika Rätsep, Kadri Karp, Ele Vool
Estonian University of Life Sciences
reelika.ratsep@emu.ee

Abstract
The experiment was carried out at the Research Centre of Organic Farming of Estonian University of Life Sciences in 2012 and 2013. The study was aimed to find out the effect of defoliation and humic acid application on the strawberry (Fragaria × ananassa Duch.) plant growth, yield, and on the ascorbic acid, total phenolic and anthocyanin contents in ‘Darselect’ yield. Defoliation decreased significantly the number of leaves and inflorescences in both experimental years, while the fertilization effect was only in one experimental year. Fruit weight ranged from 19 to 42 g, being significantly decreased by defoliation in 2012, but increased in 2013. Yield was decreased due to defoliation up to 40% in 2012 and up to 51% in 2013, but humic acid application increased the yield of the defoliated plants in both experimental years. Defoliation in combination with humic acids had a significant effect on strawberry ascorbic acid content, but the effect was different for different fruit order. Tertiary fruits contained up to 46% more of total phenolics due to defoliation in both experimental years. Defoliation increased anthocyanins in primary and secondary fruits, but decreased it in tertiary fruits; while fertilizing increased the content up to 45% in defoliated plants in primary, but decreased about 13% in tertiary strawberries.

Key words: strawberry defoliation, humic acids, yield, biochemical compounds, mulch.

Introduction
Several experiments published are related to strawberry defoliation, determining the extent of leaf removal (Albregts et al., 1992; Casierra-Posada et al., 2012) or referring to different damages caused by insect/pest incidence or accidents due to human activities (Makaraci and Flore, 2012). Partial defoliation is executed mostly manually in strawberry transplants to lower transpiration rate (Albregts et al., 1992; Casierra-Posada et al., 2012), while post-harvest defoliation as an alternative to chemical plant treatment is implemented mechanically after harvesting (Metspalu et al., 2000; Daugaard et al., 2003). More recent investigations have been aimed at determining the physical damage thresholds of strawberry leaves by using mechanical methods and understanding the plant recovery patterns better (Makaraci and Flore, 2012). Nevertheless, the mechanisms of plant reaction and recovery depend also on genotype, cultivation technologies and seasonal climatic conditions (Daugaard et al., 2003; Crespo et al., 2010; Pincemail et al., 2012; Günduz and Özdemir, 2014; Khanizadeh et al., 2014).

On the one hand, manual treatments are time consuming and demand high labor efficiency, but on the other hand, mechanical treatments can cause more plant damage. Experiments with different defoliation methods have led to more clear understanding of its relation to plant growth and development, which in turn may influence strawberry yield and quality. F. Casierra-Posada et al., (2012) have indicated the negative effect of defoliation on strawberry plant growth and yield. These kinds of impacts could be diminished by using additional fertilizing in order to improve the plant health and its recovery. M.J. Anttonen et al., (2006) claim the fertilization to be one of the most important treatments influencing fruit yield and quality. Significant effects of bio-fertilizers and growing year on strawberry yield have also been reported by M. Pešaković et al., (2013). However, besides plant growth fertilizing also affects the fruit biochemical composition. For example, U. Moor et al., (2005) have found that liquid fertilizer amendments increased the strawberry ascorbic acid content.

Strawberry defoliation has been used for different purposes and studied in many countries using various methods for the treatment. Post-harvest defoliation is a common practice in Estonia, but it is less investigated in terms of different cultivars and organic fertilizers, though being recommended in organic cultivation. The objective of this research was to determine the influence of post-harvest defoliation and humic acid application on the strawberry ‘Darselect’ vegetative growth, fruit weight, and the content of ascorbic acid, total phenolics and anthocyanins.

Materials and Methods
Experimental area and treatments
The experiment was carried out in 2012 and 2013 in a strawberry experimental plantation located at the Research Centre of Organic Farming of Estonian University of Life Sciences (58°21’N, 26°40’E, 68 m above sea level). Ecological fertilizer (NPK 4.5-2.5-8), produced from at least 30% malt germs was applied to the whole plantation area as a pre-establishment soil supplement. Strawberry cultivar ‘Darselect’ frigo plants were bought from Netherlands and planted in 2010 using 50 cm plant spacing and synthetic 0.04 mm thick black polyethylene mulch. Control plants were neither defoliated nor fertilized only watered, while two other treatments were executed. Post-harvest defoliation (D) was performed in July after...
harvesting; and in combination with D, humic (H) acid application was implemented (DH). Humic acid containing substance was applied at a concentration of 50 mL of substance per 10 L of water at a rate of 0.5 L per plant, executed during flowering and fruiting and in the middle of August. The liquid humic substance contains 12% humic acid and 3% fulvic acid. The experimental design was a randomized block design with four replications and 12 plants per replication.

