



# The potential for reducing non-target effects on parasitoids of *Ceutorhynchus obstrictus* Marsham (Coleoptera: Curculionidae) through spatially targeted insecticide applications

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Received: 4 February 2022 / Accepted: 26 June 2022 / Published online: 21 July 2022  
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## Abstract

Insecticide applications against the cabbage seedpod weevil, *Ceutorhynchus obstrictus* Marsham (Coleoptera: Curculionidae), in oilseed rape are conducted during the flowering of the crop. During the flowering period, many parasitoids of pest species are abundant in the crop, and therefore, the preservation of natural pest regulation mechanism is especially important. Parasitism often has a substantial mortality factor in pest populations and as far as possible, parasitoids need to be protected from harmful non-target effects of insecticides. In the present study, the effects of three different insecticidal products on the parasitism of cabbage seedpod weevil larvae were investigated. Additionally, the effects of a spatial targeting of insecticides, using conventional and dropleg application technique, were compared. This was done in field trials within a completely randomized block design and four replications at one trial site in 2019 and two sites in 2020. The parasitoid species *Trichomalus perfectus* Walker and *Mesopolobus morys* Walker (both Hymenoptera: Pteromalidae) were abundant in almost equal shares in 2019, whereas *M. morys* was the most important species in 2020. Only in 2019 were harmful effects of the insecticide Biscaya (a.i. thiacloprid) on parasitism rates of *C. obstrictus* larvae observed. Clear benefits, due to spatial targeting of insecticides using the dropleg technique on parasitoids, were not found. The harmfulness of insecticides is probably affected by properties of different insecticidal products and the temporal coincidence of parasitoid occurrence and insecticide application.

**Keywords** Oilseed rape · Dropleg technique · *Trichomalus perfectus* · *Mesopolobus morys*

## Introduction

Oilseed rape (*Brassica napus* L.) (OSR) is one of the most important break crops in cereal-dominated arable systems in Europe (Angus et al. 2015). It is a mass flowering crop, with a long cropping period, that offers habitat and food resources to many insects including pollinators (Westphal et al. 2003; Scherber et al. 2019). However, OSR is also the host plant to a diverse complex of insect pests (Williams 2010), whose occurrence at high densities may demand for pest control with insecticides. During the flowering period of OSR, one of the major insect pest species

in OSR is the cabbage seedpod weevil, *Ceutorhynchus obstrictus* Marsham (Coleoptera: Curculionidae) (CSW). CSW migrates into the crop at the beginning of flowering and mates after a short period of maturation feeding. The larvae live individually in the pods of OSR, where they feed on the seeds (Williams 2010). While CSW has been spreading invasively for 80 years in the USA and Canada, and is a major pest insect in OSR production (Cárcamo et al. 2001), CSW is widely controlled by endemic antagonists in its area of origin in Europe (Haye et al. 2010; Veromann et al. 2010). In Europe, however, CSW is mostly feared as the precursor for brassica pod midge, *Dasineura brassicae* Winnertz (Diptera: Cecidomyiidae). The larvae of the midge also live in OSR pods and secrete enzymes, which cause the pods to split open prematurely, and additionally, grain weights are reduced (Williams 2010). The midge itself can only oviposit into young pods and therefore uses pod injuries, caused by the maturation feeding and oviposition of CSW, to spread more widely.

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The simultaneous occurrence of both insect species can result in significant yield losses up to 30% (Hoffmann and Schmutterer 1999), which demands the application of insecticides during flowering period, according to good agricultural practice. Since the direct control of *D. brassicae* is challenging (Hausmann 2021), the CSW population is monitored and controlled instead. The damage threshold for CSW in Germany is one weevil per main raceme at the flowering stage. This threshold is lowered to 0.5 weevils per main raceme, if the brassica pod midge was present in the past (Lauenstein 1993; Ramsden et al. 2017).

In the endemic range of CSW, natural antagonists are one of the predominant natural causes of CSW mortality (Gillespie et al. 2019). All life stages of CSW are parasitized (Williams 2003). Among natural antagonists, larval ecto-parasitoids play the greatest role and parasitism rates of larvae have been reported to exceed 50% (Murchie 1996; Nissen 1997; Ulber et al. 2010b; Veromann et al. 2010). The most important parasitoid species in Europe are *Trichomalus perfectus* (Walker) and *Mesopolobus morys* (Walker) (both Hymenoptera: Pteromalidae) (Ulber et al. 2010b; Haye et al. 2018). The parasitoids overwinter as adults and females migrate into the OSR fields 2–4 weeks after the weevil (Dmoch 1975; Haye et al. 2013). It is presumed that the females feed on its host larvae to complete their egg development (Murchie 1996; Williams 2003). Host larvae are immobilized prior to oviposition (Williams 2003). Both species occur at the same time and attack mainly third (occasional second) instars of CSW larvae (Dmoch 1975; Williams 2003). However, Haye et al. (2018) found evidence that climatic conditions define the ecological niches of *T. perfectus* and *M. morys*. But in the same field, studies on the vertical distribution of both species in the flowering crop have also revealed first evidence for spatial niche partitioning, as *M. morys* is mostly abundant in the horizon of open flowers, whereas *T. perfectus* is found in lower vegetation layers (Hausmann et al. 2021). For an integrated pest management, it is necessary to preserve natural antagonists from harmful side effects of insecticides, as far as possible, and spatial targeting of insecticides is one way to achieve this (Ulber et al. 2010a). The application of insecticides during flowering may disrupt natural pest regulation and can have non-target effects on CSW parasitoids (Murchie et al. 1997; Buntin 1998). Since Haye et al. (2010) calculated that larval parasitism caused 7–15% of the generational mortality in CSW populations in Switzerland, the reduction of parasitoid populations by insecticide application could consequently reduce the natural control effect. Such negative impacts of insecticides on biological pest control were well documented for cereal aphids (Krauss et al. 2011). However, aphids have a much shorter generation period and population growth can be exponential, compared to OSR insect pests which usually have only one generation per year.

