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The challenge of targeted and effective insecticide treatment of flowering oilseed rape (*Brassica napus* L.) avoiding non-target effects on pollinators and ichneumon parasitic wasps by using dropleg technique



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**The challenge of targeted and effective insecticide treatment of flowering
oilseed rape (*Brassica napus* L.) avoiding non–target effects on pollinators
and ichneumon parasitic wasps by using dropleg technique**

Dissertation

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Submitted by

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Zusammenfassung

Raps (*Brassica napus* L.) wird von einer Vielzahl an Schadinsekten befallen und als Folge regelmäßig mit Insektiziden behandelt. Ab der Blüte treten der Kohlschotenrüssler (*Ceutorhynchus obstrictus* (Marsham)) und die Kohlschotenmücke (*Dasineura brassicae* (Winnertz)) in Erscheinung, deren Larvenstadien gemeinsam bis zu 30% Ertragsverluste verursachen können. Dabei gilt der Kohlschotenrüssler als Wegbereiter für einen starken Befall durch die Kohlschotenmücke. Aus diesem Grund richtet sich die Schadschwelle für eine Insektizid Behandlung nach dem Auftreten des Rüsslers zur Vollblüte. Es gibt jedoch kein integriertes Schädlingsmanagement, insbesondere in Bezug auf die Kohlschotenmücke. Die Applikation von Insektiziden während der Rapsblüte gefährdet potentiell Blütenbesucher und eine Vielzahl parasitoider Schlupfwespen, die zu dieser Zeit im Bestand aktiv sind. Rückstände von Pflanzenschutzmitteln können durch Honigbienen (*Apis mellifera* L.) aufgenommen werden und zu einer Belastung von Imkereiprodukten wie Honig oder Pollen führen. Mit der sogenannten Dropleg-Technik können Pflanzenschutzmittel räumlich platziert unter den Blütenhorizont ausgebracht werden. Dadurch können Pflanzenschutzmittelrückstände in Nektar und Pollen minimiert werden. Die innovative Applikationstechnik wird daher seitens der Imkerverbände begrüßt. Bislang fehlen aber Daten zur Wirksamkeit von Insektiziden nach Applikation mit Dropleg-Technik auf die Schotenschädlinge. Auch Nebeneffekte auf Nicht-Zielarten wie beispielsweise Parasitoide des Rapsglanzkäfers sowie des Kohlschotenrüsslers müssen untersucht werden.

In mehrjährigen Feldversuchen (2016–2020) wurde die Dropleg-Technik in der Umgebung von Braunschweig unter praxisüblichen Bedingungen in Großparzellen getestet und mit konventioneller Applikation von oben in den blühenden Pflanzenbestand verglichen. Dabei wurden Insektizide aus der Gruppe der Neonikotinoide (Biscaya (a.i. Thiacloprid, 72 g ha⁻¹) und Mospilan SG/SL (a.i. Acetamiprid, 40/42 g ha⁻¹)) und ein Pyrethroid (Mavrik (a.i. tau-Fluvalinat 48 g ha⁻¹)) jeweils zum Hauptflug der Kohlschotenmücke (BBCH 65–67) appliziert. Die Wirksamkeit der Insektizide auf Schotenschädlinge wurde mittels Auffangschalen untersucht, in denen von den Pflanzen fallende Adulte und Larvenstadien gefangen wurden. Zusätzlich wurden zweimal je Saison Schotenbonituren an ganzen Pflanzen durchgeführt. Effekte auf die Jungkäfergeneration wurden mit Hilfe von Photoektoren erhoben. Um mögliche

Nebeneffekte der Dropleg-Applikation auf Parasitoide abzuleiten, wurde die vertikale Verteilung der Rapsschädlinge und ihrer Parasitoide mithilfe eines tragbaren Saugers während der Blütezeit in den Jahren 2018 und 2019 untersucht. Parallel wurden in den Jahren 2016–2019 die Parasitierungsraten der Rapsglanzkäferlarven (*Brassicogethes aeneus* Fabricius) aus den Feldversuchen mit Dropleg-Technik ermittelt. In den Jahren 2019 und 2020 wurden zusätzlich die Parasitierungsraten der Kohlschotenrüsslerlarven erhoben.

Die Ergebnisse der Feldversuche haben gezeigt, dass die Wirksamkeit von Insektiziden nach einer Applikation mit Dropleg-Technik im Vergleich zu konventioneller Applikationstechnik etwas geringer war. Dies deckt sich mit den Untersuchungen zur vertikalen Verteilung der Schotenschädlinge, die zu 80% im Blütenhorizont gefangen wurden. Die vertikale Verteilung der Parasitoide war nicht unabhängig von der Pflanzenschicht und unterschied sich artspezifisch. In Bezug auf die Larvenparasitoide des Rapsglanzkäfers deutet sich eine Separierung der Arten *Phradis interstitialis* (Thomson) und *Tersilochus heterocerus* (Thomson) anhand räumlich getrennter Nischen an. Während *T. heterocerus* zu 84% im Blütenhorizont gefangen wurde, hielt sich *P. interstitialis* meist unterhalb des Blütenhorizonts auf. Die Parasitierungsraten der Rapsglanzkäferlarven durch *T. heterocerus* waren nach konventioneller Applikation in drei von vier Jahren in mindestens einer Variante des Feldversuches reduziert. Der Einsatz der Dropleg-Technik führte dagegen nur im Jahr 2019 einmalig zu einer Reduktion der Parasitierung im Vergleich zur Kontrolle. Die Stärke der Effekte lässt sich mit der Koinzidenz zwischen dem Zuflug der Parasitoide und dem Applikationstermin begründen. Bezüglich der Larvenparasitierung des Kohlschotenrüsslers wurden durchschnittliche Parasitierungsraten von 60% festgestellt. Nur im Jahr 2019 traten Behandlungseffekte nach der Applikation von Biscaya auf, wobei schwache, positive Effekte der Dropleg Applikation auf die Art *Mesopolobus morys* (Walker), nicht jedoch auf *Trichomalus perfectus* (Walker) gefunden wurden.

Die Ergebnisse zeigen, dass es bezüglich des Einsatzes der Dropleg-Technik in blühendem Raps einen Trade-off zwischen einer abfallenden Wirkung der Insektizide auf Schotenschädlinge einerseits und der Schonung bestimmter Parasitoide andererseits vorliegt. Grundsätzlich kann die Applikation von Insektiziden zur Rapsblüte notwendig sein, um Ertragsausfälle zu verhindern. Es besteht jedoch der Bedarf an verbesserten

Monitoring Methoden für die Kohlschotenmücke. Dies würde die Entwicklung von Schadschwellen ermöglichen und helfen nicht notwendige Behandlungen im Vorfeld zu unterlassen.

Abstract

Oilseed rape (*Brassica napus* L.) is damaged by plenty of insect pests and is therefore regularly treated with insecticides. During the flowering period, the cabbage seedpod weevil (*Ceutorhynchus obstrictus* (Marsham)) and the brassica pod midge (*Dasineura brassicae* (Winnertz)) migrate into the crop. Their larvae can cause crop damages of up to 30%. In this context, the cabbage seedpod weevil serves as a precursor for a strong infestation by the brassica pod midge. Therefore, the threshold for an application of insecticides is based on the extent of the cabbage seedpod weevil infestation at full flowering stage. Yet, there is a lack of integrated pest management strategies, especially regarding the brassica pod midge. The application of insecticides during the flowering of oilseed rape potentially endangers pollinators and many parasitoid ichneumon flies, which are present in the crop during this time. Residues of pesticides can be ingested by honey bees (*Apis mellifera* L.) and may result in contamination of bee products such as honey or pollen. With the so-called dropleg technique, pesticides can be applied spatially targeted below the flowering canopy of the crop. Thereby, pesticide residues in nectar and pollen can be minimized and beekeepers' associations promote this innovative application technique. However, there has been a lack of data on the effectiveness of insecticides applied using the dropleg technique on pod damaging insect pests. In addition, non-target effects on important parasitoids of the pollen beetle (*Brassicogethes aeneus* Fabricius) and the cabbage seedpod weevil need to be studied.

In five years of field trials (2016–2020), the dropleg technique was tested near Braunschweig, Germany, under standard practical conditions in large-scale plots and compared with conventional application from above into the flowering plant stand. The applications of insecticides from the group of neonicotinoids (Biscaya (a.i. thiacloprid, 72 g ha⁻¹) and Mospilan SG/SL (a.i. acetamiprid, 40/42 g ha⁻¹)) and a pyrethroid (Mavrik (a.i. tau-fluvalinate 48 g ha⁻¹)) were carried out at the peak flight of the brassica pod midge (BBCH 65–67). The efficacy of the applied insecticides on pod damaging insect pests was investigated using water trays to collect larvae and adults dropping from the plants. Additionally, full plants were examined twice in each season for damaged pods. Effects on the new generations of insect pests were examined using photoelectors. In order to derive possible side effects of the dropleg application on parasitoids, the vertical distribution of the oilseed rape insect pests and their parasitoids was investigated with the

aid of a portable aspirator during the flowering period in 2018 and 2019. In parallel, the parasitisation rates of pollen beetle larvae in the dropleg field trials were assessed from 2016–2019. Additionally, in 2019 and 2020, the parasitisation rates of cabbage seedpod weevil larvae were determined.

The results showed, that the effectiveness of insecticides applied with dropleg technique in the field trials was slightly reduced compared to conventional application technique. This is in accordance with the investigations on the vertical distribution of pod damaging insect pests, which were present in the flowering canopy at 80% of all cases. The vertical distribution of parasitoid species was not independent from the crop layers and differed species-specific. With regard to the larval parasitoids of the pollen beetle, hints for a niche separation of key species *Phradis interstitialis* (Thomson) and *Tersilochus heterocerus* (Thomson) was found. The latter was abundant at 84% of all cases in the flowering canopy, whereas *P. interstitialis* was collected mostly below the horizon of pods and open flowers. In the field trials, parasitisation rates of pollen beetle larvae by *T. heterocerus* were significantly reduced after conventional application in three out of four years. The use of dropleg-technique resulted in reduced parasitisation rates compared to the untreated control only in the year 2019. The strength of the observed effects can be explained by the coincidence between the immigration of the parasitoids into the crop and the date of application. Regarding the cabbage seedpod weevil, average parasitisation rates of the larvae were about 60%. Only in 2019, treatment effects occurred after the application of Biscaya and a weak, positive effect of dropleg application on the species *Mesopolobus morys* (Walker), but not on *Trichomalus perfectus* (Walker), was found. The evaluation shows, that there is a trade-off between a slightly reduced efficacy on pod damaging insect pests on the one hand and benefits for certain parasitoid species on the other using dropleg technique for the application of insecticides in flowering oilseed rape. In general, insecticides applied during the flowering period of oilseed rape can be crucial in preventing yield losses. However, there is a need of improved monitoring methods for the brassica pod midge. This would help to develop damage thresholds and to prevent superfluous applications in advance.

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Chapter I

General introduction

Oilseed rape (*Brassica napus* L.) is a rather young crop species (Mason and Snowdon 2016), whose origin, however, goes back to a hybridization of *B. rapa* L. and *B. oleracea* L. about 7500 years ago (Chalhoub et al. 2014). In Europe, the cultivation of oilseed rape can be traced back to the 13th century, when rapeseed oil was used as lamp oil (Frieß et al. 2020). Due to successful breeding of varieties with low contents of aliphatic glucosinolate and erucic acid, the nutritional value of oilseed rape was significantly improved (Downey 1990). Today, rapeseed oil is used for human consumption, cosmetic and pharmaceutical applications, and the meal is used as animal feed (Rivera et al. 2016; Sarwar 2013). Additionally, oilseed rape is processed to biodiesel or lubricants for industrial machinery (Friedt and Snowdon 2010). Due to its wide range of uses, oilseed rape is currently the second most important oilseed in arable farming after soybean (*Glycine max* L.) (<http://www.fao.org/faostat/>), with a total cultivation area of approx. 20.4 million hectares worldwide in 2019. The largest cultivation areas are in Canada and China, followed by the EU and Australia. Average yields are about 2000 kg ha⁻¹ in Canada and China, 1300 kg ha⁻¹ in Australia and highest yields are realized in France, Germany and the UK with 3500 kg ha⁻¹ (average of the years 2010–2019, data: FAOSTAT).

As the genetic origin of oilseed rape is Mediterranean Europe (Dixon and Dickson 2007), it is not surprising that a diverse community of herbivorous insects is damaging the crop in Europe (Alford 2003). Thus, the protection of oilseed rape from herbivorous insect pests was always a major issue and there are already detailed descriptions of different pest insects from the 19th century (Nördlinger 1869; Speyer 1925; Winnertz 1853). But still today, 150 years later, the control of insect pests in oilseed rape production is a major challenge for farmers (Andert and Ziesemer 2021; Zheng et al. 2020). Over time, a broad range of methods have been developed to get control of the pest insects e.g. mechanical devices to collect insects (Buhl and Meyer 1937), the growing of trap crops (Buntin 1998; Thöming et al. 2020), resistance breeding (Austel et al. 2021; Hervé 2018) or the use of entomopathogenic fungi (Kaiser et al. 2020). It is also known that all stages of pest insects are hosts to a large number of parasitoids. In Europe more than 80

parasitoid species of oilseed rape pest insects are abundant (Ulber et al. 2010b). The enhancement of natural pest regulation by parasitoids is an important indirect control measure, as some parasitoid species have been shown to have significant effects on pest population dynamics (Büchi 2002; Hokkanen 2008; Ulber et al. 2010b; Veromann et al. 2010). However, the mentioned control measures have in common that they are not very efficient in reducing acute pest pressure. For this reason, the application of insecticides remains the most important measure of crop protection in case of acute infestations as it is fast and highly effective (Andert and Ziesemer 2021; Kathage et al. 2018; Zhang et al. 2017). However, the active ingredients approved for insect pest control in oilseed rape in the European Union are limited to a few modes of action (Zheng et al. 2020). The selection pressure on available mode of actions, especially on pyrethroids, is high and there is increasing insecticide resistance that is related to many pest species in oilseed rape nowadays (Brandes and Heimbach 2019; Heimbach and Müller 2013; Spitzer et al. 2020; Willis et al. 2020). In order to be approved in the EU, insecticides must meet the requirements of being preferably selective, having low persistence in the environment and being safe for humans and non-target organisms, in addition to their effectiveness in controlling pests (European Union 2009). Furthermore, insecticides are in focus, as there are concerns about a decline in insect biodiversity in agricultural landscapes (Seibold et al. 2019; van Klink et al. 2020). The use of insecticides in agriculture is considered a possible cause (Tooker and Pearsons 2021). Although this aspect has been insufficiently researched so far, the European Commission has already announced the target to reduce the use of pesticides by 50% by 2030 (European Commission 2020). On the other hand, oilseed rape cannot be grown economically profitable in most years without chemical crop protection, which can be concluded from a comparable high treatment index (6.81 from 2011–2019) (Julius Kühn-Institut 2021). This is also reflected in the fact that oilseed rape is hardly cultivated in organic farming in Germany (3445 ha in 2020: < 0.5% of total organic farming area (DESTATIS 2021)). In current crop rotations, oilseed rape is an outstanding mass flowering crop that provides nutrient sources for a wide range of pollinators including the honey bee (*Apis mellifera* L.) (Carruthers et al. 2017; Genersch et al. 2010; Williams 1980). With regard to the abundance and diversity of pollinators, the density and spatial distribution of oilseed rape can enhance the early growth of bumblebee colonies and bumblebee densities at landscape scale (Westphal et al. 2003; Westphal et al. 2009) and the abundance and fitness of solitary wild bees (Holzschuh et

al. 2013; van Reeth et al. 2018). In summary, oilseed rape is a versatile and ecologically valuable crop, but its protection against pest insects is becoming increasingly difficult. High insect pest pressure and missing control options are discussed as one reason for recent declines of oilseed rape cultivation area in important growing countries like Germany (Andert et al. 2021) or the UK (Scott and Bilsborrow 2019).

Despite all the controversy in the scientific, social and political debates, stakeholders from practical farming and crop protection as well as opponents of chemical crop protection should share at least the same interest, namely the avoidance of unnecessary insecticide sprays. Following the principles of integrated pest management (IPM), future insecticide applications should possibly be environmentally friendly and economically justified. This is the background to the present thesis, which investigates sustainable control strategies of pod damaging insect pests in flowering oilseed rape using agrochemicals and a new application technique. These pests are the cabbage seedpod weevil, *Ceutorhynchus obstrictus* Marsham, and brassica pod midge, *Dasineura brassicae* Winnertz. Both are widely distributed throughout Europe and the weevil is a major pest in canola production in North America (Alford et al. 2003). Potential damage can be as high as 30% if both pest species occur in the same field. The migration of the species into the oilseed rape crop starts with the beginning of flowering. The cabbage seedpod weevil has about two weeks of maturation feeding on flowers and young pods (Kirchner 1961). After that, the females start laying eggs, for which they eat small holes in the pods into which they then lay individual eggs (Ferguson and Williams 1991). The brassica pod midge also oviposits inside pods, however, it can only pierce young, soft tissue with its ovipositor and is otherwise dependent on existing holes and damaged pods. Therefore, the cabbage seedpod weevil is regarded as the major provider of suitable pods for brassica pod midge (Free et al. 1983). The larvae of the cabbage seedpod weevil feed on the seeds whereas the larvae of *D. brassicae* feed on the pod walls of oilseed rape. Through the release of enzymes, the latter causes deformation and premature splitting of the pods (Meakin and Roberts 1991). Damage prognosis is based on the numbers of seedpod weevils per main shoot during flowering. The existing damage threshold is lowered, if damage by the midge was observed in previous years. Although the midge itself is counted among the six most important pests in oilseed rape cultivation (Alford et al. 2003), there has been little research into more targeted damage prediction and integrated control in the past 20 years. To control the pest complex of *C. obstrictus* and

D. brassicae, currently, only insecticides from the class of pyrethroids are approved in Germany. The active ingredient thiacloprid (systemic acting neonicotinoid) has been widely used for applications against the pod damaging pest insects in flowering oilseed rape until its ban of application in 2020. In addition to insecticides, the crop is treated regularly with fungicides during the flowering period to prevent yield losses due to *Sclerotinia* stem rot, which is caused by the phytopathogenic fungus *Sclerotinia sclerotiorum* (de Bary). Pesticide residues in pollen, nectar and honey typically reflect the active ingredients that are used for applications in flowering oilseed rape (Genersch et al. 2010; Karise et al. 2017). Thereby, for example thiacloprid has a comparable low toxicity for bees (Iwasa et al. 2004). However, individual studies have shown, that sub-lethal doses of thiacloprid have an effect on the orientation of foragers (Tison et al. 2017) and interact with the sensitivity of honey bees to the intestinal parasite *Nosema ceranae* (Vidau et al. 2011). Though, there is no evidence of adverse effects of chronic exposure of honey bees to field-realistic sub-lethal concentrations of thiacloprid on the performance of whole colonies and their overwintering success (Odemer and Rosenkranz 2020; Siede et al. 2017). Still, the use of pesticides during the flowering period harbours potential for conflict, especially between agriculture and beekeeping (Barnett et al. 2007). In addition to pollinators, many antagonists of pest insects e.g. ichneumon parasitic wasps are present in the crop during flowering period of oilseed rape (Ulber et al. 2010a). Parasitoids find their hosts, mainly the larval stages of the pests, in various plant parts such as the stems, but also in the flowers and later in the pods. And at least for one important pollen beetle parasitoid, it has been documented that the species also feeds on nectar of OSR (Rusch et al. 2013). Therefore, not only the targeted pests but also parasitoids can be directly or indirectly harmed by insecticide applications during the flowering period (Ulber et al. 2010a).

