

THE POSSIBILITY OF MUTUAL CONTROL OF STEM MINING WEEVILS AND POLLEN BEETLE IN OILSEED RAPE

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Abstract. Stem weevils and pollen beetle are significant pests that attack oilseed rape crops after winter hibernation. Correct timing of insecticide application is a key point in control of both pests. The aim of this research is whether the mutual control of both pests is possible in order to reduce insecticide input. The two-years experiment was set up according to the EPPO guidelines in random block design in four replications with nine different treatments. The optimal treatment period was determined by monitoring the occurrence and population density of stem weevils using yellow water traps. The decision threshold for pollen beetles was determined by beating terminal inflorescences into trays. The use of insecticides against rape stem weevils and pollen beetles is possible if thresholds are exceeded at the same time. All insecticides applied against stem mining weevils increased rapeseed yield, and in the absence of treatment, the average loss of oilseed rape seed yield was 37%.

Keywords: oilseed rape, pests, integrated pest management, Curculionidae, Nitidulidae

Introduction

Oilseed rape stem weevil (*Ceutorhynchus napi* Gyllenhal, 1837) and cabbage stem weevil (*Ceutorhynchus pallidactylus* Marsham, 1802) (Coleoptera: Curculionidae) are the first pests which attack oilseed rape plants after winter hibernation. They are often considered as a pest complex due to their biological and ecological characteristics which are similar although their life cycles are different and demand different control approach. Pollen beetle (*Brassicogethes aeneus* Fabricius, 1775) (Coleoptera: Nitidulidae) is the most important oilseed rape pest from the *Brassicogethes* genus and appears in oilseed rape fields after stem weevils when temperature exceeds 12°C (Láska and Kocourek, 1991; Alford et al., 2003; Williams, 2010). Direct damage caused by adult forms of stem weevils occurs as a result of the oviposition inside plant tissue. More significant damage occurs as a result of the larvae feeding inside leave petioles, secondary shoots and stems. In general, damages result in short and tiny plants. It is enough to find only one larva inside the plant to classify damage from the feeding of the larvae as significant. The plant deforms, stunts and decays rapidly. One day after oviposition plants begin to respond with histological changes, which are resulted by the slower development of the plant above the attacked part, the deformation of the stem and the cracking tissue at different places. Due to the destruction of the central part of the stem, a deformation of the tissue and loss of plant vigors, the number of siliques per plant is reduced. The plant produces lateral shoots and has a bushy appearance which causes a ripening maturity and makes difficulties to harvest. Feeding of adults and larvae of the pollen beetle cause bud abscission with podless stalks or siliques that can be distorted and weakened (Klukowski and Kelm, 2000; Alford et al., 2003; Krause et al., 2006; Williams, 2010). Yield losses up to 50% caused by stem weevils and up to 70% by pollen beetle have been reported (Alford et al., 2003).

The number of insecticides treatments for pest control in oilseed rape as well as the cost of treatment and insecticides in Europe have been changing over the last 30 years (Walczak and Mrówczyński, 2006). Continued frequent use of insecticides may decrease effectiveness of insecticides for stem weevils and pollen beetle control and is already present in most countries in the European Union (Heimbach et al., 2006; Thieme et al., 2010; Gotlin Čuljak et al., 2013). According to Maceljski (2002) both species of stem weevils and pollen beetle are sufficiently controlled with the single insecticide application, especially with earlier applications at the beginning of the stem elongation. In other EU countries the first treatment is performed against cabbage stem weevil and the second against rape stem weevil and pollen beetle as two insecticides application in spring significantly reduces the pollen beetle populations (Wahmhoff, 2000).

The aim of this research was to determine possibility of joint control of stem weevils and pollen beetle which might reduce insecticide input and result in slower resistance development of the oilseed rape pest.

