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Problems in cabbage stem weevil control (*Ceutorhynchus pallidacytlus* Marsh.) in winter oilseed rape

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Abstract: Due to the length of egg-laying period (> 80 days), two applications of insecticides against cabbage stem weevil (*Ceutorhynchus pallidactylus* Marsh.) are currently needed. However, resistance of pollen beetle (*Brassicogethes aeneus* F.) to pyrethroids complicates the choice of suitable insecticide for the second application. The active ingredients cypermethrin, etofenprox, pymetrozine, indoxacarb and chlorpyrifos-ethyl applied as second spring applications to winter oilseed rape crops were assessed under field conditions from 2016 to 2018 to ascertain how they could reinforce the effects of the first spring application (beta-cyfluthrin) on cabbage stem weevil. Chlorpyrifos-ethyl and etofenprox strengthened the effects of the first spring spray on cabbage stem weevil markedly more than cypermethrin. Pymetrozine and indoxacarb, effective on resistant populations of pollen beetles, showed the lowest contribution to increase the effects. Indoxacarb showed a low effect on *C. pallidactylus* in laboratory tests too. The impacts of the bans on active ingredients chlorpyrifos-ethyl and pymetrozine are discussed.

Keywords: integrated pest management; flight activity monitoring; resistance to insecticides; pyrethroids; organophosphates; indoxacarb; pymetrozine

In Central Europe, the univoltine stem weevils *Ceutorhynchus napi* Gyll. (rape stem weevil) and *Ceutorhynchus pallidactylus* Marsh. (cabbage stem weevil) (Coleoptera: Curculionidae) are two of the most damaging pests of winter oilseed rape, and both can cause significant yield losses (Kelm & Klukowski 2000; Klukowski 2006). They are often monitored as a complex of pests, although their biological characteristics are different and require different approaches for control. Adults of *C. napi* overwinter in cocoons in the soil around plants, where they complete their development (Dechert & Ulber 2004; Juran et al. 2011). They emerge from the following early spring and migrate to oilseed rape fields. On winter oilseed rape crops, weevils can appear very early, in February and March. Females usually start laying their eggs on growing stems in March/April (Dechert & Ulber 2004), exceptionally in February too (Central Institute for Supervising and Testing in Agriculture, ÚKZÚZ 2021).

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Adults of *C. pallidactylus* emerge from the soil gradually as the oilseed rape ripens and overwinter in shallow layers of soil or under leaves and other plant remains at places which surround fields and pastures (field banks, margins of field tracks, wood margins). Oviposition begins later than at *C. napi* and most females usually lay their eggs during April and often in May and June (Büchs 1998; Dechert & Ulber 2004; Juran et al. 2011). The reason for the delayed start of oviposition of *C. pallidactylus* (in comparison to *C. napi*) is the difference in progress of the male and female's migrations to crops from hibernating sites. The proportion of females was found to increase gradually in yellow water traps during the monitoring of flight activity in winter oilseed rape, so the ratio of males to females present in crops equals out substantially later in *C. pallidactylus* populations than in the case of *C. napi*. This results in a delayed start of mating, oviposition period and a shift of its peak to later dates (later growth stages of the crop) for *C. pallidactylus* (Büchs 1998).

In general, the larvae of both stem mining weevils damage plants in terms of their stability, nutrient supply and forming of pods (Büchs 1998; Dechert & Ulber 2004; Juran et al. 2011). Damage to stems induced by *C. pallidactylus* larvae also facilitates infestation by the fungal diseases, *Leptosphaeria maculans* Ces. & De Not. (*Phoma lingam*), *L. biglobosa* and *Botrytis cinerea* (Broschewitz et al. 1993; Šedivý & Kocourek 1994; Krause et al. 2006; Jedryczka et al. 2010). The egg deposition of *C. napi* females results (in some cases and seasons – not regularly) in twisting and splitting stem tissues, followed by distortion and disruption of growth (Juran et al. 2011). Both species can cause significant yield losses (Klukowski 2006; Juran et al. 2011).

In the Czech Republic a threshold of ≥ 9 adult individuals of *C. napi* and *C. pallidactylus* per yellow trap within three days is used as a common standard (Talich et al. 2013; Seidenglanz et al. 2018). However, the recommended control threshold for both species varies among European regions from four to 30 adult weevils per yellow water trap within three consecutive days (Juran et al. 2011; Eickermann et al. 2015). In Germany, the threshold is five *C. napi* and 15 *C. pallidactylus* per yellow water trap (with grid) within three days, in Austria and Luxembourg that is 10 adults per trap in three days (Alford 2008; Eickermann et al. 2020), in Croatia according to Juran et al. (2020) it is 10 adults for *C. napi* and 20 adults for *C. pallidactylus*, and in Slovakia (Central Institute for Supervising and Testing in Agriculture, ÚKSÚP 2021) it is 4–6 adults per trap in three days for both species.

In central Europe farmers regularly apply three consecutive insecticidal sprays into oilseed rape every spring: the first one against stem weevils in March/April, and the second one primarily against pollen beetles (*Brassicogethes aeneus* F.) during the second half of April or at the beginning of May. The third insecticide is usually applied at the second half of the flowering stage (second half of May – beginning of June in the Czech Republic) targeted especially at pod midge (*Dasineura brassicae* Winnertz, 1853).

This paper is based on a hypothesis that the second spring application of insecticides primarily targeted to pollen beetle can substantially influence the final level of damage caused by *C. pallidactylus* and *C. napi* larvae in seasons where females of the weevils show a prolonged egg-laying period. It concentrates on the question of whether the insecticides suitable for application against pollen beetle (usually applied as a second spring spray in central Europe), i.e. insecticides presently (indoxacarb) or not long ago (pymetrozine, organophosphates) recommended for an anti-resistance strategy (Brandes et al. 2018), are more or less helpful in reducing the damage induced by stem weevil larvae, than the insecticides whose usage against pollen beetle is not more recommended due to the phenomenon of resistance (pyrethroids).

