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M. Sc. Thesis

Insecticide application into flowering oilseed rape (*Brassica napus* L.) by using dropleg technique – Impact on blossom insect pests and the pollen beetle (*Brassicogethes aeneus* (Fabr.)) parasitoid *Tersilochus heterocerus* (Thoms.)

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Abstract

The dropleg technique is an innovative method for a safer pesticide application at flowering stage of oilseed rape that may in future replace the conventional technique of applying pesticides directly onto the flowers. The advantageous feature of the dropleg technique is that the spray nozzles are hanging below the flowering canopy. Thus, the flowering part is omitted whereby harmful effects of pesticides on beneficial insects such as honey bees or parasitoids feeding on nectar can be avoided since a much lower concentration of pesticides in nectar and pollen of oilseed rape was verified.

The aim of this study was to evaluate the efficacy of the dropleg technique compared to the conventional application technique in terms of controlling important insect pest at flowering stage of oilseed rape. Examined blossom insect pests were the seedpod weevil (*Ceutorhynchus obstrictus* Marsham), the brassica pod midge (*Dasineura brassicae* Winnertz) and the pollen beetle (*Brassicogethes aeneus* Fabricius). Additionally, a focus was placed on the effects of both techniques on the abundance and the efficacy of the pollen beetle parasitoid *Tersilochus heterocerus* (Thoms.). It was hypothesized that this parasitic wasp also benefits from lower insecticide residues in the nectar of treated oilseed rape using the dropleg technique.

The experiment was conducted in 2017 in a randomized complete block design with plots of 12 x 24 m in four replications in Wendhausen, nearby Braunschweig, Germany. The insecticides Biscaya (72 g ha^{-1} thiacloprid) and Mospilan SG (40 g ha^{-1} acetamiprid) were applied, both using the conventional and the new dropleg technique at flowering stage of oilseed rape (BBCH 65-67). Six water trays were placed on the ground of each plot to measure both infestation of adult insect pests and larvae dropping down for pupation. Once a week, the trays were emptied and insect pests were identified and counted. On two dates (BBCH 75 and 80) a pod assessment was carried out to determine pod infestation by brassica pod midge. Furthermore, two emergence traps were placed in each plot to calculate the size of the new generation of insect pests. To evaluate the effect of insecticide application on the natural abundance of parasitoids, parasitic wasps were captured with a D-vac suction sampler. Moreover, pollen beetle larvae were dissected to determine the parasitism level within the different treatments.

In the present field trial pest pressure was below the economic application thresholds and there was no significant effect of insecticide treatment found on the yield. However, the observed efficacy of Biscaya applied using the dropleg technique was comparable to the efficacy of the conventional technique regarding the reduction of brassica pod midge larvae, whereas Mospilan showed almost no effect at all. The dropleg technique exhibited slightly weaker effects on pod infestation by brassica pod midge at first date of pod assessment. Nevertheless, positive effects of the dropleg technique on the abundance and the efficacy *T. heterocerus* were revealed, indicating that the dropleg technique promotes the conservation of naturally-occuring biocontrol agents.

1. Introduction

Following cereals and maize, oilseed rape (OSR) (*Brassica napus* L.) is one of the most important arable crops in Germany (DESTATIS 2018). In 2017, OSR was cultivated on an area of 1.3 million hectare and as 78.9 million liters are consumed by private households, rapeseed oil is the most popular unsaturated edible oil (STATISTA 2018). Besides its use for culinary purposes, OSR seeds are processed to biofuel and lubricant (ALFORD 2003). Due to its structure composed of high-quality lipid acids it is not only valued in human diet but byproducts are also fed to life stock. OSR further plays an important role as break crop in crop rotations (WILLIAMS 2010).

Besides infestation by severe fungal diseases such as the economically important white mold (*Sclerotinia sclerotiorum* (Lib.) de Bary) (BOLTON et al. 2006) and phoma stem canker (*Leptosphaeria maculans* (Desm.) Ces. et de Not) (FITT et al. 2005), many insect pests are attracted to OSR wherefore crop protection measures, i.e. insecticide application, are frequently required (ALFORD 2003). The six major insect pests of OSR are the pollen beetle (*Brassicogethes aeneus* Fabr.) (Coleoptera: Nitidulidae), the brassica pod midge (*Dasineura brassicae* Winnertz) (Diptera: Decidomyiidae), the cabbage seed pod weevil (*Ceutorhynchus obstrictus* Marsham) (Coleoptera: Curculionidae), the cabbage stem weevil (*Ceutorhynchus pallidactylus* Marsham.) (Coleoptera: Curculionidae), the rape stem weevil (*Ceutorhynchus napi* Gyllenhal) (Coleoptera: Curculionidae) and the cabbage stem flea beetle (*Psylliodes chrysocephala* L.) (Coleoptera: Chrysomelidae) (WILLIAMS 2010a). At the flowering stage of OSR insecticide application is aimed at the cabbage seed pod weevil and the brassica pod midge. In North America and Canada, *C. obstrictus* is a serious pest (DOSDALL & MASON 2010) while in Germany, the brassica pod midge is said to be more important (HOFFMANN & SCHMUTTERER 1999). However, since the infestation of *D. brassicae* is linked to the occurrence of *C. obstrictus*, control of cabbage seed pod weevil infestation prevents seed weight losses up to 82 % possible due to *D. brassicae* infestation (WILLIAMS 2010).

Flowering OSR attracts not only insect pests but also various important pollinators such as the Western honey bee (*Apis mellifera* L.) and solitary bees. Therefore, the need to apply pesticides against important insect pests co-occurs with honey bees foraging in the blossoms of OSR fields. Especially the detection of insecticide residues in honey causes an atmosphere of tension between farmers, beekeepers and the general population. The maximum residue level for the neonicotinoid thiacloprid in honey is 0.05 mg kg^{-1} and the exceedance of this residue levels causes sale inability (BFR 2016).
Insecticides belonging to the class of neonicotinods are nowadays the worldwide most widely used insecticides against sucking and biting insect pests and therefore at the center of criticism concerning the worldwide increasing bee mortality (SANCHEZ-BAYO &

GOKA 2014). Due to their systemic mode of action, neonicotinoids are transported through the plant to protect all parts of the entire crop. Their selective target site is the nicotinic acetylcholine receptor of insects wherefore they possess a relatively low risk for non-target organisms such as vertebrates. When binding to their target site in the insect central nervous system they block the receptors, causing paralysis and death (KUNDOO 2018; JESCHKE 2011).

In recent years many studies are focused on the impact of pesticides on pollinators, primarily the honey bee. Even though bee toxicity of neonicotinoids is controversially discussed (YANG et al. 2008; POHORECKA 2012; ROLKE ET AL. 2016), insecticide application in general is less and less accepted by the society which is furthermore triggered through cheap propaganda (GREENPEACE 2014).

The most bee-toxic neonicotinoids are the nitro-substituted compounds such as clothianidin, dinotefuran, imidacloprid and its metabolites, thiamethoxam and nitenpyram ((DECOURTYE & DEVILLERS 2010). In 2018, the European Commission sat a final ban on clothianidin, imidacloprid and thiamethoxam (EUROPEAN COMMISSION 2018). The neonicotinoids acetamiprid and thiacloprid are still on the market. Both substances belong to the cyano-substituted neonicotinoids that are considered as less toxic to bees (DECOURTYE & DEVILLERS 2010). In consequence of the non-existing cross-resistance in neonicotioids with other insecticide classes they play a key role in pyrethroid resistance management (KUNDOO 2018).

Besides the importance to protect the honey bee as an important pollen vector, the impact of pesticides on naturally-occurring biocontrol agents arises more and more awareness (LONGLEY et al. 1996; NEUMANN et al. 2010; HANSON et al. 2015).

More than 80 different species of parasitic wasps were identified to attack at least one of the six major insect pests of OSR and out of these different species a number of twelve are determined as key parasitoids. These egg or larval parasitoids belong particularly to the braconid, chalcid or ichneumonid wasps of the Hymenopteran order (ULBER et al. 2010). They are common across Europe both in winter and spring rape with slight variations. Overall, parasitism levels between 20 % and 50 % were measured, sometimes exceeding 80 % (WILLIAMS 2010). Parasitism levels of pollen beetle larvae of already 30-40 % significantly reduce the size of the new beetle generation (HOKKANEN 2008).

The most frequent key parasitoid species of *B. aeneus* are *Tersilochus heterocerus* (Thoms.), *Phradis interstitialis* (Walker) and *Phradis morionellus* (Walker) (Hymenoptera: Ichneumonidae). The main activity period of these parasitoids within the OSR field is between the late bud stage and the end of flowering (ULBER et al. 2010).

For the sake of a safer and more eco-friendly pesticide application into flowering OSR, in this study the efficacy of the innovative dropleg technique is examined which may in future replace the traditional application technique of directly applying pesticides onto the blossoms of OSR. This technique was originally invented by the company Kuhn for pesticide application onto potatoes. The company Lechler further developed the technique for the use in other crops such as OSR (RÜEGG & TOTAL 2003). The dropleg technique is characterized by the advantageous feature of spray nozzles that are hanging on flexible tubes approx. 40 cm into the crop canopy below the flowering part (HABERLAH-KORR 2016). This leads to omitting of the blossoms since the spray is directed to the ground whereby harmful effects of pesticides on beneficial insects foraging in the flowering part may be avoided. In a study conducted by WALLNER et al. (2014) it was verified that no pesticide residues were measurable in honey from OSR fields treated with insecticides using the dropleg technique. However, systemic pesticides such as thiacloprid are still detectable in pollen but the concentrations of residues are much lower compared to conventional insecticide application. Thefore, a weaker or even no effect on honey bees and thus parasitoids compared to the conventional insecticide application technique can be assumed. Besides greatly decreased residue concentration in pollen and nectar, drift threat at least within the crop is decreased as well (WALLNER 2014).

In 2015 a study was conducted to test the efficacy of the dropleg technique against *S. sclerotiorum*. Since fungicides against white mold are also applied during flowering to prevent the spread of the fungal disease, it was investigated whether the application of fungicides below the flowering part directly onto the stems would enhance crop protection against *Sclerotinia*. It was observed that the dropleg technique was on the same level as the conventional application technique regarding the reduction of white mold infestation (HABERLAH-KORR 2016; DICKE 2018).

The JKI in Braunschweig compared the efficacy of different insecticides applied using the dropleg and the conventional application technique in field experiments against important blossom insect pests. Results revealed a slightly reduced efficacy of the dropleg compared to the traditional application technique. However, the insect pest pressure in the experimental years was too low to come to a clear conclusion (GÖDEKE 2017; HAUSMANN 2018).

The present field trial within a master's thesis was also conducted in cooperation with the JKI to verify if insecticide application using the dropleg technique ensures the same efficacy against important insect pests as the conventional technique. The insecticides Biscaya (72 g ha^{-1} thiacloprid) and Mospilan SG (40 g ha^{-1} acetamiprid) were applied, both using the conventional and the new dropleg technique at flowering stage of oilseed rape (BBCH 65-67). A main focus was additionally set on the capability of the dropleg technique to provide conservation of natural-occurring biocontrol agents such as parasitic wasps. Considering that, special attention was paid to the pollen beetle parasitoid *T. heterocerus* which was the predominant parasitoid species at the experimental site.

Due to the widely distributed pyrethroid resistance in *B. aeneus* populations throughout Europe (HEIMBACH & MÜLLER 2013), the parasitoid's capability of reducing insect pest abundance is highly promising in terms of resistance management and incorporating biocontrol strategies. In the present study it is investigated whether insecticide application using the dropleg technique provides a beneficial impact on the abundance and the efficacy of *T. heterocerus* compared to conventionally treated field plots.

1 Methodology

1.1 Study site

As testing site a field of the JKI in Wendhausen northeast of Braunschweig was selected with winter barley and one adjacent field with oilseed rape (OSR) as previous crop. The test ground was a heavy clay ground with a rating of 45 points from the German ground inventory ("Reichsbodenschätzung"). The Ap-horizon was a clayey loam (tL). On the 24th of August 2016 the variety Visby was sown in a mulch sowing procedure with 70 seeds m^{-2}. Additionally, Metarex slug pellets were spread. One day after sowing an application of the herbicide Butisan Gold was carried out. On the 19th and 30th of September the graminicide Select240 + Radimax + Targa Super was spread and Phfix5 + Karate Zeon added on the 30th of September against the cabbage stem flea beetle (*P. chrysococephala*). Approximately two months later (24th of November) another herbicide application of Kerb Flo was performed. Prior to flowering an application of the insecticide Trebon EC 30 (0.20 l ha^{-1}) was carried out against rape stem weevils (*C. napi*) on the 20th of March after application threshold was exceeded. Further, another insecticide application of Avaunt (0.17 l ha^{-1}) and Trebon (0.2 l ha^{-1}) was conducted against the pollen beetle (*B. aeneus*) and the stem weevil on the 31st of March 2017. The actual important insecticide application regarding the present investigation took place on the 15th of May 2017.

1.2 Experimental design

The field trial was conducted in a completely randomized block design. The homogeneity of the test field was evaluated by means of a drone before the experimental plots sizing 12 x 25 m were set up. Five treatments were performed with four replications concentrated in field dose (**Table 1**). To prevent infestation with white mold (*S. sclerotiorum*) all plots were treated with a fungicide.

Table 1: Treatment plan of the present field trial in Wendhausen in 2017. All treatments were spread in field dose and combined with the fungicide Propulse (125 g ha^{-1} fluopyram und 125 g ha^{-1} prothioconazol) to control *Sclerotinia sclerotiorum*. The control was also treated with Propulse.

treatment number	application	active ingredient (a.i.)	field dose	abbreviation
1	Control	-	-	C
2	Biscaya conventional	thiacloprid	72 g ha^{-1}	CB
3	Mospilan SG conventional	acetamiprid	40 g ha^{-1}	CM
4	Biscaya dropleg technique	thiacloprid	72 g ha^{-1}	DB
5	Mospilan dropleg technique	acetamiprid	40 g ha^{-1}	DM

In order to compare the efficacy of the innovative dropleg with the conventional application technique, and the two different insecticides Biscaya (a. i. thiacloprid) and Mospilan SG (a. i. acetamiprid) with each other, the insecticides were applied each with the two application methods. The experimental field was arranged as four blocks equivalent to four repetitions adding to a total of 20 plots.

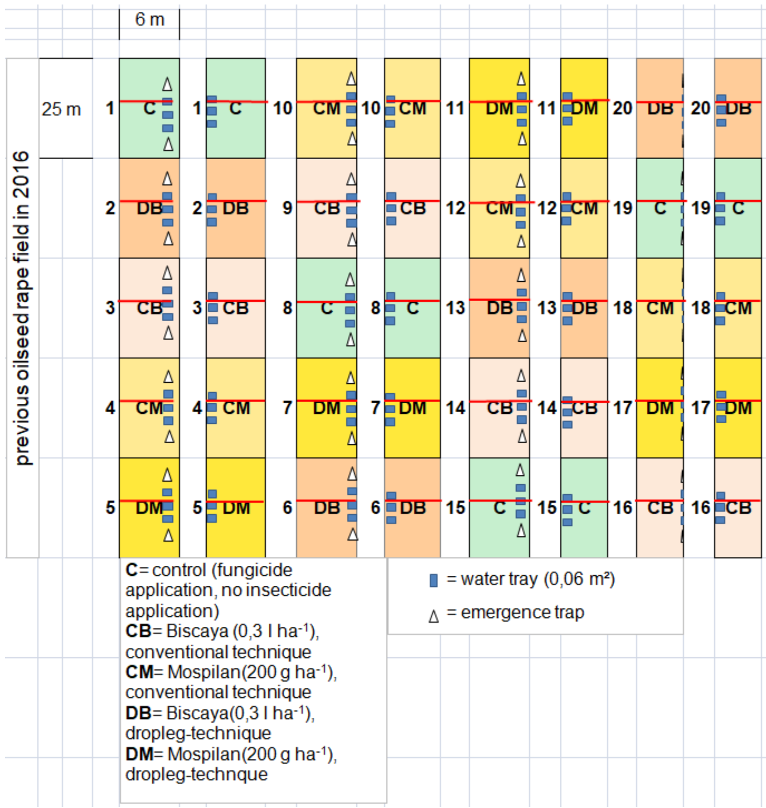

Figure 1: Experimental design for the field trial conducted in a randomized block design; plot descriptions; red lines mark cross lines within the plots; water trays and emergence traps are visualized.

Figure 1 shows the classification within the randomized block design. The plots had the size of 300 m^2 and were divided into four quarters by a vertical driving lane and a horizontal crossroad which was created with a non-selective herbicide. The driving lane was later mulched to simplify human mobility while taking samples. From the crossroads water trays the size of 10 x 60 cm were placed on the ground below the crop canopy. It is important to keep the crossroads clear of vegetation; otherwise, spotting the water trays is impaired.

1.3 Experimental application procedure

On the 15th of May the application took place during 11 and 12:30 am on a sunny and calm day when the OSR plants were in full flowering stage (BBCH 65). The temperature in the beginning was 15°C and rose to 19°C in the afternoon. During the night before it was raining wherefore the vegetation was humid until noon. The dose rate needed for Biscaya was 72 g thiacloprid ha^{-1} and for Mospilan 40 g acetamiprid ha^{-1}. The insecticides were applied with 300 l water ha^{-1} at a driving speed of 7 km h^{-1}. All insecticides were mixed with the fungicide Propulse (125 g fluopyram ha^{-1} and 125 g prothioconazol ha^{-1}) effective against white mold (*Sclerotinia sclerotiorum*). The control plots were treated only with the fungicide and the headland was left untreated.

Mospilan and Biscaya were applied with a conventionally used sprayer of the type "Rau D2" (12 m working width and tank capacity of 1000 l) and IDKN 120-4 nozzles (7 km h^{-1} at 2.0 bar). The sprayer was thoroughly rinsed before every refilling. The spray nozzles were kept 50 cm above the inflorescence. The application using the dropleg technique was performed with Lechler twin spray cab and tongue nozzles out of brass which were installed on 0.9 m long droplegs. Both nozzles joined created a spraying cone of 180°, which was directed horizontally to the side and to the ground. During insecticide application (7 km h^{-1} 3.6 bar) nozzles were kept below the flowering part, 40 cm deep into the vegetation (Figure 2).

Figure 2: Droplegs with spray nozzles hanging approx. 40 cm below the crop canopy of a flowering OSR field (HAUSMANN 2017).