In this study, we conducted field trials, testing for the effects of insecticides applied at full flowering stage of OSR on CSW and CWS parasitoids, using insecticides with different active ingredients differing in their effects on non-target organisms. To account for the spatial niches of CSW parasitoids, we applied insecticides using two different application techniques: 1) conventional, where the spray is directed from the top to bottom into the open flowers, and 2) dropleg, which spares out the horizon of open flowers and only the lower crop parts are sprayed (Hausmann et al. 2019). Using the dropleg application technique, residues of (also systemic) insecticides in nectar and pollen were reduced (Wallner 2014; Brandes 2021). Spatial targeting of insecticides, using droplegs in flowering OSR, can increase parasitism rates of pollen beetle larvae compared to conventional application (Hausmann et al. 2021). The objective of our study was, to investigate the effects of insecticide applications, application techniques on pod infestation by CSW larvae and on its parasitism rates, and on parasitoid species composition and the interaction between application technique and parasitoid species. We address the question whether the application technique or the mode of action of the insecticides is more suitable for avoiding undesirable side effects on the larval parasitoids of CSW.

## Materials and methods

### Design of the field trials

Field trials were conducted near to Braunschweig, Germany, on one field site in 2019 and two field sites in 2020, respectively (Table 1). Oilseed rape was grown in mulch tillage systems according to agricultural practice. All trials had a randomized complete block design with four blocks, each containing all the five treatments. On each trial site, treatments included an untreated control and two insecticide treatments, each applied with both conventional application technique and dropleg technique.

The insecticide Biscaya was applied in all trials, representing a neonicotinoid (IRAC class 4A) (Table 1). Additionally, in 2019 a second neonicotinoid Mospilan SL was used, which was replaced by the pyrethroid Mavrik Vita (IRAC class 3A) at both trial sites in 2020. The insecticides Biscaya and Mavrik Vita were chosen to represent different modes of action, while they also were registered as official products for the use against CSW in OSR in Germany. Mospilan SL was not approved for the control of CSW in OSR. All three insecticides are classified harmless to honey bees when used alone (BVL 2021). Mavrik Vita is also considered to be comparatively gentle on beneficial insects, e.g. pollen beetle parasitoids (Ulber et al. 2010a). Biscaya, on the other hand, can have harmful effects on

**Table 1** Field trials in 2019 and 2020 with locations, varieties, plant densities, application dates and plot sizes. Additional, the product names, active ingredients and application rates of the used insecticides are given

	2019	2020	2020
trial site	Wendhausen	Wendhausen	Groß Brunsrode
coordinates	52°19'36.03"N, 10°38'6.44"E	52°19'49.99"N, 10°37'48.18"E	52°21'14.62"N, 10°39'34.65"E
variety	DK Expansion	Avatar	DK Expansion
plant density per m <sup>2</sup>	60	30	50
date of trial application	30/04/2019	24/04/2020	24/04/2020
plot size (m <sup>2</sup> )	540	540	810
Used insecticides	Biscaya Mospilan SL	Biscaya Mavrik Vita	Biscaya Mavrik Vita
Company	Product name	Application rate and active ingredient	
Bayer Crop Science	Biscaya	72 g ha <sup>-1</sup> thiacloprid	
Cheminova Deutschland GmbH & Co. KG	Mospilan SL	42 g ha <sup>-1</sup> acetamiprid	
Adama Deutschland GmbH	Mavrik Vita	48 g ha <sup>-1</sup> tau-fluvalinate	

tersilochine parasitoids (Jansen and San Martin 2014, 2017; Hausmann et al. 2021). The applications were carried out at the full flowering stage of the OSR crop (BBCH 65–67), independent of infestation rates, with a driving speed of 7 km h<sup>-1</sup> and a pressure of 2.8 bar using water volumes of 300 l ha<sup>-1</sup>. The crop height varied between 1.20 and 1.30 m at BBCH 65. The conventional spraying was conducted with IDKN 120–4 nozzles held as usual 50 cm above the crop. The droplegs (Lechler 2020), equipped with double tongue nozzles FT 90°, were lowered 40–50 cm into the plant stand, so that the spray was beyond the horizon of open flowers, to account for the spatial distribution of CSW parasitoids.

## Sampling

To assess the abundance of cabbage seedpod weevils in the crop at the day of application, 25 plants per plot were each beaten into an individual bucket and the number of weevils per main raceme was counted. The pods for the examinations of CSW infestation rates and parasitism rates were taken from five randomly selected plants in the middle of each plot. In the year 2019, there were two sampling dates, June 16th (BBCH 80) and June 24th (BBCH 82). On the first date, five pods per main raceme and five pods of the third lateral raceme of each plant were collected. The number of pods per raceme was increased to 10 at the second sampling date. In 2020, pods were sampled on June 25th (BBCH 80/82). Due to the lower abundance of *C. obstrictus*, the number of pods per raceme was raised to 15 at the trial site Wendhausen and 20 at the trial site Groß Brunsrode, respectively. Pods that were obviously infested with larvae of the brassica pod midge were rejected.

## Assessment of parasitism rates

For each plot, the pods of the main racemes and the pods of the lateral racemes were pooled and stored in a plastic box (Bellaplast, 1275 cm<sup>3</sup>), which was enclosed by a perforated polyethylene bag (Fibrolux GmbH). The pods were placed in a climate chamber at 20° C (L:D 16:8) for at least 30 days. Parasitoids that emerged from the pods were collected and stored in 70% ethanol. The determination of the parasitoids at species level was carried out on a binocular with 60-fold magnification according to and Vidal (2003), Gibson et al. (2005), Baur et al. (2007) and Muller et al. (2007). Additionally, all pods were checked for exit holes of the weevils or the parasitoids, and then were opened and checked for any remains of weevil larvae or parasitoids. Only in 2020, exit holes were studied in detail to check whether they were from a weevil larva or a parasitoid (Fig. 1).