The so-called dropleg technique offers the opportunity for a spatial targeting of plant protection products, as the nozzles are attached to prolonged nozzle holders, which can be guided through lower crop layers (Brandes 2021) (Fig. 1). In Germany, the Julius Kühn-Institut (JKI) approved a dropleg system for the use in flowering oilseed rape for the first time in 2016 (official examination number G1994). Nozzles with 90% drift reduction are also approved for the system in Germany, which is an important prerequisite for a broader use in arable field crops. Technically, most field sprayers can be easily equipped with droplegs. However, there are problems with some type of machinery e.g.



sprayers folding the boom narrow over the wheelhouse (Brandes 2021). Originally, the technique was developed for fungicide applications in vegetables. The use of dropleg technique for applications in flowering oilseed rape was investigated within the “FITBEE” project (2011–2015) at the University of Hohenheim. The technique was shown to be suitable to significantly reduce pesticide residues in pollen and nectar (FIT BEE 2015; Wallner 2014). For this purpose, the nozzles are guided below the horizon of open flowers. In contrast to conventional application, active ingredients are mainly deposited on the leaves and stems of the plants and the flowering part of the crop is almost free of active ingredients. This is the main reason, why its use in oilseed rape is discussed and promoted by beekeepers.

Although minimizing side effects on non-target organisms is desirable, the effectiveness

of a pesticide application is the first consideration for its use in agricultural practice. This requires sufficient exposure of the target organisms to the active ingredients. In the case of the fungal disease *Sclerotinia* stem rot, ascospores infect plants often where petals have fallen onto leaves or leaf axils (Derbyshire and Denton-Giles 2016). Accordingly, fungicides applied in field trials using dropleg technique showed no differences in efficacy compared to conventional application (Dicke 2018; Haberlah-Korr et al. 2018)., Regarding pod damaging pest insects on the other hand, plant tissues like buds, flowers and young pods are not longer sprayed with insecticides using dropleg technique. Whether pest insects nevertheless come into sufficient contact with the active ingredients could not be clarified in initial studies on the control of *Sclerotinia* stem rot using dropleg technique. Because agrochemicals such as insecticides can disturb natural pest regulation, the effects of a spatial targeted application on parasitoids is of importance and needs to

be investigated. Whether there are any differences in effects on important parasitoids by using dropleg technique is not known so far.

Aims and Scopes

The main objectives of this thesis are:

- A comparison of the effectiveness of insecticides applied with conventional and dropleg application technique on the pod damaging pest insects *C. obstrictus* and *D. brassicae*. There is a gap in knowledge regarding if and where pest insects are exposed to the active ingredients after an application using dropleg technique.
- An assessment of non-target effects of insecticides, applied with dropleg technique, on parasitoids of the pollen beetle (*Brassicogethes aeneus* Fabricius) and the cabbage seedpod weevil, which have not yet been tested under field condition. It was assumed that parasitoids searching for their host larvae in the upper crop canopy containing flowers, buds and pods might benefit, if these crop layers are no longer treated with insecticides. Furthermore, it was assumed that non-target effects may interact with the mode of action of insecticides and the application technique. In addition, there is limited information about the vertical distribution of parasitoids in the flowering oilseed rape crop.
- An analysis of the status of integrated pest management of the brassica pod midge. There is no comprehensive review of the current and historic literature on *D. brassicae*. It is expected that this will help to identify important knowledge gaps related to biology and allow targeted research to further improve integrated control of the midge.

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Chapter II

Effects of dropleg application technique during flowering of oilseed rape on insect pests

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Abstract

Spraying of insecticides and fungicides during the flowering stage of oilseed rape (*Brassica napus* L.) could be necessary to control insect pests (such as *Ceutorhynchus obstrictus* (Marsham) and *Dasineura brassicae* (Winnertz)) and pathogens (*Sclerotinia sclerotiorum* (Lib.) de Bary). In contrast to the conventional application technique, dropleg nozzles are kept below the flowering canopy to restrict the exposure of pollinators to active ingredients of insecticides. The Julius Kühn-Institut (JKI) tested the efficacy of different insecticides using the dropleg technique compared to the conventional technique, on insect pests in field trials conducted from 2015 to 2017 in the area of Braunschweig. The crop was treated at full flowering (BBCH 65) for three consecutive years. The tested insecticides were Biscaya (72 g a.i. ha⁻¹ thiacloprid), Mavrik (48 g a.i. ha⁻¹ tau-fluvalinate), and Mospilan SG (40 g a.i. ha⁻¹ acetamiprid). The adults and larvae of oilseed rape pests that drop to the ground were collected using water trays. The emergence of new generation beetles (*Brassicogethes aeneus* (Fabricius), *C. obstrictus*) was recorded from BBCH 78 onwards, using photoelectors. At least twice during each season, oilseed rape plants were assessed for *D. brassicae* injury. The new dropleg application technique showed similar efficacy on pest insects compared to the conventional spraying technique. Nevertheless, the abundance of pests was not always sufficient to get clear results. Results especially on *D. brassicae* indicate that the dropleg technique had a comparable efficacy under moderate pest pressure conditions observed in our study. Further research under conditions with a higher abundance of *C. obstrictus*,

as well as research on the effects of dropleg technique on parasitoids of oilseed rape pests, is needed. The population of *B. aeneus* population was reduced slightly less using dropleg instead of conventional application technique. In all the years, the yields did not differ significantly with regard to the conventional and dropleg application techniques.

Introduction

Oilseed rape is an important crop in German agriculture with an average cultivation area of 1.3 million hectare in recent years (Statistisches Bundesamt, 2017). During flowering, the crop can be infested by insect pests, namely the cabbage seed weevil *Ceutorhynchus obstrictus* (Marsham) and the brassica pod midge *Dasineura brassicae* (Winnertz). If both species occur in the same field, they can cause great damage and up to 30% of yield losses (Hoffmann and Schmutterer, 1999). The economic threshold for cabbage seed weevil is one weevil per plant at full flowering, which drops to 0.5 weevils per plant if damage by brassica pod midge is expected (Lauenstein, 1993; Ramsden et al., 2017). For brassica pod midge, the threshold of one midge per plant at flowering (Lauenstein, 1993) is challenging for farmers to implement, since midges are tiny and difficult to identify in a field. Farmers monitor the abundance of the seedpod weevil, however insecticides targeting brassica pod midge are frequently applied without considering its density on the crop. In some years and regions, insecticide treatments are necessary for both insect pests to prevent crop damage. During flowering of oilseed rape, honeybees and other pollinators collect pollen and nectar in the fields. Plant protection products used in oilseed rape are often found as residues in honeybee products (Karise et al., 2017; Wallner, 2014b). Hence, the application of pesticides during flowering can lead to potential conflicts between farmers, beekeepers, and consumers. In addition, pollinators and also other beneficial arthropods, including parasitoids, may be affected lethally or sub-lethally by insecticides (Mänd et al., 2010). Percent parasitism of insect pests by parasitoids in oilseed rape often ranges between 20 and 50% but it can exceed 80% in some cases (Ulber et al., 2010), with many species abundant in the field during flowering. The dropleg technique provides an opportunity to improve pollinator protection (Lechler, 2017). In comparison to the conventional application technique, the nozzle carriers are not directly attached to the boom but to the so called droplegs. With a length of 90 cm, the droplegs hang down into the crop canopy and are guided below the flowering canopy during spraying. The spray of the double flood nozzles is directed to

the ground and sideways. Droplegs can be fitted to most conventional sprayers. In 2016, JKI approved the use of this technique in the flowering stage of oilseed rape in Germany (Julius Kühn-Institut, 2016). Compared to the conventional application technique, using the dropleg technique can reduce the residue level of plant protection products in flowers, nectar, and honey (Wallner, 2014b). The droplegs can be fixed at different distances on the boom and applications in different crops are possible (e.g. targeted fungicide application in vegetables, late herbicide applications in maize, or applications of liquid fertilizers). Furthermore, the droplegs may extend the window of spray opportunities because the spray liquid within the vegetation layer is less prone to drift (Weimar-Bosse et al., 2017). In contrast, the costs of additional investment for this spraying technique and further requirements concerning the sprayer operator need to be assessed. For example, the boom of the sprayer has to be raised before reversing and visual control of the nozzles during the application is not possible.

The objective of this work was to evaluate the efficacy of the dropleg technique compared to conventional foliar sprays on insect pests, as spraying below the flowering part of the plant canopy may reduce the efficacy of insecticides on insect pests damaging the pods. While effects on fungal diseases such as sclerotia stem rot have been shown to be comparable between both application techniques (Dicke, 2018, 2017; Haberlah-Korr, 2016), only limited data are available on the effects of dropleg applications on insect pests (Heimbach et al., 2016b).

Material and Methods

Field trials were conducted near Braunschweig (52°19'40'' N, 10°37'54'' E) from 2015 to 2017. The climate conditions in this area are suitable for arable crops; the average temperature is 8.8 °C with an average precipitation of 620 mm. The winter oilseed rape cultivar Visby was sown at the end of August in a mulch tillage system with row spacing of 12.5 cm and a plant density of 55 kernels m⁻² in each of the years. All trials had a randomized complete block design with 20 plots divided in four blocks containing once each of the five treatments. Plot size was 240 m² (20 m x 12 m) in 2015 and 2017 and 300 m² (25 m x 12 m) in 2016. In each year, treatments included an untreated control and two insecticide treatments, each applied with either a conventional spraying technique or the dropleg technique. Treated plots received a single application each year of a mixture of an insecticide and a fungicide. From 2015 to 2017, the neonicotinoid insecticide

Biscaya (72 g ha⁻¹ a.i. thiacloprid) was used. In addition, the pyrethroid Mavrik (48 g ha⁻¹ a.i. tau-fluvalinate) was used in 2015. In 2016, 2017, instead of Mavrik, the neonicotinoid Mospilan SG (40 g ha⁻¹ a.i. acetamiprid) was tested (Tab. 1). Each insecticide was sprayed in a tank mixture with a fungicide to control *Sclerotinia sclerotiorum*. The control plots remained untreated except in 2017 when a fungicide was applied.

Table 1 Days of application and crop growth stages in the field trials 2015–2017. Additional, application rates of tank mixtures in the different treatments

Year	2015	2016	2017
Day of application	08 th May	10 th May	15 th May
Crop Stage	BBCH 65	BBCH 65	BBCH 67
Control	no application	no application	Propulse
conventional Biscaya	Biscaya + Cantus Gold	Biscaya + Efilor	Biscaya + Propulse
conventional Mospilan	Mavrik + Cantus Gold	Mospilan + Efilor	Mospilan + Propulse
Dropleg Biscaya	Biscaya + Cantus Gold	Biscaya + Efilor	Biscaya + Propulse
Dropleg Mospilan	Mavrik + Cantus Gold	Mospilan + Efilor	Mospilan + Propulse
Company	Product name	Application rate and active ingredient	
Bayer Crop Science	Biscaya	72 g ha ⁻¹ thiacloprid	
Adama	Mavrik Vita	48 g ha ⁻¹ tau-fluvalinate	
FMC Cheminova	Mospilan SG	40 g ha ⁻¹ acetamiprid	
BASF	Cantus Gold	100 g ha ⁻¹ boscalid + 100 g ha ⁻¹ dimoxystrobin	
BASF	Efilor	133 g ha ⁻¹ boscalid + 60 g ha ⁻¹ metconazol	
Bayer Crop Science	Propulse	125 g ha ⁻¹ prothioconazol + 125 g ha ⁻¹ fluopyram	

Each plot was surrounded by an untreated crop area of at least 12 m on either side. The plant growth stages were described according to Lancashire et al. (1991). The application was carried out at the full flowering stage (BBCH 65–67) with a driving speed of 7 km h⁻¹ and a pressure of 2.8 bar using water volumes of 300 L ha⁻¹. The conventional spraying was done with IDKN 120-4 nozzles usually held 50 cm above the crop. The crop height varied between 1.20 and 1.30 m at BBCH 65. The droplegs were guided at a depth of 40–50 cm below the horizon of open flowers in the crop. Shortly after the flowering stage, the driving lines were mulched leaving a lengthwise path free of plants through the centre of each plot.

A day before application, six water trays (Backs, Rehburg, 0.06 m²) were placed at the ground in the middle of each plot to catch the adult and larval stages of insect pests dropping from the plants. To conserve the catch, the trays were filled with a 10% sodium benzoate water solution, which was replaced weekly. Except for the year 2016, the six water traps were counted individually for the first four sampling dates. In 2015, the number of traps per plot was halved two weeks after the application (22.05.2015). In the field, the content of every three traps of a plot was processed through a sieve (mesh size 0.25 mm) and transferred to the laboratory in separate vessels for each sampling date. In the year 2016 and from the 5th sampling date in 2017, the contents of each three traps per plot were pooled, resulting in two subsamples per plot. Counting and identification of the species was done in the laboratory using a binocular microscope (10-fold magnification). The water traps were emptied 1 h after application; one, three, and seven days after application; and then weekly up to BBCH 87. There were 13, 11, and 11 sampling dates in 2015, 2016, and 2017, respectively. The larvae of the pollen beetle were stored in 70% ethanol and later parasitism rates of *Tersilochus heterocerus* (Thomson) were determined according to Brandes et al. (2017). For this, the larvae were pinned with insect needles and dissected under the binocular microscope (10–20-fold magnification) and the number of dark pigmented parasitoid eggs was counted. In 2016, 2017, after pupation of larvae in the soil, new generation insect pests were trapped with photoelectors. In each plot, two photoelectors (ecoTech, Bonn, 0.25 m²) were installed and closed after the larvae of *Ceutorhynchus* spp. had dropped to the ground for pupation (between BBCH 75 and 78). The plants were retained within the closed photoelectors to allow further larval development. The emerging adults were collected in perforated polythene bags that were tied to the top of the electors and replaced twice a week from BBCH 80 until harvest day (Heimbach et al., 2016a). At least twice each season, the pod infestation was examined, namely after the first (about BBCH 75) and second generation larvae (about BBCH 82–85) of brassica pod midge had finished development. To minimize the experimental error of assessing infested pods close to harvest, which is when they tend to open accidentally, the second assessment in 2017 was carried out earlier at BBCH 80 although the final damage by brassica pod midge might have been underestimated. Since there was a large variability in the data, the number of assessed plants per plot was raised from five in 2015 to 12 in 2016–2017. The plants were collected in the centre of each plot, samples were taken from four randomly chosen sample points in the years 2016 and 2017. The number

of infested and not infested pods per plant was determined. The mature plants were harvested at 40 m² (2015/17) and 50 m² (2016) in the centre of each plot with a plot combine harvester (Haldrup C85). The yield, grain weight, and yield parameters such as plants per square meter were determined.

Statistical analysis

Statistical analysis was done with R (version 3.3.2) (R Core Team, 2016) in the graphical user surface R Studio. As a basis model a generalized linear mixed effects model (glmer) was calculated using the package lme4 (Bates et al., 2015).

Every insect pest along with its different development stages was analysed separately for each year. The basic models were executed with treatment and sampling date as fixed explanatory variables and treatment nested within the block was added as random effect.

(mod: $y \sim \text{treatment} * \text{sampling date} | (1 | \text{block} : \text{treatment})$).

For data with only one sampling date (pod infestation by cabbage seedpod weevil) only treatment was considered as fixed effect. For count data, a Poisson distribution was assumed. If the models were overdispersed, a negative binomial model was fitted. For the data of pod infestation, a binomial distribution was chosen. The selection of the best model was based on backward elimination of the full model by a comparison of the Akaike Information criterion (AIC), where the model with the lowest AIC indicates the best fit (Burnham and Anderson, 2002). With the exception of the pod infestation data in 2017 (Tab. 2), the parasitism data (Fig. 7) and the data for the new generation seedpod weevils (Tab. 3), the full model always provided the best fit. For model diagnostics the deviance residuals were plotted versus predicted values. The post-hoc analysis was done using the *emmeans* package (Lenth et al., 2018). Estimated marginal means, 95% confidence intervals and posthoc tests for pairwise comparisons at an alpha level of 0.05 with *p*-value adjustment by the Tukey method were executed. For the analysis of yield data an analysis of variance was conducted using a linear model with treatment and block as explanatory variables.

Results

Dasineura brassicae and *Ceutorhynchus obstrictus*

Dasineura brassicae occurred in all three years. In each year, significant differences were observed at the first assessment of pods infested by larvae of the first generation of *D. brassicae* (Tab. 2). In this early assessment, the application of Biscaya significantly reduced the proportion of infested pods, independent of the application technique. However, spraying with dropleg technique was less effective than conventional application in 2015 and 2016. The same tendency occurred using Mavrik in 2015 and Mospilan in 2016, but differences were not statistically significant. The pod infestation rate at the last assessment before harvest was highest in 2015 (BBCH 87) with approximately 11% infested pods in control plots; approximately 8% of the pods in 2016 (BBCH 85), and only 4% in 2017 (BBCH 80) were infested (Tab. 2). In 2015, only the conventional application of Biscaya significantly reduced the level of infested pods ($p = 0.005$). In 2016, 2017, the differences between the treatments at the second assessment were not significant.