1.4 Weather conditions

Figure 3 illustrates the monthly average temperature in 2017 and the aggregated amount of the monthly precipitation during the sampling period from April to July. The weather conditions in 2017 during the field trial were reasonably balanced in the months May to August whereas April was characterized by fluctuations in temperature and relatively little precipitation (29 mm). In April the weather conditions were cool and dry. Approximately at the 20th of April a strong late frost event occurred when the temperature decreased to -5 °C. In the mid of May daily temperature increased and reached an average of 17 °C in June and 18 °C in July. Precipitation in June (119 mm) and July (152 mm) was reasonably regular so water availability was guaranteed all the time. Additionally, there was heavy rainfall on the 19th of May four days after application.

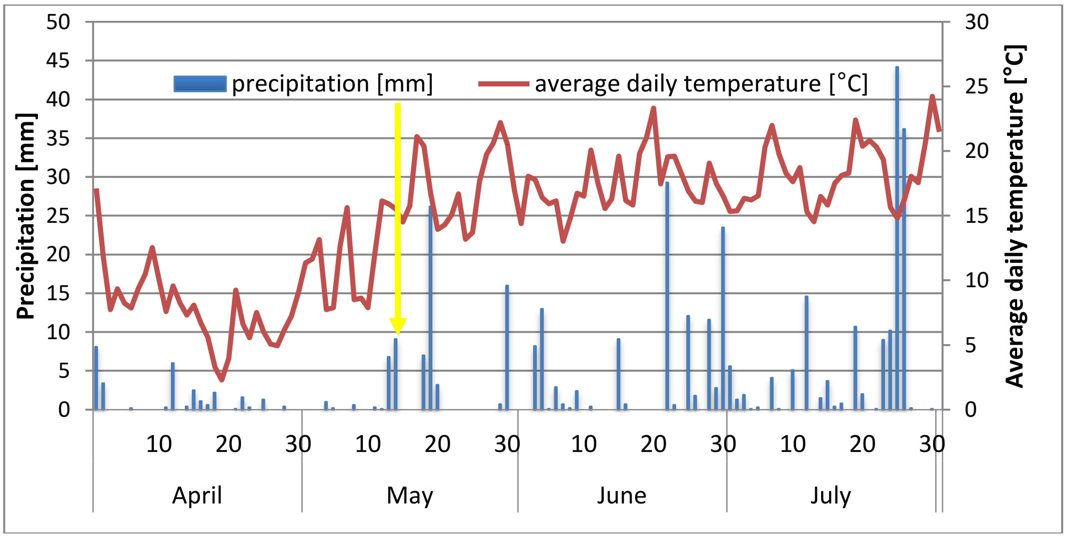

Figure 3: Weather progress during the present experiment 2017 in Wendhausen. Precipitation in mm and average daily temperature in °C are visualized. Yellow arrow indicates the application date (15th of May 2017).

1.5 Assessment of insect pest infestation

In order to estimate the efficacy of the dropleg technique regarding crop protection against insect pests of OSR, the incidence of the major insect pests was determined. In all treatments a tap monitoring, water trays, a stem and pod assessment and emergence traps were used to compare the efficacy of the two insecticides Biscaya and Mospilan and the two application techniques.

1.5.1 Tap monitoring

The flowering part of OSR is the habitat for feeding and oviposition of the cabbage seedpod weevil (*C. obstrictus)* and the pollen beetle (*B. aeneus)* (WILLIAMS 2010). For measuring the occurrence of these two insect pests a tap monitoring was conducted one day before and one day after insecticide application. In each plot, the main shoot and the first two side branches of 50 randomly picked plants were tapped into yellow traps (22.5 x 30 cm) and abundance of the two species was calculated. Only the left part of the plots was checked to not negatively influence the emergence traps on the right side of the plots.

1.5.2 Water trays

Three days before insecticide application six water trays the size of 10 x 60 cm were placed from the cross lanes at the ground directly under the plant canopy of every plot to collect all adult and larval stages of the insect pests dropping down. All water trays were carefully slid under the crop canopy after being filled up to one third with a solution of water, sodium benzoate (1:10) and detergent. The trays were covered with a wire mesh to prevent petals from falling into the solution. Three trays were placed in each case to the left and to the right side of the driving lanes. Evaluation took place one day before insecticide application, five hours after application, then one and three days after application, continuing with a weekly analysis until harvest. For analysis under the stereo microscope three trays of each side were merged to one sample (0.18 m^2). Thus, there were two technical replications in each plot that were averaged. In the laboratory, the benzoate-solution was discarded through a sieve to only keep the collected insects. The sample examination included counting of the adult and larval stages of *D. brassicae*, *B. aeneus*, *C. obstrictus*, *C. pallidactylus*, *C. napi*, *P. chrysocephala*.

1.5.3 Stem infestation assessment

In an attempt to calculate the incidence of *C. napi* and *C. pallidactylus*, a stem infestation assessment was conducted at the 1st of June when the OSR was at BBCH 75. Twelve plants per plot (three plants taken from each of the four quarters) were randomly taken and analyzed in the laboratory. Both main and side stems were sliced to look for mining larvae or feeding damage of *C. pallidactylus* and *C. napi*. Due to the marginal occurrence of these insect pests, the stem infestation was only carried out once.

1.5.4 Pod infestation assessment

On the 1st and 21st of June (BBCH 75) a pod infestation assessment was performed to estimate the infestation with *D. brassicae* and *C. obstrictus*. Data were gathered from twelve plants per plot (three plants taken from each of the four quarters). One way to avoid sampling pods from disturbed plants was to make sure that samples were only taken from the plot's inside. Then, all pods of each plant were counted and visually checked for symptoms of *D. brassicae* such as brownish and swollen pods and then opened to check for actual infestation with larvae. Additionally, pods were examined for emergence holes of *C. obstrictus* larvae leaving to pupate.

1.5.5 Emergence traps

For the sake of the evaluation of the insecticide's efficacy, emergence traps were used to measure the size of the new insect pest generation (Figure 4). At the beginning of the vegetation period, base rings of two emergence traps (0.25 m^2; EchoTech) were inserted into the right part of every plot. On the 14th of June at BBCH 75/78 all OSR stems were bended to keep them inside the base rings and close the emergence traps. Fabric tents were used to cover the whole base ring of the emergence traps. The following day perforated plastic bags were attached to the openings at the top of the emergence tents and properly fixed with elastic bands. Thus, newly emerged insects were caught in these plastic bags after they moved to the tent's opening, attracted by light. Twice a week sampling was carried out whereby plastic bags were exchanged to identify and count trapped insects in the laboratory. On the day of harvest (19th of July) insect emergence was monitored one last time. Then, tents were removed but base rings kept for investigating plant density. Furthermore, stem diameter was determined.

Figure 4: Emergence trap; base rings of 0.25 m^{-2} with fabric tents and openings on top closed with a plastic container. In the present experiment plastic bags were used instead of containers to trap emerging insect pests.

1.6 Systemic action of neonicotinoids after application using dropleg technique

To answer the question whether thicacloprid is detectable in the flowering part after application using the dropleg technique, plant samples were collected from all treatments of the present field trial one, three and seven days after application. Thus, the top 20 cm of the main shoot of one plant from each plot was cut and merged with plants from the same treatment, so there was a sample of four replicates for each treatment and sampling date. Samples were frozen and sent to the Institute for Bee protection (JKI Berlin) for analysis. Each sample was examined both for residues of acetamiprid and thiacloprid.

1.7 Impact of the insecticides and the application techniques on parasitoids

Besides testing the efficacy of the two insecticides and the two application techniques regarding crop protection against the important insect pests of OSR, a main purpose of this study was to investigate whether or not the dropleg technique has a beneficial effect on natural antagonists of the targeted insect pests. Therefore, a focus was laid on the importance of parasitoids of the pollen beetle and the possibility of the dropleg technique to positively influence the parasitic wasps by insecticide application below the flowering part

1.7.1 Parasitoid sampling

In order to determine the biodiversity of parasitoids occurring at the present test site and to understand the impact of insecticide application, a modified leaf blower (D-vac leaf blower) was utilized for sampling parasitoids directly from the flowering canopy.

Therefore, linen bags were arranged inwards at the suction hole of the leaf blower so light objects such as insects are absorbed into the linen bag when the force of the leaf blower is set on absorbing. The leaf blower was hold into the flowering part of the plot's left side to not interrupt the emergence traps on the right side of the plots, and then it was horizontally slewed through the field at normal walking speed. At the end of each plot, linen bags were changed and immediately tied up so flying insects such as parasitoids are prevented from escaping. Samples are always taken by the same person one day before, five hours, three, seven and nine days after. The bags with the collected insects were frozen overnight and afterwards all hymenopteran wasps were sighted under a stereo microscope, carefully separated from petals with tweezers and stored in 70 % ethanol before the late identification took place. Insect pest species were also counted. One way to simplify the accurate identification of chalcid wasps is to store them dry and not in ethanol since it was difficult to determine the species due to fading colors after storing in ethanol.

1.7.2 Identification of parasitoids

Parasitic wasps collected with a D-vac leave blower during suction sampling were identified under a stereo microscope. Parasitoids were identified by means of "Identification of hymenopterous parasitoids associated with oilseed rape pests" (VIDAL 2003). Due to the visible black eggs within multiple pollen beetle larvae (OSBORNE 1960), it was foreseeable that *Tersilochus heterocerus* (Thoms.) (Hymenoptera: Ichneumonnidae) was the predominant parasitoid of *B. aeneus*. In the following, morphological characteristics are presented to differentiate T. *heterocerus* and *P. interstitilis* which both parasitize pollen beetle larvae (WILLIAMS 2010a). For further explanation see VIDAL 2003.

Figure 5: Female imago *Tersilochus heterocerus* (Thoms.) with 15-17 antennal segments (own source)

Figure 6: Close up of the 2-m-cu vein leaving after the junction of veins forming the aerolet of the parasitic wasp *Tersilochus heterocerus* (Thons.) (own source)

Figure 7: Close up of the toothed and incised ovipositor tip of *Tersilochus heterocerus* (Thoms.) (own source)

Figure 8: Close up of the not incised ovipositor tip of *Phradis interstitialis* (Thoms.) (own source)

1.7.3 Determination of the parasitism level

To solve the question about the impact of insecticide and application technique on the natural occurrence and the possible efficiency of parasitoids as natural control agents of insect pests, the parasitism level of *B. aeneus* was determined within all treatments. During analysis of water tray samples black eggs (black, oval dots) of the hymenopteran wasp *T. heterocerus* were already visible in the majority of the *B. aeneus* larvae (
Figure 9). All larvae of *B. aeneus* kept from water trays were stored in 70 % ethanol and later dissected to check the actual amount of parasitoid eggs within the insect pest larvae. In detail, all *B. aeneus* larvae are fixed with needles lying on their back on a petri dish filled with hardened wax. After covering the petri dish with water and detergent to let them not get brittle, the larval abdomen was gently sliced with a thin needle and the inside was dissected to find and count all parasitoid eggs and calculate the parasitism and superparasitism level (BRANDES et al. 2017).

Figure 9: L2 larva of *Brassicogethes aeneus* (Fabr.) with visible black egg of T*ersilochus heterocerus* (Thoms.) (own source)

Figure 10: L2 larva *of Brassicogethes aeneus* (Fabr.) and a prepared egg of *Tersilochus heterocerus* (Thoms.) (own source)

1.8 Harvest

Harvest of the present field trial took place on the 19th of July 2017. A plot harvester (Haldrup C 85) took two samples à 40 m^2 per plot via core threshing lengthwise through the plot halves. For later yield analysis only samples of the plot's left part were analyzed since the plot harvester had to drive around the emergence traps that were placed in the plot's right side. The plot harvester's technology calculated yield and grain moisture and thousand kernel weights were determined using a seed grain analyzer (Marvin). Further, plant density m^{-2} at four parts of the plots was determined.

1.9 Statistics

The statistic analysis of the assessed data was conducted by means of the software SAS (software package 9.2, SAS Institute, Cary, NC, USA). For modeling the response-variables of the insect pests a logistic regression was performed by means of general linear model (GLM). The basic model was based on a completely randomized block design with five different treatments and four blocks. The application techniques and the insecticides were set as independent variables. Further, block effects and sampling dates were considered. For all counting values (number of dropped larvae and adults, number of Tersilochine and newly emerged beetles) a poisson distribution was presumed. For all other values (percentage of stems, pods, parasitism and super parasitism) a binomial distribution was assumed.

The assessed infestation levels were individually calculated depending on the insect pest and parasitism level was calculated depending on the sampling date.

Means of the assessed infestation levels were compared to the control with the LSMEANS procedure. To investigate the differences between the insecticides and the application techniques, a multiple comparison was conducted. When significances were found, a post-hoc test (tukey test) was run ($\alpha = 0.05$).

2 Results

2.1 Tap monitoring

Infestation of OSR by insect pests was low at the present field site in 2017. On average, 0.4 adult pollen beetles were found on one OSR plant one hour before insecticide application took place. None of the treatments seemed to have an effect on the number of *B. aeneus* since there were no differences between the treatments and the untreated control after a tap monitoring one day after insecticide application was conducted.

The cabbage seedpod weevil also occurred only in small numbers at the present field site. On average, 0.1 *C. obstrictus* was found on one OSR plant. A direct effect of insecticide application on cabbage seedpod weevils was only seen in the conventional Biscaya treatment one day after application (appendix). There, infestation of OSR by *C. obstrictus* was reduced by 90 % (0.01 beetle found on one plant). Anyhow, the threshold of one adult *C. obstrictus* per plant (HOFFMANN & SCHMUTTERER 1999) was not exceeded wherefore insecticide application was not necessary.

2.2 Effects of the insecticide treatments on adult beetles and larval dropping

To investigate the efficacy of the insecticides and the application techniques, water trays were used to measure the amount of dropped adult beetles and larvae within the different treatments.

2.2.1 *Ceutorhynchus obstrictus*

The occurrence of the cabbage seedpod weevil at the present field site was moderate in 2017. Compared to the control insecticide treatment did not show an effect on adult cabbage seedpod weevils dropping down. On average, 28 beetles m^{-2} dropped down over the season (appendix).

The first larvae were found on the 6th of June (22 days after application) (Figure 11). Within the following three weeks larval dropping increased and had its peak around 35 DAA (19th of June). The highest number of larvae was found in the conventional Mospilan treatment (29 larvae m^{-2}) followed by the control (23 larvae m^{-2}), the Bi-Drop (22 larvae m^{-2}) and the Mo-Drop treatment (21 larvae m^{-2}). Only the peak of the conventional Biscaya treatment occurred one week later (24 larvae m^{-2}). Dropping of *C. obstrictus* larvae decreased after the peak and on the last sampling date (17th of June) almost no larvae were found.

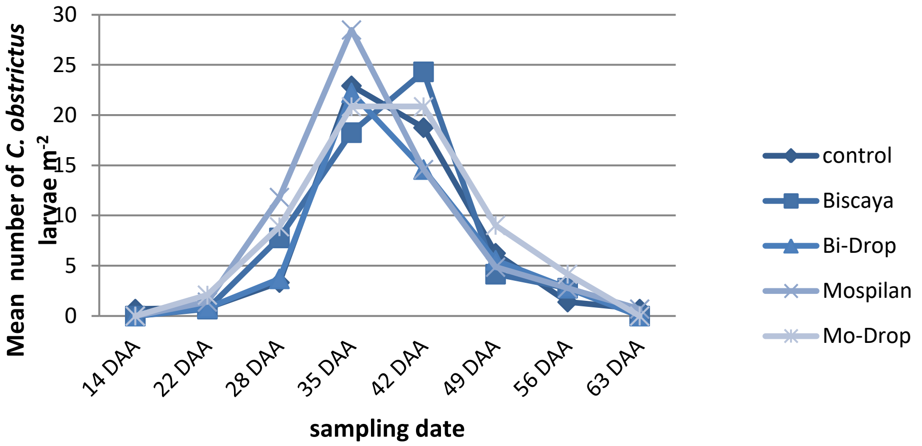

Figure 11: Mean number of *C. obstrictus* larvae m^{-2} dropped down for pupation within the different treatments on the specific days after application (DAA).

Figure 12 presents the cumulated mean number of larvae m^{-2} dropped down for pupation over all sampling dates (14-63 days after application). The control exhibited a number of 55 ± 17 larvae m^{-2}. The lowest number was found in the Bi-Drop treatment (50 ±11 larvae m^{-2}) and the highest numbers in the conventional Mospilan (65 ± 15 larvae m^{-2}) and the Mo-Drop (66 ±13 larvae m^{-2}) treatment. No differences between the insecticides or the application techniques were detected.

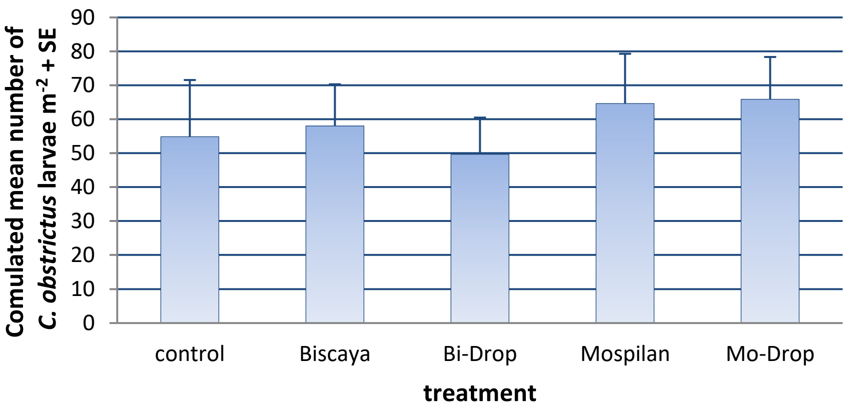

Figure 12: Cumulated mean number of *C. obstrictus* larvae m^{-2} dropped down for pupation within the different treatments from 14 until 63 days after application. Data marked with * are significantly different to the control ($p < 0.05$) + SE and letters mark significant differences between the treatments ($p < 0.05$).

