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The impact of foliar fertilization on the number of bees (*Apoidea*) on spring oilseed rape

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Abstract

Spring oilseed rape (*Brassica napus* L. var. *oleifera*) is an important oilseed crop whose cultivation area has increased significantly in Estonia. It is predominantly autogamous but cross-pollination can have several positive effects, including higher seed yield. We studied the effect of fertilization with different foliar microfertilizers on the flower density and pollen and nectar production of spring oilseed rape as well as the impact of these factors on the abundance of flower visiting bees – honey bees, bumble bees and solitary bees.

Field experiments were carried out in 2004 and 2005. The field consisted of 32 plots (10 m^2 each): control plots (no mineral fertilizers used), plots fertilized with the complex fertilizer OptiCrop (NPK 21-08-12 + S + Mg + B + Ca) alone and plots treated with OptiCrop and one of the six foliar microfertilizers (Mn, S, Cu, B, Mg, Mo). There were four replicates of each treatment. Flower visiting bees were counted twice a week on sunny days. Flowers were counted at the same time on an area of 1 m^2 on each plot. Nectar production by the flowers was measured in the field by inserting a 1 µl capillary into the corolla tube of flowers isolated for 24 h. Pollen grains were counted from previously isolated flowers after dissolving the flower tissues.

The density of flower visiting bees (honey bees, bumble bees and solitary bees) on spring oilseed rape depended mainly on flower density. Fertilization increased not only the number of flowers but also the amount of nectar and pollen per flower. Additional foliar fertilization had no effect either on the number of flowers or the amount of pollen grains per flower. Nectar production per flower seemed to be inhibited by additional manganese. Therefore, to secure higher number of pollinators for achieving higher seed yield and other benefits deriving from cross-pollination, spring oilseed rape should be given proper complex fertilization. Microfertilizers turned out to be useless in terms of increasing the number of pollinators.

Key words: *Brassica napus* L. var. *oleifera*, spring oilseed rape, *Apoidea*, flower density, pollen production, nectar production, foliar fertilization.

Introduction

Spring oilseed rape (*Brassica napus* L. var. *oleifera*) is an important oilseed crop, whose production area has increased significantly in northern countries of Europe (Treu, Emberlin, 2000), including Estonia. It is predominantly autogamous and visits of insect pollinators are not essential for the final seed yield (Williams et al., 1987). However, flower morphology favours first cross-pollination followed by self-pollination (Delaplane, Mayer, 2000). Adequate pollination 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 (Williams et al., 1987). Cross-pollination also raises the seed yield (Steffan-Dewenter, 2003; Chifflet et al., 2011).

Large fields of oilseed rape in flower are important food resources for bees enhancing both nectar and pollen reserves abundantly (Westphal et al., 2009; Mänd et al., 2010). Oilseed rape is an especially attractive food plant for bees because of the high nectar production of its flowers and its high sugar content (Pierre et al., 1999). Adult bees use nectar to satisfy their energy and water needs. Pollen is collected by bees as their only source of protein and is used as food for the larvae. The pollen of oilseed rape contains more of the three most important amino acids for bee survival and development than other field crops flowering at the same time (Cook et al., 2003).

Oilseed rape is a fast growing crop which needs more nutrients than graminaceous crops. Considering other plant species, it has been found that soil fertilizer affects the concentration of amino acids in the floral nectar of corncockle, *Agrostemma githago* (Gardener, Gillman, 2001) and soil nitrogen has a positive effect on the pollen performance of *Cucurbita pepo* (Lau, Stephenson, 1993). Many studies have focused on the effect of fertilization (Sidlauskas, Bernotas, 2003; Szulc et al., 2003; Rathke et al., 2006) and pollinators (Steffan-Dewenter, 2003; Sabbahi et al., 2005) on seed yield of oilseed rape as well as on the effect of ambient temperature conditions on honey bee foraging activity (Blažytė-Čereškienė et al., 2010). However, none of these studies have dealt with the impact of fertilization on the resource of the bee food (nectar and pollen production) provided by oilseed rape and on the number of the most important pollinators – bees. Taking into account several benefits of cross-pollination (Williams et al., 1987) and the pollinators' contribution to yield increase (Sabbahi et al., 2005), this gap in knowledge needs to be filled. In this context, the present study examines the effect of foliar fertilization on the flower density and nectar productivity of spring oilseed rape and on the number of pollen grains per flower in relation to the abundance of flower visiting bees – honey bees, bumble bees and solitary bees.