Materials and methods

The experiment was conducted in Croatia during 2017 and 2018 at two locations, Koprivnički Bregi (46°7'34.06"N 16°53'42.55"E) in Koprivnica-Križevci county and Šašínovec (45°51'2.10"N 16°11'3.01"E) in Zagreb county. Experiments were set up according to the EPPO guidelines (2014) for investigating the effectiveness of insecticides for the control of stem weevils (PP 1/219 (1)) and pollen beetles (PP 1/178 (2)). During both growing seasons, the experiment was set up according to a random block design in four replications with nine different treatments (*Table 1*). The size of each experimental plot was 30 m² with average plant density in harvest of 39 plants/m² during both investigation years. Oilseed rape had been drilled on September 5 for both experimental years with cultivar PR46W14. Row space was 14 cm and previous crop was winter wheat. The crop was fertilized with 90 kg N/ha in autumn and spring and with 40 kg S/ha in autumn. The experiment included commercial products, applied according to recommended and registered doses, with single active ingredient: lambda cyhalothrin (6.25 ml of active ingredient/ha) and deltamethrin (5 g active ingredient/ha) as well as combined products thiacloprid (55 g of active ingredient/ha) + deltamethrin (5 g active ingredient/ha) and chlorpyrifos-ethyl (450 ml active ingredient/ha) + cypermethrin (45 ml active ingredient/ha). Chlorpyrifos-ethyl belongs to organophosphates and is classified into group 1. Deltamethrin, lambda cyhalothrin and cypermethrin belong to pyrethroids and are classified into group 3. Both groups have contact action. Thiacloprid belongs to neonicotinoids and is classified into group 4 with systemic action (IRAC, 2019).

Table 1. Treatments and active ingredients during two years experiment

treatment	active ingredient/pests
1	lambda cyhalothrin/stem weevils
2	thiacloprid + deltamethrin/stem weevils
3	deltamethrin/pollen beetles
4	chlorpyrifos-ethyl + cypermethrin/pollen beetles
5	lambda cyhalothrin/stem weevils and deltamethrin/pollen beetles
6	lambda cyhalothrin/stem weevils and chlorpyrifos-ethyl + cypermethrin/pollen beetles
7	thiacloprid+deltamethrin/stem weevils and deltamethrin/pollen beetles
8	thiacloprid+deltamethrin/stem weevils and chlorpyrifos-ethyl + cypermethrin/pollen beetles
9	untreated control

Oilseed rape crop was treated using Solo Accu Power sprayer with constant pressure of 2 bar after the decision threshold of pest population was exceeded. Optimal treatment period was determined by monitoring the occurrence and population density of stem weevils using yellow water traps. The decision threshold for pollen beetle was determined by beating 50 terminal inflorescences per each treatment into trays when flower buds were but still closed with leaves (BBCH 50). The decision threshold for *C. napi* control was 10 adult forms and for the *C. pallidactylus* control 20 adult forms caught in a yellow water traps in three consecutive days (PP 1/219 (1)). The decision threshold for pollen beetle was 0.8 - 1 adults per terminal inflorescence in growth stage when the flower buds were still covered with leaves and barely noticeable (BBCH 50) (PP 1/178 (2)). The experiment was evaluated according to EPPO guidelines (PP 1/219 (1), PP 1/178 (2)) (2014) to investigate the effectiveness of insecticides. For stem weevils, the experiment was evaluated by dissecting 10 plants taken from each treatment during the flowering (BBCH 65 - 69). The number larvae and feeding tubes were recorded. The effectiveness of insecticides for the pollen beetle control was evaluated 24 hours and three days after the insecticides application. The number of live adult forms was recorded on 50 terminal inflorescences. Harvest of each experimental plot was performed by the trial combine and seed from each experimental plot was weighed and yield was converted to hectare based on 9% seed moisture. A two-way ANOVA was performed to test the difference between treatments for the number larvae and feeding tubes caused by the larval feeding, the number of live adult forms of pollen beetle and yield. The averages are compared to Tukey's rank test. Data were statistically analysed by computer program "R" (Version 3.1.2., 2014).

Results

For the first year of experiment at Koprivnički Bregi location population dynamics of stem weevils adults, from yellow water traps, is present in *Figure 1*. Decision threshold for *C. pallidactylus* was reached on February 26 and on March 21, 24, 26 and 30. Decision threshold for *C. napi* was reached on March 30. *Table 2* presents average number of pollen beetle adults recorded by beating into trays method. Decision threshold was reached on March 29 (BBCH 50).