2.2.2 *Dasineura brassicae*

At the present field site brassica pod midges had two generations in winter OSR. The dropping down of the first generation larvae for pupation occurred between the 29th of May and 12th of June with a peak on the 6th of June (22 days after application) (Figure 13). Only in the conventional Biscaya treatment the peak of larval dropping was one week delayed (35 days after application). The peak of larval dropping of the second generation was on the 3rd of July. Afterwards larval dropping came to a halt. At the first peak a clear difference between the efficacies of the two insecticides used to control *D. brassicae* can be seen. While the number of dropped larvae in both Mospilan treatments was similar to the control (1649 larvae m^{-2}), both Biscaya treatments exhibited a considerably lower number of dropped larvae m^{-2}. In the Bi-Drop 634 larvae m^{-2} and in the conventional Biscaya treatment 433 larvae m^{2} dropped to the ground for pupation. In comparison to the control both Biscaya applications decreased the number of dropped larvae more than twice, whereby the conventional application technique exhibited 200 fewer larvae than the dropleg treatment. Nevertheless, one week later the number of dropped larvae in the conventional Biscaya treatment strongly increased up to 1079 larvae m^{-2} and exceeded the other treatment's means. During the peak of the second generation the conventional Biscaya treatment had almost the same mean number as the control (1204 larvae m^{-2}) whereas the Bi-Drop and both Mospilan treatments had a 1.5 times lower number of dropped larvae m^{-2}.

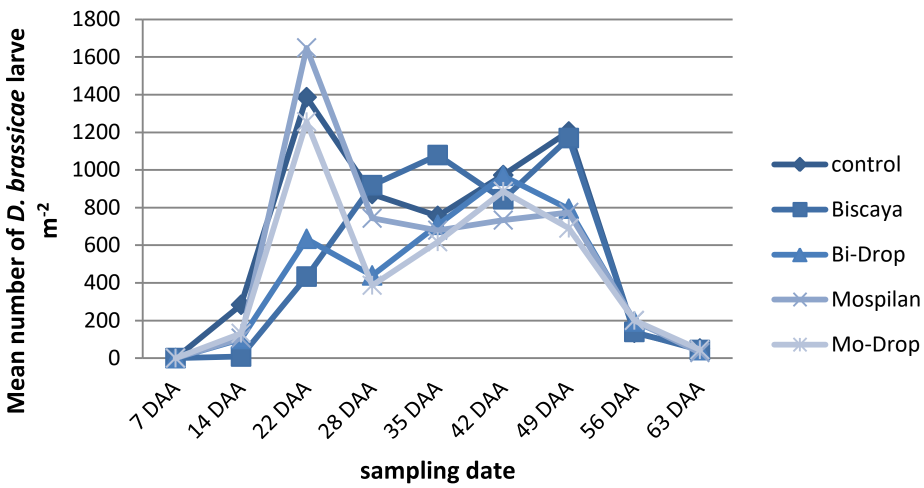

Figure 13: Mean number of *D. brassicae* larvae m^{-2} dropped down for pupation within the different treatments on the specific days after application (DAA).

In Figure 14 the cumulated mean number of dropped larvae m^{-2} over all sampling dates is illustrated. All treatments reduced the number of dropped larvae m^{-2} by at least 13 %. Moreover, the dropleg technique achieved slightly better results compared to the conventional technique. While the number of dropped larvae in the control treatments was 5659 ± 1197 larvae m^{-2}, the Bi-Drop treatment exhibited an average of 3882 ± 722 larvae m^{-2}. However, differences between the insecticides or the application techniques were not proven significant.

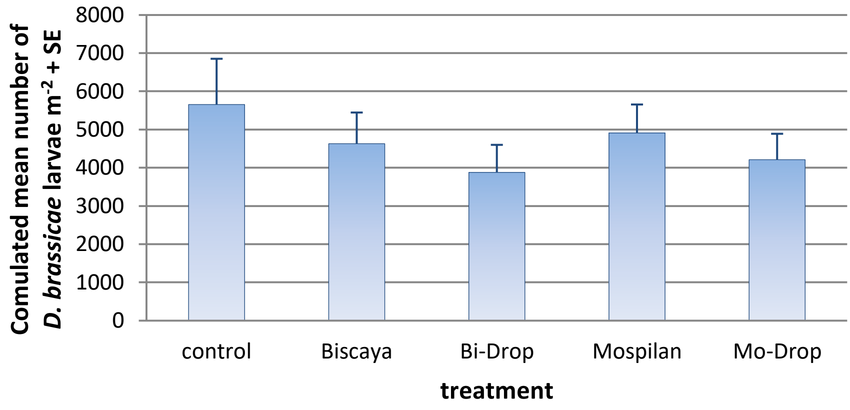

Figure 14: Cumulated mean number of *D. brassicae* larvae m^{-2} dropped down for pupation within the different treatments from 7 until 63 days after application. Data marked with * are significantly different to the control ($p < 0.05$) + SE and letters mark significant differences between the treatments ($p < 0.05$).

2.2.3 *Brassicogethes aeneus*

The direct lethal effect of the insecticides on adult pollen beetles can be seen in Figure 15. The number of *B. aeneus* adults dropped down for pupation in the insecticide treatments greatly increased starting five hours after application and lasted until three days after application. While one adult m^{-2} was found in the control three days after application, the highest number of 13 adults m^{-2} was detected in the conventional Biscaya followed by the conventional Mospilan treatment with 10 adults m^{-2}. The dropleg technique had a slightly weaker effect on adult *B. aeneus* (8 adults m^{-2} in the Mo-Drop and 7 adults m^{-2} in the Bi-Drop treatment). One week after application dropping of adult *B. aeneus* due to toxicity of insecticides decreased whereas the highest number was found in the control (3 adults m^{-2}) at this time point.

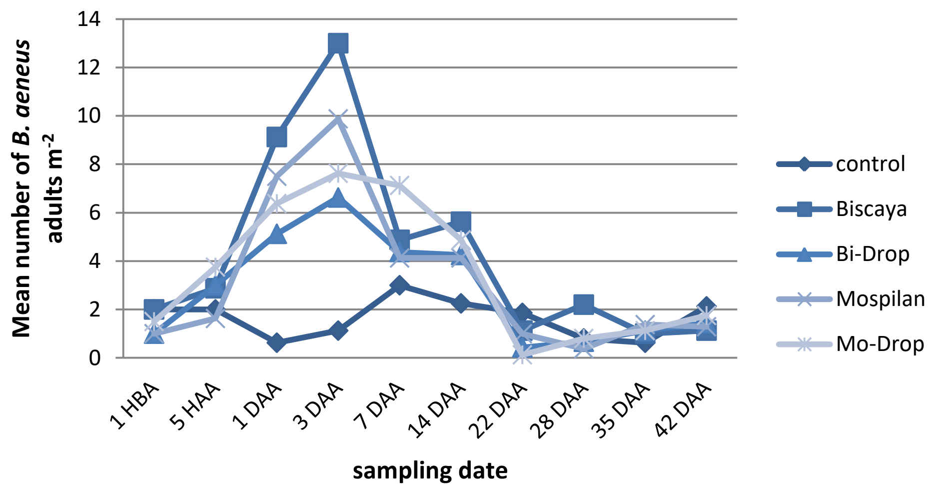

Figure 15: Mean number of adult *B. aeneus* m^{-2} dropped down within the different treatments on the specific days after application (DAA)

The cumulated mean number of adult *B. aeneus* dropped down for pupation over all sampling dates (one hour before until 42 days after application) is shown in Figure 16. No significant differences between the insecticides or the application techniques were detected. Besides the Bi-Drop treatment, all other treatments significantly increased the number of adult pollen beetles dropped down compared to the control. A number of 80 ± 25 adults m^{-2} was found in the control and 228 ±45 adults m^{-2} in the conventional Biscaya treatment which exhibited the highest number of dropped *B. aeneus* adults. The number was 2.9 times higher compared to the control. All other treatments increased the number of dropped pollen beetles at least 1.9 times.

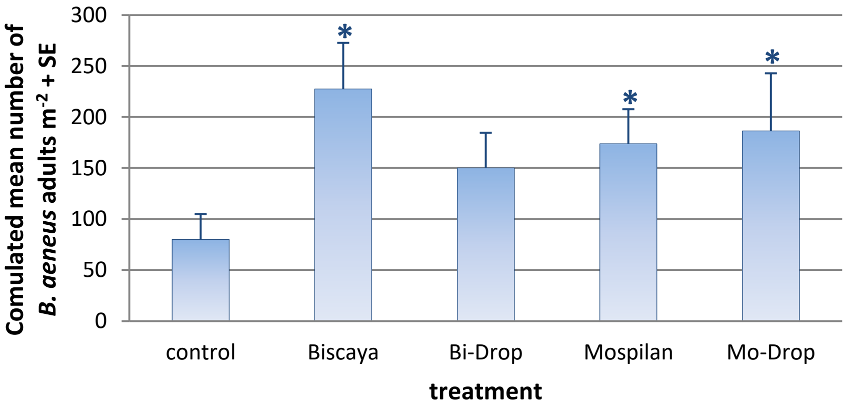

Figure 16: Cumulated mean number of dropped *B. aeneus* adults m^{-2} dropped down within the different treatments from 14 until 63 days after application. Data marked with * are significantly different to the control ($p < 0.05$) + SE and letters mark significant differences between the treatments ($p < 0.05$).

The cumulated mean number of the *B. aeneus* larvae m^{-2} dropping down for pupation over all sampling dates is illustrated in Figure 17. The two larval stages (L1 and L2) were merged. Larval dropping due to lethal effects of the applied insecticides started one day after application. There, a difference between the application techniques can be seen as both conventional treatments showed a higher mean number of dropped larvae than the control and the dopleg treatments. This difference clarifies three days after application, further showing a difference between the efficacies of the insecticides. Mospilan conventionally applied resulted in the highest number of dropped larvae (77 larvae m^{-2}), followed by Biscaya conventionally applied (57 larvae m^{-2}). The lethal effect of the insecticides in the conventionally treated plots was overlaid by the start of larvae dropping down for pupation in all other treatments. The untreated control exhibited seven days onwards the highest number of dropped larvae. 14 days after application (29th of May) the peak of larval dropping occurred in all treatments. The highest number of dropped larvae was found in the control treatment with 239 larva m^{-2} followed by both dropleg treatments (Mospilan 193 larvae m^{-2} and Biscaya 154 larvae m^{-2}).The strongest effect in terms of reducing the dropping of larvae during the peak was achieved in the conventional treatments with Mospilan having a number of 126 larvae m^{-2} and Biscaya with an even lower number of 104 larvae m^{-2}. Thus, 48 % and 55 % fewer larvae m^{-2} dropped to the ground compared to the control. Weaker effects of larval reduction were found in the dropleg treatments with Mospilan resulting in a number of 193 larvae m^{-2} and Biscaya in 153 larvae m^{-2}. Two weeks after climaxing larval dropping was completed and the number of dropped larvae rapidly decreased towards zero.

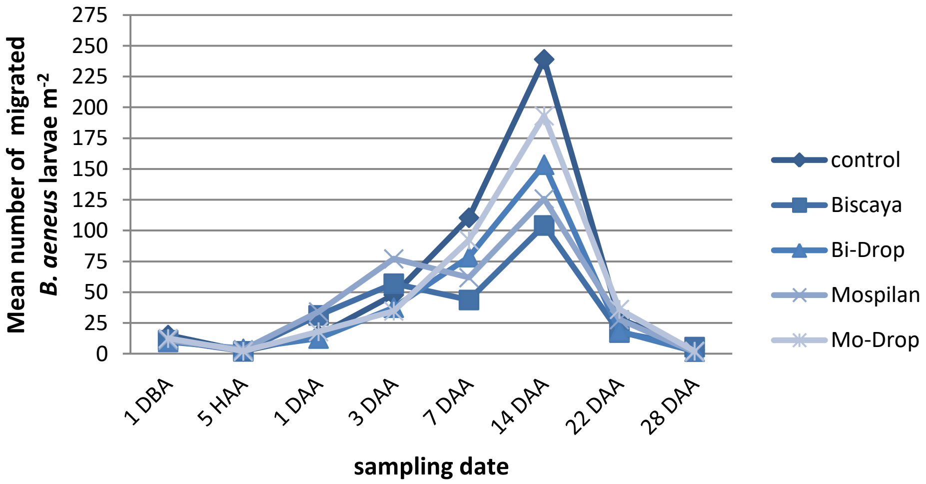

Figure 17: Mean number of *B. aeneus* larvae m^{-2} dropped down for pupation within the different treatments on the specific days after application (DAA).

Figure 18 presents the cumulated mean number of dropped *B. aeneus* larvae m^{-2} over all sampling dates within the different treatments. No significant differences between the insecticides or the application techniques were found. However, both Biscaya treatments had a significantly reducing effect on the number of dropped larvae in comparison with the control. Further, a reduction of larval dropping by Mospilan conventionally applied is observable by a tendency (p=0.0649) in comparison with the control. The control treatments exhibited the highest number of dropped *B. aeneus* larvae (447 ±55 larvae m^{-2}) and Biscaya conventionally applied reduced the number by 43 % (262 ±52 larvae m^{-2}) and the Bi-Drop treatment by 33 % (307 ± 47 larvae m^{-2}). Larval dropping in both Mospilan treatments was only slightly lower compared to the control. 332 ±67 larvae m^{-2} in the conventional Mospilan and 379 ± 52 larvae m^{-2} in the Mo-Drop treatment dropped to the ground for pupation. In general, the insecticide Biscaya and the conventional application technique showed a better reducing effect on *B. aeneus* infestation by a tendency.

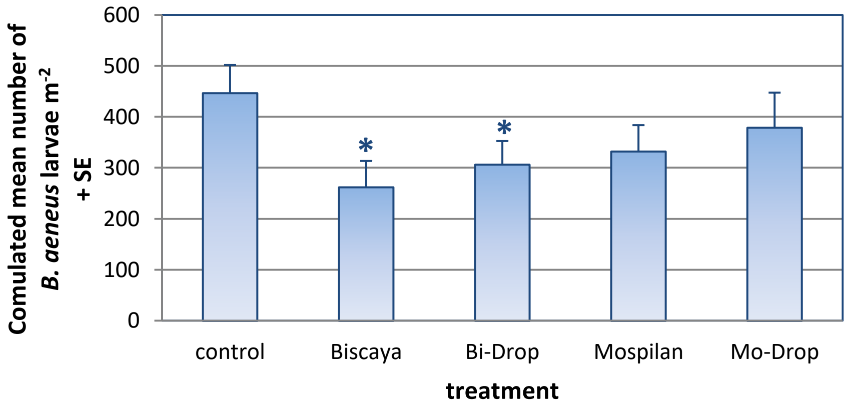

Figure 18: Cumulated mean number of *B. aeneus* larvae m^{-2} dropped down for pupation within the different treatments from 14 until 63 days after application. Data marked with * are significantly different to the control ($p < 0.05$) + SE and letters mark significant differences between the treatments ($p < 0.05$).

2.2.4 *Ceutorhynchus pallidactylus*

In 2017, the incidence of cabbage stem weevils was only marginal. A mean number of 50 beetles m^{-2} dropped down for pupation over all sampling dates (appendix). No significant differences between the insecticides or the application techniques were found.

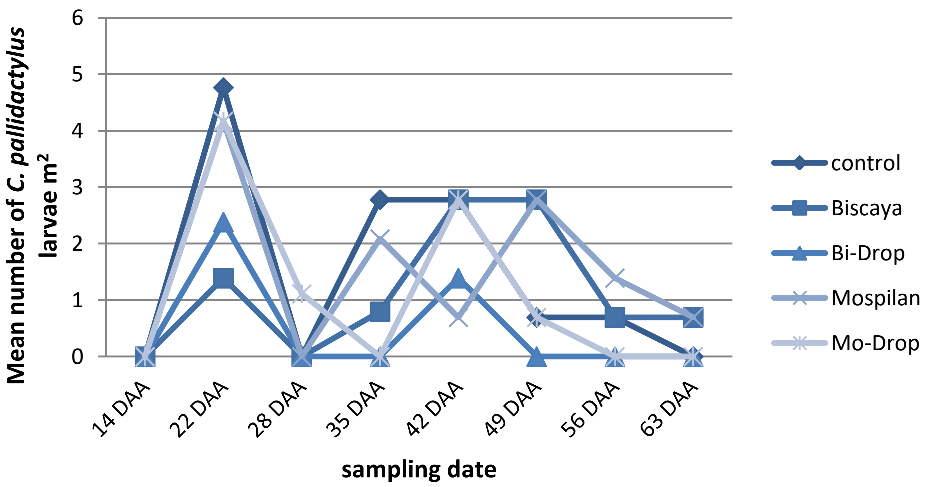

Figure 19: Mean number of *C. pallidactylus* larvae m^{-2} dropped down for pupation within the different treatments on the specific days after application (DAA).

The first larvae were found in the control on the 5th of June (22 days after application) with a mean number of 5 larvae m^{-2}. This time point also seemed to be the peak of larval dropping in the control and both Mospilan treatments. Larval dropping decreased afterwards but lasted until 63 DAA.

By looking at Figure 20 illustrating the cumulated mean number of dropped *C. pallidactylus* larvae m^{-2} over all sampling dates it can be perceived that the Bi-Drop treatment (4 ± 3 larvae m^{-2}) significantly reduced the amount of dropped larvae by 68 %

compared to the control (12 ± 4 larvae m^{-2}). A tendency of the dropleg technique achieving a better control of the cabbage stem weevil can also be assumed for the Mospilan treatments. While the number in the conventional Mospilan treatment was on the same level as the control (12 ±5 larvae m^{-2}), the dropleg treatment exhibited a number of 9 ± 5 larvae m^{-2}. In general, Biscaya seemed to have a slightly better reducing effect on *C. pallidactylus* in comparison to Mospilan. Nevertheless, no significant differences between the insecticides or the application techniques were found.

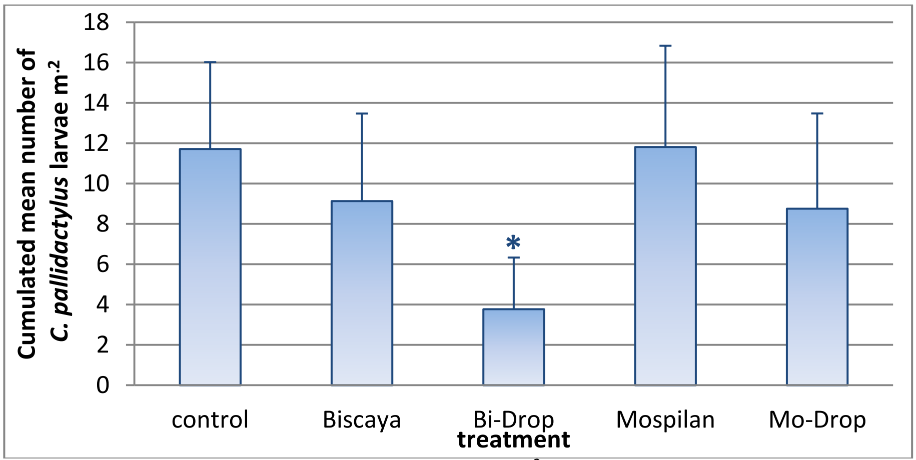

Figure 20: Cumulated mean number of *C. pallidactylus* larvae m^{-2} dropped down for pupation within the different treatments from 14 until 63 days after application. Data marked with * are significantly different to the control ($p < 0.05$) and letters mark significant differences between the treatments ($p < 0.05$).