Soil and weather conditions

The soil in the plantation was Stagnic luvisol (FAO soil classification). Soil pH KCl was 6.8 and humus content was 4.0%. The soil content of P, K and Mg was high and Ca content was sufficient (Table 1).

In 2012, the period of active temperatures (above 10 °C) started at the beginning of May and ended on the 6th October. Compared to long-term mean, the monthly mean temperatures were warmer in April, May, July and September, except in June which showed 1.3 °C lower temperature than the mean of 1971–2000 (Table 2). The sum of active temperatures in 2012 was 1967 °C. The amount of precipitation was significantly higher in April, May and June but almost at the same level in August and September in comparison with many years.

In 2013, the active plant growth period started from 7 May and ended on 23 September. The sum of active temperatures was 2263 °C which is 332 °C more than the mean of many years (1936 °C). The monthly mean temperatures were up to 3.8 °C warmer, except for April which was 0.7 °C cooler than the long term mean. The precipitation sum from 1 April to 31 October was 352 mm which was 152 mm less than in 2012, and 86 mm less than the mean (438) of 1971–2000.

Measurements and analysis

The numbers of strawberry leaves and inflorescences per plant were counted during flowering in May. The dry weight (g) of roots and crown were determined. At the end of strawberry fruit harvesting in 2013, the plants were dug out, the leaves were cut and after cleaning from soil the plant crown and the roots were dried until a constant weight was recorded. Fruits were picked according to surface color and fruit order in clusters (primary, secondary, tertiary), and stored at -20 °C until analysis. Fruit orders were analyzed separately. All the plant growth, strawberry fruit yield and quality parameters were determined and measured in both experimental years in three replications (12 plants per replication) per treatment.

Ascorbic acid content (ASC) was determined iodometrically with the modified Tillman’s method. For analysis, a mixture of meta-phosphoric and acetic acid (3% HPO3 + 8% CH3COOH) was added instantly to the pulp to avoid ascorbic acid breakdown in the air (Paim and Reis, 2000). AAC was expressed as mg 100 g-1 of strawberry fresh weight (FW). The content of total anthocyanins (ACC) was estimated by a pH differential method (Cheng and Breen, 1991).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH KCl</th>
<th>P mg kg-1</th>
<th>K mg kg-1</th>
<th>Ca mg kg-1</th>
<th>Mg mg kg-1</th>
<th>C %</th>
<th>Humus %</th>
<th>C in biomass C mg-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.6</td>
<td>256***</td>
<td>306***</td>
<td>3930***</td>
<td>600***</td>
<td>3.2</td>
<td>4.9</td>
<td>0.525</td>
</tr>
<tr>
<td>Fertilized</td>
<td>6.7</td>
<td>286***</td>
<td>347***</td>
<td>5131***</td>
<td>492***</td>
<td>3.0</td>
<td>5.2</td>
<td>0.766</td>
</tr>
</tbody>
</table>

The level of nutrients in the soil: high (***)

Table 1

<table>
<thead>
<tr>
<th>Minimum, mean and maximum temperatures (°C) from April to September of 2012, 2013 and of long-term mean of 1971–2000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>April</td>
</tr>
<tr>
<td>May</td>
</tr>
<tr>
<td>June</td>
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<tr>
<td>July</td>
</tr>
<tr>
<td>August</td>
</tr>
<tr>
<td>September</td>
</tr>
</tbody>
</table>

Data according to the Estonian Weather Service (www.ilmateenistus.ee) database.
Absorbance was measured with a Jenway 6300 spectrophotometer at 510 and at 700 nm in buffers at pH 1.0 (HCl 0.1N) and pH 4.5 (citrate buffer). The results were expressed as mg of pelargonidin-3-glucoside equivalent per 100 g of FW. The total phenolic content (TPC) was determined with the Folin-Ciocalteau phenol reagent method, using a spectrophotometer at 765 nm. The TPC was expressed as Gallic acid equivalents in mg 100 g FW of pulp.

All the results of the present experiment were tested by one-way analysis of variance (ANOVA). To evaluate the effect of treatment, the least significant difference (LSD) was calculated. Different letters on figures and tables mark significant differences at p ≤ 0.05.