Table 2 Pod infestation per plant (%) by *Dasineura brassicae* after the flight of the first generation (2015 = BBCH 80, 2016 = BBCH 76, 2017 = BBCH 75) and after the flight of the second generation (2015 = BBCH 87, 2016 = BBCH 85, 2017 = BBCH 80) for different insecticides, applied with conventional and dropleg spraying techniques. Observed data and estimated means + 95% confidential intervals obtained from the best model. Different letters within a column and year indicate significant differences, Tukey-HSD ($p < 0.05$)

Day of application	Treatment	first assessment		second assessment	
		observed	estimate means (95% CI)	observed	estimate means (95% CI)
08 May 2015		BBCH 80		BBCH 87	
	Control	4.49	4.06 (3.14–5.22) a	10.94	10.27 (7.98–13.12) a
	conventional Biscaya	0.74	0.83 (0.61–1.14) b	5.10	5.20 (3.87–6.95) b
	Dropleg Biscaya	1.93	1.90 (1.44–2.51) c	6.83	6.63 (5.07–8.65) ab
	conventional Mavrik	1.95	1.90 (1.44–2.51) c	10.60	10.06 (7.83–12.84) a
	Dropleg Mavrik	2.46	2.31 (1.76–3.03) c	7.55	7.34 (5.61–9.54) ab
10 May 2016		BBCH 76		BBCH 85	
	Control	1.86	1.97 (1.66–2.32) a	7.68	6.90 (6.02–7.89) a
	conventional Biscaya	0.63	0.63 (0.49–0.81) b	6.17	5.83 (5.06–6.70) a
	Dropleg Biscaya	1.43	1.34 (1.12–1.60) c	7.66	7.18 (6.28–8.20) a
	conventional Mospilan	1.18	1.43 (1.20–1.71) cd	6.82	5.91 (5.14–6.78) a
	Dropleg Mospilan	1.68	1.80 (1.53–2.13) ad	6.85	6.38 (5.57–7.29) a
15 May 2017		BBCH 75		BBCH 80	
	Control	1.45	1.57 (1.16–2.13) a	3.69	3.27 (2.45–4.34) a
	conventional Biscaya	0.37	0.36 (0.24–0.53) b	4.21	3.71 (2.79–4.90) a
	Dropleg Biscaya	0.66	0.74 (0.53–1.05) bc	3.50	3.05 (2.28–4.06) a
	conventional Mospilan	1.39	1.29 (0.94–1.78) ac	3.96	3.73 (2.21–4.93) a
	Dropleg Mospilan	1.04	1.07 (0.78–1.47) ac	3.05	2.86 (2.14–3.80) a

In 2015, the cumulative number of *D. brassicae* larvae of all generations dropping to the soil for pupation was about 6500 larvae m⁻². In the first week of larval migration,

the conventional application of Biscaya reduced the numbers 90-fold compared to the control and 28-fold in the second week ($p < 0.001$) (Fig. 1). The dropleg Biscaya treatment and both Mavrik treatments showed reduced efficacy and were at a similar level between the control and the conventional Biscaya treatment. At the later sampling dates, the numbers of larvae increased and differences between the treatments were not statistically significant.

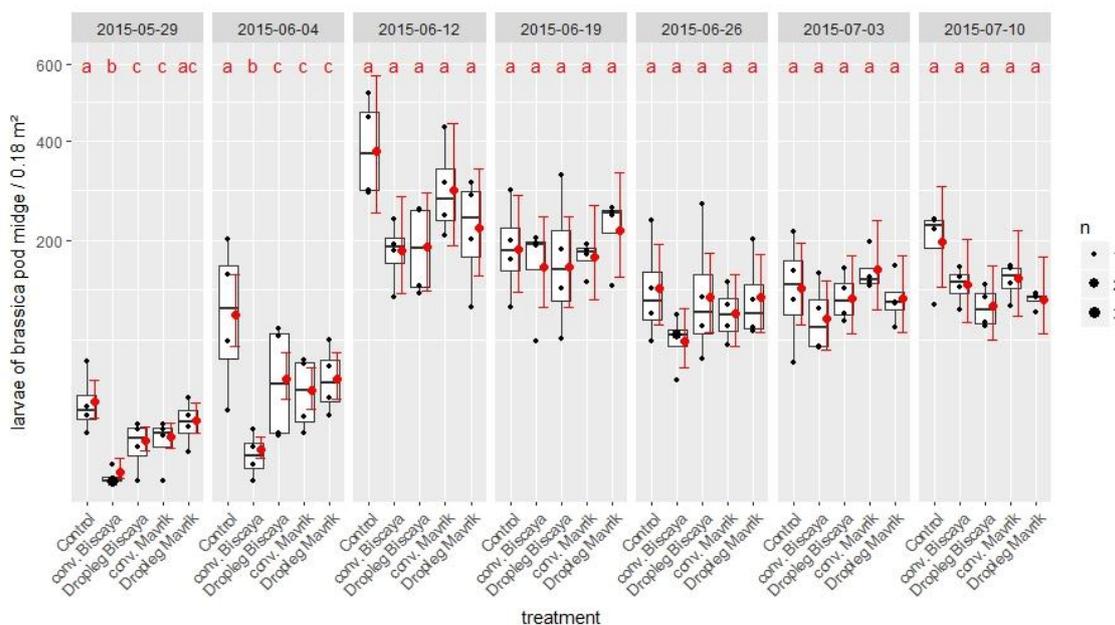


Figure 1 Brassica pod midge larvae/m² in different insecticide treatments using conventional and dropleg spraying technique in 2015. Boxplots for every sampling date with observations plotted and estimated means with confidence limits obtained from the best model are in red. The different letters indicate the significant differences between treatments within a sampling date. Tukey-HSD ($p < 0.05$)

In 2016, the numbers of larvae m⁻² were much higher than in 2015 (Fig. 2). The conventional spraying of Biscaya resulted in a significant reduction in larval numbers for the first three sampling dates compared to the control about 83% at the first ($p < 0.001$), 92% at the second ($p < 0.001$) and 66% ($p = 0.021$) at the third sampling date, whereas Mospilan only affected brassica pod midge at the first sampling date ($p = 0.001$) (Fig. 2). The use of dropleg technique caused the efficacy of Biscaya to reduce at the second sampling occasion ($p < 0.001$).

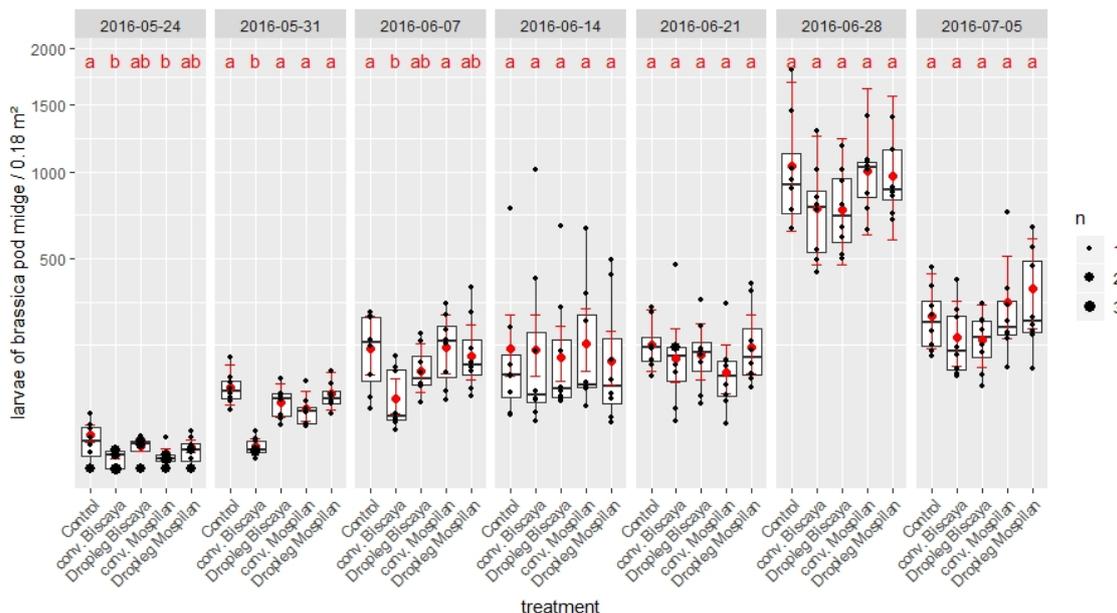


Figure 2 Brassica pod midge larvae/m² in different insecticide treatments using conventional and dropleg spraying technique in 2016. Boxplots for every sampling date with observations plotted and estimated means with confidence limits obtained from the best model are in red. The different letters indicate the significant differences between treatments within a sampling date. Tukey-HSD ($p < 0.05$)

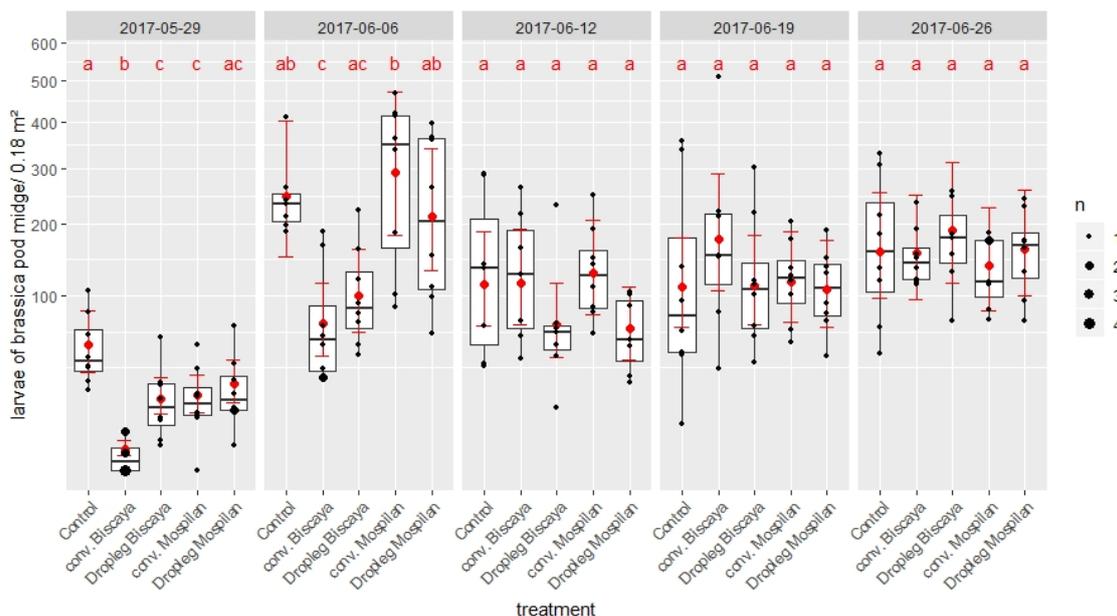


Figure 3 Brassica pod midge larvae/m² in different insecticide treatments using conventional and dropleg spraying technique in 2017. Boxplots for every sampling date with observations plotted and estimated means with confidence limits obtained from the best model are in red. The different letters indicate the significant differences between treatments within a sampling date. Tukey-HSD ($p < 0.05$)

In 2017, larval numbers of brassica pod midge significantly decreased compared to the control after conventional spraying of Biscaya at the first ($p < 0.001$) and second ($p = 0.004$) sampling date (Fig. 3). While dropleg Biscaya showed reduced efficacy at the first sampling date ($p < 0.001$), it was on the same level as the conventional Biscaya treatment in the second week when the larval migration of the first generation reached its peak. The dropleg application tended to show an even better efficacy compared to the conventional application with both the products mentioned at the third sampling date.

The abundance of cabbage seedpod weevils was below the economic threshold at full flowering in all the years. At the time of the last pod assessment in 2015 (BBCH 87), about 3% of all pods in the control contained larvae of *C. obstrictus*. The application of Biscaya reduced the number of damaged pods (conventional Biscaya 1.34% (CI 0.89–2.00), $p = 0.034$; Dropleg Biscaya 1.09% (CI 0.73–1.64), $p = 0.002$) and there was no difference between the conventional and dropleg application techniques of Biscaya and Mavrik (Fig. 4).

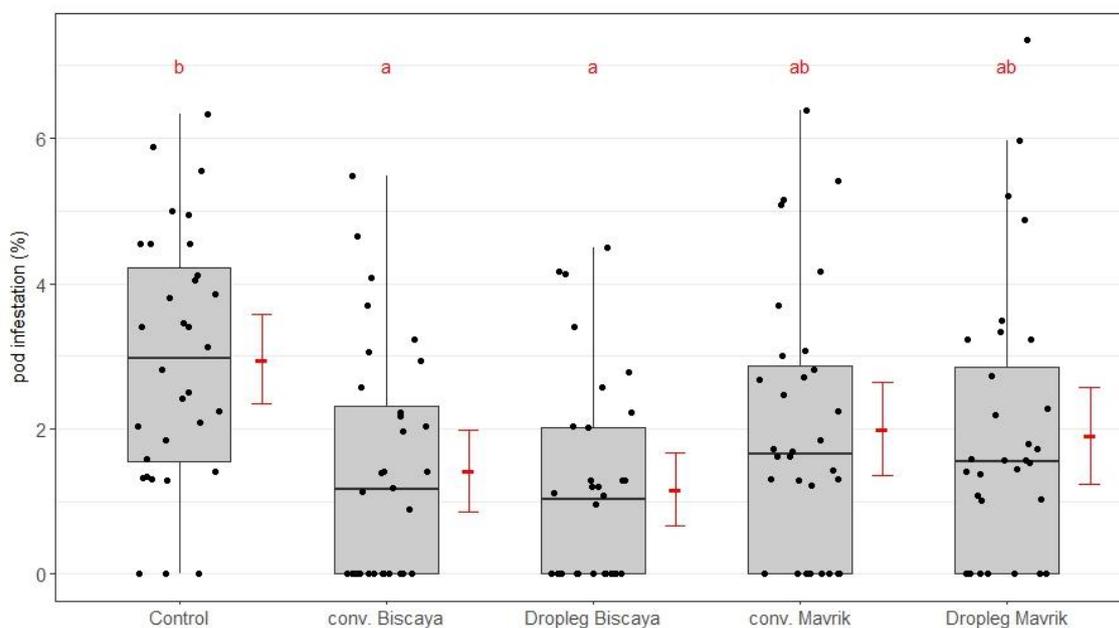


Figure 4 Jittered boxplot, median and 25/75% quantiles of the pod infestation (%) by the cabbage seedpod weevil in 2015. Estimated means from the best model with confidence limits are in red. The different letters indicate the significant differences between treatments. Each insecticide was applied with conventional and dropleg spraying techniques. Tukey-HSD ($p < 0.05$)

In 2016 and 2017, the proportion of pods damaged by *C. obstrictus* was below 0.3% and so further statistical analysis was not done. After the larvae have completed their development, they leave the pods and drop down to the soil for pupation. In 2016, while there were trends for Biscaya, irrespective of the application technique, to reduce both, larval numbers and the emergence of new generation seedpod weevils, differences compared to the untreated control were not significantly (Tab. 3). The only significant differences among emerging adults was between the conventional Biscaya treatment and the dropleg Mospilan ($p = 0.030$). There were no differences in treatment results in 2017.

Table 3 Total number of larvae dropping to soil and the emergence of new generation beetles of *Ceutorhynchus obstrictus* per m² (\pm se) in 2016 and 2017. The different letters within a column and date indicate significant differences between treatments. Tukey-HSD ($p < 0.05$)

Day of application	Treatment	larvae	new emerging adults
		mean \pm se	mean \pm se
10 May 2016	Control	50 ^a \pm 3	65 ^{ab} \pm 16
	conventional Biscaya	33 ^a \pm 4	44 ^a \pm 7
	Dropleg Biscaya	35 ^a \pm 4	47 ^{ab} \pm 7
	conventional Mospilan	52 ^a \pm 5	63 ^{ab} \pm 13
	Dropleg Mospilan	52 ^a \pm 9	88 ^b \pm 18
	Control	53 ^a \pm 12	49 ^a \pm 09
	conventional Biscaya	53 ^a \pm 05	58 ^a \pm 12
	Dropleg Biscaya	49 ^a \pm 05	77 ^a \pm 32
	conventional Mospilan	65 ^a \pm 13	52 ^a \pm 14
	Dropleg Mospilan	62 ^a \pm 06	81 ^a \pm 15

*mod1: $y \sim \text{treatment} + \text{date} + (1|\text{block:treatment})$, family=poisson, df 10, $p < 0.05$)

***Brassicogethes aeneus* and parasitism rate of its larvae**

During the three-year trial period, the number of *B. aeneus* larvae falling to the ground for pupation dropped from around 3500 larvae m⁻² in 2015 to 450 larvae m⁻² in 2017. The data for pollen beetle of the year 2015 is not shown, since there was large variability in the data with no significant difference between treatments.

Only the conventional application of Mospilan resulted in high larval mortality a day after application in 2016 compared to the control ($p = 0.026$) (Fig. 5). However, lower numbers were counted in all insecticide-treated plots when the larvae migrated to the ground for pupation (31st May). The use of the conventional application technique tended to reduce the larval numbers more, especially with the insecticide Biscaya. In the 2017

season, there was a significant increase of larvae dropping at the second sampling occasion (a day after application) in the conventionally Mospilan-treated plots ($p = 0.017$) (Fig. 6). At the third sampling date, three days after application, the numbers of larvae were 3-fold higher in the conventional Mospilan treatment compared to Dropleg Mospilan ($p = 0.011$). One week after application, the migration of larvae to the ground for pupation started in the control plots and larval numbers increased. In the conventional Biscaya treatment, the larval numbers were significantly reduced compared to both dropleg treatments (Dropleg Biscaya $p = 0.027$; Dropleg Mospilan $p = 0.012$) and the control ($p < 0.001$). One week later, larval migration climaxed and again larval numbers were reduced about 60% in the conventional Biscaya treatment compared to the untreated control ($p = 0.010$).