## Statistical analysis

For the calculation of the pod infestation by the cabbage seedpod weevil, the sum of exit holes and pods containing remains of seedpod weevil larvae or parasitoids (but no exit hole) was divided by the number of pods examined.

To calculate the parasitism rate of seedpod weevil larvae, for the 2019 data, the sum of parasitoids that emerged from the pods was divided by the sum of exit holes and pods with remains of seed weevil larvae. During the investigations in 2019, it was noticed that the exit holes of parasitoids can be distinguished from those of the CSW larvae. For this reason, in 2020, parasitism rate was calculated by dividing the sum of exit holes produced by a parasitic wasp, by the total sum of exit holes and the remains of seedpod weevil larvae. This



**Fig. 1** Exit hole of seedpod weevil larvae with frayed margins (left). Exit whole of a parasitoid with sharp margins (right)

allows us to include parasitoids, which may have already hatched before the plant sample was taken.

All statistical analyses were carried out with R (version 3.6.1 (R Core Team 2019)). As there were some changes in the trial implementation, the data were analysed year by year, using a generalized linear model (glm). The pod infestation by the cabbage seedpod weevil and the parasitism rate of its larvae were analysed with *treatment*, *raceme* (main raceme vs. third lateral raceme), *block* and in 2019 *sampling date*, respectively *field site* in 2020 as explanatory variables, assuming a binomial distribution of the data. The emergence of parasitoids from pods in the laboratory was analysed in the same way, however, assuming Poisson distribution. The full model (including all variables and interactions) was compared with simpler models and the model with the lowest AICc value was chosen as it indicates the best fit (Burnham and Anderson 2002). Count data models were checked for overdispersion. The residuals were plotted against the predicted values and the explanatory variables for model diagnostics. A post-hoc analysis was done by performing a Tukey test at an alpha level of 0.05 using the package *emmeans* (Lenth et al. 2018).

## Results

### Pod infestation by the cabbage seedpod weevil

The abundance of adult cabbage seedpod weevils per main raceme at full flowering (BBCH 65) was about 0.89 ( $\pm 0.21$ ) in the year 2019, 0.34 ( $\pm 0.15$ ) in Wendhausen 2020 and 0.18 ( $\pm 0.13$ ) in Groß Brunsrode 2020.

The average infestation of pods with CSW larvae over all treatments at BBCH 82, was 19.27% ( $\pm 1.14\%$ ) at the trial

site Wendhausen in 2019. The pod infestation with CSW was significantly lower after conventional spraying of Biscaya (15.4%,  $CI_{95\%}$  12.6–18.2%), compared to the conventional Mospilan treatment (22.6%,  $CI_{95\%}$  19.2–26.0%;  $p = 0.0122$ ) (Table 2). Dropleg spraying of Biscaya resulted in an infestation rate of 16.7% ( $CI_{95\%}$  13.7–19.8%) and differed neither to the control (17.82%,  $CI_{95\%}$  14.94–21.14%;  $p = 0.9870$ ), nor to the conventional application of Biscaya ( $p = 0.9708$ ). The infestation rate at the third lateral raceme (23.3%,  $CI_{95\%}$  21.2–25.7%) was significantly higher compared to the main raceme (14.3%,  $CI_{95\%}$  12.6–16.1%;  $p < 0.0001$ ) (Fig. 2). The interaction between treatment and raceme was not significant. However, it is noticeable that on the third lateral raceme all insecticide treatments tended to result in increased infestation rates compared to the untreated control.

In 2020, the average infestation of pods with cabbage seedpod weevil larvae was about 7.48% ( $\pm 0.80\%$ ) across all treatments in Wendhausen. In Groß Brunsrode, the infestation rate was significantly lower, about 5.30% ( $\pm 0.46\%$ ) ( $p = 0.0002$ ). Neither the treatment effect ( $p = 0.1871$ ) (Table 2) nor the effect of raceme ( $p = 0.2974$ ) were significant.

### Parasitism rates of weevil larvae

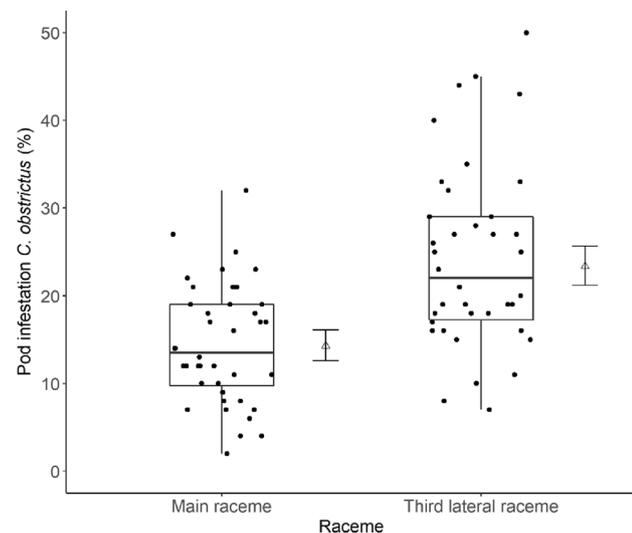
The average parasitisation rate in the untreated control was above 50%, at all trial sites in both years (Table 2). In 2019, the interaction between treatment and raceme were significant ( $p = 0.0106$ , Fig. 3a). The parasitism rate after conventional application of Biscaya, on the main raceme, was 23.2% ( $CI_{95\%}$  12.0–40.2%), which was significantly lower compared to the control (56.8%,  $CI_{95\%}$  43.6–69.2%,  $p = 0.0214$ ) and both Mospilan treatments (conventional Mospilan 69.4%,  $CI_{95\%}$  55.0–80.7%,  $p = 0.0009$ ; Dropleg