Results and Discussion
Strawberry growth and yield parameters
Defoliation (D) decreased the number of leaves and inflorescences significantly in both experimental years, while the DH affected the leaf number only in 2012 (Table 3). The considerable decrease in the number of leaves and inflorescences may refer to the negative effect of defoliation on their formation and development. Similar results were described by F. Casierra-Posada et al., (2012), who suggested that severe defoliation suppresses strawberry flower initiation, influencing leaf growth as well as the production of dry matter. After the treatments within two years, strawberry root mass was significantly increased by D, referring to high plant underground biomass production due to depressive disturbance of plant growth processes and promotion of plant recovery mechanisms. Similar mechanisms were described in experiments by A.Z. Makaraci and J.A. Flore (2012).

In the year 2012, strawberry ‘Darselect’ fruit weight was up to 42 g, and D decreased it significantly (Table 3). In 2013, on the contrary, fruit weight was increased by D, although the values remained within range from 19 to 23 g. These results are in accordance with H. Daugaard et al., (2003) who also found that post-harvest defoliation caused a significant increase in fruit size, while F. Casierra-Posada et al., (2012) found it to be decreased.

Yield per plant was decreased due to D treatment from 679 to 403 g per plant in 2012 and up from 308 to 149 g in 2013, but humic substances had a positive effect on the defoliated plants in both experimental years. F. Casierra-Posada et al., (2012) found that defoliation decreased strawberry total yield. Significant effects of bio-fertilizers and growing year on strawberry yield have been reported by M. Pešaković et al., (2013). Nevertheless, additional humic acid application increased the yield in both investigated years, showing the positive effect even in case of high soil humus content.

Ascorbic acid content (ASC)
In 2012, strawberry ASC ranged from 72 to 124 mg 100 g−1 (Figure 1A). D increased the content up to 57% in primary fruits, but decreased it in secondary and tertiary fruits. In 2013, ASC varied from 71 to 107 mg 100 g−1 (Figure 1B). D had a decreasing effect

Table 3
The effect of defoliation and fertilization on strawberry ‘Darselect’ plant growth and yield in 2012 and 2013

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatment</th>
<th>2012</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of leaves</td>
<td>Control</td>
<td>51 a</td>
<td>63 a</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>40 b</td>
<td>45 b</td>
</tr>
<tr>
<td></td>
<td>DH</td>
<td>54 a</td>
<td>52 b</td>
</tr>
<tr>
<td>Number of inflorescences</td>
<td>Control</td>
<td>18 a</td>
<td>26 a</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>12 b</td>
<td>14 b</td>
</tr>
<tr>
<td></td>
<td>DH</td>
<td>12 b</td>
<td>16 b</td>
</tr>
<tr>
<td>Root mass, g*</td>
<td>Control</td>
<td>-</td>
<td>266 c</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>-</td>
<td>404 a</td>
</tr>
<tr>
<td></td>
<td>DH</td>
<td>-</td>
<td>350 b</td>
</tr>
<tr>
<td>Fruit weight, g</td>
<td>Control</td>
<td>42 a</td>
<td>19 c</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>39 b</td>
<td>23 a</td>
</tr>
<tr>
<td></td>
<td>DH</td>
<td>42 a</td>
<td>21 b</td>
</tr>
<tr>
<td>Yield per plant, g</td>
<td>Control</td>
<td>679 a</td>
<td>308 a</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>403 c</td>
<td>149 c</td>
</tr>
<tr>
<td></td>
<td>DH</td>
<td>478 b</td>
<td>279 b</td>
</tr>
</tbody>
</table>

Treatments: (D) – defoliation; (DH) – defoliation and humic acids application.
*Root mass was determined only in 2013 by uprooting the plants.
Statistical analysis was done according to year and parameter measured, mean values followed by the same letter are not significantly different at p ≤ 0.05.
in primary, but increasing in secondary and tertiary fruits, while the influence of DH treatment was more evident in secondary fruits.

ASC values in strawberry ‘Darselect’ determined by J. Pincemail et al., (2012) reached up to 163 mg 100 g\(^{-1}\), which refer to high variations of the contents depending on the growing area and weather conditions. In the present experiment, differences in ASC were apparent from one year to another. In 2012, the reason could be related to up to 4.4 °C lower temperatures in May and June compared to year 2013 (Table 2). This is in accordance with several authors who have claimed that cooler temperatures tend to increase the content of ascorbic acid (Lee and Kader, 2000; Moor et al., 2005; Pincemail et al., 2012). Ascorbic acid content being different according to year and cultivation method indicates the fact that its content is not easily influenced by cultural practices (Moor et al., 2005; Tõnutare et al., 2009).