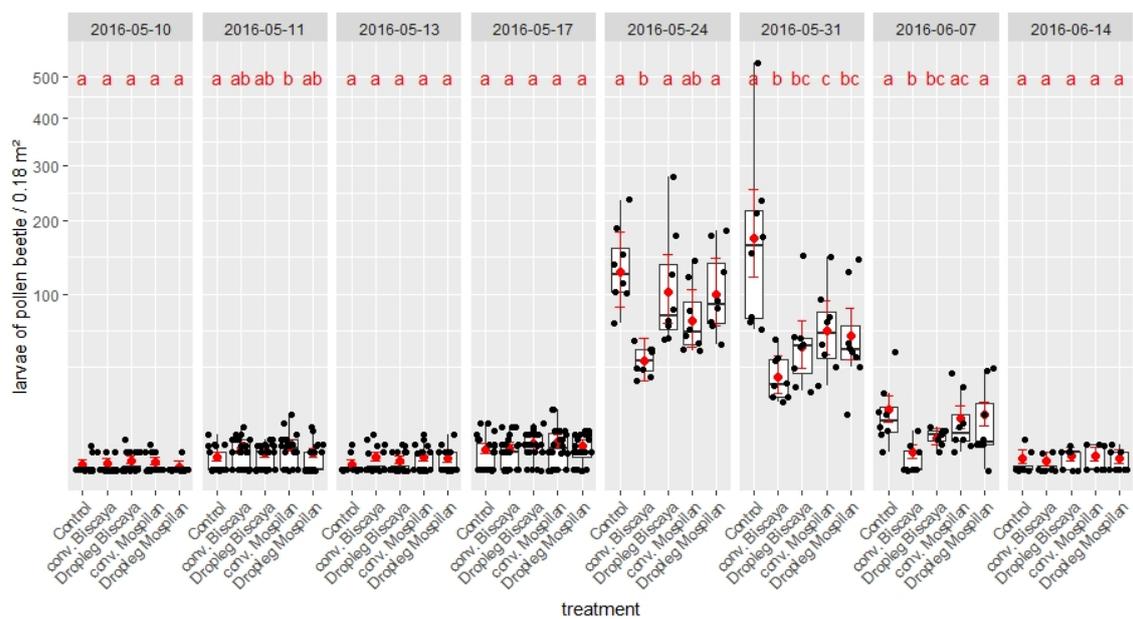


Figure 5 Pollen beetle larvae/m² in different insecticide treatments using conventional and dropleg spraying technique in 2016. Boxplots for every sampling date with observations plotted and estimated means with confidence limits obtained from the best model are in red. The different letters indicate the significant differences between treatments within a sampling date. Tukey-HSD ($p < 0.05$)

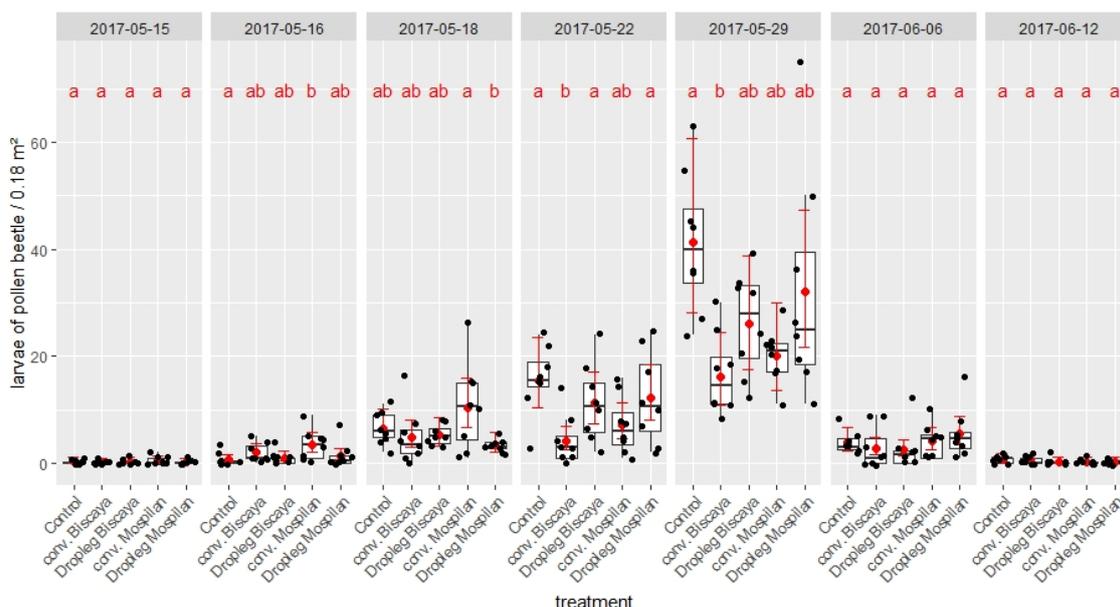


Figure 6 Pollen beetle larvae/m² in different insecticide treatments using conventional and dropleg spraying technique in 2017. Boxplots with observations plotted and estimated means with confidence limits for every sampling date obtained from the best model are in red. The different letters indicate the significant differences between treatments within a sampling date. Tukey-HSD ($p < 0.05$)

Figure 7 shows the rate of parasitism of pollen beetle larvae by *T. heteroceris* in 2017. The overall abundance of pollen beetle in this year was low with large variability in the data. Fixed effects of treatment and sampling date were significant while the interaction was not. In the first week after application, the averaged rate of parasitism was 72% and lowered to 60% two weeks after application ($p < 0.001$). Overall, the conventional Biscaya treatment resulted in an 18% lower parasitism rate than the control ($p = 0.005$) and a 22% lower rate than the Biscaya dropleg treatment ($p < 0.001$). A significantly higher level of parasitism (+18%) was seen with Dropleg Biscaya than with conventionally applied Mospilan ($p = 0.004$).

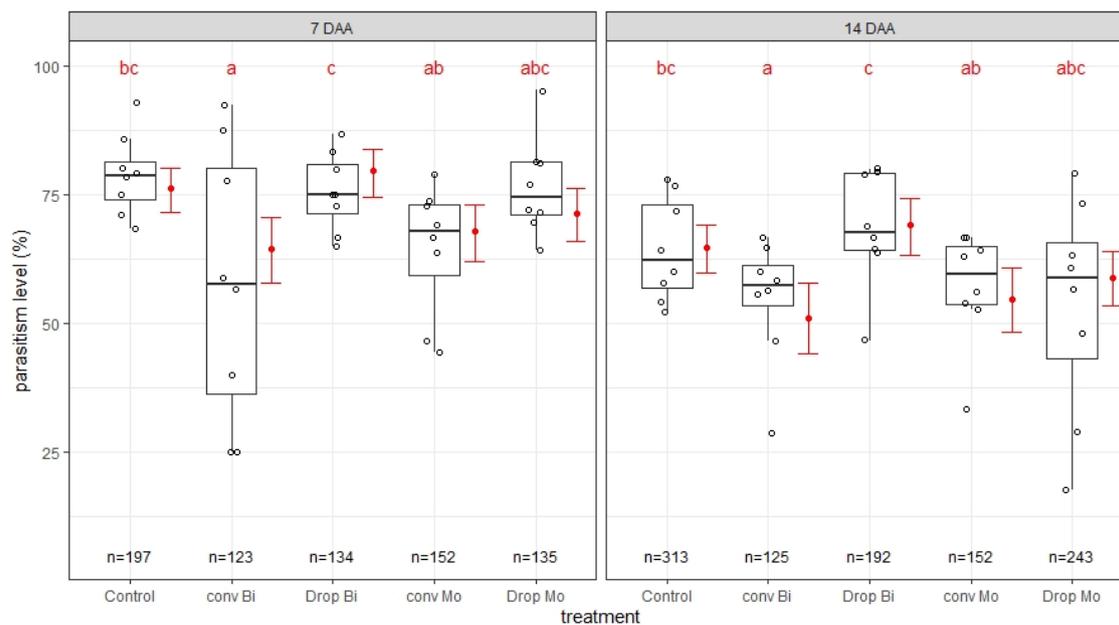


Figure 7 Parasitism level (%) in different insecticide treatments using conventional and dropleg spraying technique seven and 14 days after application (DAA) in 2017. Boxplots for each sampling date with observations plotted and estimated means with confidence limits obtained from the best model are in red. The different letters indicate the significant differences between treatments. n $\frac{1}{4}$ number of assessed larvae, Tukey-HSD ($p < 0.05$)

Yield

In 2015, there were no significant differences between treatments. In the following year, conventional spraying of Biscaya increased the yield significantly by 471 kg ha⁻¹ compared to control ($p = 0.001$). No significant differences between treatments occurred in 2017 (Tab. 4).

Table 4 Average yield (kg ha^{-1}) \pm SE in 2015–2017 using conventional and dropleg applications of Biscaya (thiacloprid), Mavrik (tau-fluvalinate), and Mospilan (acetamiprid). The different letters indicate significant differences within a year. Tukey-HSD ($p < 0.05$)

Day of application	Treatment	yield ($\text{kg} \pm \text{SE}$)
08 May 2015	Control	4528 ^a (± 92)
	conventional Biscaya	4459 ^a (± 233)
	Dropleg Biscaya	4426 ^a (± 121)
	conventional Mavrik	4749 ^a (± 233)
	Dropleg Mavrik	4372 ^a (± 40)
	F-test: ($df = 12$; $R^2 = 0.60$; $p = 0.073$)	
10 May 2016	Control	4728 ^a (± 176)
	conventional Biscaya	5200 ^b (± 58)
	Dropleg Biscaya	4974 ^{ab} (± 44)
	conventional Mospilan	5032 ^{ab} (± 114)
	Dropleg Mospilan	4910 ^{ab} (± 100)
	F-test: ($df = 12$; $R^2 = 0.73$; $p = 0.008$)	
15 May 2017	Control	3853 ^a (± 17)
	conventional Biscaya	3950 ^a (± 51)
	Dropleg Biscaya	3856 ^a (± 59)
	conventional Mospilan	4004 ^a (± 72)
	Dropleg Mospilan	3871 ^a (± 60)
	F-test: ($df = 12$; $R^2 = 0.39$; $p = 0.420$)	

Discussion

The efficacy of using dropleg technique to control sclerotia stem rot in oilseed rape is comparable to conventional application (Dicke, 2018, 2017; Haberland-Korr, 2016). After using dropleg technique, in contrast to conventional application, only traces of active ingredients were detected in the flowering part of oilseed rape (Weimar-Bosse et al., 2017). The infection of sclerotia starts in the axillary buds; therefore the application of fungicides with droplegs is suitable to manage the disease. In our field trials, the dropleg technique had an impact on insect pests attacking the flowering parts of oilseed rape. However, these effects were usually weak compared to the conventional application technique. A primary explanation for such effects may be that these insect pests hide in the lower layers of vegetation seeking shelter against unfavourable weather conditions, which is where they get in contact with insecticides using the dropleg technique. The brassica pod midge is found close to soil level if weather conditions are cold and windy (Fröhlich, 1956). The midge is also found near to the ground at nighttime (Williams et al., 1987). If weather conditions are wet and cool, cabbage seed weevils move into the flowers and remain more or less inactive inside the corolla (Williams and Free, 1978). Some of the weevils move down to the ground where they hide (Edner, 1983; Weiß,

1940). These authors observed the behaviour of weevils and pollen beetles in different weather conditions. They continually found some weevils on stems and leaves at lower canopy levels as well. Further research should focus on the position of insect pests in the vegetation layer in different weather, daytime, and environmental conditions. This may help to improve efficacy of the dropleg application system in situations with maximum exposure. Another assumption regarding how cabbage seed weevils (or other pest insects) might get in contact with insecticides is that they fall from the plant due to the mechanical vibrations caused by the droplegs moving through the crop, although we did not see evidence of this in our study. However, only a few cabbage seed weevils were active during the application period in our trials. Systemic insecticides (such as neonicotinoids) can be translocated to the pods after application. Zimmer et al. (2014) placed shoots of oilseed rape (BBCH 51–53) in a thiacloprid solution; inflorescences were infested 24 h later with pollen beetles which showed high mortality after one day. In our field trials in 2015 with Biscaya (systemic) and Mavrik (non-systemic), the systemic and non-systemic properties of the two respective insecticides did not influence the efficacy of the different application types.

Dasineura brassicae has two generations in winter oilseed rape (Williams et al., 1987). Only the first generation was directly affected by the insecticide treatments in our trials. However, the population density of the second generation is generally higher (Axelsen, 1992; Erichsen, 1982). In contrast to the first assessment, the pod infestation level at the second assessment, which focused on the second generation larvae, was similar after all treatments in the years 2016 and 2017. The cabbage seed weevil *C. obstrictus* is considered to be important for brassica pod midge infestation, as weevils increase suitable oviposition sites on oilseed rape plants through their feeding and egg-laying activities (Ankersmit, 1956; Speyer, 1921). However, it is known that brassica pod midge can also oviposit in small pods not damaged by *C. obstrictus* (Axelsen, 1992; Fröhlich, 1956) and this was probably the case in 2016 and 2017, when cabbage seed weevils densities were low. In 2015, there was an average number of 5000 pods per m² and a 3% infestation rate by cabbage seed weevil (Fig. 2). The number of pods per m² was about 7500 in 2016 and 6900 in 2017 and pod infestation rate by cabbage seed weevil was about 0.03% in 2016 and even less in 2017. Finally, in 2015 more than 10% of the pods were infested by brassica pod midge in control plots. The infestation rate was about 8% in 2016, and about 4% in 2017. The calculated number of brassica pod midge larvae

per infested pod was estimated at 12, 17, and 23 for the years 2015, 2016, and 2017, respectively. It might be possible that oviposition into several pods was limited for *D. brassicae* in 2016 and 2017 by the absence of suitable pods; in addition, multiple oviposition in the same pod was more frequent than in 2015. This means that the abundance of *C. obstrictus* may influence the number of *D. brassicae* larvae per pod. The pod infestation assessment provides no information on the number of larvae that developed in a single pod. No matter how many larvae fed on the pod walls, the visual damage was the same. If the population is sufficiently high and if there is only a limited number of suitable pods for oviposition, the application of insecticide may reduce the number of larvae per pod (i.e. the number of females laying eggs in a pod). However, this may not result in a lower pod infestation level. Apart from that, a plot size of 240 m² might be too small to avoid migration of midges of the second generation into treated plots, thereby masking the effects achieved in the first generation.

An application at the flowering stage of oilseed rape can also impact other non-target insect species present in the field. Pollen beetles are widespread all over Europe and are often abundant in the crop. Even if they rarely cause harm after flowering, they can still be a good indicator of the efficacy of an application. In our trials, the conventional spraying on the flowering canopy always resulted in a higher reduction of pollen beetle larvae than the dropleg applications. This difference can be expected because the larvae develop in the flowering and pod area, and later drop down to the ground for pupation (Williams, 2010).

Natural enemies of insect pests can play an important role in integrated pest management. Therefore, rates of parasitism of pollen beetle larvae by parasitic wasps were examined. The most abundant antagonist of *B. aeneus* was *T. heterocerus*, which emerges in the succeeding crop of oilseed rape (Ulber et al., 2010) and recolonizes oilseed rape fields at the beginning of the flowering stage (Brandes et al., 2017). Rusch et al. (2013) showed that the total amount of sugars in *T. heterocerus* increased during flowering, which indicates that this species is foraging on the nectar of oilseed rape. Through restriction of direct contact with insecticides as well as reduced level of residues in the flowering canopy (Wallner, 2014a), the dropleg technique may help to preserve these parasitoids. On the other hand, Brandes et al. (2017) found no significant effect of conventional insecticide treatments with Biscaya and Mavrik at the flowering stage

(BBCH 62) on parasitism rates, and only a slight decrease at BBCH 65. The authors argue that the host-parasitoid interaction may be host density dependent and, therefore, plots with lower larval numbers are not as attractive as control plots. However, Kaasik et al. (2014) finds that the host searching of *T. heterocerus* is density independent and the parasitoid explores open flowers, independent of the abundance of pollen beetle larvae. Similarly, in our trials, there was a reduction of the parasitism rate, especially one week after the application of the conventional Biscaya treatment. Again, the lowest larval numbers were detected in conventionally treated plots, whereas the dropleg technique resulted in more larvae and a higher rate of parasitism. Further trials are needed for a better understanding of treatment effects on parasitism and to decide if the dropleg technique is suitable for reducing such side effects and supporting IPM in oilseed rape.

The yields differed significantly only in the year 2016. Oilseed rape can compensate pod losses of 5% in an early pod stage with a higher grain weight (Diepenbrock, 2000). In 2016, there was some infection with sclerotia stem rot and about 7% of all pods were damaged by brassica pod midge, which explains some of the yield outcomes. A limitation of this study is that control plots were sprayed with a fungicide only in 2017 and were left untreated in 2015 and 2016. For the years 2015 and 2016, the effects of the fungicide and insecticide on yield were therefore confounded.

Conclusions

The application of insecticides during flowering of oilseed rape with the dropleg technique was compared to conventional spraying technique in field trials over a three-year period. The results indicate that the dropleg technique can substitute the conventional application technique and had a comparable efficacy under moderate pest pressure conditions. Further research on the efficacy of dropleg application with a higher abundance of *C. obstrictus*, as well as on the effects of dropleg technique on parasitoids of oilseed rape pests, needs to be carried out.

The effects on *B. aeneus* demonstrate that even at the flowering stage of oilseed rape, insecticides can have an effect on pollen beetle larvae and, thus, may select for insecticide resistance of this species even at a late crop stage. Therefore, if the pest threshold values are not exceeded, treatment applications should always be avoided to reduce resistance selection pressure. Overall, the dropleg technology seems to be a suitable technique for

improving protection of pollinators, and possibly parasitoids as well, in conditions which require the application of pesticides during the flowering of oilseed rape.

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Chapter III

The effect of insecticide application by dropleg sprayers on pollen beetle parasitism in oilseed rape

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Abstract

Dropleg sprayers apply pesticides below the flower horizon of oilseed rape plants and thus reduce unwanted side effects on pollinating insects. Whether this technique benefits parasitoids of seed and pollen feeding insect pests, has not been studied earlier. To answer this question, we first assessed the vertical distribution of pests and parasitoids using a portable aspirator. In addition, parasitism rates of pollen beetle, *Brassicogethes aeneus* Fabricius (Coleoptera: Nitidulidae), by the larval parasitoid *Tersilochus heterocerus* Thomson (Hymenoptera: Ichneumonidae) were compared in conventional and dropleg sprayed fields over 4 years (2016–2019), using the neonicotinoids thiacloprid and acetamiprid. Our results show that seed and pollen feeders were mainly found in the flowering canopy, while the predominant location of parasitoids was species-specific. Among pollen beetle parasitoids, *Phradis interstitialis* Thomson (Hymenoptera: Ichneumonidae) was more abundant below flowering canopy (63% of total catch), whereas *T. heterocerus* was mainly caught in the flowering canopy (84% of total catch). In the spraying experiments, average parasitism rates of pollen beetles by *T. heterocerus* ranged between 55–82% in the untreated controls. In the dropleg spray treatments, parasitism rates did not differ significantly from control levels, with the exception of thiacloprid application in 2019. In contrast, conventional spray applications resulted in a

reduction of parasitism rates by up to 37% compared to the control for at least one of the insecticides in 3 out of 4 years. The impact of conventional application differed between years, which may be explained by the temporal coincidence between spray application and the immigration of parasitoids into the crop. We conclude that dropleg spraying exerts lower non-target effects on the main biological control agent of pollen beetle.