Materials and methods

Study plots. The study was carried out in an experimental field of the Estonian University of Life Sciences near Tartu, Estonia, during the flowering period of oilseed rape in 2004 and 2005. The spring oilseed rape variety 'Mascot', bred and produced by the Swedish company "Weibull", was used. Technical data of the variety: crude fat content 40–43%, 1000 seed weight 3.5–4.5 g, glucosinolate content 20 µmol g^{-1}, lodging resistance 6–8 points, plant height 98–108 cm, growth period 90–108 days (Velička, 2003). The soil in the study area was slightly acidic (pH_{KCl} 6.2) *Stagnic Luvisol* (FAO classification LV st, 2006) with loamy texture: humus content 2.4%, P – 77.66 mg kg^{-1}, K – 169.8 mg kg^{-1}, Ca – 5648 mg kg^{-1}, S – 13.54 mg kg^{-1}.

In 2004, spring oilseed rape was sown on 5 May and in 2005 on 9 May at a rate of 200 viable seeds m^{-2}, sowing depth 2–3 (4) cm, pre-crop being potato. In 2004 and 2005, the field consisted of 32 plots (10 m^2 each). Control plots received no fertilizer; the other plots received a complex fertilizer alone or the complex fertilizer plus one of the six microfertilizers. There were four replicates of each treatment. The treatments were: 1) 0 (no mineral fertilizers), 2) OptiCrop (Opti) (only the mineral complex fertilizer OptiCrop NPK 21-08-12 + S + Mg + B + Ca, the amount of nitrogen applied 120 kg ha^{-1}), 3) Opti + HydroPlus™ Boron (Opti + B) (consumption rate 2 l ha^{-1}), 4) Opti + HydroPlus™ Micro Copper (Opti + Cu) (consumption rate 0.5 l ha^{-1}), 5) Opti + Hydromag 300 (Opti + Mg) (consumption rate 7 1 ha^{-1}), 6) Opti + HydroPlus™ Micro Manganese (Opti + Mn) (consumption rate 1 l ha^{-1}), 7) Opti + HydroPlus™ Micro Molybdenum (Opti + Mo) (consumption rate 0.25 l ha^{-1}), 8) Opti + Sulphur F3000 (Opti + S) (consumption rate 7 1 ha^{-1}).

Prior to sowing, the whole field was sprayed with the soil-applied herbicide EK Trifluralin (0.15 l ha^{-1}). The mineral complex fertilizer OptiCrop NPK 21-08-12 + S + Mg + B + Ca, the amount of nitrogen applied 120 kg ha^{-1}, was used (except for treatment 0). Liquid microfertilizers (spray volume 400 l ha^{-1}) were foliar-applied when the plants had reached the growth stage 27–31 according to the BBCH scale (Lancashire et al., 1991).

Evaluation of flower visiting bees: honey bees, bumble bees and solitary bees. Flower visiting bees were counted during the flowering period of the crop (5–22 July 2004 and 28 June to 18 July 2005) on each 10 m^2 plot twice a week (altogether 6 observation days in 2004 and 7 observation days in 2005) by walking slowly along the study plots and recording all bees visiting the flowers of oilseed rape. The observations were made on sunny days between 11:00 and 15:00 when temperature was above 16ºC and wind speed did not exceed 6 m s^{-1}.

Evaluation of flower density. Flowers were counted simultaneously with flower visiting bees on an area of 1 m^2 on each plot which was divided into 4 subplots (50 × 50 cm) and the data were summarized.