**Total phenolic content (TPC)**

TPC ranged from 155 to 236 mg 100 g\(^{-1}\) in 2012 (Figure 2A). D affected strawberry fruit TPC significantly, decreasing it in primary, but increasing it tremendously up to 46% in tertiary fruit order. DH decreased the TPC up to 14% in primary strawberry fruits. In 2013, TPC ranged from 184 to 267 mg 100 g\(^{-1}\) (Figure 2B). D had a positive effect – increasing TPC values by more than 26% in secondary and tertiary fruits. DH treatment increased the TPC up to 40% in secondary fruit order.

Significantly lower TPC in primary fruits in 2012 could be caused by dilution effect of biochemical components due to higher precipitation rate in May and June of that year. Moreover, it can be assumed that under the plastic mulch, the soil humidity could have been quite high enough during strawberry fruit ripening period. The content of phenolic compounds can be significantly affected by production systems, such as the usage of plastic mulch, but again the effects can be variable during the harvest season (Khanizadeh et al., 2014). J. Crecente-Campo et al., (2012) have indicated the decrease in phenolic acid concentrations during ripening. On the contrary, in the present experiment, significantly higher temperatures in July could have caused the increase in TPC by the time of harvesting of tertiary fruits in both years. Manyfold differences in accumulation of phenolic compounds have been determined according to fruit order by M.J. Anttonen et al., (2006). Significant variations in phenolic contents can be related to cultivar characteristics; however, the importance and influence of weather conditions cannot be underestimated (Pincemail et al., 2012; Gündüz and Özdemir, 2014).
Figure 2. The effect of defoliation (D) and humic acid application (DH) on the strawberry ‘Darselect’ total phenolic content according to fruit order in 2012 (A) and 2013 (B). Different letters on figures mark significant differences at $p \leq 0.05$.

Figure 3. The effect of defoliation (D) and humic acid application (DH) on the strawberry ‘Darselect’ phenolic content according to fruit order in 2012 (A) and 2013 (B). Different letters on figures mark significant differences at $p \leq 0.05$. 

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EFFECT OF POST-HARVEST MOWING ON STRAWBERRY ‘DARSELECT’ GROWTH AND YIELD GROWN ON PLASTIC MULCH
Anthocyanins (ACC)

In 2012, ACC ranged from 11.2 to 22.5 mg 100 g⁻¹ (Figure 3A). D increased the content in primary, but decreased it slightly in tertiary strawberries. DH increased the ACC up to 45% in primary fruits, but decreased the content for about 13% in tertiary strawberries. In 2013, ACC ranged from 8.8 to 15.8 mg 100 g⁻¹ (Figure 3B). D had a significant effect on ACC, increasing it 14% in primary and secondary fruits, but decreased it up to 44% in tertiary fruit order. DH decreased ACC in primary fruits compared to D treatment, but increased the content in secondary fruits up to 26%.

The effect of defoliation was different according to year, but it can be presumably related to the age of plantation and variable fruit weight. U. Moor et al., (2005) have pointed out that strawberry plants growing in four- to five-year-old plantations suffer under stress conditions, and this could be one cause of increased anthocyanin production in berries. T. Tõnutare et al., (2009) have also concluded that the content of anthocyanins was decreased significantly with the aging of a plantation, although the plant age effect on strawberry quality parameters is more important than that of cultivation technology. Still, anthocyanins being a significant part of total phenolics in general, depend also on genotype and cultivation technologies (Pinçemail et al., 2012; Gündüz and Özdemir, 2014). The latter indicates the fact that such interactions need to be investigated in long-term trials.

Conclusions

The results of the research revealed that the effect of defoliation and humic acid application was year dependent, and therefore, agricultural practices used for strawberry cultivation may not be of main importance. Climatic conditions can also be the major factors determining the accumulation of biochemical compounds in fruits. However, the results of the present research revealed that defoliation tends to decrease the strawberry yield, but additional fertilization with humic acids may be used in order to promote better plant growth recovery and due to the content of biochemical compounds of strawberries. Still, further research is required assessing different cultivars and the effect of defoliation and different bio-fertilizers on the vegetative growth, yield and fruit biochemical composition of strawberries in order to confirm the results obtained so far.

Acknowledgements

The research was supported by Estonian Ministry of Education and Research, project SF 0170057s09 and Estonian Ministry of Agriculture.

These studies were also supported by European Social Fund’s Doctoral Studies and Internationalization Programme DoRa which is carried out by Archimedes Foundation.

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