Introduction

Due to concerns about declining insect populations in agricultural landscapes (Beckmann et al. 2019; Habel et al. 2016; Hallmann et al. 2017), there is considerable public and political will within the European Union to reduce pesticide applications and to improve integrated pest management strategies (European Commission 2020; Hatt and Osawa 2019). In this context, biological pest control by naturally occurring parasitoids and predators is an important ecosystem service that could possibly be enhanced by new application techniques such as dropleg sprayers. Droplegs are prolonged elastic nozzle holders that hang freely floating underneath the boom of the sprayer. Depending on the nozzle setting, the spray is directed towards the ground and sideways but avoids spraying the crop canopy. This technique was initially developed to improve fungicide applications in the lower vegetation layers of vegetables (Basil 2002; Rüegg and Total 2013). Searching for new applications, its use in flowering oilseed rape, *Brassica napus* L., (OSR) is under discussion. OSR is regularly treated with fungicides and insecticides during the period of flowering to prevent yield losses by the pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary and by seed damaging insects like cabbage seedpod weevil, *Ceutorhynchus obstrictus* Marsham (Coleoptera: Curculionidae) or brassica pod midge, *Dasineura brassicae* Winnertz (Diptera: Cecidomyiidae). The active substances used for these applications reflect the most frequent residues found in honey (Karise et al. 2017) and bee bread (Rosenkranz et al. 2019), since OSR is an important forage crop for honeybees and other pollinators such as bumble bees or solitary wild bees (Garratt et al. 2014; Hayter and Cresswell 2006; van Reeth et al. 2018; Westphal et al. 2003; Westphal et al. 2009). In flowering OSR, below-canopy spraying with droplegs avoids direct exposure of pollinators with pesticides in the field. Dropleg application was also shown to decrease the level of pesticide residues of systemic and non-systemic compounds in nectar and pollen, because flowers are left unsprayed (Wallner 2014, 2015). These findings have been the main selling point for promoting the new technique in OSR. Field

studies have also shown that the control of the pathogen *S. sclerotiorum* is not affected by the type of application technique (Dicke 2018; Weimar-Bosse et al. 2017). However, the efficacy of insecticides applied with dropleg technique against the seed damaging insect pests is slightly reduced (Hausmann et al., 2019).

In addition to pollinators, parasitoids are another important insect guild in OSR, as they contribute considerably to the biological control of pests (Abram et al. 2019; Kovács et al. 2019; Ulber et al. 2010b; Veromann et al. 2010). Hence, the question arose, whether applying systemic neonicotinoid insecticides to the lower vegetation layers using dropleg sprayers (Hausmann et al. 2019) could target pests in the OSR canopy, while having no or reduced impact on their co-occurring parasitoids. Many parasitoids are abundant in the crop during the flowering period (Nissen 1997; Ulber et al. 2010a) and insecticide application in this period can have detrimental effects on the parasitism rates of OSR pests such as cabbage seedpod weevil (Murchie et al. 1997) or pollen beetle, *Brassicogethes aeneus* Fabricius (Coleoptera: Nitidulidae) (Jansen 2017; Neumann 2010). To estimate whether dropleg spraying avoids such detrimental effects on parasitoids, it is important to determine their vertical distribution in the crop. Beneficial effects of dropleg can be expected with those parasitoids that are predominantly in the flower canopy where they may not be exposed to pesticides. The first objective of this work was therefore to assess the vertical distribution of several pest species of OSR reproductive parts and their parasitoids in the different vegetation layers of the crop during flowering.

Based on these observations, the second objective of this study was to elucidate whether dropleg spraying could potentially benefit the biological control of pollen beetle by reducing non-target effects on its larval parasitoids. Adults of *B. aeneus* damage the buds of OSR and are the major pest during the green bud stage (Alford et al. 2003). The larvae develop inside the bud and drop to the ground for pupation after one month (Williams 2010). Since pollen beetles have developed widespread resistance against the insecticidal class of pyrethroids (Heimbach and Müller 2013), and are also less sensitive to neonicotinoids (IRAC 2019), chemical control options for farmers in Germany and other European countries have become more and more restricted, while biological control is gaining in importance. The most relevant and widespread parasitoids of the pollen beetle in winter OSR in Germany are *Tersilochus heterocerus* Thomson (Hymenoptera: Ichneumonidae) and *Phradis interstitialis* Thomson (Hymenoptera: Ichneumonidae)

(Ulber et al. 2010b). Both species are univoltine, koinobiont endoparasitoids of the larvae. The adults of these parasitoids overwinter in their pupal cocoons in the soil and after emergence in spring, migrate to the current year's OSR fields (Nilsson 2003). *Phradis interstitialis* often colonizes the crop in the bud stage of the plants. The arrival of *T. heterocerus* is 1-2 weeks later and coincides with the beginning of flowering. Female parasitoids are already sexually mature at this time and no maturation feeding is necessary (Nilsson 2003). However, at least *T. heterocerus* is known to feed on nectar during the flowering period of OSR (Rusch et al. 2013). This parasitoid species could benefit if neonicotinoids applied during this time are not ingested in high concentration with this food resource. Both parasitoid species differ in their temporal abundance, their host finding behaviour and their host stage preferences, indicating niche separation between species (Berger et al. 2015). Field experiments were conducted over a four-year period, comparing the effects of two systemic insecticides on pollen beetle parasitism by *T. heterocerus*, when applied conventionally or by dropleg technique. Our investigations ultimately focused on this species, as in contrast to *P. interstitialis*, *T. heterocerus* was the dominating parasitoid of pollen beetle that was mainly present in the flowering canopy. The strongest effect of dropleg application was therefore expected for this species.

With this work we provide further insight into the vertical distribution of pests and parasitoids in OSR crops and first evidence regarding the effects of dropleg spraying on the biological control of pollen beetle. Our results show that parasitism rates in dropleg sprayed fields were generally as high as in unsprayed control fields, or at least higher than in the conventional spray treatment, suggesting a potential benefit of this novel application technique for biological control under certain conditions.

Material and Methods

Field Sites

Experiments were conducted in 2016–2019 in four conventionally managed OSR fields near Braunschweig, Germany (52°19'36.89"N, 10°38'2.91"E). Meteorological data of the site were obtained from the 5 km distant weather station Braunschweig Flughafen of the Deutscher Wetterdienst (Fig. 1). A different field was used each year with field areas of 3.3 ha in 2016–2018 and 24 ha in 2019. The vertical distribution of insect pests and their parasitoids was studied in the years 2018 and 2019. Sampling was carried out

in the same OSR field used for the dropleg spraying trial, but adjacent to the treated area (Supplementary Information SI 1). As the damage threshold for pollen beetles (>10 beetles per main shoot in the bud stage) was exceeded in 2018 and 2019 the whole OSR crop was treated with contact insecticides Avaunt (a.i.: 25.5 g ha⁻¹ indoxacarb) and Trebon 30 EC (a.i.: 57.7 g ha⁻¹ etofenprox) on 11th April 2018 and 1st April 2019, respectively. The applications reflect common agricultural practice and were indispensable to ensure an undamaged development of the inflorescences. OSR is growing very rapidly from the bud stage (plant height of approx. 50 cm) to its final height (1.2–1.5 m) and all new plant tissues should be free of insecticide residues. Sampling for the assessments of the vertical distribution started 13 days after overall insecticide application (DAA) in 2018 and 7 DAA in 2019. However, in 2019 parasitoid immigration was not observed until 21 days after application. Thus, an influence of insecticide residues on the vertical distribution of insects at this time is unlikely but cannot be ruled out entirely.

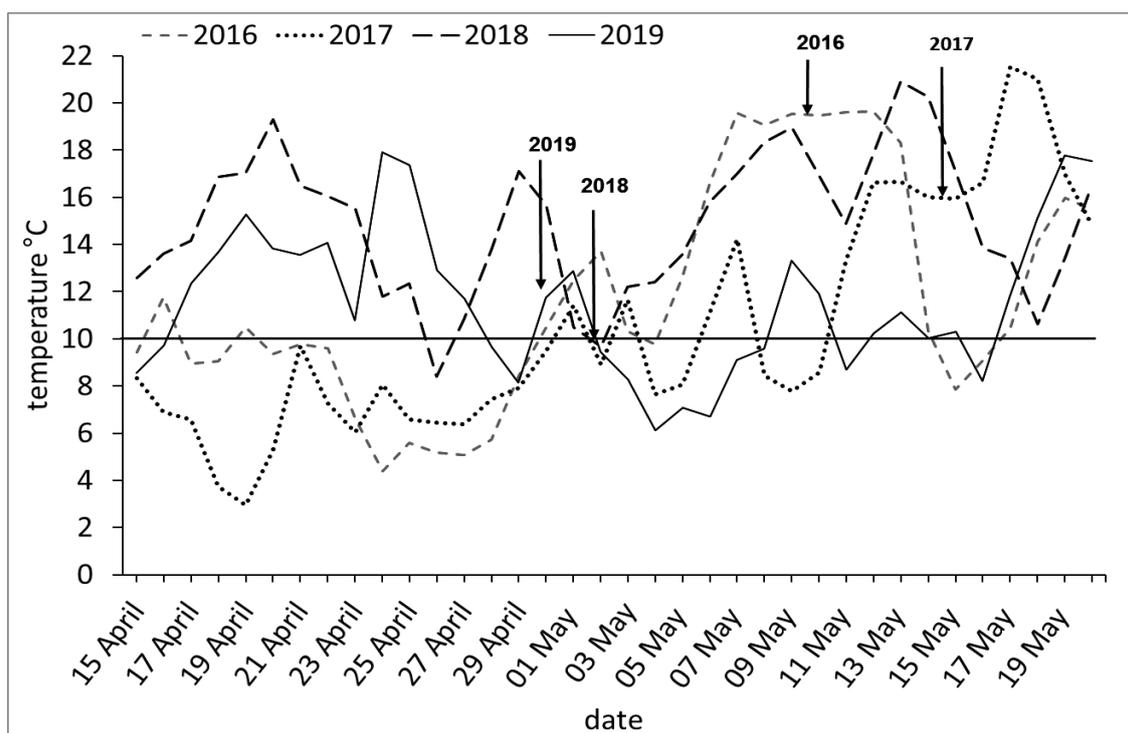


Figure 1 Average daily temperatures (°C) from 15th April to 20th May in the years 2016–2019. The arrows indicate the date of insecticide applications in the dropleg spraying trial in the specific year. The horizontal line indicates the basic temperature for flight activity of *T. heterocerus*. Data Source: Deutscher Wetterdienst, station: Braunschweig Flughafen

Assessment of the vertical distribution of insects

Insects were sampled from the end of bud stage to the beginning of pod stage (BBCH 58–72, (Lancashire et al. 1991)). In 2018, insects were collected on 13 sampling dates between 24th April (BBCH 59/60) and 17th May (BBCH 72) and in 2019 on 15 sampling dates between 8th April (BBCH 58) and 30th May (BBCH 72), respectively. All assessments were carried out in dry or dewy crop stands as frequently as possible, thereby focussing on the main flowering period. Timing of the sampling was flexible to cover any diurnal patterns.

Suction sampling was conducted by using a modified leaf vacuum sampler (STIHL SH 85C, nominal maximum air flow rate: 625 m³ h⁻¹; diameter of the aspiration port: 20 cm). Insects were collected at two different heights, namely in the “flowering canopy” and “below canopy”. In the treatment “flowering canopy”, the aspiration port was guided at a slow walking pace through the upper layer of flowers and buds along a 5 m transect. In the topmost layer, the port was held almost horizontally and orthogonal to the direction of movement at a distance of approximately 1 m from the experimenter’s body. In the treatment “below canopy”, suction samples were collected from stems and leaves at 30 cm below the flowering canopy including buds, flowers and pods. In this case, the aspiration port was held approximately 1 m in front of the experimenter’s body in the direction of movement. This was done to prevent catching any insects that respond to vibrations by dropping from buds and flowers. One sample each from the flowering canopy and the below flowering canopy was taken from two adjacent 5 m transects. This was repeated at four randomly selected locations in the field at each sampling date. All field samples were taken by the same person to minimize individual variability. Since OSR branches out during pod set, small pathways through the field (width 0.5 m, treated with glyphosate at the beginning of vegetation period) and tramlines (pathways used by machines) through the OSR field were used for sampling. A minimum distance of 25 m from the field border and 6 m from the dropleg trial area was adhered. Insect samples were collected in linen bags and then frozen at -20 °C. The frozen insects were separated from petals and plant material, counted and stored in 70% ethanol. All insect pests of OSR, as well as their key parasitoids, were identified at species level using keys by Ferguson et al. (2010), Vidal (2003) and in addition Muller et al. (2007) for the chalcid wasps. Regarding the species *T. heterocerus* and *P. interstitialis*, only the females were identified. This had ecological reasons, since males are abundant at high densities

especially at the field borders and only the females are important for larval parasitism. Moreover, it is not trivial to identify males at species level.

Field trials comparing effects of conventional and dropleg spraying

The trials were designed in a completely randomized block design with four replicates and five different treatments, including an untreated control (Tab. 1, further information see also Hausmann et al. 2019). Insecticides were applied once per year at full flowering, using both conventional spraying and dropleg spraying technique (Lechler 2020). Biscaya (a.i.: 72 g thiacloprid ha⁻¹, Bayer Crop Science) was applied in each year, while Mospilan SG (a.i.: 40 g acetamiprid ha⁻¹, FMC) was sprayed in the years 2016 and 2017 and Mospilan SL (a.i.: 42 g acetamiprid ha⁻¹, FMC) in 2018 and 2019. Both active ingredients belong to the insecticide class of the neonicotinoids (IRAC 2020) and have systemic properties.

Table 1 Design of the dropleg trials from 2016 to 2019 with applied insecticides, application dates and crop growth stages

	2016	2017	2018	2019
Trial design	randomized complete block design (RCBD), 4 replicates			
Treatments	untreated Control Conventional Biscaya Conventional Mospilan SG Dropleg Biscaya Dropleg Mospilan SG		Conventional Mospilan SL Dropleg Mospilan SL	
Date of application	10/05/2016	15/05/2017	02/05/2018	30/04/2019
Growth stage	65	65-67	64	65
Plot size (m ²)	240 (12 x 20m)	300 (12 x 25m)	240 (12 x 20m)	540 (27 x 20m)

Insecticides were applied at a driving speed of 7 km h⁻¹ with 300 l ha⁻¹ water. Weather was clear and wind speed was about 1.5–2.2 m s⁻¹ except from 2016 (3.4–3.9 m s⁻¹). All insecticides were applied at recommended field rates

The larvae of pollen beetles were collected by six white water trays per plot (Backs, Rehbeck, 60 x 10 x 10 cm) placed on the ground between the plant rows in the centre of each plot, one hour before insecticide application. The trays were filled with a 10% (w/v) solution of sodium benzoate in water and were emptied weekly, beginning 1 day after the application until the end of June (BBCH 80). Pollen beetle larvae were counted and stored in 70% ethanol at plot level. To determine parasitisation by *T. heterocerus*, the larvae were dissected and examined for parasitoid eggs (cf. Brandes et al. (2018) and Hausmann et al. (2019)). The number of larvae examined varied between years and sampling dates within a year (Supplementary Information SI 2 and Fig. 4). Parasitism by *T. heterocerus* was chosen because it is the most abundant parasitoid of *B. aeneus* in the area of

Braunschweig (Brandes et al. 2018; Ulber et al. 2010b). In the year 2016, 160 larvae per treatment and sampling date were assessed. Due to low levels of pollen beetle infestation in 2017 larval numbers ranging between 229 and 527 individuals per treatment were pooled over three sampling dates. In 2018 and 2019 at least 200 larvae per treatment were assessed for each sampling date.

Statistical analysis

All statistical analyses were performed using R (version 3.6.1) (R Core Team, 2019) in the graphical user interface R Studio. The vertical distribution of insect pests and their parasitoids in the crop layer is presented as stacked bar plot based on the counts of each species over all sampling dates and years in the flowering canopy and the below flowering canopy, respectively. Independence of the pest species observations and crop layer was tested using a χ^2 -test. For the parasitoid species observations Fisher's exact test was used to test independence from crop layer.

The parasitism rates of pollen beetle larvae by *T. heterocerus* were analysed separately for each year, because our aim was to present differences between insecticide treatments but not differences between years. A generalized linear model (GLM) explaining the parasitism rate of pollen beetle larvae by the treatment, the sampling date, the interaction between treatment and sampling date and the block was fitted using a logit link function. A binomial error distribution was assumed and models were tested for overdispersion. The full model including all variables and interactions was systematically compared with the reduced models via the AICc. The model with the lowest AICc value was chosen as it indicates the best fit (Burnham and Anderson, 2002). The effect of different variables was tested via an analysis of variance using the "F-test". For model diagnostics the residuals were plotted against the predicted values and the explanatory variables. If some of the variables showed significance, a post-hoc analysis was done by performing a Tukey test at an alpha level of 0.05 using the package *emmeans* (Lenth et al., 2018). The R-script for the parasitism data is available on demand. Figures were created using the package *ggplot2* (Wickham, 2016). Regression lines were generated using LOESS (locally estimated scatterplot smoothing) method implemented in the package.

Results

Vertical distribution of pests and parasitoids in the crop

Results on the vertical distribution of cabbage seedpod weevil (*C. obstrictus*), brassica pod midge (*D. brassicae*) and pollen beetle (*B. aeneus*) are shown as stacked bar plot (Fig. 2a). The location of the three pest species in the crop was not independent from the crop layer ($\chi^2 = 53.53$, $df = 2$, $p < 0.001$). *Dasineura brassicae* and *C. obstrictus* were mainly found in the flowering canopy. *Brassicogethes aeneus* was caught most frequently and was present only in 68% of catches in the flowering canopy. Compared to *C. obstrictus* and *D. brassicae*, *B. aeneus* was found significantly more often below the flowering canopy.

Different parasitoid species were detected during the flowering period in OSR (Fig. 2b). The pollen beetle parasitoid, *T. heterocerus* was the most abundant species. Females of this parasitoid were mainly found in the flowering canopy of the crop. Only 16% of all specimen collected were observed below flowering canopy. The second key parasitoid of the pollen beetle, *P. interstitialis*, was less abundant and occurred predominantly below flowering canopy (63%). Three larval parasitoid species of the cabbage seed weevil *C. obstrictus* were caught by suction sampling. These were, in declining order, *Trichomalus perfectus* Walker, *Mesopolobus morys* Walker and *Stenomalina gracilis* Walker (Hymenoptera: Pteromalidae). *Trichomalus perfectus* was disproportionately more frequent below canopy, whereas *M. morys* was found almost exclusively in the flowering canopy. The tersilochine parasitoids of stem weevils (*Ceutorhynchus pallidactylus* Marsham and *Ceutorhynchus napi* Gyllenhaal, Coleoptera: Curculionidae) could not be identified at species level. They were mostly sampled in the vegetation layer below the flowering canopy of the crop. The distribution of the parasitoid species sampled was not independent from the crop layer ($p < 0.001$, Fisher's exact test).