Evaluation of nectar production. Nectar was collected from five flowers in each plot three times during the flowering period of the crop in 2004. The collection was carried out in late morning at full flowering of the plants. Each flower was previously covered with a voile bag for 24 h to exclude floral visitors and to prevent nectar consumption the day before nectar measurement. Nectar production was measured in the field by inserting a 1 µl capillary into the flower corolla tube. It should be noted that nectar productivity can only be measured when there is no rainfall during 24 h. As in 2005 there was little rain on almost all days of flowering period of spring oilseed rape, nectar production was analysed only for 2004.

Evaluation of pollen production. In 2004 and 2005, after anthesis, pollen production was quantified for 5 flowers in each plot at the same time as flower visiting bees and flowers were counted. The flowers were collected randomly from the plant main raceme and stored separately. These racemes were previously isolated to avoid consumption of the pollen by pollen beetles (*Meligethes* sp.). The flowers with pollen were later acetolysed (Faegri, Iverson, 1989) to digest both the floral tissue and pollen content, leaving pollen exines intact. Separated pollen was dispersed in distilled water (1 ml). The pollen grains were counted with a light microscope using a Fuchs-Rosenthal chamber (3.2 mm^3). These data were used to calculate the number of pollen grains per flower.

Climate conditions. The flowering period of spring oilseed rape was warmer in 2004 (July 19.6ºC) and colder in 2005 (July 16.5ºC) than the mean of the past ten years (July 17.3ºC). Ambient temperature was measured every time before the evaluation of the number of flower visiting bees and flowers and nectar and pollen production at the level of the flowers. In 2004, air temperature fluctuated from 21.5ºC on the first observation day (5 July) to 26ºC on the forth observation day (15 July). In 2005, the lowest air temperature was recorded on first and last observation days (28 June and 18 July; 17.8ºC and 18.8ºC, respectively). In 2005, the flowering period was rainy with only two days without any precipitation. In 2004 15 days were without any rain. The amount of precipitation in July 2004 was 87.8 mm and in July 2005, 113.2 mm. The mean of the past ten years was 81 mm in July.

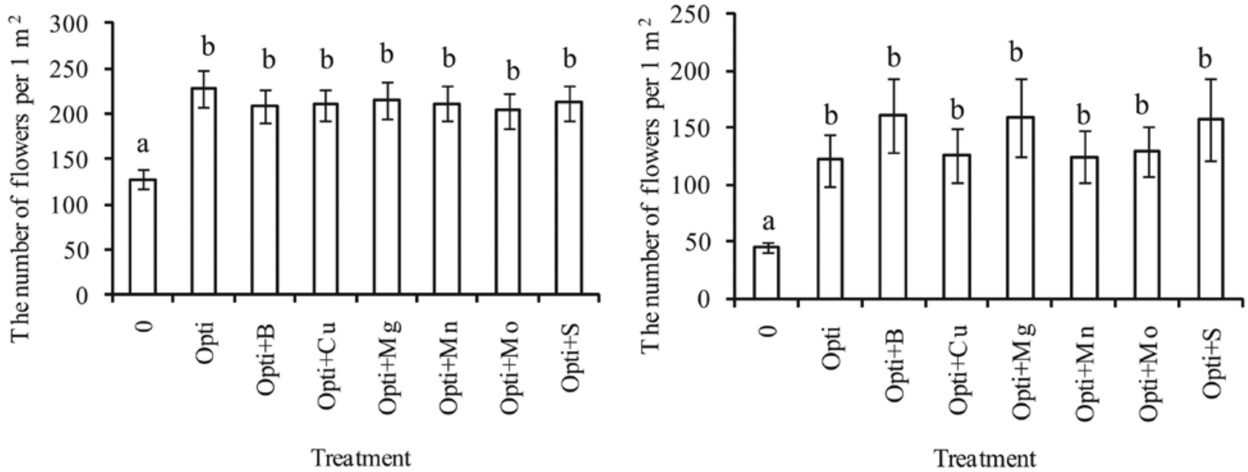
Statistical data analysis. Statistical analyses were performed using *Statistica 7*. The impact of different treatments on the number of flowers, nectar and pollen production and on the number of flower visiting bees was analysed with *ANOVA* – where necessary data were normalised. The differences between means were inspected using Fisher's protected significant difference post hoc analysis. The significance of interactions between year and treatment on pollen production and the number of bees were analysed with *factorial ANOVA*. The relationship between bees and the food resource was analysed with Pearson correlation analysis – where necessary data were normalised.