The main objectives were: (1) to identify if the second spring application regularly targeted at pollen beetles is also important for decreasing the damage caused by *C. pallidactylus* and *C. napi* larvae; (2) to determine if insecticides commonly applied as a second spring spray to winter oilseed rape differ substantially in contribution to cabbage stem weevil (and rape stem weevil) control; (3) to find out how the fact of pollen beetle's resistance to pyrethroids, which is widely spread in Europe (Zimmer & Nauen 2011; Heimbach & Müller 2013), and variability in susceptibility of *C. pallidactylus* (*C. napi*) to different types of insecticides complicate the choice of a suitable insecticide for the second spring application; (4) to analyse how the ban on organophosphates and pymetrozine affects the possibility of controlling cabbage stem (rape) weevil and pollen beetle in oilseed rape crops.

MATERIAL AND METHODS

Small plot trials. Small plot (25 m^2 per plot) trials containing 12 treatments in four repetitions were established in 2016, 2017, and 2018 (Table 1). In all three years, the trials were managed at fields not too distant from one another (max. 2 km) located nearby the town of Šumperk (north-eastern part of the Czech Republic, 49°58'02.4"N 16°58'40.0"E). The winter oilseed rape variety Orava was used. Three yellow water traps were distributed through the untreated crop immediately adjacent to the trial every year (untreated crop area: 80 m × 80 m). The traps were placed on the crop in about mid-February (February 9, 2016; February 16, 2017; February 19, 2018) and emptied twice a week (3–4 days long periods between the assessments were kept) until the end of June. In all three years air temperatures (daily means, maximums, minimums in 2 m above ground), soil temperatures (daily means in 5 cm underground), and precipitation (daily sums) were measured with an automatic meteorological station. The meteorological station used is part of the Czech Hydrometeorological Institute station network (https://www.chmi.cz/files/portal/docs/poboc/OS/stanice/ShowStations_CZ.html) and it is located at Agritec Ltd, Šumperk, north-eastern part of the Czech Republic (49°58'26.4"N 16°58'02.7"E). The distance between the localities where the field trials were founded, and the meteorological station position was no bigger than 2 km in any of the three years. All the stem weevil individuals caught in the traps were transferred to the laboratory. For each of the sampling dates, the number of adults of *C. pallidactylus* and *C. napi* (males and females counted separately for both species) was counted and expressed as a number per trap and three-day period. This data was used to record the total length of flight activity separately for *C. pallidactylus* and *C. napi*. That is the period between the date of the first record and the date of the last record of *C. pallidactylus*/*C. napi* adults in traps. Furthermore, for each of the sampling dates, the number of females (for both species separately again) with ripe eggs in ovaries (dissection and assessment of ovaries carried out in accordance with Büchs 1998 and Seidenglanz et al. 2009) was stated and expressed as a number per trap and three days. The female with ripe eggs in ovaries = female able to lay eggs. Based on this data, it was possible to estimate the total length of the egg-laying period for both *C. pallidactylus* and *C. napi* (= period between the date of the first and the date of the last record of a female with ripe eggs in ovaries in traps), as the presence of such females in traps confirms their occurrence in crop too.

The insecticides used in the trial were applied on two different dates. The date for the first application (tr. 1–5, 11) was decided according to the total number (= *C. pallidactylus* + *C. napi*) of adults caught (expressed as average per one trap within three days) and the results of dissecting female weevils present in traps. In each of the three years the insecticide intended for the first spraying (Bulldock 25 EC) was applied after ≥ 9 adults of *C. pallidactylus* + *C. napi* per yellow trap within three days were recorded (= recommended control threshold for both species in CZ: Talich et al. 2013) and at the same time after the date when the first females with ripe eggs in ovaries were caught in the yellow water traps. Both preconditions had to be met for the insecticide application. Therefore, the trial application did not need to be made immediately after recording the threshold value (1st precondition for spraying), but with some delay after that, when the first females able to lay eggs were recorded in the yellow water traps (2nd precondition for spraying), (Table 2).

Furthermore, the abundance of pollen beetles on inflorescences was assessed. The monitoring was used for determination of the date of the second insecticide application. The beetles were counted in plots sprayed on the first date (tr. 1–5 and 11) and the monitoring started shortly after this application every year. The second sprays (tr. 1–10), were applied when the mean abundances of pollen beetles in the monitoring plots reached CZ thresholds [one or three adults per plant at BBCH 51–53 respectively BBCH 55–57(59), Table 2].

All trial applications were made with the usage of a self-propelled small plot sprayer Hege (operation pressure: 300 kPa, nozzle type: flat fan XR TEEJET, nozzle spacing: 30 cm, boom length: 3 m, application amount: 312.5 L/ha).

The levels of damage to plants caused by weevil larvae were assessed when the crop achieved green maturity stage (BBCH 74–76; June 14, 2016; June 9, 2017; June 11, 2018). Twenty plants from each plot (= 80 per treatment) were randomly selected and for each of them the total length of feeding tubes inside the stem caused by weevil larvae was measured (= the length of injured part of the stem).