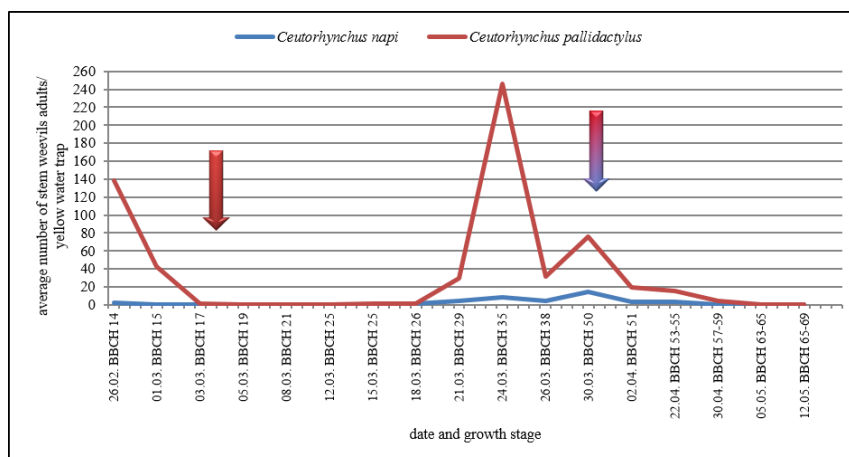


Figure 1. Average number of adult forms of *C. pallidactylus* and *C. napi* per yellow water trap at Koprivnički Bregi site (arrows present date of insecticide application)

Table 2. Average number of the pollen beetle adults per plant at Koprivnički Bregi site, BBCH 50

replication treatment	I	II	III	IV
1	0.9	1.2	1.4	0.9
2	1	1.2	1.4	0.9
3	0.8	1.2	1.1	0.9
4	1.3	1.2	1.2	0.9
5	1	1.1	1.3	1.3
6	1.2	1.1	1.5	1
7	0.9	1.2	1.3	0.9
8	0.9	1.4	1.3	0.9
9	1.9	0.8	1.5	0.8

First insecticides application was conducted on treatments 1, 2, 5, 6, 7, and 8 on March 2 after threshold for *C. pallidactylus* was exceeded. Second insecticide application was conducted on March 30 after thresholds for both species of stem weevils and pollen beetle were exceeded.

For the second year of experiment at Šašínovec location population dynamics of stem weevils adults, from yellow water traps, is present in Figure 2. Decision threshold for *C. pallidactylus* was reached on March 1 and 5. Decision threshold for *C. napi* was reached on March 26. The average number of pollen beetle adults recorded by beating into trays method are present in Table 3. Decision threshold was reached on March 30 (BBCH 50) at treatments 3, 4 and 9.

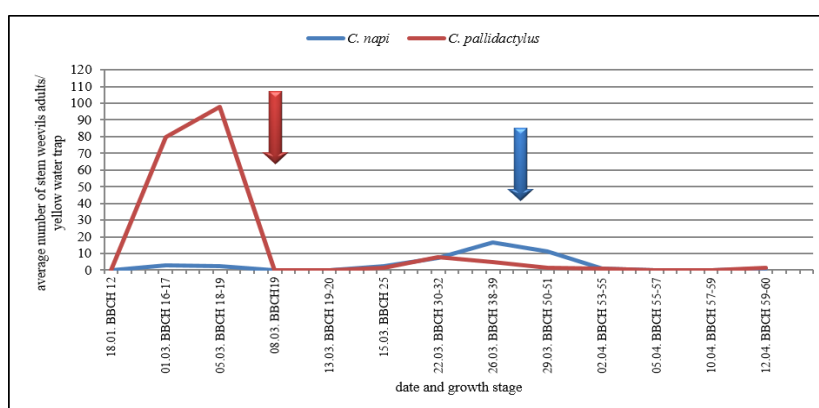


Figure 2. Average number of adult forms of *C. pallidactylus* and *C. napi* per yellow water trap at Šašínovec site (arrows present date of insecticide application)

Table 3. Average number of the pollen beetle adults per plant at Šašínovec, BBCH 50

replication treatment	I	II	III	IV
1	0.06	0.1	0.1	0.08
2	0.04	0.08	0.06	0.1
3	0.8	0.8	0.8	0.8
4	0.8	0.98	0.8	0.8
5	0.08	0.42	0.04	0.1
6	0.02	0.1	0.12	0.14
7	0.04	0.04	0.1	0.08
8	0.02	0.02	0.04	0.08
9	0.9	0.82	0.8	0.9

First insecticides application was conducted on treatments 1, 2, 5, 6, 7, and 8 on March 7 after threshold for *C. pallidactylus* was exceeded. Second insecticide application was conducted on March 27 after threshold for *C. napi* was exceeded. Third insecticide application was conducted on treatments 3 and 4 on March 30 after decision threshold for pollen beetle was exceeded.