Results and discussion

Flower density on plots with different treatments. In both years (2004 and 2005), the abundance of flowers was significantly higher on fertilized than on unfertilized plots ($F(7, 184) = 2.83$, $p = 0.01$ in 2004; $F(7, 216) = 2.85$, $p = 0.01$ in 2005). Oilseed rape is a fast growing crop which needs a high amount of nutrients from the soil; otherwise its growth will slow down and, as a result, the number of flowers produced is also lower. In the case of resource deficiency, oilseed rape plants probably preserve the size of flowers rather than the number of flowers (Cresswell et al., 2001).

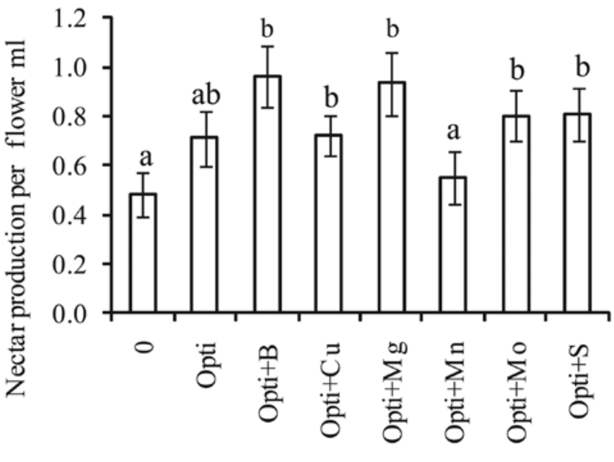
There were no significant differences between differently fertilized plots, including plots fertilized with the complex fertilizer OptiCrop alone (Fig. 1). Thus, the number of flowers depended directly on complex fertilization and addition of different foliar microfertilizers to the complex fertilizer OptiCrop did not have any significant impact on increasing the number of flowers.

Nectar production on plots with different treatments. Fertilization influences the nectar production of oilseed rape flowers. Except for fertilization with manganese or with the complex fertilizer OptiCrop alone, the production of nectar in 2004 was significantly higher on fertilized than on unfertilized plots (F (7, 312) = 2.48, *p* = 0.02). Unlike flower productivity, nectar production gains from foliar fertilization, except that supplementary manganese appeared to inhibit nectar production (Fig. 2). Plots fertilized with OptiCrop plus manganese had significantly lower nectar production than those fertilized with OptiCrop plus one of the other five microfertilizers. Flowers from plants fertilized with manganese had even less nectar than those fertilized with pure OptiCrop. Manganese increases plant height, leaf area per plant and dry weight of the aerial parts (Ali et al., 2011), and apparently, plants contribute less to nectar production.



Note. The letters above the boxes indicate statistically significant differences between treatments (*ANOVA*, Fisher LSD test). The boxes indicate the mean value and the whiskers indicate the standard error of the mean.

Figure 1. The number of flowers on plots with different treatments in 2004 (left) and 2005 (right)