Table 1. Insecticides applied on the first and second dates (2016–2018)

Treatment No.	First spray		Second spray		
	insecticide	recommended field rate (g a.i./ha)[1]	insecticide (active ingredient)	recommended field rate (g a.i./ha)[2]	comment to the second spray
1	Bulldock 25 EC (beta-cyfluthrin 25.8 g/L)	7.74	Cyperkill 25 EC (cypermethrin 250 g/L)	25	presently not recommended due to resistance of pollen beetles to pyrethroids
2			Trebon OSR (etofenprox 287.5 g/L)	57.5	the same comment as for tr. 1; but etofenprox is less influenced with the resistance
3			Plenum (pymetrozine 500 g/kg)	75	suitable for antiresistant strategy in pollen beetle control; it is banned from usage (from 2020)
4			Avaunt 15 EC (indoxacarb 150 g/L)	25.5	suitable for antiresistant strategy in pollen beetle control
5			Dursban delta (chlorpyrifos-ethyl 200 g/L)	350	suitable for antiresistant strategy in pollen beetle control; it is banned from usage (from 2020)
6	unsprayed	–	Cyperkill 25 EC (cypermethrin 250 g/L)	25	the same comment as for tr. 1
7			Trebon OSR (etofenprox 287.5 g/L)	57.5	the same comment as for tr. 2
8			Plenum (pymetrozine 500 g/kg)	75	the same comment as for tr. 3
9			Avaunt 15 EC (indoxacarb 150 g/L)	25.5	the same comment as for tr. 4, 5
10			Dursban delta (chlorpyrifos-ethyl 200 g/L)	350	the same comment as for tr. 4, 5
11	Bulldock 25 EC (beta-cyfluthrin 25.8 g/L)	7.74	unsprayed	–	–
12	unsprayed	–	unsprayed	–	–

[1] According to the list of insecticides registered for use against cabbage and rape stem weevils in winter oilseed rape in CZ for years 2016–2018 (Central Institute for Supervision and Testing in Agriculture)

[2] According to the list of insecticides registered for usage against pollen beetles in winter oilseed rape in CZ for years 2016–2018 (Central Institute for Supervising and Testing in Agriculture)

Table 2. The dates of sprays, description of growth stages of crop on the dates of spray and the results of *Ceutorhynchus pallidactylus* and *Ceutorhynchus napi* flight activity monitoring in the three seasons (2016–2018)

Season	2016	2017	2018
Date of first spray (growth stage)	April 1 (BBCH 31–50; height 12 cm)	March 31 (BBCH 31; height 10 cm)	April 12 (BBCH 50; height 10 cm)
Date of second spray (growth stage)	April 22 (BBCH 55; height 95 cm)	May 3 (BBCH 57–59; height 100 cm)	April 26 (BBCH 53–55; height 45 cm)
First record of ≥ 9 adults of *C. pallidactylus*/yellow trap within three days[1]	March 23	March 28	April 3
First record of ≥ 9 adults of *C. napi*/yellow trap within three days[1]	threshold was not achieved in the season	threshold was not achieved in the season	April 6
C. pallidactylus max. flight activity in the season (sex: date, average number of adults per trap within three days)	males: April 8, 35.67; females: April 8, 9.00	males: April 3, 45.33; females: April 3, 12.33	males: April 6, 116.33; females: April 25, 11.67
C. napi max. flight activity in the season (sex: date, average number of adults per trap within three days)	males: April 8, 0.67; females: April 8, 1.33	males: April 3, 1.67; females: April 3, 2.00	males: April 6, 4.33; females: April 6, 5.00
Total length of *C. pallidacytlus* flight activity	March 14–June 1	March 16–June 11	March 29–May 30
Total length of *C. pallidactylus* egg-laying period	April 1–May 10	March 28–June 5	April 9–May 30
Total length of *C. napi* flight activity	March 17–April 14	March 2–May 26	March 29–April 25
Total length of *C. napi* egg-laying period	April 1–14	March 28–May 22	April 9–25
Proportion of *C. pallidactylus* (*C. napi*) females with ripe eggs in ovaries present in yellow water traps at the date of first spraying (%)	7.69 (33.33)[2]	26.00 (100)[3]	26.67 (42.86)
Proportion of *C. pallidactylus* (*C. napi*) females with ripe eggs in ovaries present in yellow water traps at the date of second spraying (%)	71.35 (already not present in traps)	100 (100)	86.49 (100)

[1] Common Eropean threshold (also used in Czech Republic) of ≥ 9 adult individuals of *C. pallidactylus* (*C. napi*)/yellow trap within three days

[2] One of three *C. napi* females recorded in traps before the date of spraying had ripe eggs in ovaries; too low occurrence for valuable assessment

[3] Only one *C. napi* female recorded in traps before the date of spraying; too low occurrence for valuable assessment

Adult vial tests. In each of the three seasons, one *C. pallidactylus* and one *B. aeneus* population were tested on susceptibility to insecticides under laboratory conditions. All the insect populations were sampled from the untreated crops adjacent to the trial every year (the same crop where the yellow water traps were placed). Samplings of *C. pallidactylus* adults were always carried out as soon as enough adults for testing could be obtained (approximately 800 adults). This always happened approximately at the time of maximum flight activity (Table 2). Pollen beetles were sampled one day (2017, 2018) or immediately (2016) before the second trial application (April 22, 2016; May 2, 2017 and April 25, 2018; oilseed rape growth stages described in Table 2). For collecting pollen beetles, sweep net was used. In the case of cabbage stem weevils, it was more complicated and more time-consuming to gather enough adults. A combination of techniques using sweep net and beating tray was used. In none of the three seasons, the numbers of *C. napi* adults sampled from the untreated crop were sufficient, therefore *C. napi* adults were not tested.

Adult vial tests recommended by the Insecticide Resistance Action Committee (IRAC 2021) were used for testing both cabbage stem weevil and pollen beetle susceptibility against lambda-cyhalothrin (IRAC test No. 011 version 3), chlorpyrifos-ethyl (IRAC test No. 025) and indoxacarb (IRAC test No. 027). Instead of the cypermethrin used for second date applications in field trials (tr. 1, 6), lambda-cyhalothrin was used as a representative of type II pyrethroids for testing under laboratory conditions. Susceptibilities of the pests to pymetrozine were not tested. No commonly acceptable laboratory method for pymetrozine was available at the time when the trials were carried out. Susceptibilities to etofenprox (a member of type I pyrethroids) were not assessed either, although an acceptable laboratory method suitable for testing pyrethroids was available (IRAC test No. 011, description is below). Frequent inconsistencies between the results of laboratory and field trials recorded in previous studies aimed at monitoring pollen beetle resistance to pyrethroid etofenprox in CZ (Rubil et al. 2018) were the reason for excluding the active ingredient from laboratory testing for the purposes of this study.