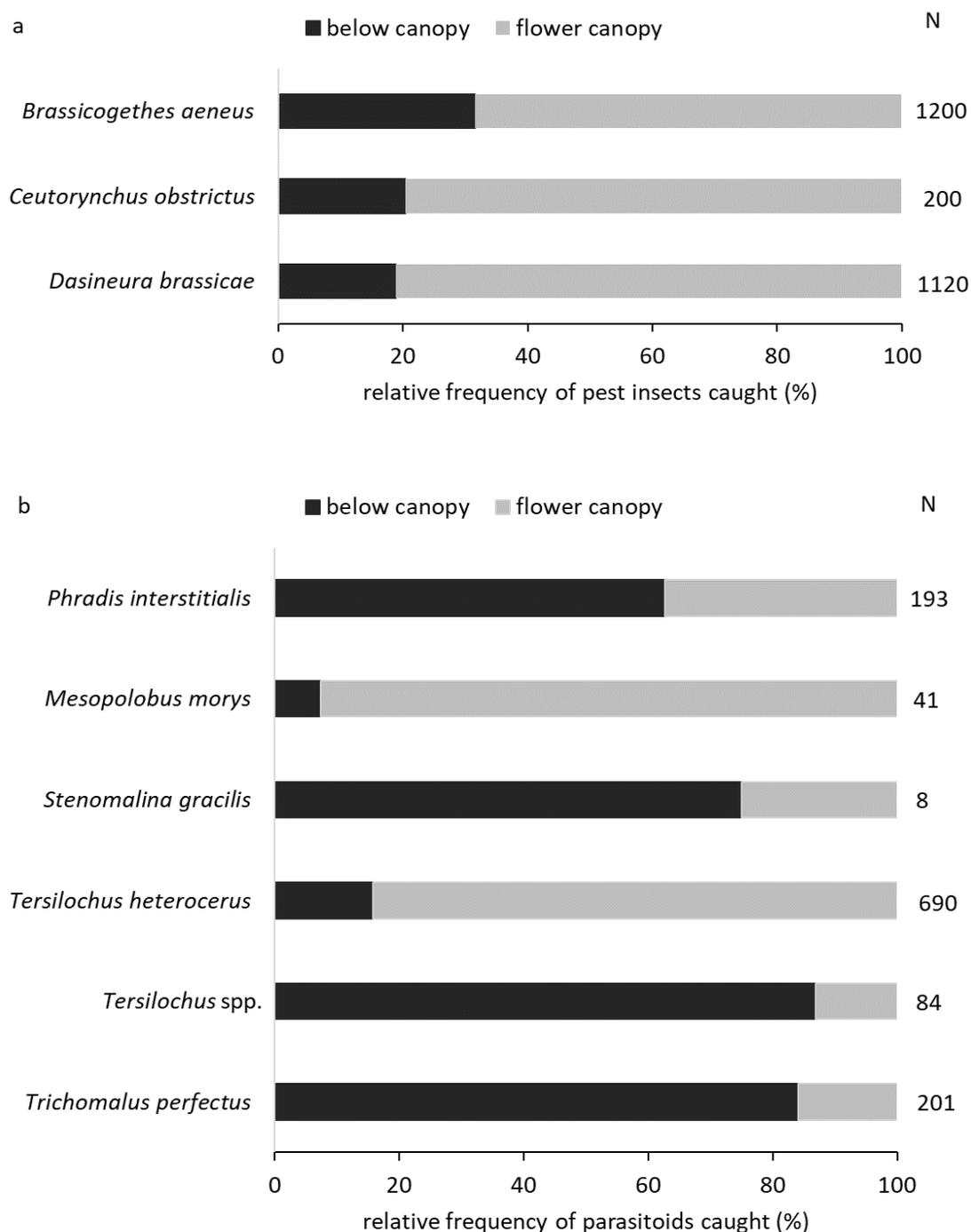


Figure 2 Vertical distribution of **a** insect pests and **b** parasitoids of insect pests in oilseed rape, sampled with a portable aspirator during the flowering period in 2018 and 2019. Bars show the relative frequencies of species in the flowering/below flowering canopy. N the total number of individuals caught during the two seasons

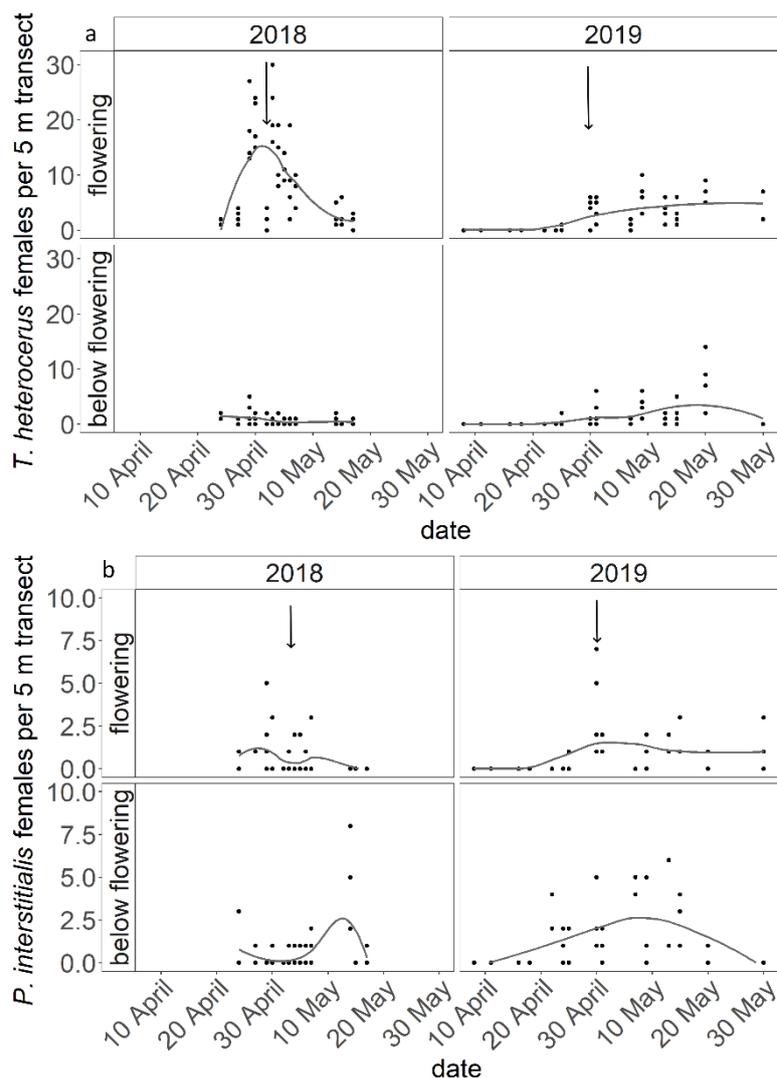


Figure 3 Number of individuals (dots) sampled with an aspiration trap on a 5 m transect of **a** *T. heterocerus* and **b** *P. interstitialis* females in the flowering canopy and below flowering canopy of oilseed rape at different sampling dates during the period of flowering in 2018 and 2019. Curves are corresponding to loess regression lines. Arrows correspond to the dates of insecticide application in the adjacent dropleg spraying trial

Temporal abundance of pollen beetle parasitoids

In 2018, monitoring was initiated at the end of bud stage (BBCH 59) on 24th April. At this time, immigration of parasitoids had already started as *P. interstitialis* and *T. heterocerus* were caught on the first sampling date (Fig. 3). In 2019, the development of inflorescences was earlier and sampling started at the end of bud stage (BBCH 58) in the second week of April. First females of *P. interstitialis* were caught below flowering canopy on the 22nd April at the beginning of flowering (BBCH 62). The first females of *T. heterocerus* were trapped three days later when the crop had reached almost the full

flowering stage (BBCH 64). The main period of activity for *T. heterocerus* was at full flowering in 2018, and in this year 93% of all individuals were found in the flowering canopy. By contrast, only 64% of all *T. heterocerus* specimen were present in the flowering canopy in the following year. The abundance was lower and the period of activity was about one month, without a clear peak. *Phradis interstitialis* was less numerous in both years and occurred during the whole sampling period in both years. Figures about the temporal abundance of the pest species can be found in the Supplementary Figure S2.

Parasitism of pollen beetle larvae in field trials using dropleg technique

The average density of pollen beetle larvae dropping to the ground for pupation was lowest in 2017 ($443 \pm \text{SE } 44$ larvae m^{-2}) and increased up to $6857 (\pm \text{SE } 648)$ larvae m^{-2} in 2019. The number of larvae per m^2 varied between the different blocks within a given year. Number of larvae in the untreated control plots was not correlated with *T. heterocerus* parasitism rates in these plots (Pearson $r = -0.15$, $df = 14$, $p = 0.578$).

The parasitism rate of pollen beetle larvae by *T. heterocerus* in the untreated control increased from 56% ($\pm \text{SE } 3\%$) in the year 2016 up to 82% ($\pm \text{SE } 1\%$) in 2018 and fell back to 55% ($\pm \text{SE } 2\%$) in 2019 (Fig. 4). The effects of treatments differed within the years 2017 ($\chi^2 = 19.71$, $df = 4$, $p = 0.0006$), 2018 ($F_{4, 149} = 3.49$, $p = 0.0093$) and 2019 ($F_{4, 87} = 48.34$, $p < 0.0001$). In all years, parasitism rates by *T. heterocerus* in the dropleg Mospilan treatment was not significantly different from the control, while the dropleg Biscaya treatment showed a significantly lower parasitism rate only in 2019 ($44\% \pm \text{SE } 2\%$, $p = 0.0054$). In the years 2017-2019, lower parasitism rates were found in the conventional treatment compared to control and dropleg treatments. More specifically, conventional application of Biscaya significantly reduced parasitism rates in 2017 ($63\% \pm \text{SE } 3\%$) compared to the control (parasitism rate $73\% \pm \text{SE } 2\%$, $p = 0.0351$) and the dropleg Biscaya treatment (parasitism rate $77\% \pm \text{SE } 2\%$, $p = 0.0021$). In 2019, the conventional application of Biscaya (parasitism rate $34\% \pm \text{SE } 2\%$) resulted in a significantly lower parasitism rate compared to the control (parasitism rate $55\% \pm \text{SE } 2\%$, $p < 0.0001$) and both dropleg treatments (dropleg Biscaya $44\% \pm \text{SE } 2\%$, $p = 0.0123$; dropleg Mospilan $52\% \pm \text{SE } 2\%$, $p < 0.0001$). The conventional Mospilan treatment significantly reduced the parasitism rate of pollen beetles in 2018 ($74\% \pm \text{SE } 2\%$) in comparison to the control (parasitism rate $82\% \pm \text{SE } 1\%$, $p = 0.0069$) and the dropleg

Mospilan treatment (parasitism rate $81\% \pm \text{SE } 3\%$, $p = 0.0288$). The same was found in 2019 with $37\% \pm \text{SE } 2\%$ parasitism in conventional Mospilan treatment (control $p < 0.0001$; dropleg Mospilan $p < 0.0001$). Results on average parasitism rates and multiparasitism by *Phradis* in four years of field trials are presented in the Supplementary Tab. S2.

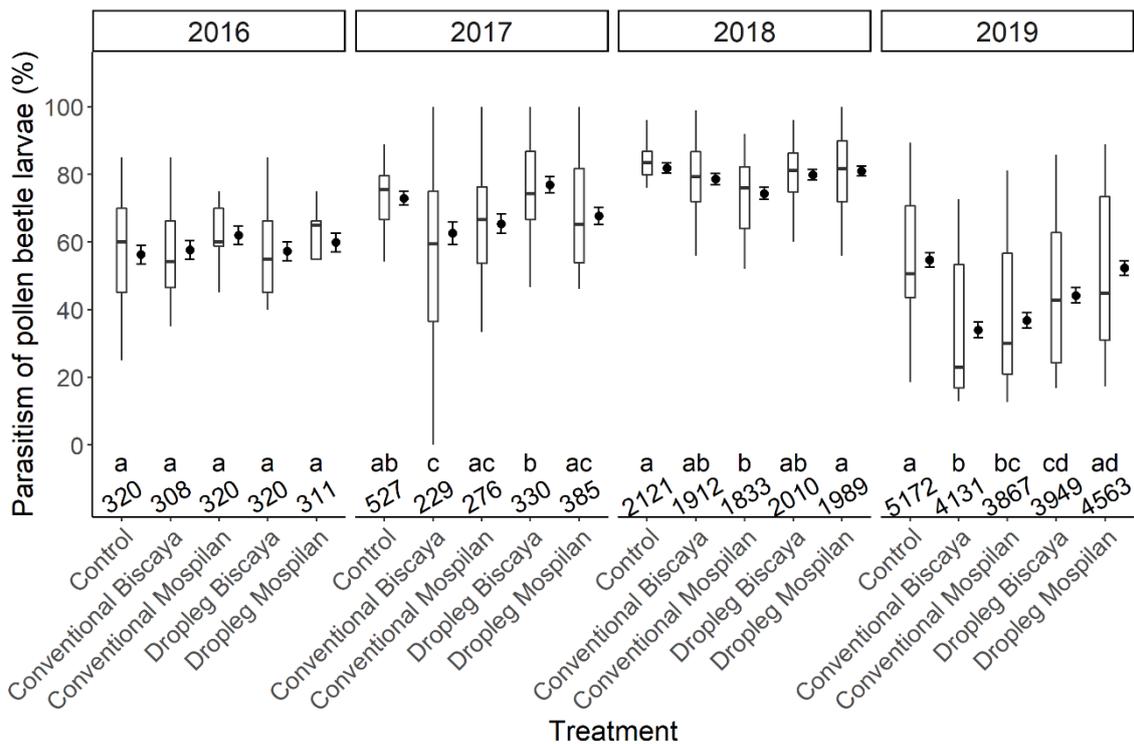


Figure 4 Boxplots showing the median value (solid line), the 25th and 75th percentile of parasitism rates of pollen beetle larvae by *T. heterocerus* per replicate and sampling date in the years 2016–2019; the error bars below and above the box indicate the 10th and 90th percentile, respectively. Additionally, calculated means \pm SE obtained from the best model are shown. Treatments with different letters within a given year differ significantly from each other ($p < 0.05$). Numbers below the boxplots show the total number of larvae investigated per treatment and year

Discussion

After insecticide application by using dropleg technique the flowering canopy of the crop is left unsprayed and the residue levels of the pesticide in nectar and pollen are relatively low compared to conventional spray application (Wallner, 2014; Weimar-Bosse et al., 2017). In our study, we tested whether dropleg technique is suitable to avoid non-target effects on parasitoids. Therefore, the vertical distribution of insect pests and parasitoids was studied during the flowering period of OSR. Our results demonstrate that

both, the seed damaging pest insects *C. obstrictus* and *D. brassicae*, as well as the bud damaging beetle *B. aeneus* are active predominately in the flowering canopy (Fig. 2a). The latter was the most frequent insect pest in our study (55% of all insect pest catches) and was partially found below canopy (32% of all pollen beetle catches). This might be explained, because females of *B. aeneus* feed on pollen and lay their eggs in medium sized, closed buds of OSR (Hervé et al., 2015). In OSR the main raceme is the first to produce flowers (Tayo and Morgan, 1975). At this growth stage (BBCH 61-65), the side branches are still at a lower height and in the bud stage, which renders them suitable for oviposition and might attract *B. aeneus* beetles (Supplementary Figure S2).

The parasitoid species of OSR herbivores found in our study differed significantly in their vertical distribution (Fig. 2b). The most frequently caught parasitoid species were *T. heterocerus* (56% of all parasitoid catches) and *P. interstitialis* (16% of all parasitoid catches), both of which parasitize the larvae of *B. aeneus*. The two parasitoid species can avoid competition as *P. interstitialis* parasitizes eggs and L₁ larvae in the buds, whereas *T. heterocerus* prefers L₂ larvae in the open flowers (Berger et al., 2015; Nilsson, 2003). The latter is also known to feed on nectar during flowering of OSR (Rusch et al., 2013). The vertical distribution pattern of these two parasitoids shown in our study fits this, as *T. heterocerus* was found predominantly in the flowering canopy, while *P. interstitialis* was instead found below flowering canopy on most sampling dates. These findings are in agreement with previous studies, in which parasitoids were caught in yellow water traps that were placed at soil level and at top height of the flowering canopy (Neumann, 2010; Nitzsche, 1998). *Tersilochus heterocerus* was mainly found at top height, while females of *P. interstitialis* were equally distributed at crop height and soil level (Neumann, 2010). Overall, these observations fit the niche separation hypothesis (Berger et al., 2015) as both parasitoid species, although competing for the same host, use their environment and resources differently. Based on these results, we hypothesized that the pollen beetle parasitoid *T. heterocerus* should benefit most from the new dropleg technique. Although effects on other parasitoid species have not been assessed in this study, we assume that the *C. obstrictus* parasitoid, *M. morys*, could also benefit from the use of the dropleg technique, because this species was frequently found in the flowering canopy, albeit in low numbers. In contrast, the pollen beetle parasitoid *P. interstitialis*, the chalcid wasp *T. perfectus*, which parasitizes *C. obstrictus* and parasitoids of the stem weevils, were

collected mostly below canopy. A positive impact of the dropleg application technique on these species seems therefore unlikely.

In four years' field trials, we tested the hypothesis that dropleg spraying causes less side-effects on *T. heterocerus*. The rate of pollen beetle larvae parasitized by *T. heterocerus* in the untreated control in all four years exceeded 50%, which is high enough to have a significant impact on pollen beetle population development (Hokkanen, 2008; Jansen, 2017). The parasitism rates in treatments with dropleg technique never differed from the unsprayed control with the exception of the Dropleg Biscaya treatment in 2019, where a significantly lower parasitism rate was found. On the other hand, in 2016-2019, four out of eight insecticide treatments, using conventional spray application, significantly reduced parasitism rates compared to the control.

Since conventional insecticide application greatly reduced the number of pollen beetle larvae in the crop, it is possible that lower host densities could explain the decreased parasitism rates, when compared to the untreated plots. However, other studies found no relationship between host-density and parasitism rates in pollen beetle parasitoids (Ferguson et al., 2003; Kaasik et al., 2014). Furthermore, in our control plots we observed no interaction between parasitism rates and larval densities over the four years. Hence, we assume that direct toxic effects of the insecticides on adult parasitoids rather than reduced host density caused lower parasitism rates in the conventional treatments.

Strikingly, the effects of the conventional application in our field trials were quite variable between years and also between the two neonicotinoid insecticides. The persistence of insecticide activity in the field can be affected by environmental conditions such as temperature, air humidity and UV light (Holmstrup et al., 2010). Higher post-exposure temperatures can increase the toxicity of acetamiprid to psyllids and green lacewings (Boina et al., 2009; Mansoor et al., 2015). The differences between the insecticide effects in our study therefore might be related to different temperature regimes in individual years (Fig. 1). In 2018, insecticides were applied in the field trial at 10 a.m. at approximately 20 °C and the following days were warm and sunny. In 2019, however, the application was carried out in the evening at approx. 16 °C, with low temperatures on

the following days (Fig. 1). Such different conditions may possibly explain why Mospilan in 2018 showed a stronger impact on larval parasitoids than Biscaya.