**Table 2** Pod infestation with *Ceutorhynchus obstrictus* larvae (%) and proportion of parasitized larvae (%) for different insecticides, applied with conventional and dropleg spraying techniques

Year	Location	Sampling dates, Growth stage	Treatment	N	Pod infestation		Parasitism	
					Observed $\pm$ se	Estimate means (95% CI)	Observed $\pm$ se	
2019	Wendhausen	June 16th and June 24th, BBCH 80–82	Control	300	18.13 $\pm$ 1.88	17.83 (CI 14.73–20.93)	ab	64 $\pm$ 8
			conventional Biscaya	300	16.62 $\pm$ 2.88	15.40 (CI 12.56–18.23)	a	38 $\pm$ 8
			conventional Mospilan	300	23.50 $\pm$ 2.37	22.59 (CI 19.16–26.02)	b	63 $\pm$ 6
			Dropleg Biscaya	300	17.27 $\pm$ 1.99	16.72 (CI 13.65–19.78)	ab	37 $\pm$ 8
			Dropleg Mospilan	300	20.62 $\pm$ 3.16	20.01 (CI 16.82–23.20)	ab	62 $\pm$ 7
2020	Wendhausen	June 25th, BBCH 80–82	Control	300	6.12 $\pm$ 1.01	6.09 (CI 4.79–7.73)	a	68 $\pm$ 12
			conventional Biscaya	300	10.38 $\pm$ 1.89	8.64 (CI 7.03–10.57)	a	85 $\pm$ 5
			conventional Mavrik	300	8.25 $\pm$ 2.74	6.92 (CI 5.51–8.65)	a	73 $\pm$ 9
			Dropleg Biscaya	300	5.62 $\pm$ 1.05	7.00 (CI 5.58–8.74)	a	62 $\pm$ 11
			Dropleg Mavrik	300	7.00 $\pm$ 1.55	7.33 (CI 5.87–9.11)	a	54 $\pm$ 14
2020	Groß Brunsrode	June 25th, BBCH 80–82	Control	400	4.62 $\pm$ 0.73	4.32 (CI 3.37–5.51)	a	50 $\pm$ 13
			Conventional Biscaya	400	5.38 $\pm$ 1.07	6.17 (CI 4.99–7.60)	a	42 $\pm$ 11
			Conventional Mavrik	400	4.38 $\pm$ 0.94	4.91 (CI 3.89–6.18)	a	60 $\pm$ 12
			Dropleg Biscaya	400	6.25 $\pm$ 1.00	4.97 (CI 3.94–6.25)	a	60 $\pm$ 10
			Dropleg Mavrik	400	5.88 $\pm$ 1.43	5.21 (CI 4.15–6.52)	a	51 $\pm$ 13

Observed data  $\pm$  se and estimated means + confidential intervals were obtained from the best model. Different letters indicate significant differences between treatments within year and trial site, Tukey-HSD ( $p < 0.05$ )

Mospilan 73.6%,  $CI_{95\%}$  59.0–84.4%,  $p = 0.0003$ ). The



**Fig. 2** Boxplots showing the median value (solid line), the 25th and 75th percentiles of pod infestation with larvae of *Ceutorhynchus obstrictus* (%) at BBCH 82 at the main raceme and the third lateral raceme, respectively in Wendhausen, 2019. The error bars below and above the box indicate the 10th and 90th percentile. Estimated means ( $\Delta$ ) and 95% confidence intervals obtained from the best model

dropleg application of Biscaya resulted in parasitism rates of 31.7% ( $CI_{95\%}$  19.0–47.9%), which was significantly reduced compared to the Mospilan treatments (conventional

Mospilan,  $p = 0.0065$ ; Dropleg Mospilan,  $p = 0.0021$ ), but not to the control ( $p = 0.1267$ ). At the third lateral raceme, the parasitism rate increased to 71.4% ( $CI_{95\%}$  57.6–82%) in the control. Compared to the control and irrespective of the application technique, Biscaya reduced the parasitism rate significantly (conv. Biscaya 37.7%,  $CI_{95\%}$  26.8–50.0%,  $p = 0.0039$ ; Dropleg Biscaya 44.9%,  $CI_{95\%}$  32.6–57.8,  $p = 0.0482$ ). As the parasitism rates of the Mospilan treatments tended to be above the control rate at the main raceme, it was the opposite at the third lateral raceme.