Results of a two-way ANOVA conducted to test differences between treatments for stem weevil larvae for all locations and years are present in *Figure 3*. Statistical analysis showed a significant difference between years ($F = 504.8$; $DF = 1.8$; $P < 0.001$) and between treatments ($F = 23.4$; $DF = 8.8$; $P < 0.0001$). Tukey's HSD post hoc test showed significant differences between interaction of years and treatments.

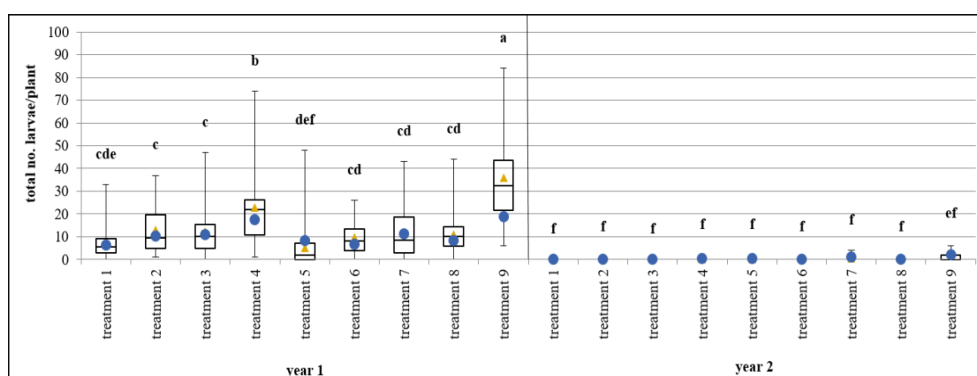


Figure 3. Two-way ANOVA for number of stem weevils larvae per plant (each treatment is presented by upper and lower quartile, minimum and maximum values; median is presented by middle line, middle value by triangle and standard deviation by circle; ranges not connected with the same latter are significantly different)

Results of a two-way ANOVA conducted to test differences between treatments for feeding tubes of stem weevils for all locations and years are present in *Figure 4*. Statistical analysis showed a significant difference between years ($F = 536.06$; $DF = 1.8$; $P < 0.0001$) and between treatments ($F = 8.4$; $DF = 8.8$; $P < 0.0001$) as well as interaction between treatments and years ($F = 4.7$; $DF = 8.8$; $P < 0.0001$). Tukey's HSD post hoc test showed significant differences between interaction of years and treatments.

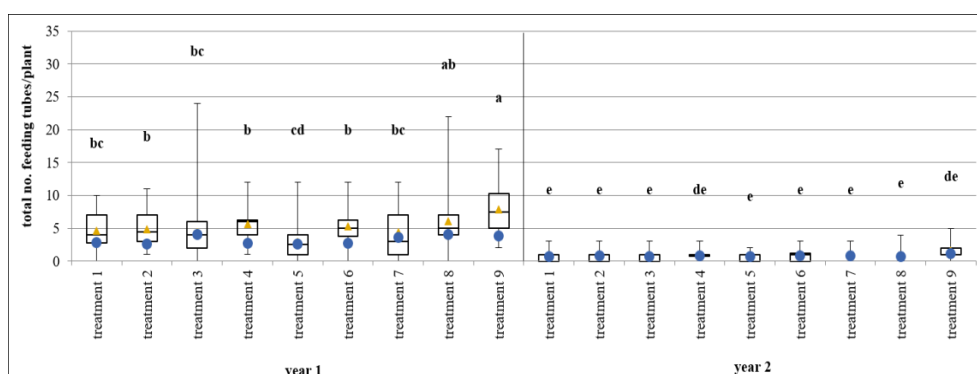


Figure 4. Two-way ANOVA for number of stem weevils feeding tubes per plant (each treatment is presented by upper and lower quartile, minimum and maximum values; median is presented by middle line, middle value by triangle and standard deviation by circle; ranges not connected with the same latter are significantly different)