Unlike the IRAC methodology, the number of compared concentrations was increased in the case of all three tested insecticides to make the calculation of lethal concentrations (LC values) more precise. The concentrations of the three active ingredients tested in this study are listed in Table 3.

For every population, three replicates were used for each tested concentration (= three testing vials). 8–12 adult pollen beetles or 6–10 adults of *C. pallidactylus* were placed in each testing vial. The vials with adults were stored in constant environment facilities at 20 ± 2 °C and 16 : 8 h light : dark. After 24 h, the adults were tipped out of the vials and scored on filter discs with a diameter of 15 cm in the case of lambda-cyhalothrin and indoxacarb and 8 cm in the case of chlorpyrifos-ethyl.

Lambda-cyhalothrin (analytical standard; batch number: HUD6A 3514) was obtained from Syngenta Czech s.r.o. (Czech Republic), and chlorpyrifos-ethyl (analytical standard; batch number: PE1377-2ML) was obtained from Sigma-Aldrich (MO, USA). In the case of indoxacarb, a commercial liquid EC formulation containing 150 g/L of active ingredient (Avaunt 150 EC) was used for the preparation of the solutions tested.

Statistical analysis. The primary data from small plot trials was analysed using Statistica software, version 12. For all sets of data, one-way ANOVA tests were performed. The differences between the means were evaluated using Tukey's HSD test ($P < 0.05$). For the ANOVA test, the homogeneity of variance was previously checked using Bartlett tests ($P < 0.05$).

Table 3. Concentrations (g a.i./ha) of the three insecticide active ingredients tested in laboratory tests (2016–2018)

Adult vial tests		
Indoxacarb	chlorpyrifos-ethyl	lambda-cyhalothrin
0	0	0
0.04	0.092	0.06
0.08	0.290	0.30
0.14	0.920	1.50
0.2	2.900	7.5*
0.94	9.400	37.50
3.19	30.000	112.50
6.38	96.000	–
9.05	307.2**	–
25.5*	–	–

*Recommended field rate in Europe; **recommended field rate in CZ (2016–2018), recommended field rate in many other EU countries was substantially lower for chlorpyrifos-ethyl: 187 g a.i./ha

In Adult vial tests, adults incapable of coordinated movement were scored as dead. Based on this primary data the values of LC_{50} and LC_{90} were estimated for each of the three active ingredients. Probit regression was used (Polo Plus version 2; LeOra Software, CA, USA) for the calculations.

RESULTS

Field trials. Air and especially soil temperatures started to grow above the zero values (0 °C) earlier in 2018 than in 2016 and 2017. In 2018 the warmest January was recorded of the three seasons. Contrary to that, February and especially March (in this case especially soil temperatures) were markedly colder in 2018 than in 2016 and 2017. Maximum daily air temperatures (measured in 2 m above ground) exceeded the value of 10 °C for the first time earlier in 2016 – it was already at the beginning of February. In the two other years it was recorded markedly later, in 2017 it was in the middle of February and in 2018 the maximum air temperatures attacked the value of 10 °C during the second week in March for the first time. The 2016 season was characteristic with the warmest February and the 2017 season with warmest March (Figure 1).

In all three years, *C. pallidactylus* adults dominated over *C. napi* adults in the yellow water traps at the locality. *C. pallidactylus* abundances exceed-