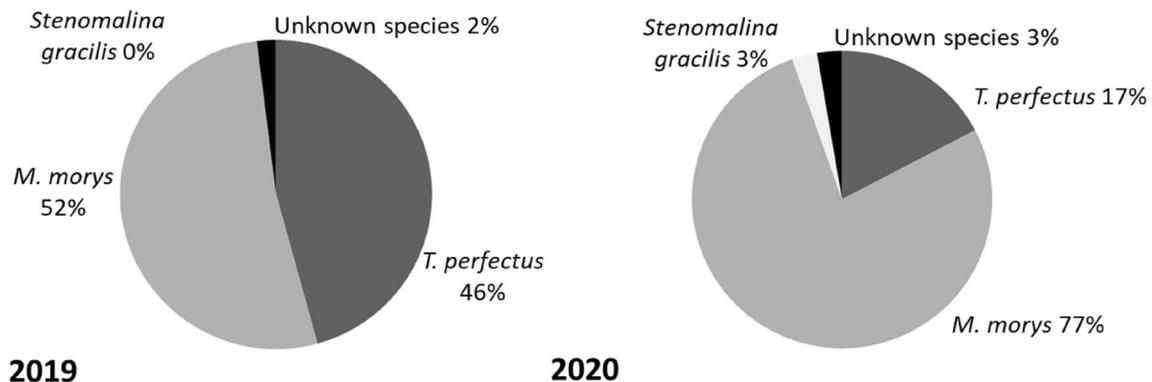
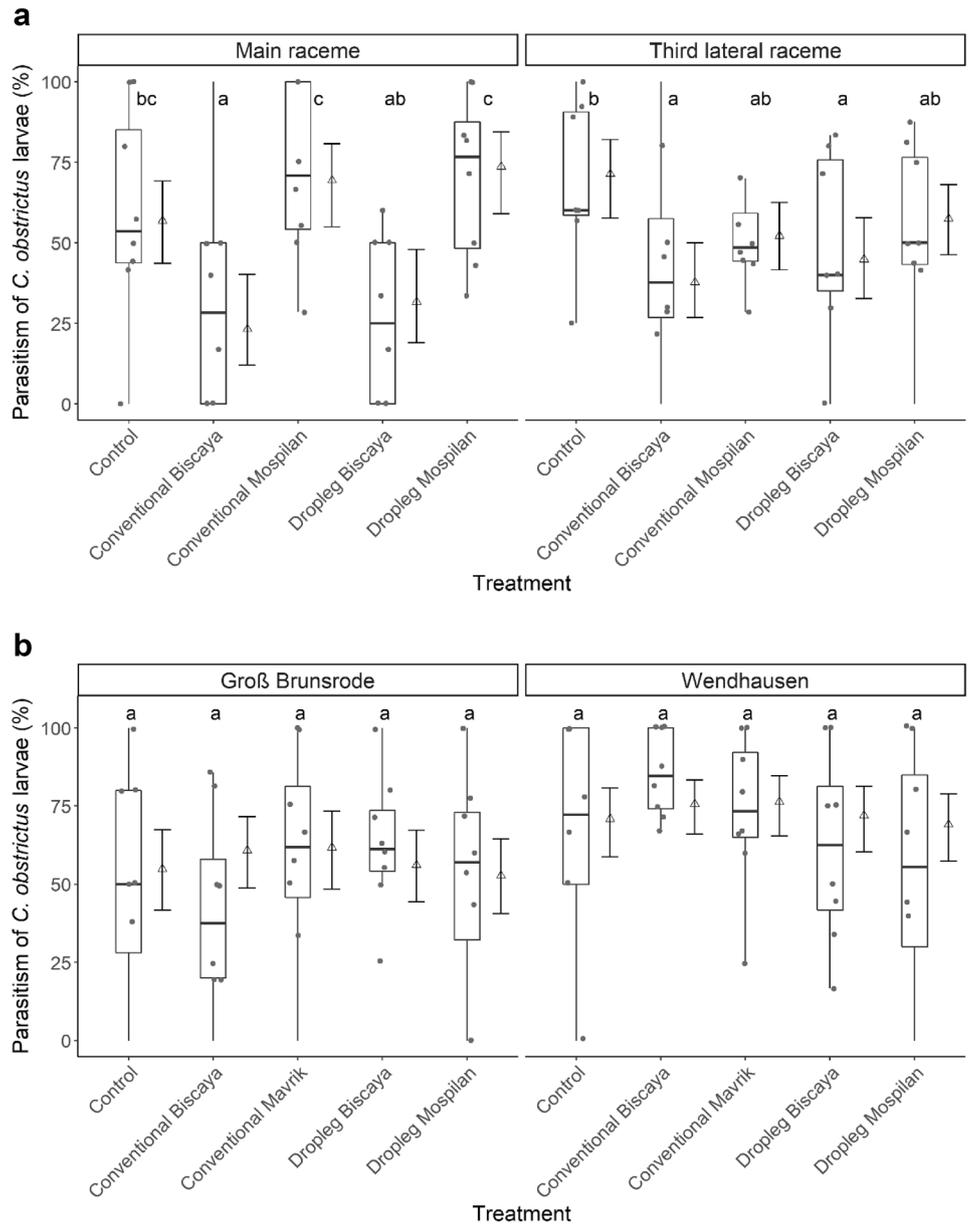
In the year 2020, the average parasitism rates differed significantly between the trial sites (Wendhausen 72.9%,  $CI_{95\%}$  66.3–78.6% and Groß Brunsrode 57.3%,  $CI_{95\%}$  50.2–64.1%;  $p = 0.0011$ ). The different treatments and the position of the pods, at the main raceme or third lateral raceme, did not affect parasitism rates (Fig. 3b).

### Effects of the insecticide applications on the different parasitoid species

In 2019, 3067 pods were collected, and 304 parasitoids emerged out of them in the laboratory. The prevalent species were *M. morys* (52%) and *T. perfectus* (46%) (Fig. 4). Six individuals could not be identified at species level, due to their preservation status.