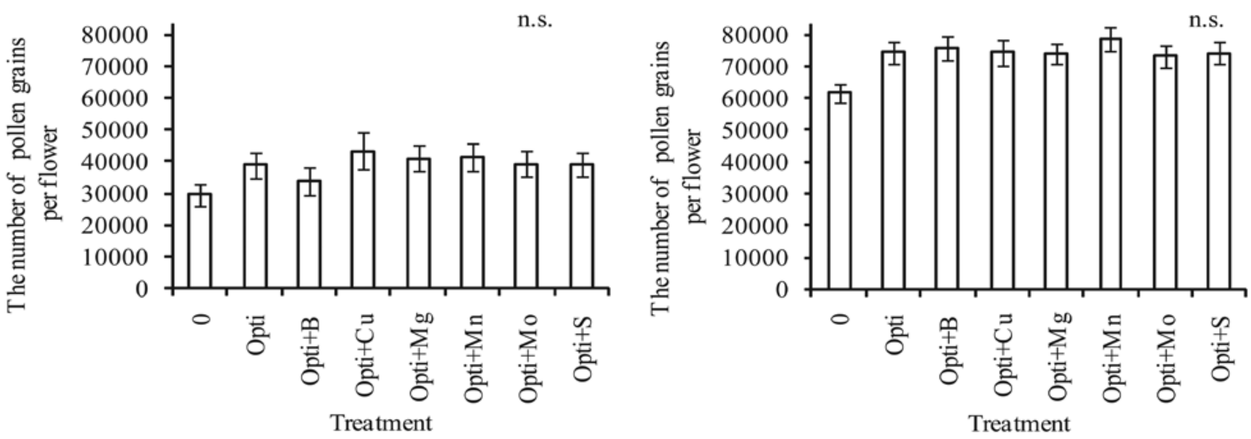
Note. The letters above the boxes indicate statistically significant differences between treatments (*ANOVA*, Fisher LSD test). The boxes indicate the mean value and the whiskers indicate the standard error of the mean.

Figure 2. Nectar production of spring oilseed rape flowers on plots with different treatments in 2004

Several authors have studied the nectar production of oilseed rape flowers depending on the varietal (Mohr, Jay, 1990; Kotowski, 2001) and genetic differences (Pierre et al., 1999) but not the effect of fertilization on nectar production. As several factors affecting nectar production and nectar standing crop are documented, e.g., evaporation and absorption (Corbet, 2003), final conclusions cannot be drawn on the basis of one study year, although a preliminary trend is evident. The topic of the effect of fertilization on nectar production needs further research.

Pollen production on the plots with different treatments. In both years (2004 and 2005), there were no significant differences in pollen production between differently treated plots (F (7, 248) = 1.15, *p* = 0.33 in 2004; F (7, 344) = 2.02, *p* = 0.05 in 2005). However, in both years, especially in 2005, the pollen production was higher on fertilized than on unfertilized plots (Fig. 3). Still, the difference was not statistically significant, probably because of the high variability of pollen production. When summarizing over the two years, the effect of treatment became significant (Table 1). In addition, there was no statistically significant interaction between year and treatment on the number of pollen grains produced per flower, which means that the impact of different treatments followed the same trend in both years being higher on fertilized than on unfertilized plots.

Pollen dissemination by pollinators (Hayter, Cresswell, 2006) and the influence of other factors on pollen transfer and gene flow (Beckie et al., 2003; Devaux et al., 2008) have received considerable attention recently in connection with potential problems associated with the adoption of genetically modified oilseed rape. However, as pollinators visit flowers to have some reward, the effect of fertilization on pollen production, which in turn can affect the number of pollinators, deserves attention as well.



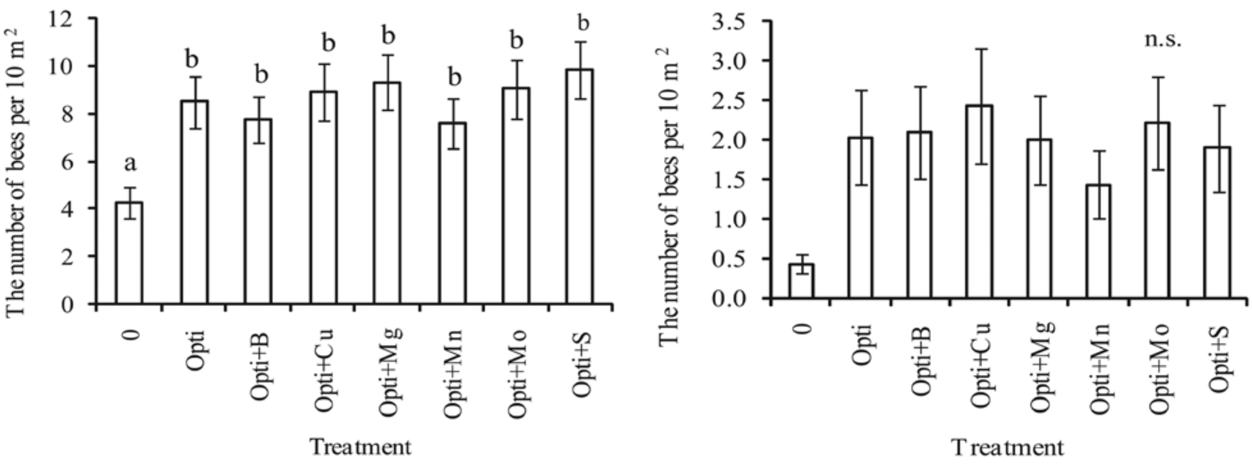
Note. The boxes indicate the mean value and the whiskers indicate the standard error of the mean; n.s. – statistically not significant.