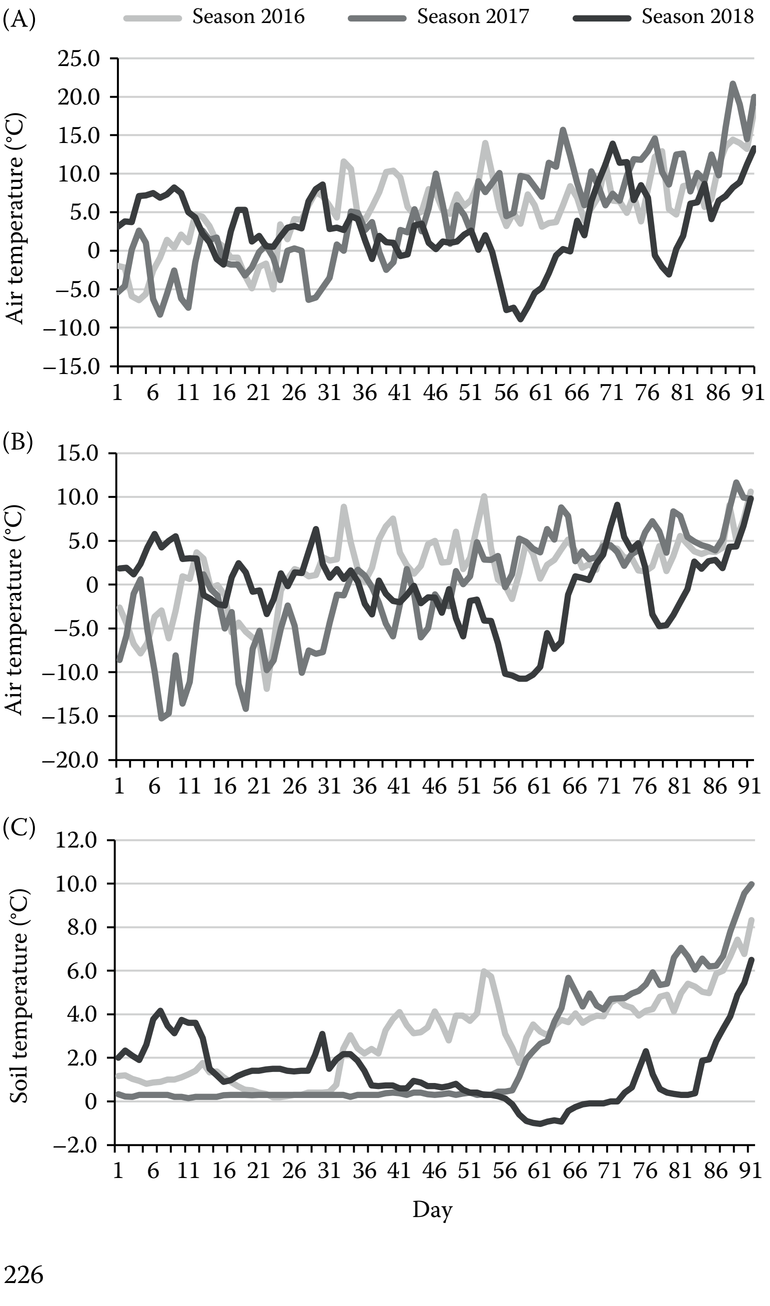

Figure 1. Progress in air and soil temperatures during the first three months of seasons 2016, 2017 and 2018

(A) Maximal air temperature per day; (B) average air temperature per day; (C) average soil temperature per day ed the CZ threshold every year, though *C. napi* just exceeded the value once, in 2018. Regardless of the differences in the progress of air and soil temperatures in January and February, in each of the three years the flight activity was recorded during March for the first time (March 14–29) and the females of both species were able to lay eggs from the end of March (2017), respectively from the beginning of April (2016, 2018). In each of the three seasons, the females of both species began laying eggs at the same time – the oviposition periods of *C. napi* did not start earlier than those of *C. pallidactylus* but in all three years the egg-laying periods of *C. pallidactylus* lasted significantly longer. In 2016 the egg-laying period of *C. pallidactylus* was 26 days longer than the oviposition period of *C. napi*, in 2017 and 2018 the difference was 14 and 36 days. Females of *C. pallidactylus* capable of laying eggs were still present in the traps and thus probably also in crops until the beginning of May (2016) or even up to May/June (2017, 2018). In the case of both species the longest egg-laying period was the season 2017. The 2018 season was characteristic with the highest mean counts of *C. pallidactylus* adults recorded in traps at the time of maximal flight activity and also the total count (total number of adults caught per season) was the highest in 2018 (sums are not shown). In this season, *C. napi* adults were also more numerous in traps and so the insect pest was more dangerous in 2018 than in the two previous seasons – *C. napi* counts slightly exceeded the CZ threshold value only one time, on April 6, 2018 (Table 2).

The interannual variabilities in numbers of *C. pallidactylus* adults caught in traps (means recorded at the times of maximum flight activity as well as seasonal sums) were caused, in particular, by differences in the counts of males: on average 35.67 males in 2016, 45.33 males in 2017 and 116.33 males in 2018 recorded per trap on the date of maximum flight activity. Interannual differences in the counts of *C. pallidactylus* females did not vary so much: on average 9–12.33 females per trap on the date of maximum flight activity (Table 2).

Differences in the final levels of stem damage were significant among the treatments in many cases in each of the three years. On the dates of assessment, only negligible numbers of stem weevil larvae (only *C. pallidactylus*) were still present in plants, most of them already left the plants to pupate in the soil. Therefore, it is possible to consider the levels of stem damage induced by larvae recorded on these dates to be final. In all three years, the contribution of the second application to increasing the effectiveness of the first application (beta-cyfluthrin) was high in cases where chlorpyrifos-ethyl or etofenprox were applied as the second spray and substantially lower, but not negligible, in the case of cypermethrin application. Contrary to that, pymetrozine and indoxacarb applications, which followed the first beta-cyfluthrin spray, did not increase the effects of the first spray on the stem weevils at all, or the benefit of the applications was markedly lower than in the cases of both the pyrethroids (cypermethrin and etofenprox) and organophosphate chlorpyrifos-ethyl (Table 4).

Laboratory trials. A comparison of the three-year mean lambda-cyhalothrin LC_{50} values estimated both for pollen beetles (three-year mean of LC_{50}: 7.92 g a.i./ha) and cabbage stem weevils (three-year mean of LC_{50}: 0.15 g a.i./ha) sampled at the trial locality indicates an approximately 53-fold lower susceptibility of pollen beetles to the active ingredient than cabbage stem weevils. LC_{90} values for the active ingredient are markedly above the recommended field rate (7.5 g a.i./ha) in pollen beetles. Cabbage stem weevils showed high susceptibility to the active ingredient in all three years at the locality (Table 5).

In the case of indoxacarb, the situation contrasts with lambda-cyhalothrin. Pollen beetles showed high susceptibility to indoxacarb (three-year mean of LC_{50}: 0.18 g a.i./ha), whereas cabbage stem weevils proved to be distinctly less susceptible to the active ingredient (after 24 h of contact exposure; three-year mean of LC_{50}: 14.88 g a.i./ha). LC_{90} values for the active ingredient are markedly above the recommended field rate (25.5 g a.i./ha) in cabbage stem weevils (Table 5). From Figure 2, it is clear how differently both insect species reacted to the insecticide in the three years.

Both groups of insect pests showed high susceptibility to chlorpyrifos-ethyl. The cabbage stem weevil population seemed to be somewhat more susceptible to the active ingredient than the pollen beetles at the locality. LC_{90} values estimated for the active ingredient were lower than the recommended field rate commonly used in Europe before the year 2020 (187 g a.i./ha) for cabbage stem weevils (three-year mean of LC_{90}: 4.21 g a.i./ha) as well as pollen beetles (three-year mean of LC_{90}: 20.71 g a.i./ha), (Table 5).