The abundance of *C. obstrictus* was lower in 2020 and the proportion of damaged pods decreased from 19% in 2019 to 6% in 2020. The number of parasitoids decreased as well. However, 260 individuals from both trial sites,

**Fig. 3 a** Boxplots showing the median value (solid line), the 25th and 75th percentiles of parasitism rates of *Ceutorhynchus obstrictus* larvae (%) at BBCH 82 after different insecticide treatments in oilseed rape at full flowering (BBCH 65/67) in Wendhausen in 2019. The error bars below and above the box indicate the 10th and 90th percentile. Points show the mean parasitism over blocks and sampling dates. Estimated means ( $\Delta$ ) and 95% confidence intervals were obtained from the best model. Treatments with different letters are significantly different, Tukey-HSD ( $p < 0.05$ ). **b** Boxplots showing the median value (solid line), the 25th and 75th percentiles of parasitism rates of *Ceutorhynchus obstrictus* larvae (%) at BBCH 82 after different insecticide treatments in oilseed rape at full flowering (BBCH 65/67) in Groß Brunrode and Wendhausen in 2020. The error bars below and above the box indicate the 10th and 90th percentile. Points show the mean parasitism over blocks. Estimated means ( $\Delta$ ) and 95% confidence intervals were obtained from the best model. Treatments with different letters are significantly different, Tukey-HSD ( $p < 0.05$ )

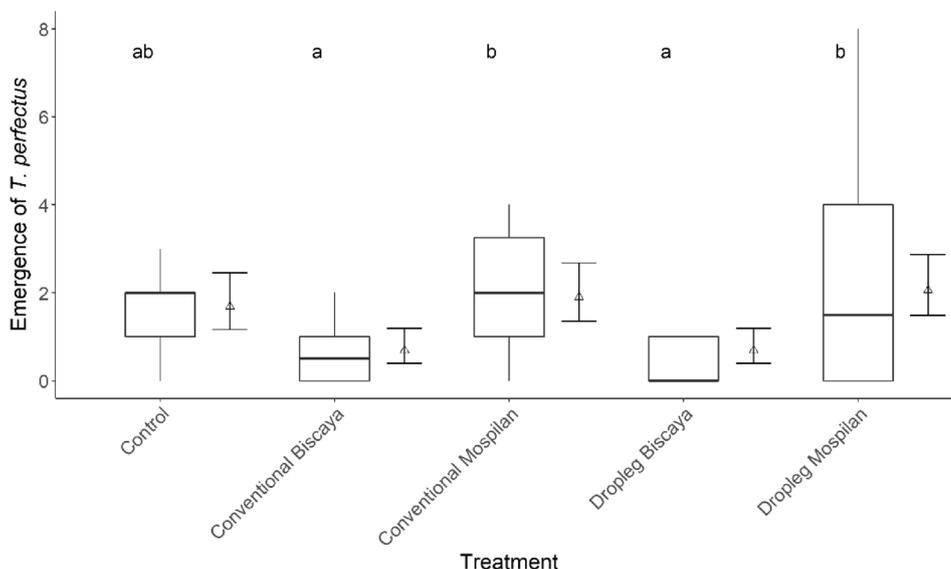


**Fig. 4** Species composition (%) of parasitoids (Pteromalidae) that emerged from oilseed rape pods collected at BBCH 82 (maturing of pods) at one trial site in 2019 and two sites in 2020

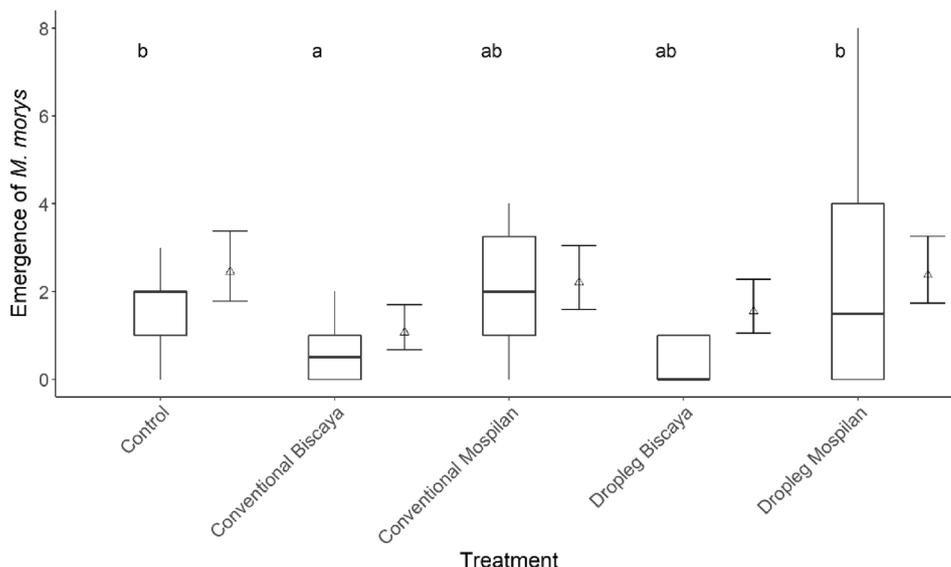
emerged from 7000 collected pods in the laboratory. It is noticeable that the proportion of *M. morys* in the year 2020 increased by 25%, compared to the previous year. Additionally, in this year seven species of *Stenomalina gracilis*, Walker (Hymenoptera: Pteromalidae) emerged from the pods. There was no difference in the species composition between both trial sites in 2020. The relative abundance of *M. morys* per plot was significantly higher in Wendhausen (2.18, CI<sub>95%</sub> 1.76–2.70,  $p=0.0050$ ) compared to Groß Brunsrode (1.39, CI<sub>95%</sub> 1.07–1.80). Also, the relative abundance of *M. morys* per plot was higher at the third lateral raceme of the OSR plant (2.30, CI<sub>95%</sub> 0.87–2.83), compared to the main raceme (1.32, CI<sub>95%</sub> 1.01–1.72,  $p=0.0006$ ).