2.2.5 *Ceutorhynchus napi*

The occurrence of rape stem weevils was low at the present field site in 2017. The average of the cumulated mean number of dropped *C. obstrictus* over all sampling dates was three adults m^{-2} (Figure 21).

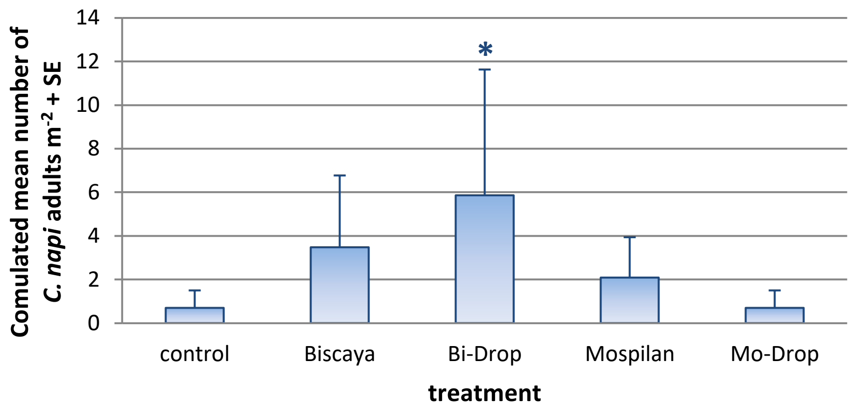

Figure 21: Cumulated mean number of *C. napi* adults m^{-2} dropped down within the different treatments from 14 until 63 days after application. Data marked with * are significantly different to the control ($p < 0.05$) and letters mark significant differences between the treatments ($p < 0.05$).

The lowest number was found in the control and the Mo-Drop (1 ± 1 adults m^{-2}) treatment. The highest number and significant difference compared to the control was observed in the Bi-Drop (6 ± 6 adults m^{-2}) followed by the conventional Biscaya

(3 ± 3 adults m^{-2}) and the conventional Mospilan treatment (2 ± 2 adults m^{-2}). No significant differences between the insecticides or the application techniques were observed. However, Bi-Drop showed a significantly reducing effect on *C. napi* adults compared to the control.

The first larvae were found in the water trays on the 15th of May (seven days after application) in the control (7 larvae m^{-2}) (appendix). Afterwards, dropping of larvae decreased but continued until the 26th of June (42 days after application). The number of larvae dropping down was variable in all treatments but never exceeded 6 larvae m^{-2} (both Mospilan treatments). The highest number was 4 larvae m^{-2} in the conventional Biscaya treatment whereas the Bi-Drop treatment reduced the number steadily below 3 m^{-2}.

Figure 22 illustrates the cumulated mean number of *C. napi* larvae dropped down for pupation. Even though there were no significant differences found between the insecticides or the application techniques, an effect of Biscaya applied using the dropleg technique on larval dropping can be observed. The number of dropped larvae was reduced by 55 % (5 ± 2 larvae m^{-2}) compared to the control (11 ± 6 larvae m^{-2}). All other treatments had approx. the same number as the control.

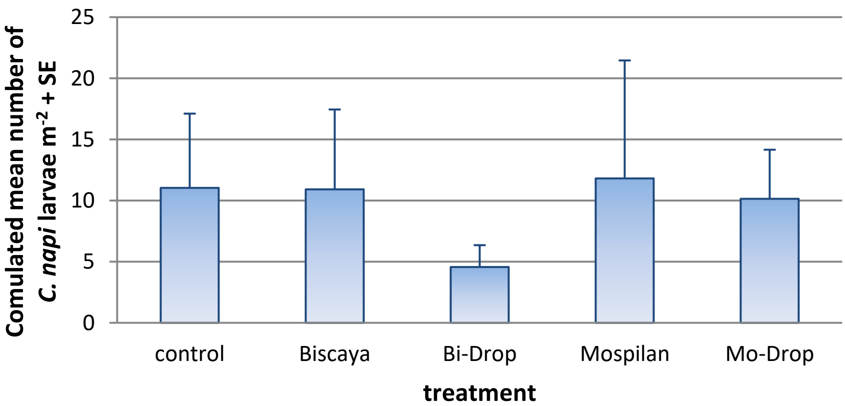

Figure 22: Cumulated mean number of *C. napi* larvae m^{-2} dropped down for pupation within the different treatments from 14 until 63 days after application. Data marked with * are significantly different to the control ($p < 0.05$) and letters mark significant differences between the treatments ($p < 0.05$).

2.2.6 *Psylliodes chrysocephala*

During the present field study only a small part of *P. chrysocephala's* life cycle was investigated. Figure 23 illustrates the cumulated mean number of dropped adults m^{-2} within the different treatments over all sampling dates. A number of 51 ± 17 adults m^{-2} was found in the control, an even higher number of 60 ± 17 adults m^{-2} in the Bi-Drop treatment and 52 ± 14 adults m^{-2} in the Mo-Drop treatment. Both conventional applied insecticides showed a slightly better control of *P. chrysocephala* adults. A number of 46 ± 12 adults m^{-2} was found in the Biscaya and 38 ± 16 adults m^{-2} in the Mospilan treatment. However, no significant differences between the insecticides or the application techniques were found.

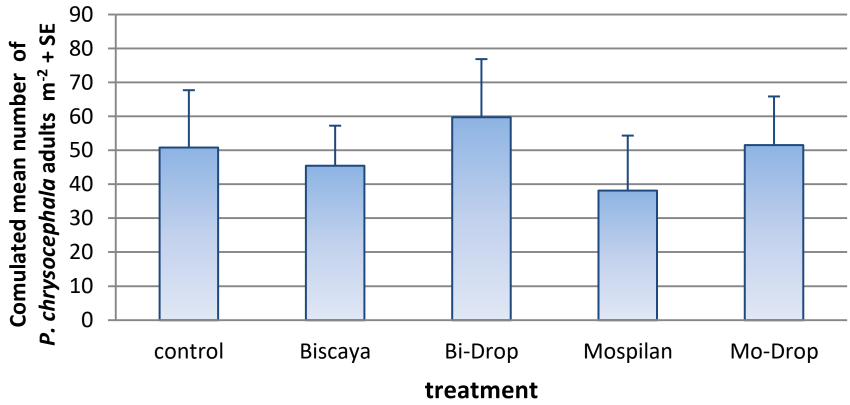

Figure 23: Cumulated mean number of *P. chrysocephala* adults m^{-2} dropped down within the different treatments from 14 until 63 days after application. Data marked with * are significantly different to the control ($p < 0.05$) + SE and letters mark significant differences between the treatments ($p < 0.05$).

2.3 Stem assessment

To calculate the infestation with stem-boring weevil larvae (*C. pallidactylus* and *C. napi*) a stem assessment took place (Figure 24).

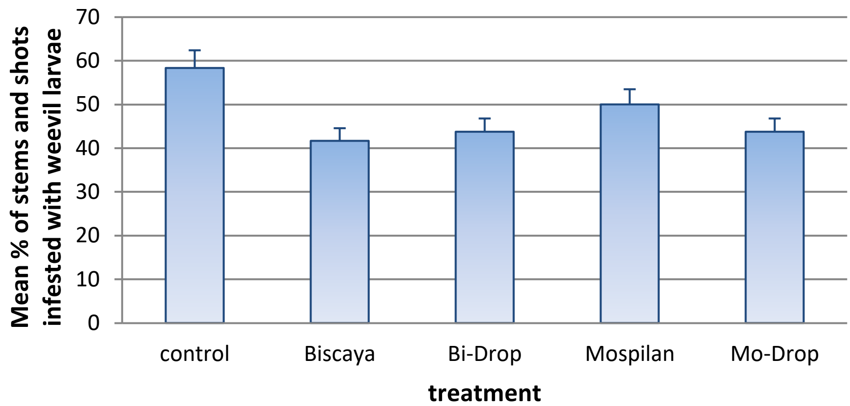

Figure 24: Mean % of stem and shoot infestation with mining weevil larvae (*C. pallidactylus* and *C. napi*) within the different treatments. Data marked with * are significantly different to the control ($p < 0.05$) + SE and letters mark significant differences between the treatments ($p < 0.05$).

A reducing tendency of the insecticides on weevil infestation can be observed in the insecticide treatments. The insecticides reduced weevil infestation by at least 14 % compared to the control (58 ± 4 % stem infestation). Biscaya conventionally applied achieved the best reduction of weevil infestation followed by both dropleg treatments (44 ± 3 % stem infestation) and the conventional Mospilan treatment (50 ± 3 % stem infestation). No significant differences between the insecticides or the application techniques were detected.

2.4 Newly emerged beetles

Besides measuring larval dropping another way to evaluate the efficacies of the insecticides and the application techniques is to analyze the size of the new beetle generation that was able to withstand the impact of insecticide treatment and emerged in the test field later in the season.

2.4.1 *Ceutorhynchus obstrictus*

The chronological trend of the emergence of cabbage seedpod weevil adults m^{-2} over all sampling dates is presented in Figure 25. Emergence of adult seedpod weevils started on the 15th of June (31 days after application) with a peak on the 29th of June and was finished on the 10th of July.

During the peak of emergence the Bi-Drop treatment exhibited the highest number (39 adults m^{-2}), followed by the Mo-Drop (29 adults m^{-2}) and the conventional Biscaya treatment (26 adults m^{-2}). The control only had a number of 19 adults m^{-2} and the lowest number of adult seedpod weevils during the peak was found in the Mospilan treatment (15 beetles m^{-2}).

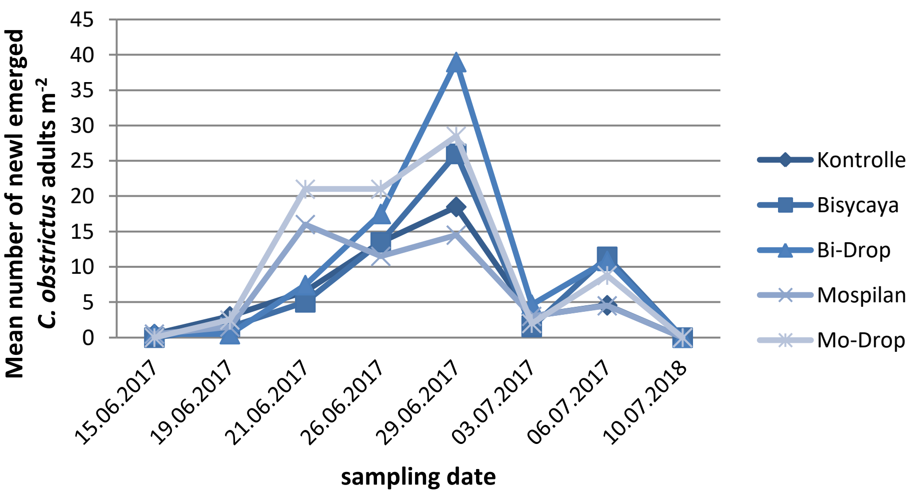

Figure 25: Mean number of newly emerged *C. obstrictus* adults m^{-2} within the different treatments on the specific days after application (DAA).

Considering the cumulated mean number of the newly emerged *C. obstrictus* adults m^{-2} over all sampling dates (figure 33), it can be seen that no significant differences between the insecticides or the application techniques were detected. However, the lowest number was found in the control (50 ± 19 adults m^{-2} and in the conventional Mospilan treatment (52 ± 19 adults m^{-2}). The conventional Biscaya treatment exhibited a total of 60 ±1 7 adults m^{-2} whereas the size of the new generation in the dropleg treatments was at least 25 % higher (Bi-Drop 80 ± 38 adults m^{-2} and Mo-Drop 84 ± 26 adults m^{-2}).

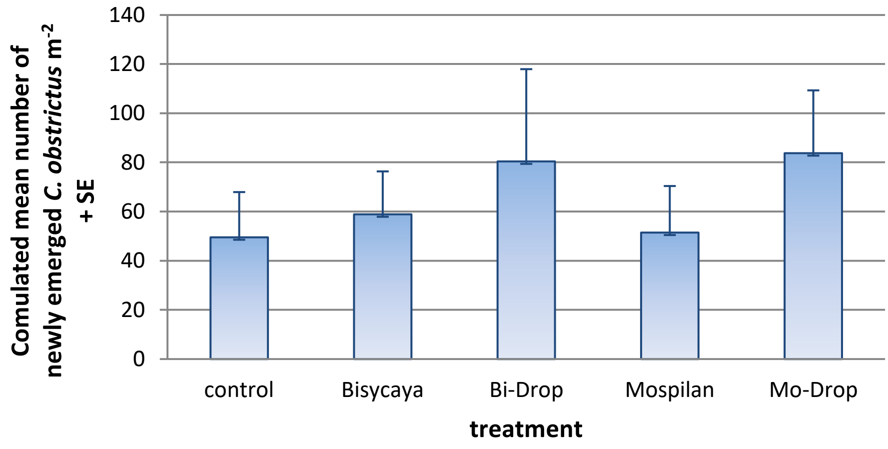

Figure 26: Cumulated mean number of newly emerged *C. obstrictus* adults m^{-2} within the different treatments from the 15th of June until the 10th of July. Data marked with * are significantly different to the control ($p < 0.05$) + SE and letters mark significant differences between the treatments ($p < 0.05$).

2.4.1 *Brassicogethes aeneus*

The emergence of the new generation of pollen beetles started on the 15th of June (Figure 27). Four days later (19th of June), an emergence peak occurred in the control with a number of 11 adults m^{-2}. On the contrary, emergence in the insecticde treatments stayed below 5 adults m^{-2}. In both dropleg treatments a slightly higher amount of pollen beetles emerged (Bi-Drop 5 adults m^{-2} and Mo-Drop 4 adults m^{-2}). While the majority of new generation beetles in the control treatment emerged around the 19th of June, both Mospilan and the conventional Biscaya treatments had another peak around the 26th of June. In the conventional Mospilan treatment a number of 7 adults m^{-2} was detected and in the other insecticide treatments a number between 4 and 5 adults m^{-2}. After this peak, emergence decreased and marginally continued until the 6th of July.

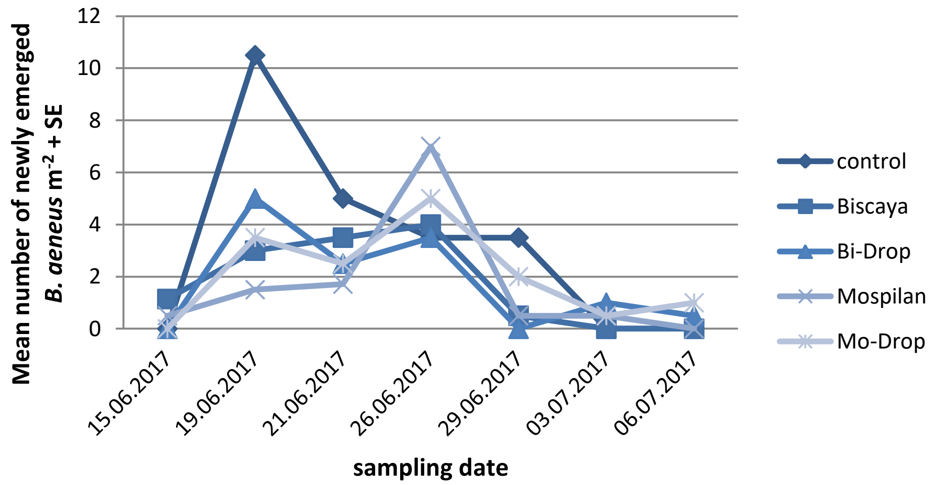

Figure 27: Mean number of newly emerged *B. aeneus* adults m^{-2} within the different treatments on the specific days after application (DAA).

By looking at (Figure 28) giving the cumulated mean number of the new *B. aeneus* generation within the different treatments over all sampling dates, a reducing effect of all insecticide treatments by approx. 50 % can be observed compared to the control (23 ± 9 adults m^{-2}). However, no significant differences between the insecticides or the application techniques were found.

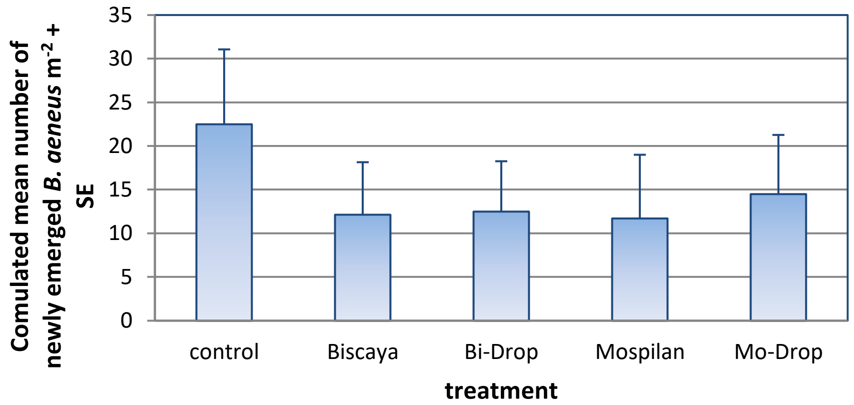

Figure 28: Cumulated mean number of newly emerged *B. aeneus* m^{-2} within the different treatments from the 15th of June until the 6th of July. Data marked with * are significantly different to the control ($p < 0.05$) + SE and letters mark significant differences between the treatments ($p < 0.05$).

2.4.2 *Ceutorhynchus pallidactylus*

Since infestation of OSR by *C. pallidactylus* was only of moderate size at the present field trial in 2017, the new generation of cabbage stem weevils was low. An emergence peak occurred on the 19th of June in the control with a number of 5 beetles m^{-2} (appendix).

By looking at (Figure 29**Fehler! Verweisquelle konnte nicht gefunden werden.**), it can be figured that no significant differences between the insecticides or the application techniques were detected.