Table 4. Mean levels of stem damage induced by the weevil larvae (mainly by *Ceutorhynchus pallidactylus* and partly by *Ceutorhynchus napi*) recorded in the trials and the levels of effectiveness stated for the compared insecticide applications (2016–2018)

Treatment No.	First spray	Second spray	Damage of stems induced by *C. pallidactylus* and *C. napi* larvae											
			2016				2017				2018			
			mean length of stem damage (cm)	SD	effectiveness (%)[1]	contribution of the second spray (%)[2]	mean length of stem damage (cm)	SD	effectiveness (%)[1]	contribution of the second spray (%)[2]	mean length of stem damage (cm)	SD	effectiveness (%)[1]	contribution of the second spray (%)[2]
1	beta-cyfluthrin	cypermethrin	7.08^{gh}	12.68	79.85	49.93	11.90^{d}	9.00	49.06	14.38	16.30^{efg}	11.57	56.22	23.15
2		etofenprox	5.04^{hi}	7.80	85.65	64.36	5.51^{e}	6.01	76.41	60.36	12.28^{gh}	8.25	67.02	42.10
3		pymetrozine	10.48^{fg}	14.07	70.17	25.88	14.24^{cd}	11.89	39.04	−2.45	19.18^{def}	11.77	48.48	9.57
4		indoxacarb	12.75^{ef}	12.58	63.71	9.83	15.60^{cd}	11.15	33.22	−12.23	22.13^{cd}	12.04	40.56	−4.34
5		chlorpyrifos-ethyl	2.45^{i}	4.32	93.03	82.67	7.56^{e}	8.73	67.64	45.61	9.71^{h}	8.12	73.92	54.22
6	unsprayed	cypermethrin	25.86^{bc}	17.69	26.39		20.23^{ab}	9.44	13.40		26.13^{bc}	10.41	29.82	
7		etofenprox	21.79^{cd}	19.44	37.97		18.78^{abc}	10.71	19.61		22.28^{cd}	11.04	40.16	
8		pymetrozine	31.98^{ab}	18.19	8.97		23.13^{ab}	15.57	0.99		36.90^{a}	15.79	0.89	
9		indoxacarb	33.09^{ab}	23.51	5.81		25.65^{a}	18.74	−9.80		30.49^{ab}	17.31	18.10	
10		chlorpyrifos-ethyl	17.61^{de}	17.10	49.87		17.05^{bc}	12.40	27.01		15.84^{fg}	10.49	57.45	
11	beta-cyfluthrin	unsprayed	14.14^{def}	17.80	59.75	0.00	13.90^{cd}	11.62	40.50	0.00	21.21^{de}	10.77	43.03	0.00
12	unsprayed	unsprayed	35.13^{a}	20.94	0.00		23.36^{a}	11.12	0.00		37.23^{a}	15.99	0.00	
			$F = 38.525; P < 0.001$				$F = 22.401; P < 0.001$				$F = 41.661; P < 0.001$			

[a–i] Mean values placed in the same column are significantly different when they are marked with different letters

[1] Effectiveness expressed according to Abbott's formula; tr. 12 = 0.00%

[2] Contribution of the second spray to increasing the first spray effectiveness; counted as effectiveness expressed according to Abbott's formula; tr. 11 = 0.00%

Table 5. Log dose probit mortality data was obtained for lambda-cyhalothrin, chlorpyrofos-ethyl and indoxacarb from adult vial tests (IRAC method 011 version 3, IRAC method 025 and IRAC method 027) against *Ceutorhynchus pallidactylus* and *Brassicogethes aeneus* collected in untreated winter oilseed rape in 2016, 2017 and 2018: lethal concentrations for 50% and 90% of the beetles (LC_{50}, LC_{90}; g/ha) and corresponding 95% confidence limits (95% CL; g/ha)

Population (season)	Lambda-cyhalothrin (g/ha)				Chlorpyrifos-ethyl (g/ha)				Indoxacarb (g/ha)			
	LC_{50}	95% CL	LC_{90}	95% CL	LC_{50}	95% CL	LC_{90}	95% CL	LC_{50}	95% CL	LC_{90}	95% CL
C. pallidactylus (2016)	0.19^{a}	0.13–0.27	0.74^{a}	0.48–1.57	2.18^{bc}	1.56–3.01	6.43^{bcd}	4.42–11.67	13.46^{c}	9.33–22.88	99.05^{c}	47.86–385.23
C. pallidactylus (2017)	0.16^{a}	0.12–0.21	0.58^{a}	0.38–1.30	1.44^{ab}	1.03–2.01	4.24^{abc}	2.89–7.88	13.10^{c}	9.12–22.03	96.41^{c}	46.89–371.03
C. pallidactylus (2018)	0.09^{a}	0.03–0.18	1.01^{a}	0.50–3.82	0.95^{a}	0.72–1.30	1.97^{a}	1.42–3.71	18.09^{c}	13.19–30.56	79.48^{c}	42.35–298.40
B. aeneus (2016)	8.12^{b}	5.08–12.72	69.03^{b}	39.27–156.82	3.35^{cd}	2.51–4.52	12.12^{de}	8.37–20.76	0.24^{b}	0.20–0.31	0.60^{b}	0.44–0.98
B. aeneus (2017)	7.70^{b}	4.74–12.18	46.55^{b}	27.07–104.50	2.86^{bcd}	1.57–5.51	12.56^{cde}	6.32–47.81	0.12^{a}	0.11–0.13	0.18^{a}	0.16–0.22
B. aeneus (2018)	7.93^{b}	5.13–12.23	40.11^{b}	23.81–90.40	5.70^{d}	3.34–10.32	37.44^{e}	18.66–117.86	0.19^{b}	0.16–0.24	0.40^{b}	0.30–0.72