Figure 3. The number of pollen grains per flower on the plots with different treatments in 2004 (left) and 2005 (right)

Table 1. Factorial *ANOVA* table of F-values showing the effect of year and treatment on the number of pollen grains per flower in 2004 and 2005

Effect	df	SS	F	*p*
Year	1	19543	374.5	<0.01
Treatment	7	3643	374.5	<0.01
Interaction between year and treatment	7	6709	0.4	0.92

The number of flower visiting bees on the plots with different treatments. In 2004, the number of flower visiting bees was significantly higher on fertilized than on unfertilized plots ($F (7, 184) = 2.62, p = 0.01$). Similar results were obtained in 2005 but the differences were not statistically significant ($F (7, 216) = 1.24, p = 0.28$) (Fig. 4). When summarizing over the two years, the effect of treatment was significant (Table 2). Again, there was no statistically significant interaction between year and treatment on the number of flower visiting bees, which means that the impact of different treatments followed the same trend in both years being higher on fertilized than on unfertilized plots.



Notes. The letters above the boxes indicate statistically significant differences between different treatments (*ANOVA* Fisher LSD); n.s. – statistically not significant. The boxes indicate the mean value and the whiskers indicate the standard error of the mean. Note that there are differences in the scale values of the y-axes.

Figure 4. The mean number of flower visiting bees on the plots with different treatments in 2004 (left) and 2005 (right)

Table 2. Factorial *ANOVA* table of F-values showing the effect of year and treatment on the number of bees in 2004 and 2005

Effect	df	SS	F	*p*
Year	1	4155	239.8	<0.01
Treatment	7	487	4.0	<0.01
Interaction between year and treatment	7	138	1.1	0.34

Relations between flower visiting bees and the food resource of spring oilseed rape. A significant positive correlation between the number of flower visiting bees and the number of flowers was found in both years ($r = 0.59$, $p < 0.01$ in 2004; $r = 0.69$, $p < 0.01$ in 2005). There was also a moderate correlation between nectar production and the number of flower visiting bees ($r = 0.41$, $p < 0.01$). For pollen production, a weak correlation was found in 2005 ($r = 0.21$, $p < 0.01$), but not in 2004 ($r = -0.01$, $p = 0.93$).

The economically most important and abundant pollinators of spring oilseed rape are bees (Klein et al., 2007). Considering the fact that bees visit flowers in search of food, the number of bees in the field is affected by existing food resources: the density of flowers and nectar and pollen content in them. Most bees collect only two food items from flowers: nectar, which provides bees with energy, and pollen, which provides them with protein necessary for growth of larvae (Rasheed, Harder, 1997). According to an optimal foraging theory, bees try to maximize the benefit and minimize the costs (Pettersson, Sjödin, 2000). Hence the food collected from the flower – the reward – has to exceed the energy spent on flying.

The positive correlation between the number of flower visiting bees and the number of flowers found in this experiment shows that bees consider the abundance of the food resource while looking for food, preferring areas with higher flower density. Karise et al. (2007) also found that the density of oilseed rape flowers most likely played a major role in choice of foraging area. It is energetically more profitable to choose denser flower areas in order to expend less energy in flying between flowers (Cartar, Real, 1997). As the nectar of oilseed rape flowers can be replenished within half an hour of depletion (Pierre et al., 1999), encountering empty flowers is unlikely. Oilseed rape is a favourable food plant for bees because its flowers provide copiously pollen and nectar. High-density flower patches may serve as a sign of presence of vigorous plants which are able to provide abundant food for bees (Karise et al., 2007).