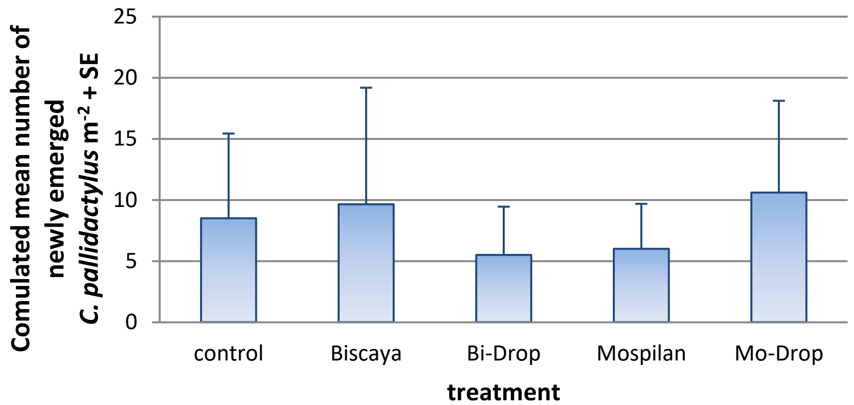

Figure 29: Cumulated mean number of newly emerged *C. pallidactylus* adults m^{-2} within the different treatments from the 15th of June until the 10th of July. Data marked with * are significantly different to the control ($p < 0.05$) + SE and letters mark significant differences between the treatments ($p < 0.05$).

2.4.3 *Psylliodes chrysocephala*

During the period of the present field experiment a new generation of cabbage flea beetle emerged. The peak of emergence was around the 6th of July (Figure 30).

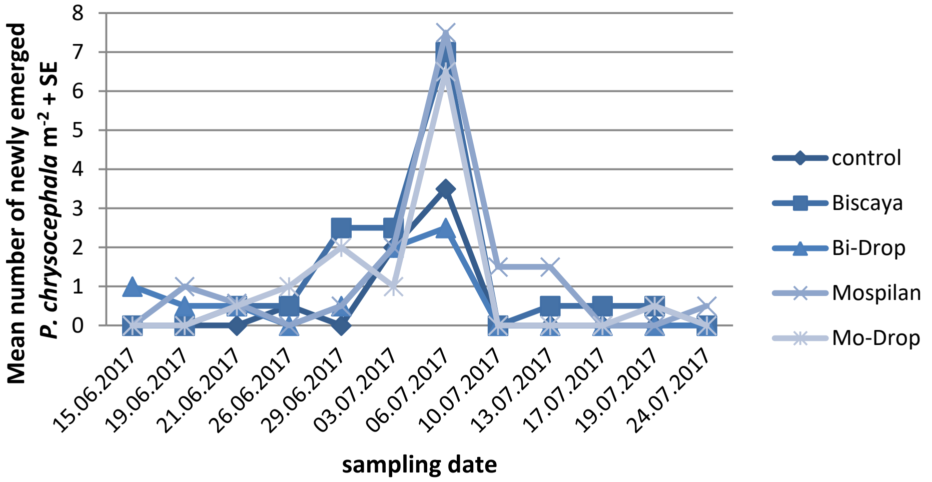

Figure 30: Cumulated mean number of newly emerged *P. chrysocephala* adults m^{-2} within the different treatments on the specific days after application (DAA).

The highest cumulated mean number of adults m^{-2} over all sampling dates was found in both conventional treatments (15 ± 6 adults m^{-2}) followed by the Mo-Drop (11.5 ± 5 adults m^{-2}) and the Bi-Drop (7 ± 4 adults m^{-2}) treatment (Figure 31). A number of only 6 ±3 adults m^{-2} was found in the control. No significant differences between the insecticides or the application techniques were detected.

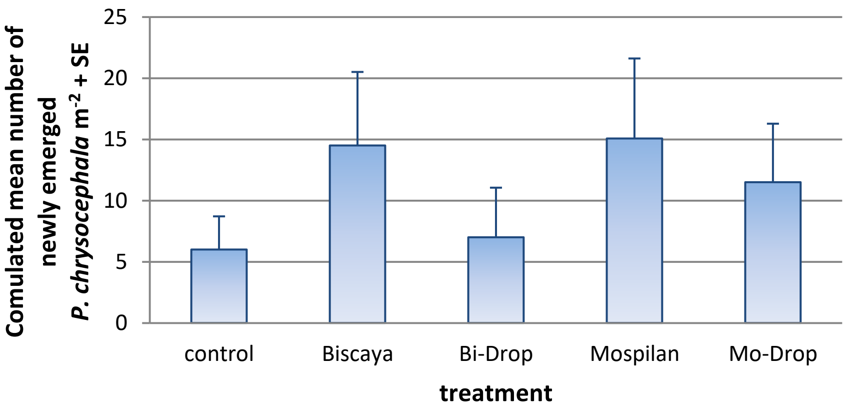

Figure 31: Cumulated mean number of newly emerged *P. chrysocephala* adults m^{-2} within the different treatments from the 15th of June until the 10th of July. Data marked with * are significantly different to the control ($p < 0.05$) + SE and letters mark significant differences between the treatments ($p < 0.05$).

2.5 Pod assessment

A pod assessment to investigate midge-infested pods within the different treatments was firstly conducted on the 1st of July (18 days after application) at BBCH 75 when dropping of brassica pod midge larvae increased in the field (Figure 32). The second assessment took place on the 21st of July (36 days after application) at BBCH 80 (Figure 33). At the second date of the pod assessment most larvae of the second generation were already visible indicating a slightly too late setting of the assessment date.

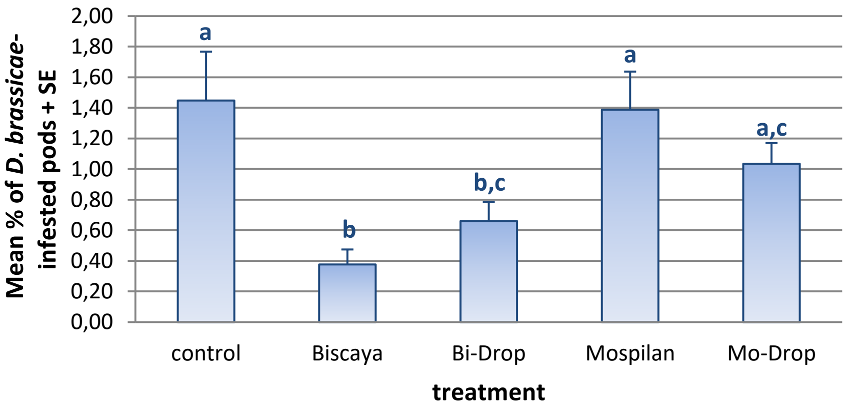

Figure 32: Mean percentage of *D. brassicae*-infested pods within the different treatments on the 1st pod assessment 17 days after application (1st of June; BBCH 75). Data marked with * are significantly different to the control ($p < 0.05$) + SE and letters mark significant differences between the treatments ($p < 0.05$).

In general, pod infestation was low at the present field site. The highest percentage of infested pods was 4.2 % in the conventional Biscaya treatment on the second pod assessment date. On the first assessment date 17 days after application (BBCH 75) both Biscaya treatments exhibited a significantly lower pod infestation with *D. brassicae*. The best result was found in the conventional Biscaya treatment with only 0.4 % pods infested, significantly different to the Bi-Drop treatment (0.7 % pod infestation). Thus, infestation was reduced by 74 % and 54 % in comparison to the control (1.5%). There were no effects of both Mospilan treatments detectable (Mo-Drop 1 % and conventional Mospilan 1.4 %). The second pod assessment 36 days after application (BBCH 80) did also not result into significant differences between the insecticides or the application techniques (Figure 33). In general, pod infestation was higher compared to the 1st pod assessment. Infestation by the brassica pod midge in the treatments was similar to the control (3.7 %).

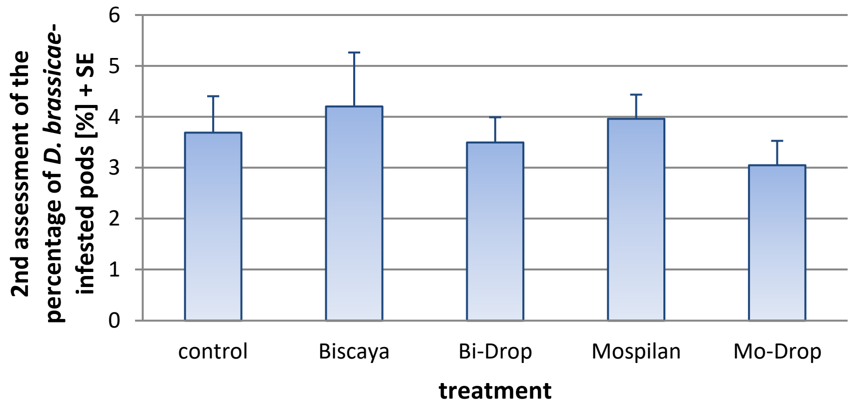

Figure 33: Mean percentage of *D. brassicae*-infested pods within the different treatments on the 2nd pod assessment 17 days after application (1st of June; BBCH 75). Data marked with * are significantly different to the control ($p < 0.05$) + SE and letters mark significant differences between the treatments ($p < 0.05$).

2.6 Suction sampling of *Dasineura brassicae*

To evaluate the effect of the insecticides and the application techniques on the natural abundance of *D. brassicae*, adult midges were caught with a D-vac suction sampler (1 day before until 9 days after application) (Figure 34). In comparison with Figure 35 giving the cumulative mean number of adult *D. brassicae* a slight reducing tendency of the insecticide treatments can be perceived with Biscaya conventionally applied showing the lowest number of *D. brassicae* (4 ± 1 adults m^{-2}) while the control had 5 ± 1 adults m^{-2}. Nonetheless, there were no significant differences found between the insecticides and the application techniques.

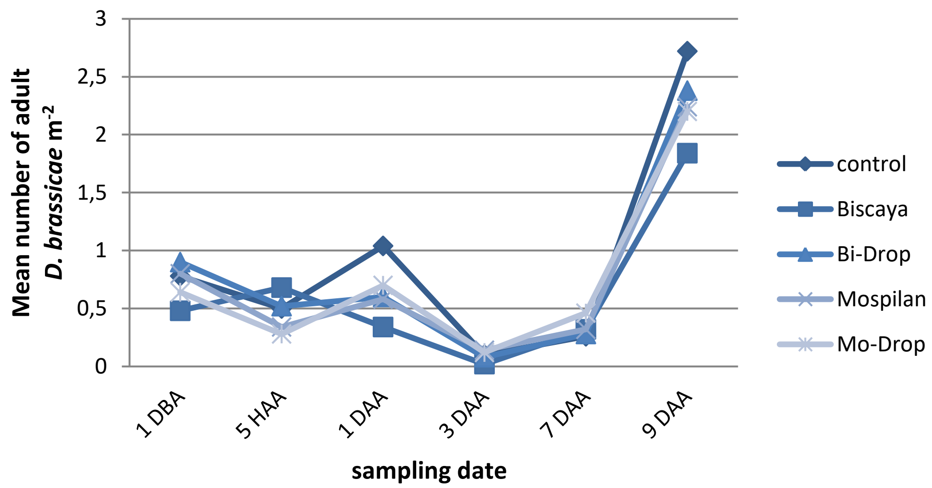

Figure 34: Mean number of *D. brassicae* adults m^{-2} caught my means of suction sampling within the different treatments over the days after application (DAA).

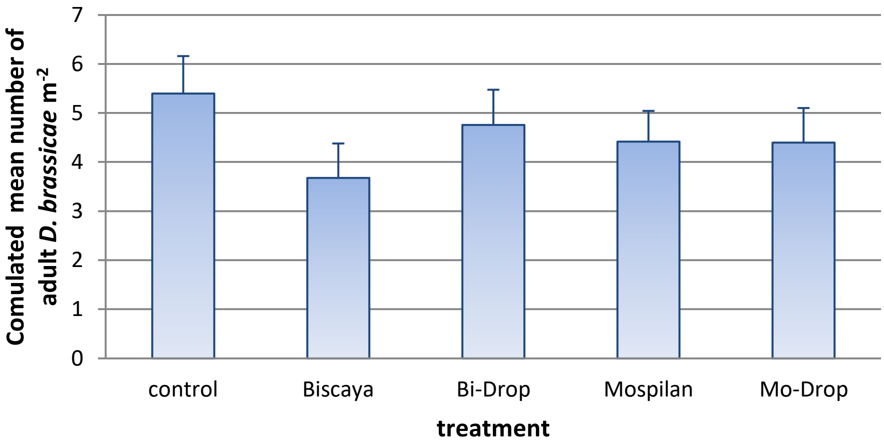

Figure 35: Cumulated mean number of *D. brassicae* adults m^{-2} caught by means of suction sampling within the different treatments from one day before until nine days after application. Data marked with * are significantly different to the control ($p < 0.05$) + SE and letters mark significant differences between the treatments ($p < 0.05$).

2.7 Systemic action of neonicotinoids after application using the dropleg technique

To analyze the systemic action of neonicotinoids applied using the dropleg technique, the top 20 cm of the main shoot of one plant from each plot was cut and merged with plant samples from the same treatment, so there was a sample of four replicates for each treatment and sampling date. The concentration of thiacloprid and acetamiprid was measured (Figure 36). It is observable for both insecticides that their concentration decreases over time. In general, concentrations were highest in the conventional treatments, but one day after application the concentration of thiacloprid in the Bi-Drop treatment (1484 µg kg^{-1}) was unexpected twice as high as in the conventional Biscaya treatment (722 µg kg^{-1}). Further, there was a much lower concentration of acetamiprid found in the plant samples compared to thiacloprid.

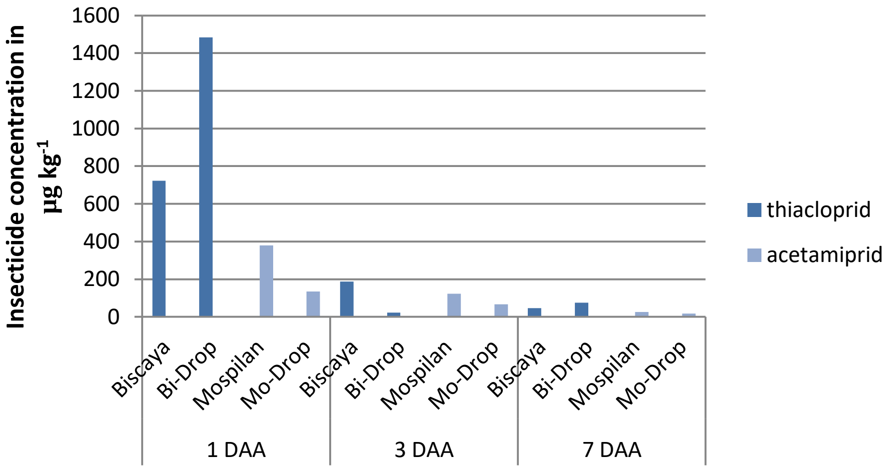

Figure 36: Concentration of insecticide residues of thiacloprid and acetamiprid in the flowering part of oilseed rape within the different treatments on the days after application (DAA)

2.8 Impact of the insecticides and the application techniques on parasitoids

To investigate whether the dropleg technique contributes a beneficial effect on natural antagonists of target insect pests the occurrence of parasitic wasps, especially the pollen beetle parasitoid *T. heterocerus*, was investigated and the parasitism and superparasitism level of pollen beetle larvae was determined.

2.8.1 Occurrence of parasitoids

Two species of the family Tersilochine were identified which are determined as parasitoids of *B. aeneus*. Besides some individuals of *Phradis interstitialis*, *Tersilochus heterocerus* was the dominant species within the present field trial. The male individuals of *P. interstitialis* and *T. heterocerus* cannot be distinguished wherefore they were merged with the females of *T. heterocerus* to calculate the mean number of Tersilochine m^{-2} within the different treatments over the sampling dates (one day before until nine days after application) (Figure 37). The chronological trend points out that the number of Tersilochine m^{-2} decreased five hours after insecticide application in comparison to the day before application. The number of Tersilochine m^{-2} was also affected in the control five hours after application. The number of parasitic wasps one day after application increased again in all treatments but then decreased until seven days after application. Afterwards, an increase of the parasitoid abundance in the treatments was observed while the control showed a decrease. The number of Tersilochine was always the highest in the untreated control.

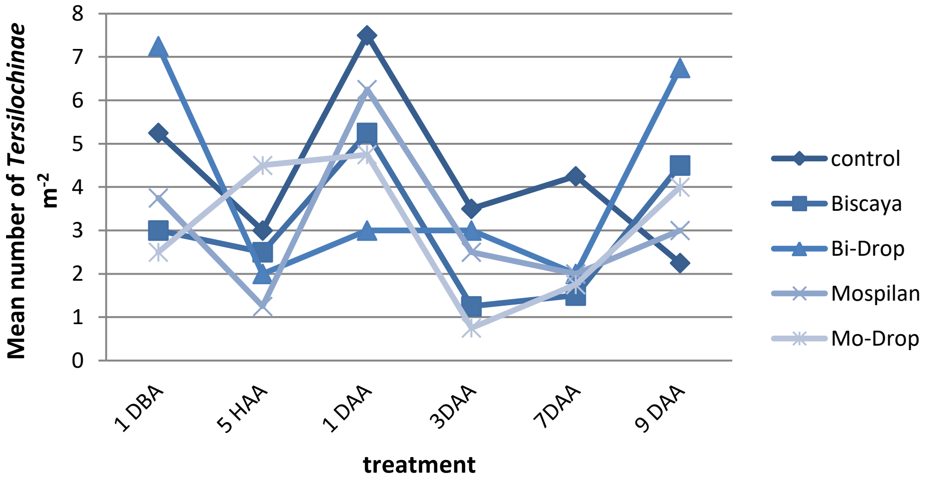

Figure 37: Mean number of *Tersilochinae* m^{-2} caught my means of suction sampling within the different treatments over the sampling dates

In (Figure 38) the cumulated mean number of the Tersilochine m^{-2} within the different treatments over the sampling dates (one day before until nine days after application) is presented. There were no significant differences found between the insecticides or the application techniques, but a clear tendency of the insecticides reducing the number of

Tersilochine m^{-2} can be observed. All insecticide treatments exhibited at least 33 % fewer Tersilochine m^{-2} compared to the control (18 ± 4 Tersilochine m^{-2}).