In 2019, there were significant differences between insecticide treatments. The average number of *T. perfectus* (Fig. 5) that emerged per plot and sampling date was 0.69 (CI<sub>95%</sub> 0.40–1.19) in the conventional Biscaya treatment. This was significantly lower, compared to both Mospilan treatments (conventional Mospilan 1.90, CI<sub>95%</sub> 1.35–2.68,  $p=0.0142$  and Dropleg Mospilan 2.06, CI<sub>95%</sub> 1.48–2.87,  $p=0.0055$ ), but not to the control (1.68, CI<sub>95%</sub> 1.16–2.45,  $p=0.0532$ ). Also, the application of Biscaya with dropleg technique (0.69, CI<sub>95%</sub> 0.40–1.19) resulted in significantly reduced numbers of *T. perfectus*, compared to conventional Mospilan ( $p=0.0142$ ) and Dropleg Mospilan ( $p=0.0054$ ). For the second parasitoid species *M. morys*, a similar trend was observed in 2019 (Fig. 6). The conventional application of Biscaya decreased the number of emerged parasitoids

**Fig. 5** Boxplots showing the median value (solid line), the 25th and 75th percentiles of *Trichomalus perfectus* emergence from oilseed rape pods collected at BBCH 82 after different insecticide treatments during flowering (BBCH 65). The error bars below and above the box indicate the 10th and 90th percentile. Different letters indicate significant differences between treatments, Tukey-HSD ( $p < 0.05$ )



**Fig. 6** Boxplots showing the median value (solid line), the 25th and 75th percentiles of *Mesopolobus morys* emergence from oilseed rape pods collected at BBCH 82 after different insecticide treatments during flowering (BBCH 65). The error bars below and above the box indicate the 10th and 90th percentile. Different letters indicate significant differences between treatments, Tukey-HSD ( $p < 0.05$ )



per plot and sampling date significantly to 1.07 (CI<sub>95%</sub> 0.67–1.70), compared to the control (2.45, CI<sub>95%</sub> 1.78–3.38,  $p=0.0301$ ) and the Dropleg Mospilan treatment (2.38, CI<sub>95%</sub> 1.74–3.26,  $p=0.0393$ ). None of the parasitoid species was affected by the insecticide treatments in 2020.

## Discussion

It is a basic understanding of integrated pest management that pesticides are applied only after pest monitoring and the usage of damage thresholds (Barzman et al. 2015). In this study, the respective threshold for CSW was only exceeded in the year 2019. Regarding the effects of the applied insecticides on the pod infestation with CSW larvae, the conventional application of Biscaya resulted in significantly lower infestation rates, compared to the conventional application of Mospilan in 2019. However, in both years, insecticide treatments did not reduce pod infestation rates compared to the untreated control. Assuming that the active ingredients are potentially effective, further immigration into the insecticide-treated plots must therefore have occurred after application. To circumvent this shortcoming, future investigations should focus on the abundance of CSW adults and parasitoids in the days after application.

The insecticidal treatments affected parasitism rates of seedpod weevil larvae, similar to the effects on pod infestation with CSW larvae, only in 2019. Insecticides differ in their non-target effects due to the properties of the active ingredients (Sánchez-Bayo 2012) and its formulations (Pereira et al. 2009). All active ingredients used have in common that they act as contact and feeding poisons. While Biscaya (a.i. thiacloprid) and Mospilan (a.i. acetamiprid) can distribute systemically in the plant, Mavrik Vita (a.i. tau-fluvalinate) does not translocate and acts only locally. Since CSW feed and reproduce on the generative plant organs, a better effect would have been expected from conventional application, since here flowers and young pods are sprayed with active ingredients. Instead, no difference was found with respect to the application technique. This may be because the weevils fall from the plants to protect themselves from predators, when they sense vibrations or sudden shading. They are then exposed to the active ingredients even after dropleg application. Another reason could be the systemic translocation of the active ingredients in the plants, whereby effects against pest insects and non-targets in the flower and pod canopy could also occur after dropleg application. However, no general conclusions can be drawn from the results of our field trials, because despite the systemic properties of Biscaya and Mospilan, effects on CSW and its parasitism rates were observed only after application of Biscaya. Going more detailed into chemical structures, thiacloprid belongs to the five-membered neonicotinoids, whereas

acetamiprid belongs to the non-cyclic neonicotinoids (Schäfer 2008; Jeschke and Nauen 2019). The chemical structures influence their physical properties, e.g. acetamiprid penetrates the cuticle of insects poorly, but is translaminar and acropetally active. Thiacloprid is very stable on the plant surface and is translocated acropetally (Jeschke and Nauen 2019). However, chemical structure also shapes biochemical properties and determine the intrinsic toxicity of the active ingredients to groups of organisms. Therefore, the application of different insecticidal products in the field can result in completely different non-target effects (Jansen and San Martin 2014, 2017). In this study, only the application of Biscaya significantly reduced parasitism rates of CSW larvae in 2019. However, neither Mospilan nor Mavrik Vita had significant effects on CSW larval parasitism. This is in accordance with Jansen and San Martin (2017), who found that the application of Biscaya soon before flowering of OSR significantly reduced adult parasitoids of the Tersilochinae sub-family, while Mavrik Vita showed no effects. However, Jansen and San Martin (2017) observed no harmful effects on Pteromalidae from any product.

Besides its toxicity, the exposure of an organism with the respective active ingredients is another important aspect. In their study, Murchie et al. (1997) found that an insecticide (a.i. triazophos) applied after flowering, during pod maturation, directly killed adults of *T. perfectus* and CSW larvae. Instead, an insecticide (a.i. alpha-cypermethrin) applied at flowering stage of OSR, against the adults of CSW, had no adverse effects on parasitism rates, which the authors trace back to the missing temporal coincidence between the application and the abundance of the parasitoids (Murchie et al. 1997). The aspect of temporal coincidence is discussed in the following. CSW migrates into the crop when temperatures exceed 16 °C, at the beginning of flowering (Williams 2010). Before females start with oviposition, there is a period of maturation feeding that lasts at least two weeks (Kirchner 1961; McCaffrey et al. 1991). For oviposition, young or smaller pods seem to be preferred (Williams and Free 1978; Dossdall and Moisey 2004). As OSR first flowers at the main raceme (bottom to top) and lateral racemes start flowering gradually (Tayo and Morgan 1975), there is a little delay in formation and ripening of pods, comparing the main raceme and the third lateral raceme. In the present study, we found significantly increased CSW infestation at the third lateral raceme, compared to the main raceme in 2019. This indicates that the main period of egg-laying was after full flowering in 2019, and effects of the applied insecticides did not last long enough to cover that period. Accordingly, second and third instar larvae, which are suitable for parasitism, should not be present before pods have reached their final size (> BBCH 71) (Dossdall and Moisey 2004). The parasitoid species *T. perfectus* migrates into the OSR fields when temperatures exceed 17 °C, however, main flight only starts