Furthermore, temperature and environmental conditions also determine the time of immigration of parasitoids from their place of emergence into the new crop (Johnen et al., 2010; Nilsson, 2003), which was recorded by sampling with a portable aspirator around the dropleg field trials in 2018 and 2019 (Fig. 3a). *Tersilochus heterocerus* starts to migrate when the average daily temperature exceeds 10 °C, combined with sunshine and a maximum daily temperature above 14 °C (Johnen et al., 2010). This was the case at the beginning of OSR flowering in both years. In 2018, migration into the field was within a few days and the main period of activity was during full bloom. In the field trial, the insecticides were applied at the peak of *T. heterocerus* abundance, however, its main activity period started already one week before (Fig. 3a). In contrast, due to lower temperatures (Fig. 1), the flowering period in 2019 was much longer and the abundance of *T. heterocerus* increased only slowly without showing a specific peak. The insecticide application was conducted during the first wave of immigration, leading to a significant reduction of parasitism rates by conventional spraying. In 2018, the average rate of pollen beetle larvae parasitized by *T. heterocerus* was as high as 80% six days after application. In 2019, parasitism rate was only 24% at day 9 after application. Thus, the temporal coincidence of insecticide application and an increasing abundance of *T. heterocerus* may have resulted in greater effects in the year 2019 compared to 2018. It is likely that the insecticides did not have any impact in the year 2016, because the warm days preceding the application (Fig. 1) had already allowed the parasitoids to nearly complete egg laying. Similar findings were presented by Brandes et al. (2018), who investigated the effects of insecticide applications during the bud stage (BBCH 55) or the flowering stage (BBCH 62-65) of OSR on parasitism rates of pollen beetle larvae by *T. heterocerus*. The authors were unable to detect larval parasitism by *T. heterocerus* before BBCH 65 and insecticide applications at this growth stage tended to reduce parasitism rates compared to applications at the bud stage (BBCH 55). Overall, the results indicate that the timing of an insecticide application is a major factor influencing the side effects on parasitoids. However, detrimental effects of insecticides on *T. heterocerus* were also moderated by using the dropleg technique.

In conclusion, many parasitoid species are abundant in high numbers during the bloom of OSR. The vertical distribution patterns of parasitoid species in the crop showed that many species prefer a specific crop layer, presumably because of niche separation between species based on the distribution of their hosts. Although parasitism rates of pollen beetle larvae varied between years, our data revealed that only in a single year dropleg spraying affected parasitism rates by the most important parasitoid of pollen beetle larvae. This was in contrast to conventional application, which had a stronger detrimental impact on *T. heterocerus*. Whether other parasitoid species might benefit from dropleg application, needs further investigation. Additionally, the trade-off between a somewhat lower efficacy of insecticides applied with dropleg technique against pests and the reduced side effects on beneficial parasitoids, should be evaluated under real-world farming conditions. Overall, our study suggests that to some extent, dropleg spray application can reduce non-target effects of neonicotinoid insecticides on the most important parasitoid of pollen beetle.

Supplementary information

The supplementary files mentioned in this chapter can be found in the appendix A.

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Chapter IV

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

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Abstract

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

insecticides is probably affected by the temporal coincidence of parasitoid occurrence and insecticide application, followed by properties of different insecticidal products.

Introduction

Oilseed rape (*Brassica napus* L.) (OSR) is one of the most important break crops in cereal dominated arable systems in Europe (Angus et al., 2015). It is a mass flowering crop with a long cropping period that offers habitat and food resources to many insects including pollinators (Scherber et al., 2019; Westphal et al., 2003). However, OSR is also the host plant to a diverse complex of insect pests (Williams, 2010), whose occurrence at high densities may demand for pest control with insecticides. During the flowering period of OSR, one of the major insect pest species in OSR is the cabbage seedpod weevil, *Ceutorhynchus obstrictus* Marsham (Coleoptera: Curculionidae) (CSW). CSW migrates into the crop at the beginning of flowering and mates after a short period of maturation feeding. The larvae live individually in the pods of OSR, where they feed on the seeds (Williams, 2010). While CSW has been spreading invasively for 80 years in the USA and Canada and is a major pest in OSR production (Cárcamo et al., 2001), CSW is widely controlled by endemic antagonists in its area of origin in Europe (Haye et al., 2010; Veromann et al., 2010). Here, however, CSW is mostly feared as the precursor for brassica pod midge, *Dasineura brassicae* Winnertz (Diptera: Cecidomyiidae). The larvae of the midge also live in OSR pods and secrete enzymes, which cause the pods to split open prematurely and additionally grain weights are reduced (Williams, 2010). The midge itself can only oviposit into young pods and therefore uses pod injuries caused by the maturation feeding and oviposition of CSW to spread more widely. The simultaneous occurrence of both pest species can result in significant yield losses up to 30% (Hoffmann and Schmutterer, 1999), which demands the application of insecticides during flowering period according good agricultural practice. Since the direct control of *D. brassicae* is challenging (Hausmann, 2021), the CSW population is monitored and controlled instead. The damage threshold for CSW in Germany is one weevil per main raceme at flowering stage. This threshold is lowered to 0.5 weevils per main raceme, if the brassica pod midge was present in the past (Lauenstein 1993; Ramsden et al. 2017).

In the endemic range of CSW, natural antagonists are one of the predominant natural causes of CSW mortality (Gillespie et al., 2019). All life stages of CSW are parasitized (Williams, 2003). Among natural antagonists, larval ecto-parasitoids play the greatest

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

In this study, we conducted field trials testing for the effects of insecticides applied at full flowering stage of OSR on CSW and CWS parasitoids, using insecticides with different active ingredients differing in their effects on non-target organisms. To account for the spatial niches of CSW parasitoids we applied insecticides using two different application techniques: 1) conventional, where the spray is directed from the top to bottom into the open flowers, and 2) dropleg, which spares out the horizon of open flowers and only the lower crop parts are sprayed (Hausmann et al., 2019). Using dropleg

application technique, residues of (also systemic) insecticides in nectar and pollen are reduced (Brandes 2021; Wallner 2014). Spatial targeting of insecticides using droplegs in flowering OSR can increase parasitism rates of pollen beetle larvae compared to conventional application (Hausmann et al. 2021). The objective of our study was, to investigate the effects of insecticide applications and application techniques on pod infestation by CSW larvae and on its parasitism rates, but also on parasitoid species composition and the interaction between application technique and parasitoid species. We address the question whether rather the application technique or the mode of action of the insecticides are more suitable for avoiding undesirable side effects on the larval parasitoids of CSW.

Material and Methods

Design of the field trials

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

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

	2019	2020	2020
trial site	Wendhausen	Wendhausen	Groß Brunsrode
coordinates	52°19'36.03"N, 10°38'6.44"E	52°19'49.99"N, 10°37'48.18"E	52°21'14.62"N, 10°39'34.65"E
variety	DK Expansion	Avatar	DK Expansion
plant density per m²	60	30	50
date of trial application	April 30 th	April 24 th	April 24 th
plot size (m²)	540	540	810
Used insecticides	Biscaya Mospilan SL	Biscaya Mavrik Vita	Biscaya Mavrik Vita

Company	Product name	Application rate and active ingredient
Bayer Crop Science	Biscaya	72 g ha ⁻¹ thiacloprid
Chemnova Deutschland GmbH & Co. KG	Mospilan SL	42 g ha ⁻¹ acetamiprid
Adama Deutschland GmbH	Mavrik Vita	48 g ha ⁻¹ tau-fluvalinate

The insecticide Biscaya was applied at recommended field rate of 72 g a.i. thiacloprid ha⁻¹ in all trials, representing a neonicotinoid (IRAC class 4A) (Tab. 1). Additional, in 2019 a second neonicotinoid Mospilan SL was used, which was replaced by the pyrethroid Mavrik Vita (IRAC class 3A) at both trial sites in 2020. The insecticides

Biscaya and Mavrik Vita were chosen to represent different mode of actions, while they also were registered as official products for the use against CSW in OSR in Germany. Mospilan SL was not approved for the control of CSW in OSR. All three insecticides are classified as harmless to honey bees when used alone (BVL, 2021). Mavrik Vita is also considered to be comparatively gentle on beneficial insects, e.g. pollen beetle parasitoids (Ulber et al., 2010a). Biscaya, on the other hand can have harmful effects on tersilochine parasitoids (Hausmann et al., 2021; Jansen and San Martin, 2017, 2014). The applications were carried out at the full flowering stage of the OSR crop (BBCH 65–67), independent of infestation rates, with a driving speed of 7 km h⁻¹ and a pressure of 2.8 bar using water volumes of 300 l ha⁻¹. The crop height varied between 1.20 and 1.30 m at BBCH 65. The conventional spraying was conducted with IDKN 120-4 nozzles held as usual 50 cm above the crop. The droplegs (Lechler, 2020), equipped with double tongue nozzles FT 90°, were lowered 40–50 cm into the plant stand, so that the spray was beyond the horizon of open flowers, to account for the spatial distribution of CSW parasitoids.

Sampling

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

Assessment of parasitism rates

For each plot, the pods of the main racemes and the pods of the lateral racemes were pooled and stored in a plastic box (Bellaplast, 1275 cm³), which was enclosed by a perforated polyethylene bag (Fibrolux GmbH). The pods were placed in a climate chamber at 20° C (L:D 16:8) for at least 30 days. Parasitoids that emerged from the pods

were collected and stored in 70% ethanol. The determination of the parasitoids at species level was carried out on a binocular with 60-fold magnification according to Baur et al. 2007; Gibson et al. 2005; Muller et al. 2007 and Vidal 2003. Additionally, all pods were checked for exit holes of the weevils or the parasitoids and then were opened and checked for any remains of weevil larvae or parasitoids. Only in 2020, exit holes were studied in detail to check whether they were from a weevil larva or a parasitoid (Fig. 1).



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

Statistical analysis

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

To calculate the parasitism rate of seedpod weevil larvae, for the 2019 data, the sum of parasitoids emerged from the pods was divided by the sum of exit holes and pods with remains of seed weevil larvae. During the investigations in the year 2019, it was noticed that the exit holes of parasitoids can be distinguished from those of the CSW larvae. For this reason, in 2020, parasitism rate was calculated by dividing the sum of exit holes produced by a parasitic wasp by the total sum of exit holes and the remains of seedpod weevil larvae. This allows to include parasitoids, which may have already hatched before the plant sample was taken.

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

Results

Pod infestation with cabbage seedpod weevil

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

The average infestation of pods with CSW larvae over all treatments at BBCH 82 was 19.27% ($\pm 1.14\%$) at the trial site Wendhausen in 2019. The pod infestation with CSW was significantly lower after conventional spraying of Biscaya (15.4%, CI_{95%} 12.6–18.2%) compared to the conventional Mospilan treatment (22.6%, CI_{95%} 19.2–26.0%; $p = 0.0122$) (Tab. 2). Dropleg spraying of Biscaya resulted in an infestation rate of 16.7% (CI_{95%} 13.7–19.8%) and differed neither to the control (17.82%, CI_{95%} 14.94–21.14%; $p = 0.9870$) nor to the conventional application of Biscaya ($p = 0.9708$). The infestation rate at the third lateral raceme (23.3%, CI_{95%} 21.2–25.7%) was significantly higher compared to the main raceme (14.3%, CI_{95%} 12.6–16.1%; $p < 0.0001$) (Fig. 2). The interaction between treatment and raceme was not significant. However, it is noticeable that on the third lateral raceme all insecticide treatments tended to result in increased infestation rates compared to the untreated control.

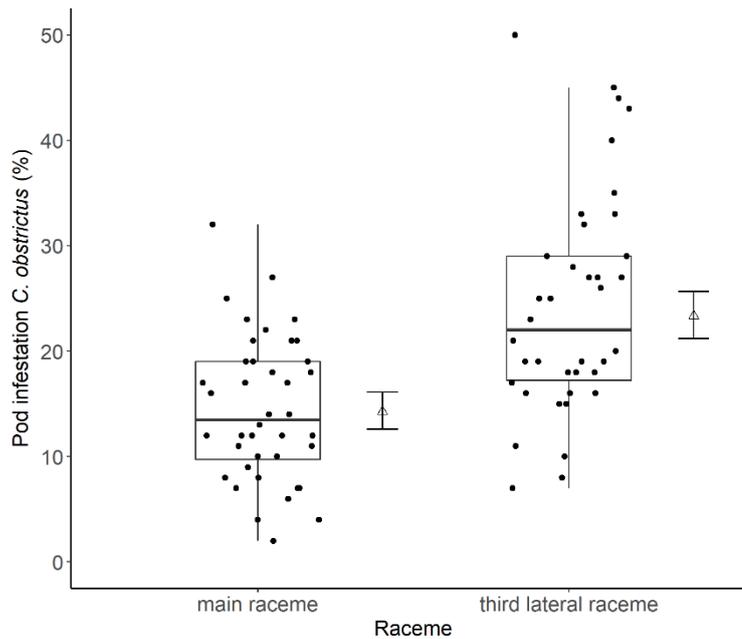


Figure 2 Boxplots showing the median value (solid line), the 25th and 75th percentiles of pod infestation with larvae of *Ceutorhynchus obstrictus* (%) at BBCH 82 at the main raceme and the third lateral raceme, respectively in Wendhausen, 2019. The error bars below and above the box indicate the 10th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

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

Table 2 Pod infestation with *Ceutorhynchus obstrictus* larvae (%) and proportion of parasitized larvae (%) for different insecticides, applied with conventional and dropleg spraying techniques. Observed data \pm se and estimated means with confidential intervals obtained from the best model. Different letters indicate significant differences between treatments within year and trial site, Tukey-HSD ($p < 0.05$)

Year	Location	Sampling dates, Growth stage	Treatment	N	pod infestation		parasitism	
					observed \pm se	estimate means (95% CI)	observed \pm se	
2019	Wendhausen	16/06/2019, 24/06/2019, BBCH 80–82	Control	300	18.13 \pm 1.88	17.83 (CI 14.73–20.93)	ab	64 \pm 8
			conventional Biscaya	300	16.62 \pm 2.88	15.40 (CI 12.56–18.23)	a	38 \pm 8
			conventional Mospilan	300	23.50 \pm 2.37	22.59 (CI 19.16–26.02)	b	63 \pm 6
			Dropleg Biscaya	300	17.27 \pm 1.99	16.72 (CI 13.65–19.78)	ab	37 \pm 8
			Dropleg Mospilan	300	20.62 \pm 3.16	20.01 (CI 16.82–23.20)	ab	62 \pm 7
2020	Wendhausen	25/06/2020, BBCH 80–82	Control	300	6.12 \pm 1.01			68 \pm 12
			conventional Biscaya	300	10.38 \pm 1.89	treatment effect not significant		85 \pm 5
			conventional Mavrik	300	8.25 \pm 2.74			73 \pm 9
			Dropleg Biscaya	300	5.62 \pm 1.05			62 \pm 11
			Dropleg Mavrik	300	7.00 \pm 1.55			54 \pm 14
2020	Groß Brunsrode	25/06/2020, BBCH 80-82	Control	400	4.62 \pm 0.73			50 \pm 13
			conventional Biscaya	400	5.38 \pm 1.07	treatment effect not significant		42 \pm 11
			conventional Mavrik	400	4.38 \pm 0.94			60 \pm 12
			Dropleg Biscaya	400	6.25 \pm 1.00			60 \pm 10
			Dropleg Mavrik	400	5.88 \pm 1.43			51 \pm 13

Parasitism rates of weevil larvae

The average parasitisation rate in the untreated control was above 50% at all trial sites in both years (Tab. 2). In 2019, the interaction between treatment and raceme was significant ($p = 0.0106$, Fig. 3).

The parasitism rate after conventional application of Biscaya on the main raceme was 23.2% (CI_{95%} 12.0–40.2%), which was significantly lower compared to the control (56.8%, CI_{95%} 43.6–69.2%, $p = 0.0214$) and both Mospilan treatments (conventional Mospilan 69.4%, CI_{95%} 55.0–80.7%, $p = 0.0009$; Dropleg Mospilan 73.6%, CI_{95%} 59.0–84.4%, $p = 0.0003$). The dropleg application of Biscaya resulted in parasitism rates of 31.7% (CI_{95%} 19.0–47.9%), which is significantly reduced compared to the Mospilan treatments (conventional Mospilan, $p = 0.0065$; Dropleg Mospilan, $p = 0.0021$), but not to the control ($p = 0.1267$). At the third lateral raceme, the parasitism rate raised up to 71.4% (CI_{95%} 57.6–82%) in the control. Compared to the control and irrespective of the application technique, Biscaya reduced the parasitism rate significantly (conv. Biscaya 37.7%, CI_{95%} 26.8–50.0%, $p = 0.0039$; Dropleg Biscaya 44.9%, CI_{95%} 32.6–57.8, $p = 0.0482$). As the parasitism rates of the Mospilan treatments tended to be above the control rate at the main raceme, it was the opposite at the third lateral raceme.

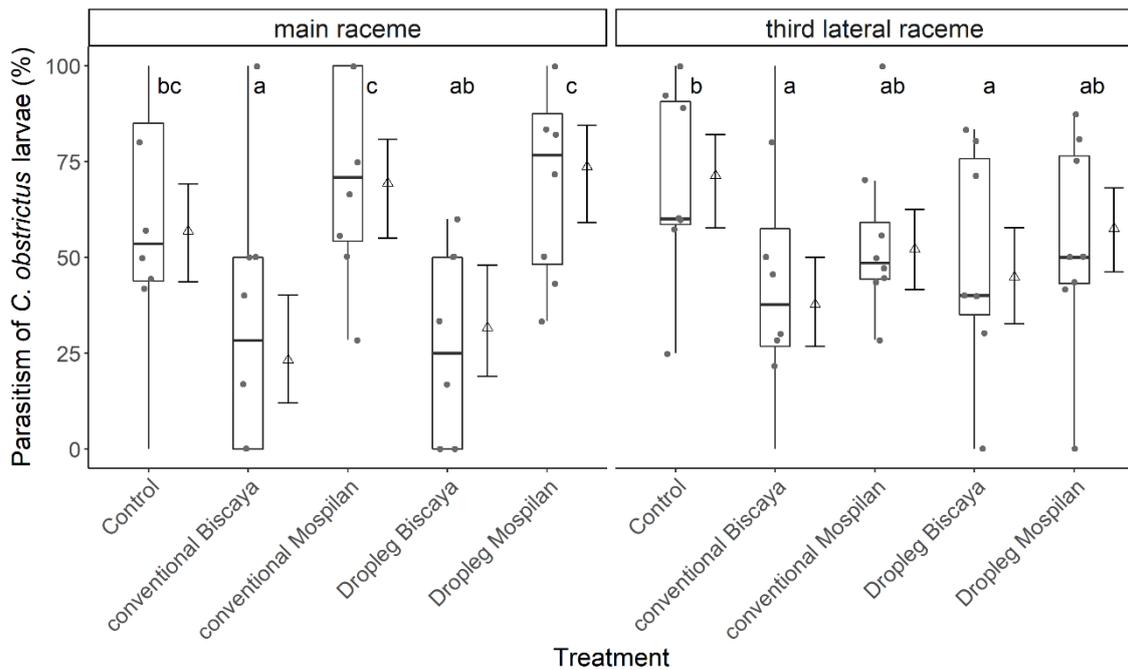


Figure 3 Boxplots showing the median value (solid line), the 25th and 75th percentiles of parasitism rates of *Ceutorhynchus obstructus* larvae (%) at BBCH 82 after different insecticide treatments in oilseed rape at full flowering (BBCH 65/67) in Wendhausen in 2019. The error bars below and above the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

In the year 2020, the average parasitism rates differed significantly between the trial sites (Wendhausen 72.9%, CI_{95%} 66.3–78.6% and Groß Brunsrode 57.3%, CI_{95%} 50.2–64.1%; $p = 0.0011$). The different treatments and the position of the pods at the main raceme or third lateral raceme did not affect parasitism rates (Fig. 4).