Conclusions

Spring oilseed rape (*Brassica napus* L. var. *oleifera*) is an important oilseed crop, whose production area has increased significantly in northern Europe, including Estonia. Spring oilseed rape is predominantly autogamous but cross-pollination can have several positive effects, including higher seed yield. Hence it is profitable to encourage high number of pollinators in oilseed rape fields. The results of the current study allowed us to make the following conclusions:

1. The density of flower visiting bees – honey bees, bumble bees and solitary bees on spring oilseed rape depended mainly on flower density.

2. Fertilization increased not only the number of flowers but also the amount of nectar and pollen per flower.

3. Additional foliar fertilization had no effect either on the number of flowers or on the amount of pollen grains per flower. Nectar production per flower appeared to be inhibited by additional manganese.

4. To secure a higher number of pollinators for achieving higher seed yield and other benefits deriving from cross-pollination spring oilseed rape should receive proper complex fertilization. Microfertilizers turned out to be useless in terms of increasing the number of pollinators.

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Tręšimo per lapus įtaka bičių (*Apoidea*) kiekiui vasariniuose rapsuose

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Santrauka

Vasarinis rapsas (*Brassica napus* L. var. *oleifera*) yra svarbus aliejinis augalas, kurio auginimo plotai Estijoje smarkiai padidėjo. Šis augalas yra savidulkis, ir tai yra teigiamas veiksnys, ypač jo sėklų derliui. Tirta tręšimo įvairiomis lapų mikrotrąšomis įtaka vasarinių aliejinių rapsų žiedų tankumui ir žiedadulkių formavimuisi bei nektaro išsiskyrimui, taip pat šių veiksnių įtaka žiedus lankančių bičių gausai.

Lauko bandymai vykdyti 2004 ir 2005 m. Lauką sudarė 32 laukeliai (10 m^2): kontroliniai (netręšti mineralinėmis trąšomis), tręšti tik kompleksinėmis trąšomis OptiCrop (NPK 21-08-12 + S + Mg + B + Ca), ir tręšti OptiCrop bei vienomis iš šešių mikrotrąšų (Mn, S, Cu, B, Mg, Mo). Kiekvienas variantas turėjo keturis pakartojimus. Žiedus lankančios bitės skaičiuotos du kartus per savaitę saulėtomis dienomis. Žiedai skaičiuoti tuo pačiu metu kiekvieno laukelio 1 m^2 plote. Nektaro išsiskyrimas matuotas lauke, 1 µl kapiliarą įstačius į 24 valandas izoliuotų žiedų vainikėlio vamzdelį. Žiedadulkių grūdeliai skaičiuoti ištirpinus prieš tai izoliuotų žiedų audinius.

Žiedus lankančių medunešių, kamanių ir pavienių bičių tankumas ant vasarinių rapsų daugiausia priklausė nuo žiedų tankumo. Tręšimas padidino ne tik žiedų skaičių, bet ir nektaro bei žiedadulkių kiekį viename žiede. Papildomas tręšimas per lapus neturėjo įtakos nei žiedų, nei žiedadulkių grūdelių kiekiui. Vieno žiedo nektaro skyrimąsi slopino papildomas tręšimas manganu. Todėl, siekiant užtikrinti didesnį kiekį apdulkintojų ir gauti didesnį sėklų derlių bei kitą kryžmadulkos teikiamą naudą, rapsus reikėtų tręšti tinkamomis kompleksinėmis trąšomis. Apdulkintojų kiekiui mikrotrąšos nebuvo efektyvios.

Reikšminiai žodžiai: *Brassica napus* L. var. *oleifera*, vasariniai rapsai, *Apoidea*, žiedų tankumas, žiedadulkių formavimasis, nektaro išsiskyrimas, tręšimas per lapus.