Results of a two-way ANOVA conducted to test differences between treatments for live adults of pollen beetle 24 hours after insecticides application for all locations and years are present in *Figure 5*. Statistical analysis showed a significant difference between years ($F = 86.06$; $DF = 1.8$; $P < 0.0001$) and between treatments ($F = 54.8$; $DF = 8.8$; $P < 0.0001$) as well as interaction between treatments and years ($F = 25.9$; $DF = 8.8$; $P < 0.0001$). Tukey's HSD post hoc test showed significant differences between interaction of years and treatments.

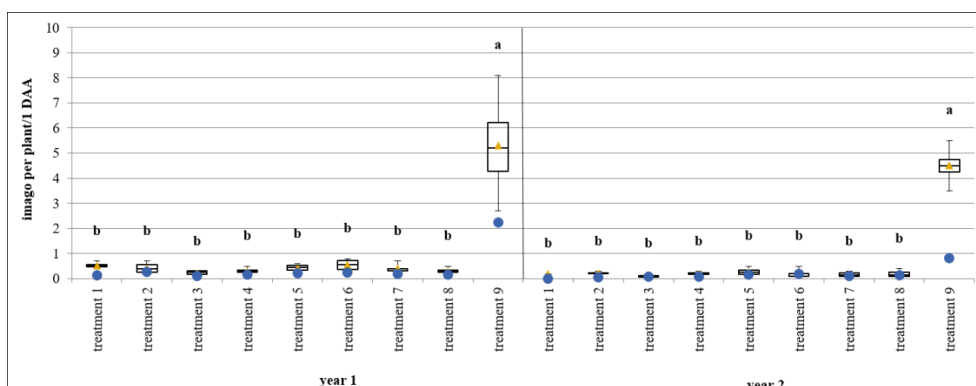


Figure 5. Two-way ANOVA for number of live pollen beetle adults 24 hours after application (each treatment is presented by upper and lower quartile, minimum and maximum values; median is presented by middle line, middle value by triangle and standard deviation by circle; ranges not connected with the same latter are significantly different)

Results of a two-way ANOVA conducted to test differences between treatments for live adults of pollen beetle 3 days after insecticides application for all locations and years are present in *Figure 6*. Statistical analysis showed a significant difference between years ($F = 89.5$; $DF = 1.8$; $P < 0.0001$) and between treatments ($F = 44.1$; $DF = 8.8$; $P < 0.0001$) as well as interaction between treatments and years ($F = 16.5$; $DF = 8.8$; $P < 0.0001$). Tukey's HSD post hoc test showed significant differences between interaction of years and treatments.

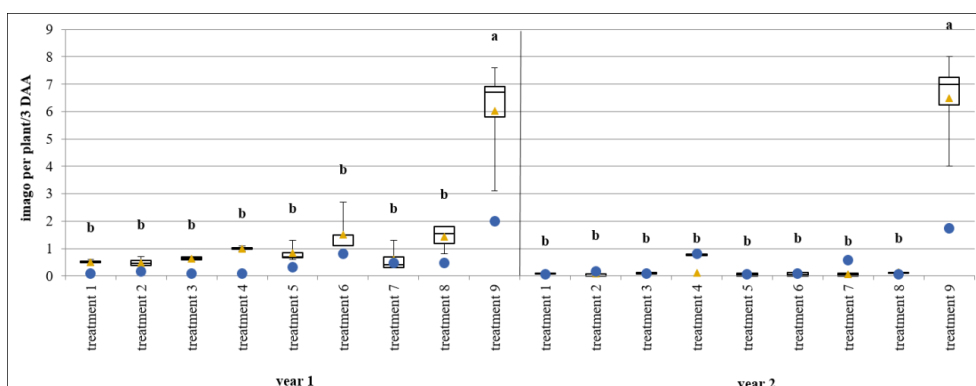


Figure 6. Two-way ANOVA for number of live pollen beetle adults 3 days after application (each treatment is presented by upper and lower quartile, minimum and maximum values; median is presented by middle line, middle value by triangle and standard deviation by circle; ranges not connected with the same latter are significantly different)

Results of a two-way ANOVA conducted to test differences between treatments for yield of oilseed rape for all locations and years are present in *Figure 7*. Statistical analysis of data showed a significant difference between years ($F = 163.3$; $DF = 1.8$; $P < 0.0001$). No statistically significant differences between treatments ($F = 1.5$; $DF = 8.8$; $P < 0.1922$) and interaction between treatments and years ($F = 1.2$; $DF = 8.8$; $P < 0.3005$) were recorded.