when mean temperature is above 14 °C and maximum temperature reaches 23 °C (Johnen et al. 2010). In most years, this is 2–4 weeks after the weevils have colonized the OSR fields (Dmoch 1975; Haye et al. 2018). In conclusion, if insecticides are applied at full flowering stage, direct effects on the parasitoids of CSW seem unlikely, because often there is no temporal coincidence with the parasitoid emergence in the crop. This was probably also the case in 2020.

The main larval parasitoids of CSW, in our trials, were the parasitoid wasps *T. perfectus* and *M. morys*. While they were abundant in almost equal proportions in 2019, *M. morys* was the predominant species in the following year. Previous studies found *T. perfectus* to be the dominant parasitoid of CSW in northern Germany (Ulber and Vidal 1998; Haye et al. 2018). In comparison, *M. morys* is better adapted to warm, continental climates and is more frequent in southern Germany, Switzerland and Austria (Haye et al. 2018). The weather around Braunschweig was warmer and drier than average in the period from May 2018 to July 2019, which might have favoured *M. morys* during the two years period. As this species was mainly found in the upper crop layers in a previous study (Hausmann et al. 2021), we assumed, in advance, that *M. morys* could benefit from spatial targeted insecticide sprays using dropleg technique. Results show that the emergence of *M. morys* in the Dropleg Biscaya treatment was not statistically different from any other treatment, while after conventional application of Biscaya, significantly less parasitoids emerged compared to the control and the Dropleg Mospilan treatment in 2019. Instead, the application technique had no influence on the emergence of *T. perfectus*. Besides direct effects, which seem unlikely as discussed before, parasitoids can be affected by sub-lethal insecticide concentrations, e.g. due to contact or ingestion of lower concentrations of insecticide residues (Desneux et al. 2007). There are major gaps in our knowledge regarding the biology of the individual parasitoid species and their feeding behaviour. Still, it is likely that CSW parasitoids seek for food and its hosts in the upper crop canopy, with open flowers and pods. It is presumed that females of *T. perfectus* feed on their hosts, however, this has not been directly observed so far (Williams 2003). Host feeding was indicated in some studies, because up to 50% of CSW larvae found within the pods were dead (Murchie 1996; Ulber and Vidal 1998). However, this was hardly observed in the present study. Whether CSW parasitoids additionally take up nectar or pollen for energy supply remains unclear. Ultimately, the results from only one trial do not allow a clear statement on whether *M. morys* benefits from lower pesticide residue levels in the upper crop canopy, after applications using dropleg technique.

Our study design does not allow conclusions to be drawn as to whether the observed effects on CSW larvae parasitism was due to direct mortality, sub-lethal or indirect effects

such as reduced host densities in treated plots. In a previous study, the exposure of artificially induced host densities of CSW larvae to the natural parasitoid community revealed no evidence of host density-dependent parasitism by *T. perfectus* (Ulber and Vidal 1998). Female parasitoids locate their host larvae in a short range, perceiving stimuli with their antennae while walking along the pods (Dmoch 1998). Oviposition by the parasitoid is later elicited by the frass of third instar CSW larvae (Williams 2003). This points out that the females have to check all pods individually for the presence of host larvae, which makes host density-dependent effects less likely. On the other hand, Buntin (1998) reported partly contradictory effects in U.S. fields, by observing that pod infestation by CSW larvae and also absolute numbers of the parasitoid *T. perfectus* (later identified as *T. lucidus* by Gibson (2005)) were significantly reduced after insecticide treatments in flowering OSR. However, parasitism rates in insecticide-treated plots were increased compared to the untreated control (Buntin 1998). Compared to European conditions, overall parasitism rates found by Buntin (1998) were rather low and did not exceed 7%. This suggests that parasitism in North America tends to be parasitoid-limited, probably because CSW parasitoids, like their host, are endemic and more abundant in Europe (Gibson et al. 2005). In the present study, larval parasitism rates ranged between 50 and 68% in the untreated control in both years. This is in accordance with recent studies (Laborius 1972; Dmoch 1975; Murchie 1996; Haye et al. 2010; Veromann et al. 2010). Such high parasitism rates suggest that parasitoids possibly have a substantial effect on pest population dynamics (Hawkins and Cornell 1994; Hokkanen 2008; Thies et al. 2008; Gillespie et al. 2019).

In the present study, we found minor effects of insecticides applied in flowering OSR on parasitism rates of CSW larvae. The present results indicate that spatial targeting of insecticides did not affect non-target effects on larval parasitoids of CSW. In general, it can be assumed that CSW larvae parasitism as a valuable ecosystem service, is rather influenced by more general conditions such as climate (Haye et al. 2018) or landscape composition (Kovács et al. 2019) rather than by insecticide treatments during flowering.

**Acknowledgements** We would like to thank HORSCH Maschinen GmbH for the support during the conversion of a field sprayer with dropleg technique. Also, many thanks to our colleagues Christoph Joachim and Max Paluch, for their useful comments and discussions about our manuscript.

**Funding** Open Access funding enabled and organized by Projekt DEAL. No funding was received for conducting this study.

**Declaration**

**Competing interests** The authors have no competing interests to declare that are relevant to the content of this article.

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