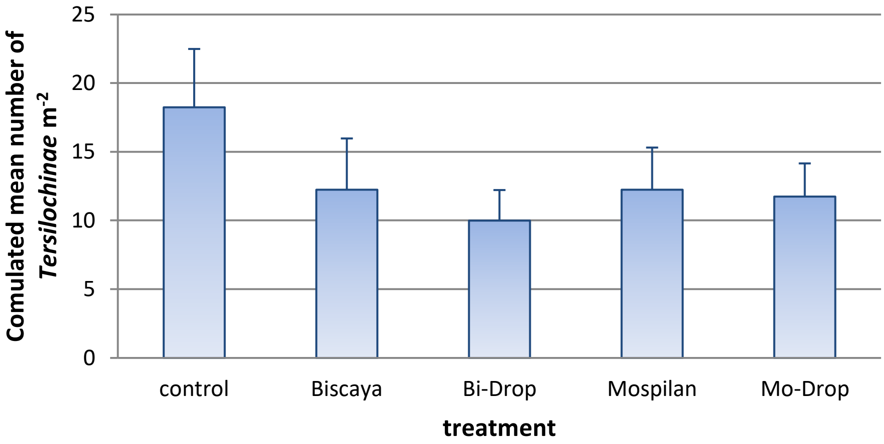

Figure 38: Cumulated mean number of Tersilochine m^{-2} caught by means of suction sampling within the different treatments from one day before until nine days after application. Data marked with * are significantly different to the control ($p < 0.05$) + SE and letters mark significant differences between the treatments ($p < 0.05$).

The identifying of further parasitic wasps belonging to the Chalcidoidea was entrusted to Prof. Dr. Stefan Vidal. As a result two species of cabbage seedpod weevil parasitoids, *Mesopolobus morys* (Walker) and *Trichomalus perfectus* (Walker) (Hymenoptera: Pteromalidae) were identified (HOFFMANN & SCHMUTTERER 1999). Since the occurrence of these chalcid wasps was only marginal, the impact of the insecticide treatments was not further considered.

2.8.2 Parasitism level of *Brassicogethes aeneus* larvae

The number of black eggs of the parasitoid *T. heterocerus* was determined by dissecticting the larvae of its host *B. aeneus* to calculate the parasitism level within the different treatments over the sampling dates (one day until 22 days after application).

The sum of dissected pollen beetle larvae caught on the different sampling dates can be seen in (Figure 39). Since some of the collected L1 larvae from the water trays were too small for dissection they were not kept. Therefore, the amount of dissected larvae differentiates from the cumulative mean number of pollen beetle larvae calculated from the water trays. During seven and 14 days after application larval dropping had its peak which can also be seen in Figure 18. Further, it can be perceived that the conventional technique better reduces the amount of dropped larvae in comparison to the dropleg technique.

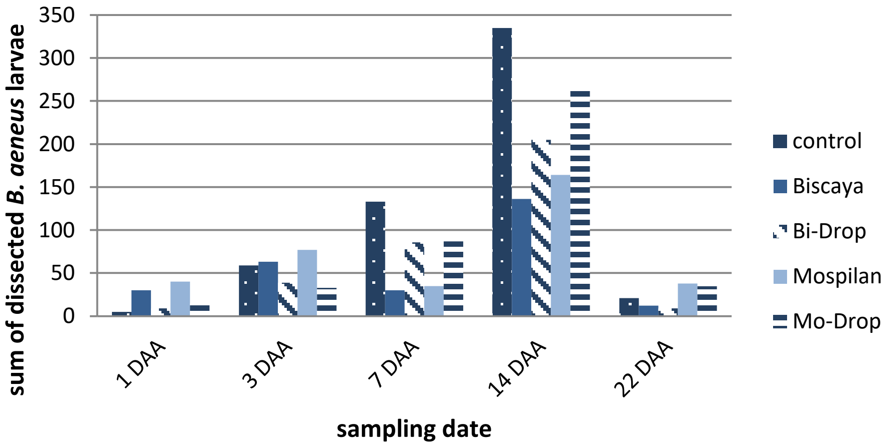

Figure 39: Sum of dissected *B. aeneus* larvae within the different treatments over the sampling dates for the calculation of the mean parasitism levels.

The mean parasitism level of pollen beetle larvae parasitized by *T. heterocerus* during the first three weeks after insecticide application can be seen in (Figure 40). During the first two weeks of sampling the parasitism level of pollen beetle larvae was about 50 % in all treatments

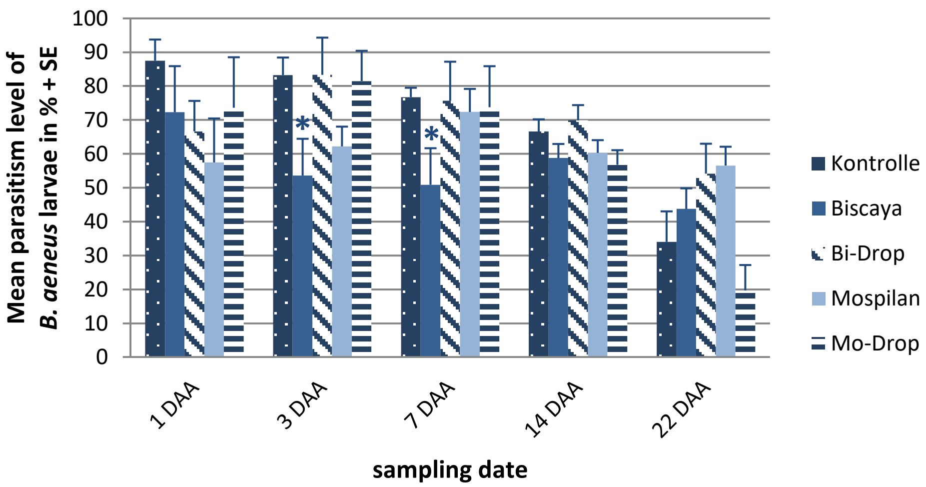

Figure 40: Mean parasitism level of *B. aeneus* larvae in % within the different treatments over the sampling dates. Data marked with * are significantly different to the control ($p < 0.05$) + SE and letters mark significant differences between the treatments ($p < 0.05$).

One day after application the control had a parasitism level of 88 %. Even though significant differences between the insecticides and the application techniques were not detected, a tendency of insecticide application reducing the parasitism level by at least 16 % is observable. Three days after application the first difference between the application techniques can be seen. The parasitism level in the conventional Mospilan was reduced by 24 % and in the conventional Biscaya treatment significantly reduced by 35 %. The parasitism levels in the dropleg treatments increased to the same level as the control (82 %). Seven days after application the parasitism level in the conventional Mospilan treatment also reached control level. Only the conventional Biscaya treatment still had a significantly lower parasitism level (51 %) seven days after application whereas all other treatments had a mean above 70 %. Two weeks after application parasitism rate was on the same level in all treatments, but in general slightly lower compared to the first dates of sampling.

Figure 41 illustrates the cumulated mean parasitism level over the first two trial weeks and in conclusion with the previously described observations, shows that Biscaya conventionally applied reduced the parasitism level of pollen beetle larvae by 20 % compared to the control. The parasitism level in both Mospilan treatments was approx. 61 ± 7 % and Bi-Drop (70 ± 9 %) was on the same level as the control. No significant differences between the insecticides and the application techniques were detected.

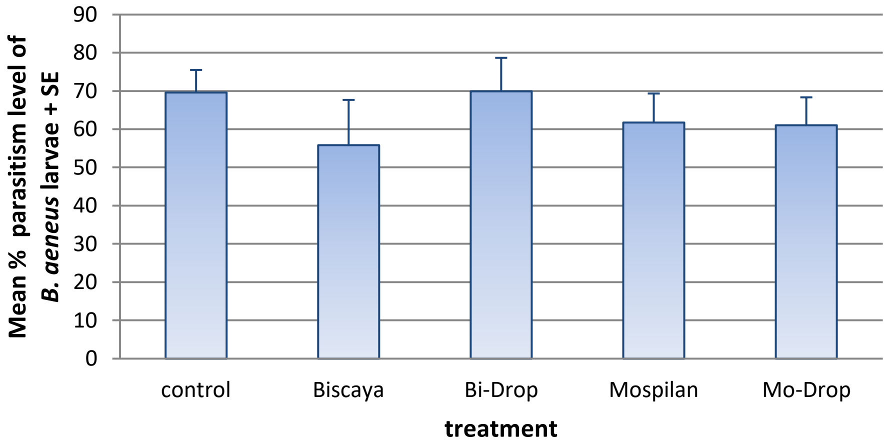

Figure 41: Mean percentage of the parasitism level of *B. aeneus* larvae within the different treatments from one day before until 22 days after application. Data marked with * are significantly different to the control ($p < 0.05$) + SE and letters mark significant differences between the treatments ($p < 0.05$).

2.8.3 Superparasitism of *Brassicogethes aeneus* larvae

To investigate the superparasitism level of pollen beetle larvae the mean percentage of larva with more than one egg of *T. heterocerus* was calculated over the first three experimental weeks (Figure 42). In the control superparasitism occurred in 65 ± 10 % of all analyzed larvae. A slight tendency of insecticide treatment reducing the superparasitism level can be observed. Appropriate to the data of the parasitism level, Biscaya conventionally applied caused in the lowest level that was reduced by 32 % compared to the control. Further, the dropleg technique had a slightly weaker effect on reducing the superparasitism level compared to the conventional technique. No significant differences between the insecticides and the application techniques were detected.

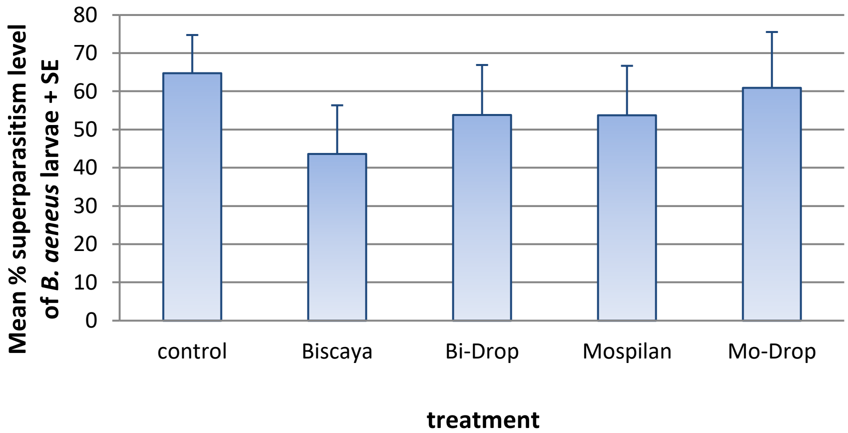

Figure 42: Mean superparasitism level of *B. aeneus* larvae within the different treatments from one day before until 22 days after application. Data marked with * are significantly different to the control ($p < 0.05$) + SE and letters mark significant differences between the treatments ($p < 0.05$).

2.9 Yield parameters

Yield value was calculated for 9 % grain moisture. At the present field site a yield of 3853 ± 16 kg ha^{-1} was achieved in the untreated control. Besides the conventional Mospilan treatment, yield of insecticide treatments showed slightly higher values. The highest yield was obtained in the Bi-Drop treatment (4003 ± 59 kg ha^{-1}) followed by the conventional Biscaya (3950 ± 51 kg ha^{-1}) and the Mo-Drop (3870 ± 60 kg ha^{-1}) treatment. The lowest yield was obtained from the conventional Mospilan treatment (3853 ± 72 kg ha^{-1}). By a tendency, using the insecticide Biscaya achieved better yields than using Mospilan. Further, the dropleg technique had a slightly better effect on the yield. Nevertheless, not significant differences between the insecticides and the application techniques were detected.

Table 1: Mean number of yield parameters in the different treatments(+ SE). No significant differences detected.

	yield [kg/ha]	TKM [g]	plant density m^{-2}	pods per plant
control	**3853** ± 16	**5,51** ± 0,02	**23,88** ± 1,81	**262,77** ± 6,53
Biscaya	**3950** ± 51	**5,54** ± 0,03	**25,11** ± 2,13	**268,72** ± 1,74
Bi-Drop	**4003** ± 59	**5,44** ±0,01	**25,05** ± 2,05	**242,79** ±5,25
Mospilan	**3853** ± 72	**5,44** ± 0,07	**22,92** ± 2,24	**248,09** ± 27,22
Mo-Drop	**370** ± 60	**5,47** ± 0,05	**25,52** ± 1,58	**272,15** ± 7,68

The thousand kernel weight in the control was on average 5.51 g. All other treatments exhibited only a marginally higher value with Mo-Drop resulting in the heaviest seeds (5.47 g) followed by the conventional Biscaya (5.54 g) and both the Bi-Drop and the conventional Mospilan treatments with seeds of 5.44 g.

A plant density of 24 plants m^{-2} was counted in the control. Highest plant density was found in the Mo-Drop treatment (26 plants m^{-2}) whereas the conventional Mospilan treatment (23 plants m^{-2}) had a slightly lower density than the control. The conventional Biscaya and Bi-Drop treatment's plant density was almost the same (25 plants m^{-2}).

A mean of 263 pods per plant were counted in the control. In comparison, number of pods per plant was higher in the conventional Biscaya (267 pods per plant) and Mo-Drop treatment (272 pods per plant). The conventional Mospilan (248 pods per plant) and Bi-Drop treatment (243 pods per plant) exhibited lower means compared to the control. There were no significant differences found between the insecticides and the application techniques regarding the different yield parameters.

3 Discussion

3.1 Efficacy of the different insecticide treatments regarding insect pest control

In the present study the efficacy of two neonicotinioids (thiacloprid and acetamiprid) applied using the innovative dropleg technique was compared to the efficacy of the conventional application technique in terms of controlling important insect pests of OSR. The main focus was set on the blossom insect pests the cabbage seedpod weevil (*C. obstrictus*) and the brassica pod midge (*D. brassicae*).

3.1.1 *Ceutorhynchus obstrictus*

The infestation of OSR by cabbage seedpod weevils was low at the present test field site in 2017. Only an average of 0.1 *C. obstrictus* adult per plant was found one hour before application. Thus, the economic threshold of one *C. obstrictus* per plant (Hoffmann & Schmutterer 1999) was not exceeded and control measures were not necessary. In the control, a cumulated mean number of 55 weevil larvae m^{-2} dropped to the ground for pupation (Figure 12). There were no significant differences found between the tested insecticides or the application techniques in terms of reducing the larval dropping of *C. obstrictus.*

Bonnemaison (1965) stated that *C. obstrictus* exits its hibernation place when the air temperature approaches 15 °C and the overwintering sites are sufficiently humid. Since the beginning of April was of moderate temperatures around 15° C, early flight activity of *C. obstrictus* was most likely triggered. The late frost event around the 19th of April might have caused a strong reduction in weevil density. According to Càrcàmo et al. (2009) temperature has the greatest influence on the mortality of overwintering cabbage seedpod weevils. When temperatures decrease to -5°C the mortality significantly increases which might have been the case in the present study and impaired the viability of not yet emerged weevils. In general, the present field site seems not suitable for the cabbage seedpod weevil since previous years were also characterized by low infestation by *C. obstrictus* (Gödeke 2016; Hausmann 2017).

In a study conducted by Dosdall et al. (2002) the distribution of adult seedpod weevils within a crop field was analyzed over 24 h at full flowering stage of OSR. Results showed that weevil distribution was primarily concentrated on the inflorescences and fewer on stems and leaves, independently on the day time. During an investigation about the feeding behavior of cabbage seedpod weevils, Williams & Free (1978) also found that they spend most of their time feeding on the upper flowering plant part.

After emergence, seedpod weevils immigrate to flowering OSR fields where they feed on and oviposit into developing pods. Feeding of adult cabbage seedpod weevils is rather insignificant Due to the main immigration of *C. obstrictus* at the flowering stage of OSR, control measures are conducted at BBCH 64 (Hoffmann & Schmutterer 1999).

Females usually deposit one egg per pod and mark these with a pheromone in order to prevent multiple deposition and egg laying by female competitors (ALFORD 2003). This residence and feeding behavior in the crop indicates that the site of action of insecticides against *C. obstrictus* has to be concentrated on the flowering part of OSR for a sufficient control of this insect pest. Therefore, it was hypothesized that systemically acting neonicotinoids applied below the flowering part using the dropleg technique might also be effective against *C. obstrictus* foraging in the upper flowering part, likewise as the conventional technique. In fact, there were no differences found between the application techniques regarding insect pest reduction but treatments were also not significantly different to the untreated control. However, the residue analysis of systemically acting neonicotinoids in the flowering part applied using the dropleg technique revealed very low concentrations of the tested insecticides (Figure 36). Thus, this indicates that the control of the cabbage seedpod weevil in terms of insecticide application using the dropleg technique might not be sufficient enough.

When comparing the size of the new beetle generation m^{-2} (Figure 25) with the number of dropped larvae m^{-2} (Figure 12), contradictory results have been observed. Only the untreated control showed a slightly lower amount of newly emerged beetles compared to the actual number of dropped larvae m^{-2}. Moreover, these findings are not in agreement with previous studies regarding weevil mortality. HAYE et al. (2010) detected mortality rates of *C. obstrictus* higher than 99.6 % in a three-year study with overwintering mortality contributing 50 % and factors influencing the immature (egg, larval, pupal) stages the remaining 50 % for the total mortality rate. Fungi, bacteria, nematodes and carabid beetles play an important role in the mortality of *C. obstrictus* larvae during pupation. Furthermore, parasitoids can decrease the insect pest density (BONNEMAISON 1965; HAYE et al. 2010). After all, temperature is said to play the most important role regarding mortality of *C. obstrictus* (CÁRCAMO et al. 2009).

Since the size of the new beetle generation was almost in accordance with the amount of dropped larvae m^{-2} in the control and both conventional treatments, it can be concluded that the mortality rate of cabbage seedpod weevils was extremely low. Actually, only a small number of the most important cabbage seedpod weevil parasitoids *Trichomalus perfectus* (Walker) and *Mesopolobus morys* (Walker) were found during suction sampling. Both parasitoids as well as *Stenomalina gracilis* were determined during the MASTER project as the three key parasitoid species (ULBER et al 2010). All three pteromalid wasps are ectoparasitoids which means they lay their eggs on the surface of the host larvae and after the parasitoid larva has hatched, it feeds externally on the immobilized host larva. Without forming a cocoon, pupation takes place alongside the host's body. Later, the parasitoid imago bites a hole through the pod wall to exit into the environment. Parasitism levels exceeding 50 % have been reported in Germany (ULBER et al. 2010) but also levels up to 96 % are found in Estonia (VEROMANN ET AL. 2011). However, as the parasitism level of *C. obstrictus* was not investigated in this study, a low mortality rate cannot be explained by the absence of parasitic wasps as biocontrol agents.