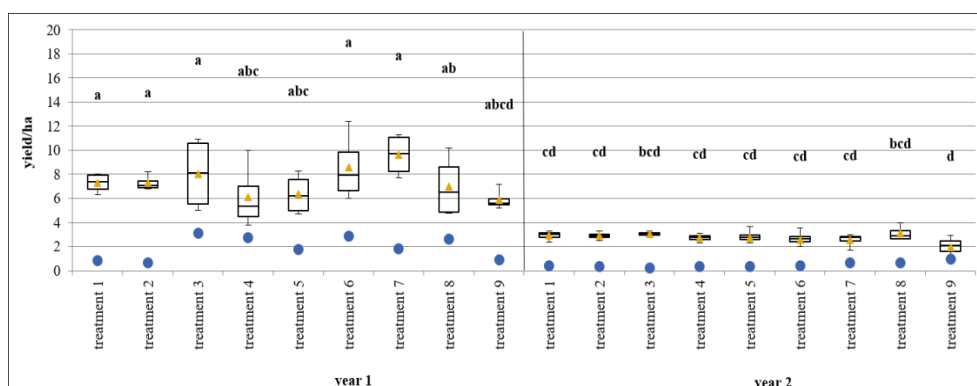


Figure 7. Two-way ANOVA for oilseed rape seed yield per hectare (each treatment is presented by upper and lower quartile, minimum and maximum values; median is presented by middle line, middle value by triangle and standard deviation by circle; ranges not connected with the same letter are significantly different)

Discussion

During both years of research, the first insecticide application was performed against adult forms of *C. pallidactylus*, which is coincided by the biology of this species. Another insecticide application was performed against *C. napi* and *Brassicogethes* sp. as both species reached the decision threshold at the same time.

The main indicator of the effectiveness of insecticides is the number of stem weevils larvae in plants and the yield of rapeseed (EPPO, 2014). The number of larvae and the number feeding tubes caused by the diet of stem weevil larvae in rapeseed plants, were significantly higher in the first experimental year compared with second year. The main reason is the later positioning of the yellow water traps in the crop of the first year and it is very likely that, by the time of the first application of the insecticide, the decision threshold for the control of *C. pallidactylus* had already been reached. As both sexes of *C. pallidactylus* tend to appear simultaneously (Juran et al., 2011) there is a possibility that copulation has occurred and that females have laid eggs before insecticide application, which is contrary to the results of Büchs (1998), who states that treatment of adult forms of *C. pallidactylus* may be delayed two weeks after the decision threshold is exceeded. Due to the large differences in the abundance of stem weevil larvae in plants in both years, it can be concluded that insecticides should be applied immediately after decision threshold for *C. pallidactylus* control is reached and not after two weeks when females become sexually mature as proposed by Kostal (1992), Büchs (1998) and Seidenglanz et al. (2009). The decision threshold should be considered as the number of females caught in yellow water traps, as suggested by Wahmhoff (2000). This is especially important when pests appear in high adult population and when the number of females exceeds the critical number of 10 for *C. napi* or 20 adult forms for