$^{a-e}LC_{50}$ and LC_{90} values placed in the same column are significantly different when they are marked with different letters

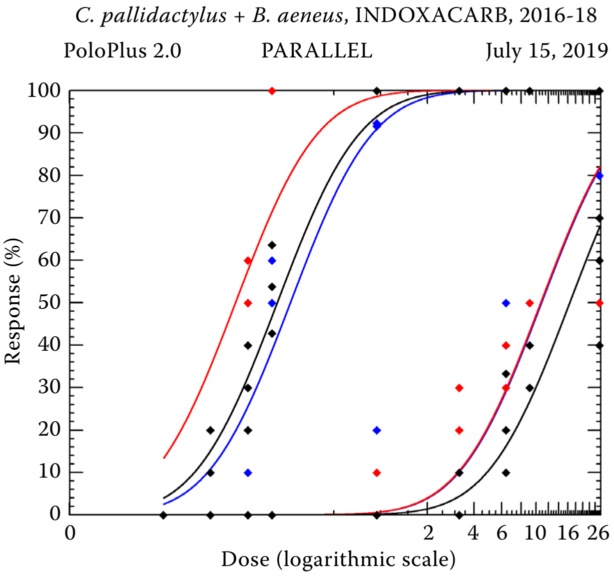

Figure 2. The curves show the differences between the growth of mortality (axis *y*) against the tested rate of indoxacarb (axis *x*) in the three tested populations of *Brassicogethes aeneus* (2016, 2017, 2018; the three curves located on the left) and the three tested populations of *Ceutorhynchus pallidactylus* (2016, 2017, 2018; the three curves located on the right)

DISCUSSION

The results of the field trials show that in situations where the abundance of adults of *C. pallidactylus* (*C. napi* was not so important in this study) exceeds the common threshold value (Seidenglanz et al. 2009; Eickermann et al. 2015, 2020) and when the length of flight activity and oviposition period is long at a locality, the effects of only one spray, where no other application follows, can be unsatisfactory. The effectiveness of the first spring application (when assessed separately), even though it was made in time and in accordance with common recommendations, exceeded a value of 50% effectiveness (according to Abbott's formula) only in one season. That was in 2016, the season with the shortest *C. pallidactylus* egg-laying period – it lasted 40 days: April 1–May 10, 2016 (treatment 11 represented this option in the field trials). Such results indicate that another spring application can be important for reaching low levels of stem damage. Therefore, the insecticide intended for the second application should be effective not only on pollen beetle but also on the stem weevils which usually still occur in crop at that time. Despite this fact, in practice the second spring application is perceived as a spray targeted at pollen beetles only because farmers and even advisors mostly perceive stem weevils and pollen beetles as two temporally separate problems which need different approaches for sufficient control (Büchs 1998; Seidenglanz et al. 2009). In fact, the period of pollen beetle presence in crop (at least partly) coincides with the time when *C. pallidactylus* and *C. napi* lay eggs in the crop (Büchs 1998; Seidenglanz et al. 2018). However, the need for high effectiveness against both stem weevils and pollen beetle makes the choice of suitable insecticide for the second spring application somewhat complicated because the group of convenient and at the same time available insecticides is limited.

Pyrethroids are not a suitable option for such application due to the widespread resistance of pollen beetles to them (Hansen 2003; Derron et al. 2004; Thieme et al. 2008; Wegorek et al. 2009; Philippou et al. 2011; Zimmer & Nauen 2011; Heimbach & Műller 2013; Brandes et al. 2018; Rubil et al. 2018). However, as is evident from the results of this study, the contributions of pyrethroids applied as a second spring spray (cypermethrin, etofenprox) to increase the effect of the first spray on stem weevils are markedly higher (especially in the case of etofenprox) than the contributions of presently recommended alternative for pollen beetle control, insecticide indoxacarb. The same is true for the previously preferred alternative, pymetrozine, banned from usage in 2020. In the case of indoxacarb, low susceptibility of cabbage stem weevil to the insecticide was confirmed in laboratory tests too. Regardless of their high effects on pollen beetle, both active ingredients proved not to be a good choice for the second spring application. For that reason, the widespread resistance of pollen beetles to pyrethroids is a more serious problem than usually perceived, as it complicates the possibility of controlling cabbage stem (rape) weevils too.

Several other alternatives to pyrethroids were suggested for use and applied against pollen beetles in (winter) oilseed rape in recent years: organophosphates (mainly chlorpyrifos-ethyl and chlorpyrifos-methyl), spinetoram and neonicotinoids (thiacloprid, acetamiprid). All of them show different modes of action than pyrethroids, thus they can easily be included in anti-resistance strategies (Philippou et al. 2011). From the group, only chlorpyrifos-ethyl was tested in this study and seemed to be the convenient choice for the second spring application according to the recorded levels of effectiveness. It markedly strengthened the effects of the first spray on the stem weevils and, at the same time, pollen beetles showed high susceptibility to the insecticide. All European populations of pollen beetle have shown susceptibility to chlorpyrifos-ethyl (Wegorek et al. 2009; Heimbach & Müller 2013; Seidenglanz et al. 2017). However, the insecticide (and all other organophosphates) was banned from usage in 2020. Organophosphates can probably disturb and destroy populations of natural enemies of oilseed rape insect pests through their long-term effects more than other insecticides (Jansen & Gomez 2014). Recently a large section of growers used various commercial formulations containing these insecticides (chlorpyrifos-ethyl or a similar ingredient, chlorpyrifos-methyl) for the first or the second spring applications into winter oilseed rape crops in the Czech Republic (Seidenglanz et al. 2018). A significant number of growers also used these insecticides repeatedly during the spring. After the ban of the group of insecticides, high portion of farmers expressed concerns about availability of some other effective control options for cabbage stem (rape) weevils and pollen beetle in crops. And the first seasons after the ban showed that the fears must be assessed with high seriousness (Seidenglanz et al. 2021a).