Both the dropleg treatments exhibited an at least 40 % higher number of newly emerged beetles m^{-2} in comparison with the control, and the number was at least 23 % higher than the actual number of dropped larvae m^{-2}, leading to contradiction (Figure 26). The striking standard errors of the number of *C. obstictus* may be explained by the effect of heterogeneous distribution of cabbage seedpod weevils within the field indicated by BLAKE et al. (2010). Their study points out that beneficial oviposition sites characterized by high levels of sulfur and low levels of nitrogen are most likely not homogenously distributed within the crop field, causing increased spatial clustering of *C. obstrictus*. This reason would imply that water trays are not efficient enough to evaluate the efficacies of insecticides and application techniques to control cabbage seedpod weevils.

3.1.2 *Dasineura brassicae*

Even though the infestation of OSR by cabbage seedpod weevils was low, the brassica pod midge occurred abundantly at the present field site. It is known that *D. brassicae* infestation is linked to the abundance of cabbage seedpod weevils since midges prefer to oviposit into pods previously damaged by weevil feeding or emergence holes of weevil larvae (HOFFMANN & SCHMUTTERER 1999, WILLIAMS 2010a). However, AXELSEN 1992 and GRAORA ET AL. 2015 stated that *D. brassicae* is also able to oviposit into healthy young pods hence infestation can be independent of cabbage seedpod weevil abundance. This seems to have been the case at the present field site in 2017.

Flight activity of brassica pod midges starts when OSR begins to flower with a main period during the full flowering stage. Control measures are therefore conducted at full flowering of OSR (HOFFMANN & SCHMUTTERER 1999).

The oviposition site of the brassica pod midge is the upper part of the plants, more precisely, the pods of OSR. Egg deposition occurs mainly during the later afternoon at temperatures above 19°C (HOFFMANN & SCHMUTTERER 1999). The residue analysis of neonicotinoids in the flowering part of OSR after application using the dropleg technique (Figure 36) revealed that the concentration e.g. of thiacloprid is much lower compared to the concentration of thiacloprid conventionally applied. Thus, it can be assumed that the conventional insecticide application directly onto the flowers would be more effective due to direct lethal effects on *D. brassicae*. The foraging of brassica pod midges is not well examined. While SPEYER (1921) observed midges feeding on nectar and wound sap, FRÖHLICH (1956) did not detect any food uptake. Thus, it cannot be assumed that midges might take up systemically transported insecticides applied using the dropleg technique.

WILLIAMS et al. (1978) investigated the vertical distribution of *D. brassicae* and caught midges on different levels within the crop. Further, FRÖHLICH (1956) stated that midges reside at ground level at high wind conditions. In the present study during the suction sampling there were also some midges found in the lower plant canopy before the new generation of *D. brassicae* hatched. Thus, it can be assumed that brassica pod midges also reside in the lower plant part and hence can be affected by insecticide application using the dropleg technique.

Interestingly, during the first peak of larval dropping 19 days after application both Biscaya treatments strongly decreased the number of dropped larvae in comparison to the control (1386 larvae m^{-2}) (Figure 11). In the Bi-Drop treatment 54 % and in the conventional Biscaya treatment 69 % fewer larvae m^{-2} dropped to the ground for pupation. However, larval dropping in the conventional Biscaya treatment strongly increased during the following weeks and was at the same level as the control when the second larval generation dropped to the ground. In the end, no significant differences between the insecticide treatments in terms of reducing larval dropping were found (Figure 12). Nonetheless, it can be observed that larval dropping was at least 13 % reduced in all treatments compared to the control and that the conventional application technique and the insecticide Biscaya achieved a slightly better reduction. Furthermore, the number of adult *D. brassicae* was assessed during suction sampling. Although not significant, all treatments showed a reduced number of brassica pod midges with Biscaya conventionally applied resulting in the lowest number compared to the control.

The first pod assessment took place 17 days after application (1st of June; BBCH 75) and revealed significant differences between the applied insecticides (Figure 21).

The insecticide Biscaya significantly reduced the percentage of midge-infested pods. In detail, Biscaya conventionally applied achieved the best result in reducing the pod midge infestation by 74 % compared to the control. In general, percentage of midge-infested pods was low in the control (1.5 % pod infestation) at the first pod assessment date.

By comparing the percentage of infested pods and the first peak of larval dropping (6th of June) (Figure 13) it can be noticed that larval dropping in the conventional Biscaya treatment duplicated one week after the peak in the control whereas it decreased in all other treatments. Therefore, it seems most likely that the date of the first pod assessment was set too early to detect the total infestation by the first *D. brassicae* larvae in the conventional Biscaya treatment and caused a maybe too low percentage of midge-infested pods in the conventional Biscaya treatment.

Besides lethal effects on brassica pod midges, a deterrent mode of action by insecticides on insects is known (STAPEL et al. 2000). When considering the reduced number of adult *D. brassicae* from the suction sampling in (Figure 35) which was the lowest in the conventional Biscaya treatment, lethal or deterrent effects of Biscaya directly applied on the foraging and oviposition site of the pod midges can be assumed. This might have caused a reduced oviposition, i.e. less larvae m^{-2}. Nevertheless, NEUMANN 2010 stated that deterrent effects of thiacloprid on parasitoids decrease after some days. This may also apply for *D. brassicae* and may have led to immigration of midges from adjacent into the

conventional Biscaya treated plots. The deterrent effect may therefore have resulted in a shift in oviposition and hence larval dropping of the first generation. Therefore, it is suggested that the plot size of 300 m^{-2} might be too small to test the efficacy of insecticides due to the possible immigration of pod midges.
The second pod assessment on the 21st of June did not show a significant difference between the insecticide treatments in terms of reducing pod infestation (Figure 33). This time point of assessment was set more accurate since it includes the delayed peak of larval dropping in the conventional Biscaya treatment. However, immigration of pod midges into treated plots cannot be excluded and the larval dropping of the second generation also started.
Due to the fact that a major part of the first generation of brassica pod midge larvae do not emerge in the same year but in the following 5 years newly emerged midges were not recorded (NILSSON et al. 2004).

3.1.3 *Brassicogethes aeneus*

Damage caused by pollen beetles already occurs before OSR starts to flower. When temperature reaches 12-15°C pollen beetles emerge and immigrate into OSR at green bud stage (ALFORD 2003). Adults feed bite through buds to feed on pollen which causes may podless stalks when infestation level is high (HOFFMANN & SCHMUTTERER 1999). Later, adults oviposit 2-3 eggs into a single bud. Feeding of larvae on pollen within the buds and later on pollen of open flowers is rather insignificant (HOFFMANN & SCHMUTTERER 1999). Damage caused by adult pollen beetles at low infestation level can be compensated by the plant through developing new buds. Since OSR is most susceptible during early bud stage and becomes less susceptible when the plant development progresses, winter OSR can often escape pollen beetle damage before the main invasion occurs. In general, insecticides are applied when economic threshold levels are exceeded at green bud stage (HOFFMANN & SCHMUTTERER 1999; ALFORD 2003b).
Even though insecticide application during flowering is aimed at the blossom insect pests *C. obstrictus* and *D. brassicae* (HOFFMANN & SCHMUTTERER 1999), the present study also investigated the efficacy of the tested insecticides applied using the dropleg compared to the conventional application technique in terms of controlling pollen beetles.
All treatments showed a direct lethal effect on adult pollen beetles compared to the control (80 beetles m^{-2}) and these differences were significant except in the Bi-Drop treatment. Especially, conventional application of Biscaya resulted in a three-fold reduction of pollen beetle density (228 beetles m^{-2} dropped to the ground). Dropping of adult pollen beetles started directly after insecticide application and showed an increase until day 3 after application.
A tendency of acetamiprid in reducing larval dropping of pollen beetles was noticeable but only thiacloprid significantly decreased larval dropping in comparison with the

control. KUPFER & SCHRÖDER (2015) likewise detected a lower efficacy of acetamiprid on *B. aeneus* infestation. Moreover, an in vivo study on the metabolism of acetamiprid in honey bees indicated a low toxicity due to its rapid degradation. In less than 30 min 50 % of the insecticide was metabolized. This indicates a very short half-life of this insecticide and thus a lower toxicity (BRUNET et al. 2005).

In contrast to a widely distributed resistance of pollen beetles against pyrethroids (HEIMBACH & MÜLLER 2012; ZIMMER et al. 2014; STARÁ 2017) resistance development has not yet been reported for neonicotinoids in laboratory experiments. Nevertheless, a decrease in the efficacy of thialoprid was already noticed in field studies conducted by KUPFER & SCHRÖDER (2015). Further, development of neonicotinoid resistance has already been detected in other insect species (NAUEN & DENHOLM 2005).

BRANDES ET AL. (2018) compared the pyrethroid tau-fluvalinate with the neonicotinoid thiacloprid and found that despite of both insecticides causing the same level of reduction in pollen beetle density, thiacloprid treatment resulted in a highly lower bud infestation. Additional greenhouse experiments indicated sublethal effects of thiacloprid on egg laying and beetle behavior. Impairing effects of insecticides on insect behavior are demonstrated several times in various studies (LONGLEY et al. 1996; STAPEL et al. 2000). The better reduction effect of thiacloprid on larval dropping compared to acetamiprid may therefore be explained by stronger sublethal effects on pollen beetles during their oviposition period resulting in a lower number of damaging larvae within the buds.

The effect of insecticides on pollen beetles is also observable when looking at the size of the newly emerged beetle population (Figure 28). Although no significant differences were detected, the new beetle generation was reduced by at least 50 % in all treatments compared to the control. Interestingly, by comparing the number of dropped larvae m^{-2} (Figure 18) with the actual number of the newly emerged beetles, a mortality of at least 95 % can be noticed in all treatments.

High mortality rates of pollen beetle larvae in the ground up to 96 % were also discovered by BÜCHI (2001). In field experiments in the years 1996-1999 different factors that contribute to the mortality of pollen beetle larvae were analyzed. Predators, parasitoids and unspecific factors were evaluated. BÜCHI (2001) discovered that a mortality of 16-27 % can be caused by predators, 1-2 % by parasitoids and 46-72 % by unspecified factors such as weather conditions. In terms of parsitoids, the MASTER project (2001-2005) revealed that parasitism levels of pollen beetle larvae in Germany were up to 97 % with average levels between 25-50 % and *T. heterocerus* being one of the predominant species (WILLIAMS 2010).

Larval parasitization by the ichneumonid wasp *T. heterocerus* was also determined in the present study and revealed an average parasitism level exceeding 56 % in all treatments. Further, parasitism levels up to 88 % in the untreated control were detected (Figure 40).

HOKKANEN (2008) found in a study about the correlation between parasitism level and pollen beetle density reduction that parasitism levels of 30-40 % can already significantly reduce the insect population. The high average of parasitism level in the present experiment clearly contributed to a reduction in the new generation of pollen beetles. However, NITZSCHE & ULBER (1998) documented that despite of parasitization, a percentage of 9.1 of parasitized pollen beetle larvae were able to suppress parasitoid development to continue their own development to the adult stage. This was also noticed by OSBORNE (1960) who found the shells of *T. heterocerus* eggs within the fat-body of adult pollen beetles. In the first hours after parasitization, a defense reaction of the pollen beetle larva against the eggs of *T. heterocerus* is triggered. Haemocytes aggregate on the parasitoid eggs or around the first-instar larvae. After a four-day period encapsulation is terminated, i.e. the haemocytes become a thin, hard, dark-brown capsule inhibiting parasitoid development (ALFORD 2003). Even though only one parasitoid larva per host will survive in the end, the common multiple egg laying (superparasitism) of *T. heterocerus* is not a wasted process since it weakens the encapsulation defense reaction and enhances the parasitoid's survival within the host larva. Moreover, the competition between *T. heterocerus* and *P. interstitialis* larvae within the same host larva (multiparasitism) also has an effect on reducing the host's defense reaction (NILSSON 2003).

Additionally, weather conditions also play an important role in the mortality of larvae and pupae in the soil (BÜCHI 2002). During the first two weeks after insecticide application when larval dropping for pupation took place, precipitation was irregular and even absent for more than a week (Figure 3). Therefore, dry ground conditions might have caused desiccation of larval cocoons and therefore a reduced new beetle generation.

The main purpose of the present study was to compare the efficacy of the dropleg and the conventional insecticide application technique. Even though statistic analysis did not detect any significant differences, a slightly weaker effect of insecticides applied using the dropleg technique on larval dropping of *B. aeneus* can be observed. Interestingly, the evaluation of the new pollen beetle generation did not reveal any tendencies and no significant differences between the application techniques (Figure 28).

Even though insecticides are applied below the flowering part of OSR resulting in skipping of the foraging site of pollen beetles, neonicotinoids are systemically transported into the flowering part where the beetles feed on pollen or bite through buds to lay their eggs (ALFORD 2003b). In a bioassay, ZIMMER et al. (2014) proved the toxic effect of systemically transported thiacloprid on *B. aeneus* feeding on the flowering part of OSR that was immersed in a thiacloprid solution. However, WALLNER (2014) proved a substantial lower insecticide concentration within the pollen after insecticide application using the dropleg technique. Further, the present residue analysis of insecticides in the flowering part of OSR treated with systemically acting neonicotinoids using the dropleg technique also revealed much lower concentrations in the upper plant canopy. This may be the reason in the present field trial for a slightly weaker effect of insecticides applied using the dropleg technique on pollen feeding larvae (Figure 18).

3.1.4 *Ceutorhynchus pallidactylus* and *Ceutorhynchus napi*

Even though the focus of the present study was set on the efficacy of insecticide application using the dropleg technique against blossom insect pests, the cabbage stem weevil (*C. pallidactylus*) and the rape stem weevil (*C. napi*) were also considered.
Both weevils belong to the most important stem-boring insect pests of OSR. The immigration of *C. pallidactylus* starts at the bud stage of winter OSR (ALFORD 2003). The female weevil prefers to lay a bunch of eggs into the underside of the petioles and sometimes also in young shoots. Larvae initially mine in the petioles before they migrate to the stems and lateral shoots to continue feeding. As soon as larval development is completed, they exit the stems to pupate in the ground (WILLIAMS 2010). In winter OSR direct mining damage caused by *C. pallidactylus* is rarely of significance but wounds caused by adults or larvae are entry gates for the fungal disease stem canker (*Phoma lingam*) (ALFORD 2003a).
On the contrary, the rape stem weevil is of economic importance in winter OSR. First adult weevils occur in February and March and migrate to winter OSR during the period of stem elongation. There, eggs are laid in young developing stems. Due to secretions by the females or eggs during oviposition, stem tissue twists and splits causing growth impairment. The wounds caused by this disruption open the gates for *P. lingam* as well. Larvae mine in the stem pith before they migrate to the ground to pupate. The adult rape stem weevil does not emerge in the same year but in the following spring (ALFORD 2003a).

Knowing that the foraging site of both mining insect pests is concentrated on petioles and stems, hence below the flowering part, it was assumed that insecticide application using the dropleg technique would be of accurate efficacy to limit the damage of the occurring generation and to decrease the size of the new generation. Further, the systemic mode of action of the applied neonicotinoids would also enhance the efficacy against mining weevil larvae when applied directly onto the plant part where larvae mine below.
In a study conducted by HABERLAH-KORR (2016) it has already been proven that insecticide application using the dropleg technique against white mold exhibits the same efficacy as the conventional technique since it is concentrated directly on the infection site of the fungus.
The abundance of the rape stem weevil in the present field study was low and evaluation of water tray samples started after immigration of rape stem weevils into the field during the period of stem elongation in early spring. Since larvae hatch after 1-2 weeks after egg-deposition and mine inside the plants for about 3-5 weeks before they pupate in the soil

(WILLIAMS 2010), larval dropping was already well advanced when the insecticide application experiment started. Biscaya applied using the dropleg technique resulted in the lowest number of dropped larvae and the highest number of dropped adults (Figure 21; Figure 22). However, it is assumed that most of the larvae found in the water trays determined as *C. napi* actually were larvae of *C. pallidactylus* since larval dropping of *C. napi* was supposed to have been finished when the present experiment started. Therefore, the evaluation of *C. napi* in the present experiment can be neglected.
The results of the water tray evaluation of *C. pallidactylus* larval dropping revealed no significant differences between the insecticides or the application techniques (Figure 20) However, Bi-Drop (4 larvae m^{-2}) was the only treatment that significantly reduced the number of dropped larvae by 67 % compared to the control (12 larvae m^{-2}). Further, a slightly better effect of the insecticide Biscaya in comparison to Mospilan can be observed in terms of reducing larval dropping (Figure 22).
Interestingly, the cumulated mean number of dropped *C. pallidactylus* adults was moderate, 60 adult weevils m^{-2} were found in the control treatment (Figure 21). Nonetheless, a much smaller number of larvae was assessed in the water trays. This difference may have been caused by imprecise identification of *C. pallidactylus* and *C. napi* larvae as they have a quite similar appearance (see JURAN et al 2011).
Besides the estimation of the efficacy of insecticides and the application techniques on weevil infestation, a stem assessment was carried out at BBCH 75. There, weevil damage was not differentiated between *C. pallidactylus* and *C. napi* since many larvae already left the stems for pupation in the soil. Anyhow, *C. napi* occurred only marginal within the experimental field wherefore most of the stem damage can be attributed to *C. pallidactlus*. In the control treatment, 58 % of dissected stems were infested. For all treatments a tendency of reduced weevil damage can be seen since infestation was decreased by at least 14 %. However, no significant differences were detected.

3.2 Impact of insecticide application using the dropleg technique on the pollen beetle parasitoid *Tersilochus heterocerus*

In the present field study the ichneumonid wasp and pollen beetle endoparasitoid *T. heterocerus* was the predominant species found most frequently in the suction samples. The parasitization evidence was only determined via dissection of pollen beetle larvae for this parasitic wasp. In the suction samples only few females of *Phradis interstitialis* were caught. The number of this parasitoid was only marginal and during larval dissection no proof of parasitism was found. However, detection of *P. interstitialis* larvae within the host larvae is very challenging since they are of the same color as the fat body of the larvae which might have caused missing detection (OSBORNE 1960). Further, the activity period of this parasitoid starts already before flowering (ALFORD 2003) because this parasitoid prefers to oviposit into eggs or first-instar larvae within the buds (BERGER 2015). Thus, it can be assumed that the date of insecticide application in the present experiment would be at least of lower impact on *P. interstitialis* adults since parasitism of first-instar larvae begins before the oilseed rape field is at full flowering stage (ALFORD 2003) and the investigated insecticide application experiment took place.