C. pallidactylus per yellow water trap. Considering different temporal occurrence of adult stages of both stem weevil species, insecticides application at two deadlines during the spring could significantly reduce the population of adult forms (Wahmhoff, 2000). The highest efficacy in reducing larval abundance in rapeseed plants in the first year was observed with the use of a lambda cyhalothrin against adult forms (treatments 1 and 5). Systemic active ingredients should have good efficacy on larvae within plants (Tomlin, 1994) but on treatments where thiacloprid (treatments 2, 7 and 8) was used in combination with deltamethrin, the number of stem weevil larvae was not significantly reduced (Gratina et al., 2011). This may be due to lower temperatures at the first insecticide application against adult forms of *C. pallidactylus* (Seta and Wolski, 2006) at which neonicotinoids have lower efficacy (Tomlin, 1994). During second year, no significant differences were observed in the number of stem weevils larvae in rapeseed plants. Differences are not present even on treatments three and four where stem weevils were not controlled and as one reason may be the different distribution of these pests across the field (Green et al., 1991; Kuhne, 1997). Uneven distribution across the surface has also been recorded for adult forms of pollen beetle (Ferguson et al., 2003, 2005). At treatments where pollen beetle was controlled with the chlorpyrifos-ethyl and cypermethrin (treatment 4), satisfactory effects on stem weevil larvae inside the stem were not achieved during both years of the study, although the organophosphorus component penetrate deeper into plant tissue (Corteva, 2019) which is partially confirmed by Milovac et al. (2017). This is in contradiction with the results of Indić et al. (2011), but the applied insecticide dose per hectare (1l), in their investigation was twice higher than recommended. The same authors conclude that there are no statistically significant differences in the number of larvae in the plant between the treatment of adult forms of stem weevils with chlorpyrifos-ethyl + cypermethrin and deltamethrin.

The results of the ANOVA for the number of larvae are also accompanied by the number of the feeding tubes which is to be expected since the damage depends on their presence and their number in the plant. The differences in the number of feeding tubes between treatments 1 and 6 (lambda cyhalothrin was used in both treatments to control stem weevils) could be explained by the different spatial distribution of adults across the field. Thiacloprid did not have a satisfactory effect on the number of feeding tubes in the plant due to the lower efficacy at lower temperatures (mean daily temperature ranged from 3.4 to 6.7°C) at the time of application of the insecticide (CMHS, 2019). During both years of the study, no statistically significant differences were found between treatments against adult forms of pollen beetle, although a year later, resistance to pyrethroids was confirmed in Croatia (Gotlin Čuljak et al., 2013).

Although no statistically differences were found between treatments for rapeseed yield in both years, it is important to observe the increase in yield from a biological importance, not a statistical significance level (Lovell, 2013). In all treatments where insecticides were applied, during the two years of the study, a relative increase in the yield of rapeseed ranged from 4 to 63% compared to the untreated control. Although the highest insecticide efficacy, for number of stem weevil larvae and feeding tubes was observed for the lambda-cyhalothrin (treatment 1 and 5 against stem weevils) did not ultimately affect the seed yield, as confirmed by Seta and Wolski (2006). The highest yield increasing in the first year of investigation was recorded on treatment 7 (63% increase in yield) where stem weevils were controlled with combination of thiacloprid and deltamethrin, and pollen beetle with deltamethrin, which is in contrast with the results of the statistical analysis for the number of larvae. A significant uniformity of the rapeseed yield across all treatments

was observed during second year, which again demonstrates that insecticide application against adults of *C. pallidactylus* is required immediately after decision threshold is exceeded. Any application of insecticides against stem weevils results in an increase in yield regardless results cannot be statistically significant, which is confirmed by Seta and Mrówczyński (1999), Seta and Wolski (2006), Petraitienė et al. (2012). The results show that stem weevils can reduce rapeseed seed yield by up to 60% and 37% on average, as confirmed by several authors (Walczak et al., 1997; Kelm and Walczak, 1998; Kelm and Klukowski, 2000; Seta and Mrówczyński, 2000; Alford et al., 2003; Dechert and Ulber, 2004).

Conclusions

Based on the results of the control and considering the life cycle of stem weevils, the first application of insecticide against adult forms of *C. pallidactylus* is critical point in protecting rapeseed against these pest. *C. napi* can be controlled with pollen beetles as the decision thresholds for these two pests coincide. Considering the confirmed resistance of pollen beetle to pyrethroids and possible resistance to organophosphorus insecticides in joint control of *C. napi* and *Brassicogethes* sp. producers should pay attention of the proper selection of insecticides and insecticides rotation to achieve effective control. For the future research, it is highly recommended to investigate the impact of stem weevils on different components of oilseed rape yield and to determine which component has the most significant impact on yield lose.

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