Neonicotinoids (acetamiprid, thiacloprid) or spinosyns (spinetoram) were not included in the trials, although they have been frequently mentioned among the suitable alternatives to pyrethroids and recommended for use in controlling pollen beetles (Thieme et al. 2008; Zimmer & Nauen 2011; Heimbach & Müller 2013; Brandes et al. 2018). It was decided not to include spinosyns (spinetoram) in the trials, because this group has not been registered for use in oilseed rape crops in the Czech Republic and in many other European countries at the time the trials were managed. Regarding the neonicotinoids, there were two main reasons why they were not included in the trials; the first was that they are regularly applied against pod midges, usually as a third spring application. After the ban of thiacloprid in 2020, control of pod midges in crops is fully dependant on acetamiprid applications. Its usage as a second spring application would pose the risk of overuse in crops and this is not desirable because neonicotinoids show relatively high negative effects on non-target organisms in oilseed rape crops. Even if it relates more to the banned thiacloprid than acetamiprid (Jansen & Gomez 2014).

The second reason: neonicotinoids are also threatened by resistance. Significant shifts in the pollen beetle's susceptibility to thiacloprid have been reported in Europe (Kaiser et al. 2018; Rubil et al. 2018). Some studies also demonstrated low effects of thiacloprid on stem weevils under field conditions (Milovac et al. 2017). All these reasons indicate the unsuitability of these insecticides (neonicotinoids, now represented only with acetamiprid) for use as a second spring spray in oilseed rape crops. Even if they were not tested in this study.

According to the results presented by Milovac et al. (2017), the first spring application targeted at stem weevils may not be efficient enough when another effective application does not follow. In their study, insecticides applied according to common recommendations (as first spring spray) exceeded the 50% level of effectiveness (expressed according to Abbott's formula) on the stem weevils only in some cases (bifenthrin, chlorpyrifos-ethyl + cypermethrin, alpha-cypermethrin) and not in all years. Some of the insecticides did not achieve the level of effectiveness in any season of their four-year study (tau-fluvalinate, thiacloprid). These findings coincide with the present results, although only one treatment representing the one-spray option (beta-cyfluthrin, tr. 11) was included.

Some studies (Junk et al. 2012; Eickermann et al. 2014) predict a more complicated timing of insecticidal sprays against some insect pests on brassicaceous host plants due to the shifts in their migration linked to climate change. According to Junk et al. (2012), for *C. pallidactylus*, a prolonged period of flight activity or crop invasion can be expected because of climate change. Eickermann et al. (2014) predicted the same for *C. napi*, the periods of crop invasion will start earlier but, in addition, the time spans of possible crop invasion will be prolonged, potentially making additional insecticide applications necessary. If soon such predictions are confirmed, the ban of organophosphates, the unsuitability of indoxacarb for second spring applications and continuing overuse of pyrethroids (regardless the fact of pollen beetle resistance and the first records of decreased susceptibility in cabbage stem weevils to the insecticides, Heimbach & Müller 2013; Seidenglanz et al. 2021b) will probably result in higher levels of damage induced by the stem weevils and in a rapid development of their resistance to pyrethroids. Additionally, crops showing higher damage induced by stem weevil larvae are more predisposed to fungal infections (especially *L. maculans* or *L. biglobosa*) (Broschewitz et al. 1993; Šedivý & Kocourek 1994; Krause et al. 2006; Jedryczka et al. 2010).

Problems with resistance coupled with a declining availability of new pesticide active ingredients due to both decreasing discovery rates and tighter legislation surrounding product approval (Jensen 2015) represent a real threat to the efficacy of pesticide use and, consequently, to the productivity of pesticide-reliant agricultural systems. Hence, in response to these issues, there is an urgent need to develop more sustainable integrated pest management strategies for crop protection. As Skellern and Cook (2018) stated, near-future research must aim at such factors in oilseed rape growing as crop rotation and crop design including the effect of crop diversity, sowing dates and growth stage profiles, plant density, weed management, usage of growth regulators, crop nutritional status, modifications in the insecticides regime and use varieties with higher levels of tolerance to (a)biotic stresses.

In the case of stem weevils and pollen beetles, not only new insecticides but (perhaps mainly) new approaches to their control are needed. There seems to be no advantage in separating the control of the insect pests. In the case of stem weevils, monitoring should be aimed rather at the females only and at the development of eggs in their ovaries to determine a precise time for spraying against them. Such approaches should make possible the well-founded delay of the first spray to the time when pollen beetles already occur in crops – at least in some years (Büchs 1998; Seidenglanz et al. 2009). It is possible that the first insecticidal application is often made too early in practice (Büchs 1998; Klukowski 2006; Seidenglanz et al. 2018) and the importance of the second insecticidal application is not correctly evaluated by many farmers and advisors because they do not consider the real lengths of the egg-laying period of cabbage stem weevils in many seasons.

CONCLUSION

The second spring application regularly targeted at pollen beetles is also important for decreasing the damage caused by *C. pallidactylus* (*C. napi*) larvae.

Insecticides commonly applied as a second spring spray to winter oilseed rape differ substantially in contribution to cabbage stem weevil (and rape stem weevil) control.

The fact of pollen beetle's resistance to pyrethroids and variability in susceptibility of *C. pallidactylus* to different types of insecticides complicate the choice of a suitable insecticide for the second spring application.

Organophosphates, represented by chlorpyrifos-ethyl in this study, proved to be an effective option when the application is intended both against pollen beetles and stem weevils. Presently, oilseed rape growers find themselves in complicated situation when the option is not available.

Despite the suitability of indoxacarb for the control of pollen beetles, it is less suitable for the second spring application because it shows a low contribution to increasing the effectiveness of the first spring insecticidal application on stem weevils.

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