To evaluate the abundance of parasitoids after insecticide application by means of suction sampling, the number of identified *T. heterocerus* females and not distinguishable male parasitoids either belonging to *T. heterocerus* or *P. interstitialis* were merged. Both insecticides reduced the number of Tersilochine parasitoids compared to the control in the first nine days after application (Figure 37). JANSEN et al. (2004) also evaluated the impact of thiacloprid on the natural abundance of adult parasitoids within the field and found a reduction by 59-72 %. Nevertheless, the differences between the treatments and the control were not statistically validated in the present study (Figure 36).
Further, it was suggested in ALFORD (2003) that sweep-net catches cannot be well correlated to parasitism level, i.e. efficacy of parasitoids. On the one hand, male parasitoids emerge before females and swarm above the crop for a much shorter life span. Thus, sampling time to detect parasitoid abundance needs to be extended. On the other hand, the random effect of the pollen beetle density influences the abundance of parasitoids and weather factors such as wind direction also have an impact on their distribution (ALFORD 2003).
In the present study the parasitism levels within the different treatments were determined to examine the effect of the tested insecticides on the efficacy of parasitic wasps as biocontrol agents of *B. aeneus*. The calculation of the mean parasitism level within the different treatments during the first three experimental weeks showed that parasitization in the conventional Biscaya treatment exhibited the strongest reduction in parasitism level compared to the other treatments (Figure 40). While the control had an average parasitism level of 70 %, thiacloprid conventionally applied resulted in a reduction of 20 % revealing an average parasitism level of 56 %. More precisely, at the sampling point three and seven days after application, the parasitism level in the conventional Biscaya treatment was significant different compared to the control. This points out a greater influence of the insecticide thiacloprid compared to acetamiprid and corresponds to the findings of BRUNET et al. (2005) who conducted an in vivo study about the metabolism of acetamiprid in the honey bee (*Apis mellifera*). Results indicate a rapid metabolism of acetamiprid which may be correlated with a lower toxicity compared to thiacloprid. However, the tendencies of insecticides in terms of reducing the mean parasitism levels within the different treatments over the sampling period were not significantly different.

Some studies have verified an impairing impact of pyrethroids on parasitic wasps and thus their efficacy as naturally-occurring biocontrol agents of insect pests (ULBER ET AL. 2010; NEUMANN 2010). Yet in 1996, LONGLEY et al. pointed out a repellent effect of deltamethrin on aphid parasitoids that was present up to two days after application and significantly reduced parasitism levels. Moreover, these authors found that with increasing deltamethrin concentration the parasitoids resided shorter on aphid honeydew-treated areas which they are attracted to find their hosts. Further, when pre-exposed to field doses of deltamethrin parasitoids also showed a shorter residence on honeydew-treated areas and behaved abnormally in comparison with control parasitoids. Only after 12 h a "normal" behavior was observed again (LONGLEY et al 1996).

For neonicotinoids many studies also proved impairing effects on the behavior of parasitic wasps (JANSEN et al. 2004; NEUMANN 2010; TAPPERT et al. 2017).

The impact of thiacloprid on parasitic wasps has been evaluated in a study by JANSEN ET AL. (2014) who discovered a significant decrease in the parasitism level of pollen beetle larvae compared to the untreated control. This corresponds to the finding of the present study showing that only thiacloprid conventionally applied caused a significantly reduced parasitism level up to seven days after application compared to the control (Figure 40). On the sampling date 14 days after application no more differences were present between the parasitism levels obtained from the different treatments compared to the control. These findings are comparable to the results obtained by NEUMANN (2010). In field experiments in the years of 2006-2007 the effect of thiacloprid on parasitism levels of pollen beetle larvae was investigated and demonstrated a significant reduction in the parasitism level of pollen beetle larvae. When repellent effects of thiacloprid were diminished, parasitoid immigration into treated fields increased again which was much lower in the first days after application. Additionally, in a laboratory experiment pollen beetle larvae exposed to thiacloprid only showed a lower parasitism level 3 to 5 days after application which also indicates a fading effect of insecticides on parasitoids after a few days (NEUMANN 2010).

The reason behind the reduction of parasitism levels due to insecticides is not only based on lethal effects on parasitic wasps, which can come in contact with pesticides directly or through the uptake of insecticide residues from the plant surface or nectar and honeydew and further through the host (WILLIAMS 2010). Moreover, insecticides may have sublethal effects such as a repellent action on parasitoids because the parasitic wasps are able to differentiate between treated and untreated plants (NEUMANN 2010). Consequently, treated plants are avoided, retention time of females on treated plants to search for hosts or food is reduced and ovipositor probing is also performed less. A recent study from 2017 by TAPPERT et al. found that the finding of a suitable host and the sexual orientation of the parasitoid wasp *Nasonia vitripennis* was highly impaired by the neonicotinoid imidacloprid. It was observed that females had problems in using male sex pheromones as cues to find mating partners resulting in a mating rate reduction by up to 80 %. Moreover, olfactory cues could no longer be used to detect their hosts (NEUMANN 2010). Nevertheless, the plot size of 300 m^2 may have been too small to detect actual deterrent effects of insecticides on parasitoids since parasitic wasps from field margins and untreated plots may have immigrated after the repellent effect of insecticides had faded.

It is well known that the main activity period of parasitoids of pollen beetles is between the late bud stage and the end of flowering (WILLIAMS 2010). Consequently, insecticide treatments before or during flowering against the important blossom insect pests have the greatest influence on parasitic wasps.

A study about the niche separation of pollen beetle parasitoids conducted by BERGER ET AL. 2015 outlined that *T. heterocerus* was active during the whole season with a preference for *B. aeneus* infested flower odors since the wasp favors to oviposit into large, second-instar larvae which they find in high numbers in open flowers.

Furthermore, BRANDES et al. (2017) found that parasitism of *B. aeneus* larvae by the parasitoid *T. heterocerus* was not found before full flowering and also JÖNSSON et al. (2015) proved that *T. heterocerus* is attracted to the odors of flowering of OSR. Consequently, it can be hypothesized that there is a great influence of conventionally applied insecticides during flowering stage of OSR on the parasitic wasp *T. heterocerus*.

The aim of this study was to verify if the innovative the dropleg technique in comparison to the conventional technique is of valuable benefit to parasitic wasps since insecticides are applied below the flowering part. When looking at the parasitism levels during the first three weeks of the present field experiment, different effects of the conventional and the dropleg technique on parasitization of *B. aeneus* larvae can be perceived (Figure 40). The parasitism levels in all treatments were decreased one day after application which may be explained by the increased dropping of yet unparasitized *B. aeneus* larvae due to insecticide application. However, levels in the dropleg treatments increased again to the same level as the control three days after application. On the contrary, both conventional treatments caused lower parasitism levels. The difference in the conventional thiacloprid treatment was even significant until seven days after application compared to the control. By using the dropleg technique, insecticides are not applied directly into the flowering part. Hence, it can be assumed that direct lethal effects of insecticides applied using the dropleg technique on parasitoids that search for host insects in the flowering part are lower compared to the conventional spraying technique.

Further, studies found a lower insecticide concentration in the nectar of OSR when insecticides were applied using the dropleg technique (WALLNER 2014), pointing out that systemic activity of products should not be overestimated. This statement is supported by the lower insecticide concentration in the flowering part after application using the dropleg technique, analyzed in this study (Figure 36). Thus, the reduced insecticide concentration in the nectar of OSR is most likely not only beneficial for honey bees but can also promote the conservation of parasitic wasps as biocontrol agents.

This suggestion is supported by a study of RUSCH et al. in 2013. The research team conducted an experiment about the nutritional state of *T. heterocerus* during its foraging in the field between emergence and throughout the season. By means of high-performance anion-exchange chromatography it was figured that this parasitoid emerges with a very low sugar amount which increases during its activity in the field. Consequently, the sugar concentration at the end of flowering was always higher than in the beginning, which indicates that *T. heterocerus* increases its sugar content by feeding on OSR nectar or flowers in the surrounding.

Besides the determination of the parasitism level of pollen beetle larvae by *T. heteroceurs*, the percentage of superparasitized larvae was also assessed. Even though *T. heterocerus* is a solitary parasitoid, superparasitism, i.e. more than one egg is deposited into the same host larva, is a frequently occurring phenomenon (WILLIAMS 2006). In the present study, *B. aeneus* larvae with up to seven black eggs of *T. heterocerus* were found. It is known, that the parasitic wasp cannot differentiate between already parasitized and not parasitized host eggs (NITZSCHE 1998). However, superparasitism is believed to be of benefit for the successful hatching of parasitoids. Actually, despite of parasitization, 9.1 % of pollen beetle larvae are able to suppress the development of the parasitoid and reach their final instar (NITZSCHE 1998). The underlying process is the encapsulation of the parasitoid eggs within the host larvae. However, the success of the encapsulation can be reduced by laying more than one egg into the host larva hence decreasing the host`s vitality (ALFORD 2003).

The beneficial effect of superparasitism was also observed in the present study. In the control, the superparasitism level was 65 % (Figure 42). It can be assumed that this high level is also responsible for the high mortality rate of dropped pollen beetle larvae which was assessed through the size of the new beetle generation (Figure 28). In comparison to the control, superparasitism levels were slightly reduced in all treatments. More precisely, thiacloprid conventionally applied reduced the superparasitism level by 32 % compared to the control (p=0.593). This finding can be related to the previously detected tendency of thiacloprid conventionally applied reducing both the parasitoid abundance and the parasitism level (Figure 41).

HEGAZI & KHAFAGI (2005) observed in their study about the interaction of the endoparasitoid *Microplitis rufiventris* and its host *Spodoptera littoralis* that the success of parasitoid development to its final instar increases with the amount of eggs laid into the host larva. Thus, it can be assumed that the success of *T. heterocerus* as natural biocontrol agent of the pollen beetle decreases within the years when Biscaya is conventionally applied due to the fact that superparasitism is reduced.

3.3 Conclusion and prospects

The innovative dropleg technique provided a comparable control of OSR insect pests as the conventional technique at the present field site in 2017. However, the cabbage seedpod weevil, which is one of the targeted blossom insect pests, occurred in small numbers, only. Therefore, no certain conclusion can be drawn about the efficacy of insecticides applied using the dropleg technique against cabbage seedpod weevils. It remains doubtful whether the low insecticide concentrations in the flowering part of OSR after application of systemically acting neonicotinoids (thiacloprid and acetamiprid) by using the dropleg technique are sufficient enough to control a pest that is mainly focused on the inflorescences. The efficacy of the dropleg technique needs to be evaluated at a different field site with a higher infestation level of *C. obstrictus*. Furthermore, seedpod weevils are not homogenously distributed within OSR fields wherefore water trays as evaluation tools are not sufficient to measure the actual infestation level of *C. obstrictus*.

The infestation of OSR plants by the brassica pod midge was moderate at the present field site in 2017. The number of larvae m^{-2} dropped down for pupation did not reveal differences between the application techniques. However, the insecticide Biscaya seemed to reduce larval dropping slightly more compared to Mospilan SG. The first pod assessment clearly showed a better effect of the insecticide Biscaya and the conventional application technique in terms of reducing the level of midge-infested pods. Nonetheless, the pod assessment seemed to be timed too early because of the delayed peak of *D. brassicae* larvae dropping down in the conventional Biscaya treatment wherefore the percentage of midge-infested pods might have been rated too low. For future experiments it seems reasonable to conduct more pod assessments around the peak of the first midge generation to detect the actual infestation level more accurately.

It is yet not fully understood how systemically acting insecticides applied using the dropleg technique work in terms of controlling insect pests that forage in the flowering part of OSR. A much lower insecticide concentration in the flowering part of OSR after application using the dropleg technique was verified which was also seen during the residue analysis of the present study. The latter, however, was not properly conducted wherefore it is recommended to repeat this residue analysis with repetitions to determine the actual concentration of insecticides in the flowering part after application using the dropleg technique. Furthermore, an evaluation of the spatial distribution of the brassica pod midges within the crop canopy needs to be conducted to clarify whether and at which conditions *D. brassicae* resides in the lower canopy of OSR and, further whether the midge forage in the inflorescence to take up insecticide residues. These clarifications may help to improve the setting of the accurate timing to apply insecticides using the dropleg technique to optimally expose brassica pod midges to insecticides.

Even though insecticide application during flowering is aimed at *C. obstrictus* and *D. brassicae*, the present study has proven that insecticide application at flowering stage of OSR also affects pollen beetles. The dropleg technique revealed slightly lower effects on pollen beetle larvae feeding in the upper plant part in comparison to the conventional technique.

Nevertheless, the combination of the eco-friendly dropleg application technique and the systemically acting neonicotinoids may positively contribute to the conflict between beekeepers and farmers coping with the consequences of insecticide residues in honey and the widespread pyrethroid resistance of pollen beetles. Besides various other studies, the present field trial also demonstrated that insecticides conventionally applied at flowering stage of OSR have a strong impact on the efficacy of naturally-occurring biocontrol agents such as parasitic wasps. Moreover, this study demonstrated that insecticides applied using the dropleg technique interfere less with the efficacy of parasitoids as biological control agents and offer a potential to conserve biocontrol agents to increase the efficacy of insect pest control. Nevertheless, it would be beneficial to test whether insecticide application below the flowering part of OSR influences parasitoids of stem-boring insect pests such as the cabbage stem weevil, and predatory beetles below the plant canopy.

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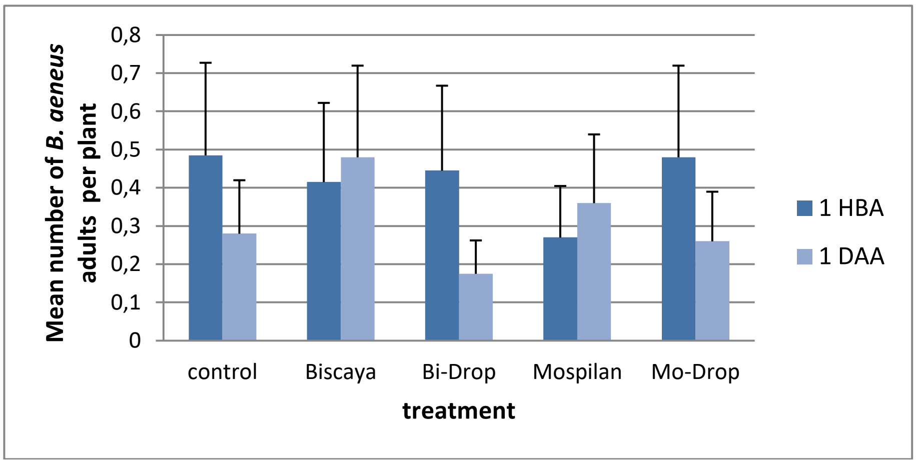
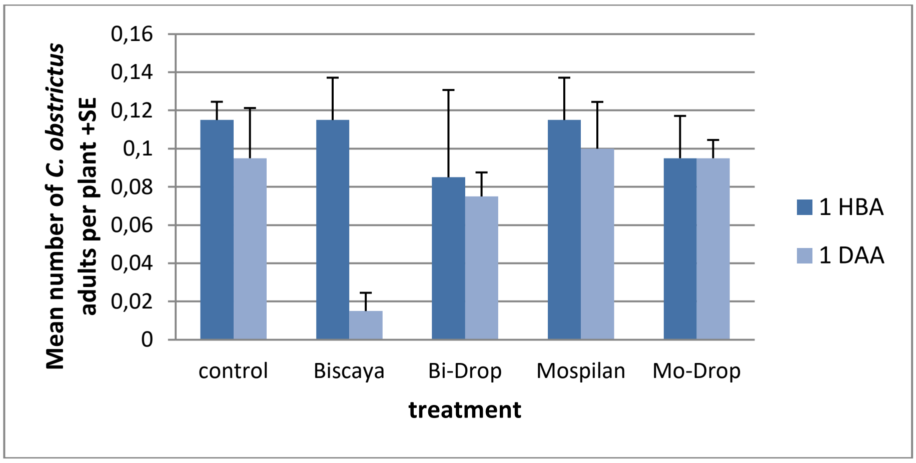
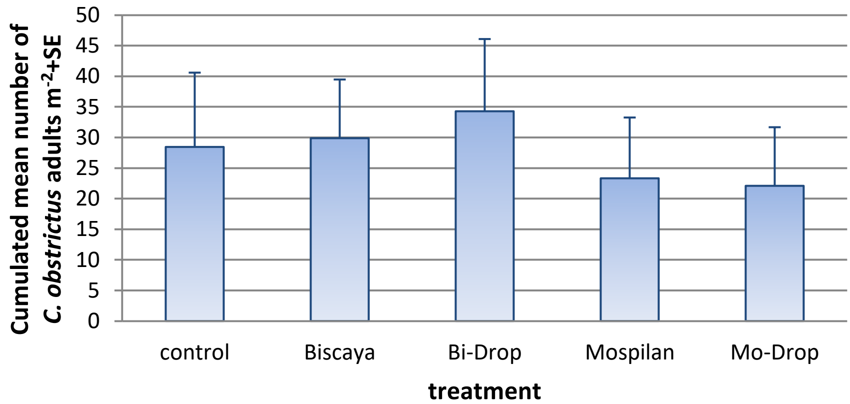
5 Annex



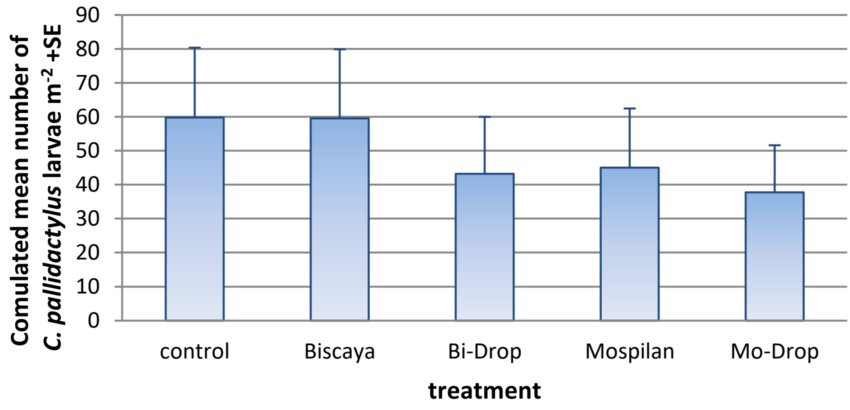
1: Mean number of *B. aeneus* adults per plant within the different treatments one hour before and a day after application. + SE



2: Mean number of *C. obstrictus* adults per plant within the different treatments one hour before and a day after application + SE



3: Cumulated mean number of *C. obstictus* m^{-2} within the different treatments over the sampling dates (16.06-10.07.2017) + SE



4: Cumulated mean number of *C. pallidactylus* larvae m^{-2} dropped down for pupation within the different treatments over the sampling dates + SE

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