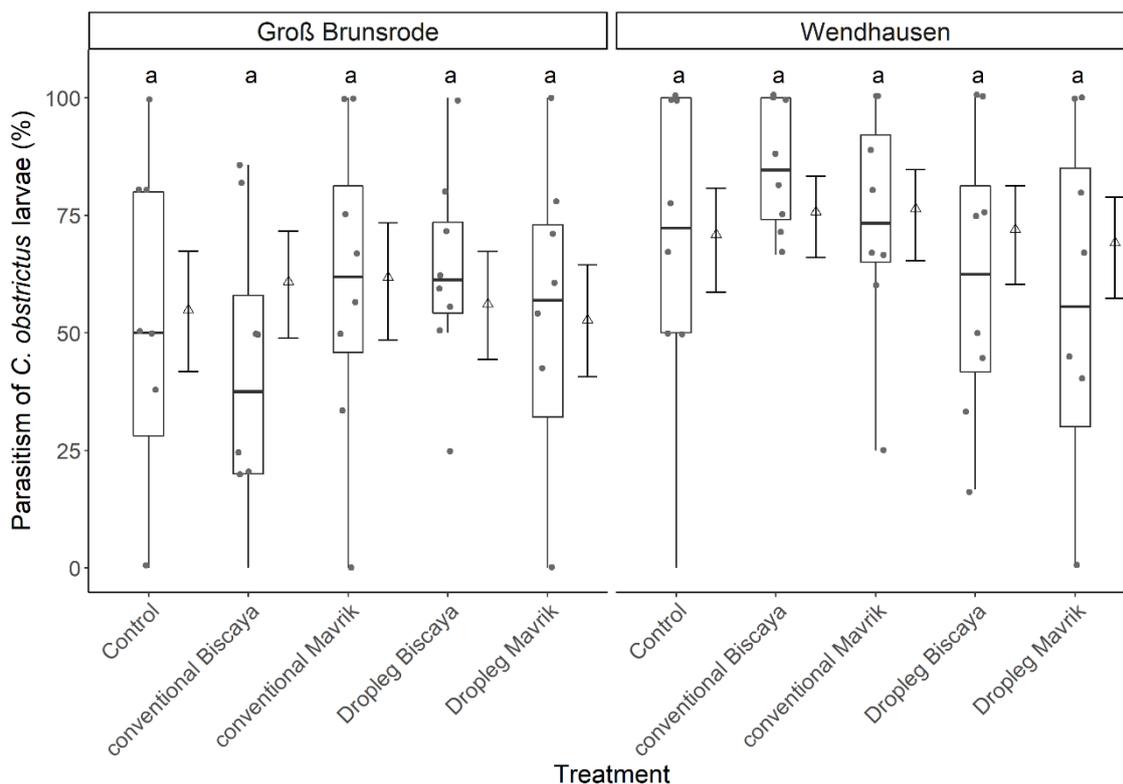


Figure 4 Boxplots showing the median value (solid line), the 25th and 75th percentiles of parasitism rates of *Ceutorhynchus obstrictus* larvae (%) at BBCH 82 after different insecticide treatments in oilseed rape at full flowering (BBCH 65/67) in Groß Brunsrode and Wendhausen in 2020. The error bars below and above the box indicate the 10th and 90th percentile. Points show the mean parasitism over blocks. Estimated means (Δ) and 95% confidence intervals obtained from the best model. Treatments with different letters are significantly different, Tukey-HSD ($p < 0.05$)

Effects of the insecticide applications on the different parasitoid species

In 2019, 3067 pods were collected and 304 parasitoids emerged out of them in the laboratory. The prevalent species were *M. morys* (52%) and *T. perfectus* (46%) (Fig. 5). Six individuals could not be identified at species level, due to their preservation status.

The abundance of *C. obstrictus* was lower in 2020 and the proportion of damaged pods decreased from 19% in 2019 to 6% in 2020. The number of parasitoids decreased as well. However, a number of 260 individuals from both trial sites emerged from 7000 collected pods in the laboratory. It is noticeable that the proportion of *M. morys* in the year 2020 increased by 25% compared to the previous year. Additionally, in this year seven species of *Stenomalina gracilis*, Walker (Hymenoptera: Pteromalidae) emerged from the pods. There was no difference in the species composition between both trial sites in 2020. The relative abundance of *M. morys* per plot was significantly higher in Wendhausen

(2.18, CI_{95%} 1.76–2.70, $p = 0.0050$) compared to Groß Brunsrode (1.39, CI_{95%} 1.07–1.80). Also, the relative abundance of *M. morys* per plot was higher at the third lateral raceme of the OSR plant (2.30, CI_{95%} 0.87–2.83) compared to the main raceme (1.32, CI_{95%} 1.01–1.72, $p = 0.0006$).

In 2019, there were significant differences between insecticide treatments. The average number of *T. perfectus* that emerged per plot and sampling date, was 0.69 (CI_{95%} 0.40–1.19) in the conventional Biscaya treatment (Fig. 6). This was significantly lower compared to both Mospilan treatments (conventional Mospilan 1.90, CI_{95%} 1.35–2.68, $p = 0.0142$ and Dropleg Mospilan 2.06, CI_{95%} 1.48–2.87, $p = 0.0055$), but not to the control (1.68, CI_{95%} 1.16–2.45, $p = 0.0532$). Also, the application of Biscaya with dropleg technique (0.69, CI_{95%} 0.40–1.19) resulted in significantly reduced numbers of *T. perfectus* compared to conventional Mospilan ($p = 0.0142$) and Dropleg Mospilan ($p = 0.0054$). For the second parasitoid species *M. morys*, a similar trend was observed in 2019 (Fig. 7). The conventional application of Biscaya decreased the number of emerged parasitoids per plot and sampling date significantly to 1.07 (CI_{95%} 0.67–1.70) compared to the control (2.45, CI_{95%} 1.78–3.38, $p = 0.0301$) and the Dropleg Mospilan treatment (2.38, CI_{95%} 1.74–3.26, $p = 0.0393$). None of the parasitoid species was affected by the insecticide treatments in 2020.

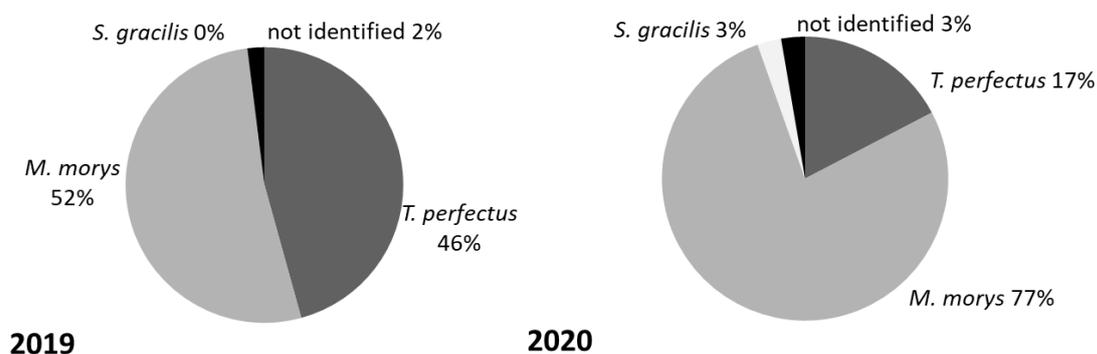


Figure 5 Species composition (%) of parasitoids (Pteromalidae) that emerged from oilseed rape pods collected at BBCH 82 (maturing of pods) at one trial site in 2019 and two sites in 2020

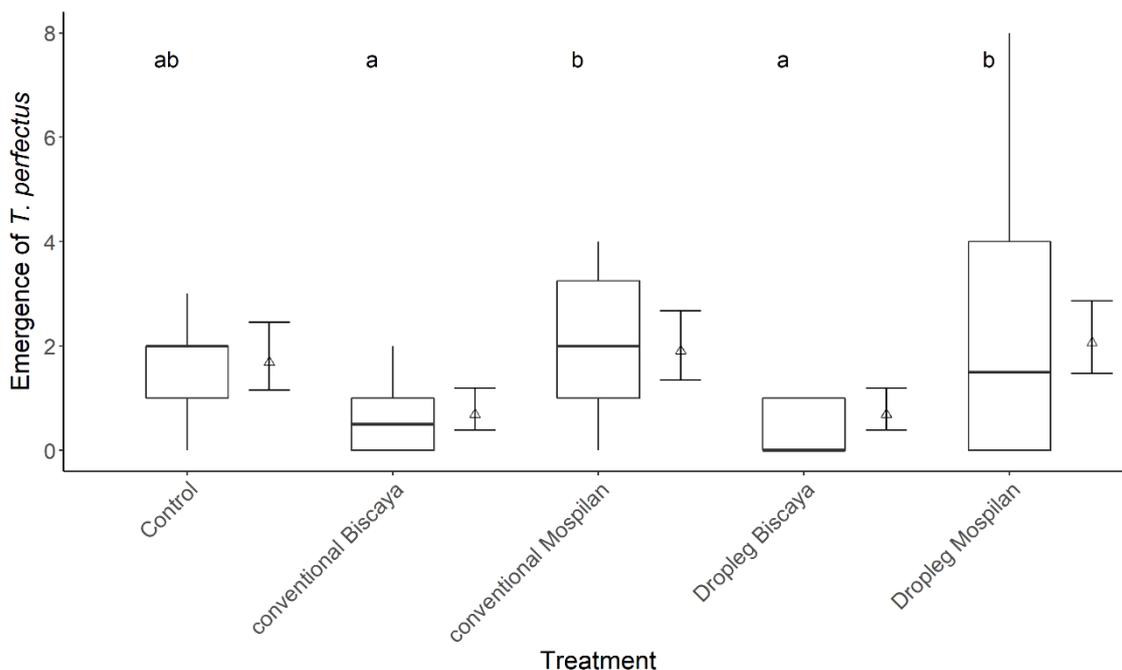


Figure 6 Boxplots showing the median value (solid line), the 25th and 75th percentiles of *Trichomalus perfectus* emergence from oilseed rape pods collected at BBCH 82 after different insecticide treatments during flowering (BBCH 65). The error bars below and above the box indicate the 10th and 90th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

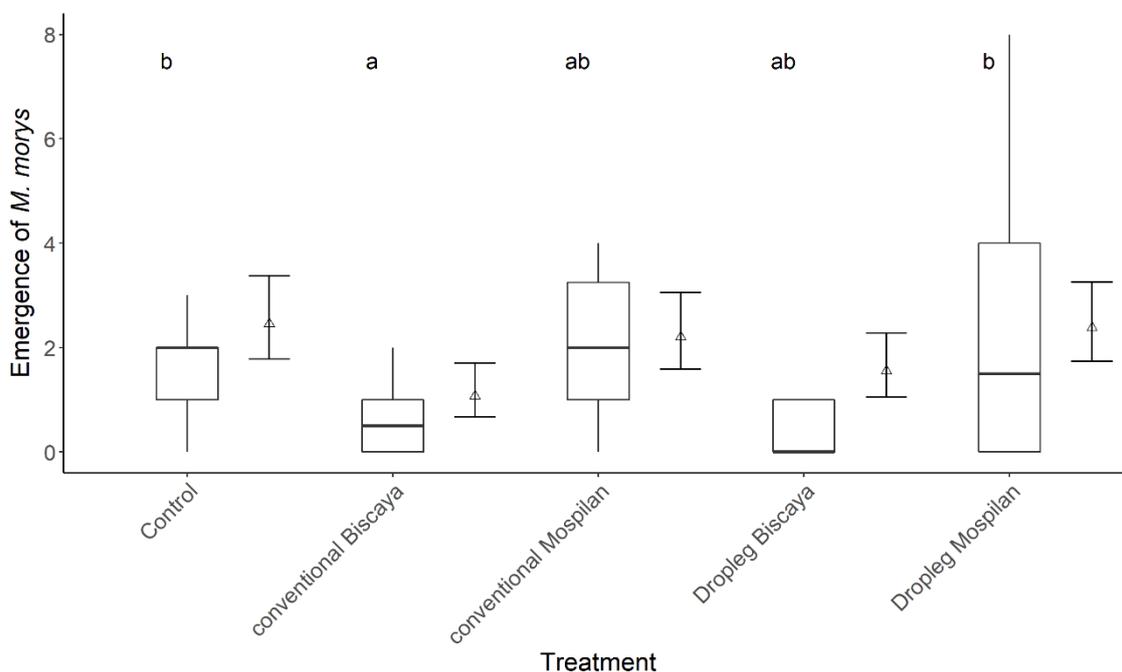


Figure 7 Boxplots showing the median value (solid line), the 25th and 75th percentiles of *Mesopolobus morys* emergence from oilseed rape pods collected at BBCH 82 after different insecticide treatments during flowering (BBCH 65). The error bars below and above the box indicate the 10th and 90th percentile. Different letters indicate significant differences between treatments, Tukey-HSD ($p < 0.05$)

Discussion

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

The insecticidal treatments affected parasitism rates of seedpod weevil larvae, similar to the effects on pod infestation with CSW larvae, only in 2019. Insecticides differ in their non-target effects due to the properties of the active ingredients (Sánchez-Bayo, 2012) and its formulations (Pereira et al. 2009). All active ingredients used have in common that they act as contact and feeding poisons. While Biscaya (a.i. thiacloprid) and Mospilan (a.i. acetamiprid) can distribute systemically in the plant, Mavrik Vita (a.i. tau-fluvalinate) does not translocate and acts only locally. Since CSW feed and reproduce on the generative plant organs, a better effect would have been expected from conventional application, since here flowers and young pods are sprayed with active ingredients. Instead, no difference was found with respect to the application technique. This may be because the weevils fall from plants to protect themselves from predators when they sense vibrations or sudden shading. They are then exposed to the active ingredients even after dropleg application. Another reason could be the systemic translocation of the active ingredients in the plants, whereby effects against pests and non-targets in the flower and pod canopy could also occur after dropleg application. However, no general conclusions can be drawn from the results of our field trials, because despite the systemic properties of Biscaya and Mospilan, effects on CSW and its parasitism rates were observed only after application of Biscaya. Going more detailed into chemical structures, thiacloprid belongs to the five-membered neonicotinoids, whereas acetamiprid belongs to the non-cyclic neonicotinoids (Jeschke and Nauen 2019; Schäfer 2008). The chemical structures

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

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

and Moisey, 2004). The parasitoid species *T. perfectus* migrates into the OSR fields when temperatures exceed 17 °C, however, main flight only starts when mean temperature is above 14 °C and maximum temperature reaches 23 °C (Johnen et al., 2010). In most years, this is 2–4 weeks after the weevils have colonized the OSR fields (Dmoch, 1975; Haye et al., 2018). In conclusion, if insecticides are applied at full flowering stage, direct effects on the parasitoids of CSW seem unlikely, because often there is no temporal coincidence with the parasitoid emergence in the crop. This was probably also the case in 2020.

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

nectar or pollen for energy supply remains unclear. Ultimately, the results from only one trial do not allow a clear statement on whether *M. morys* benefits from lower pesticide residues in the upper crop canopy after applications using dropleg technique.

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

In the present study, we found minor effects of insecticides applied in flowering OSR on parasitism rates of CSW larvae. The present results indicate that spatial targeting of insecticides did not affect non-target effects on larval parasitoids of CSW. In general, it can be assumed that CSW larvae parasitism as a valuable ecosystem service is rather

influenced by more general conditions such as climate (Haye et al., 2018) or landscape composition (Kovács et al., 2019) rather than by insecticide treatments during flowering.

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Chapter V

Challenges for integrated pest management of *Dasineura brassicae* in oilseed rape

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Abstract

The use of insecticides in flowering oilseed rape (*Brassica napus* L.) against pest insects such as the brassica pod midge (*Dasineura brassicae* W.) often conflicts with the protection of pollinating and beneficial insects. *Dasineura brassicae* is a major pest insect in European oilseed rape production. However, a comprehensive and sustainable pest control strategy within the framework of integrated pest management (IPM) does not exist and little research on the insect has been published during the past two decades. This paper reviews the existing knowledge about *D. brassicae* along its life cycle and is intended to form the basis for further research activities on pod damaging pest insects in oilseed rape. Important knowledge gaps are identified, regarding the significance of natural enemies, diapause induction and predictions on damage potential, based on initial pest insect population. The short lifespan of the adults is particularly challenging in praxis. The implementation of IPM for *D. brassicae* is discussed on the basis of the four IPM steps (set an economic threshold, establish pest monitoring, preventive measures and direct control measures) and remaining hurdles, as well as potential solutions for a better IPM, are identified. For *D. brassicae*, there is no science-based economic threshold and no applicable monitoring methods for farmers, which hinders a field specific damage forecast and the precise timing of insecticide applications. Research into improved monitoring (e.g. selective attractants, real time monitoring using remote sensing technologies) appears to be a promising step towards an integrated pest management of *D. brassicae*.

Introduction

Integrated pest management (IPM) is considered to be the standard in modern agriculture (Dara 2019; European Commission 2020). It aims to establish sustainable crop management systems, with effective regulation of pest organisms by natural antagonisms and environmentally friendly measures. There are various approaches how to implement IPM strategies in practice, all covering the following four steps as core elements (Barzman et al. 2015; Ehler 2006; Hokkanen 2015). At first, an economic threshold is set, then a pest monitoring is established, preventive measures are taken, and only as the last option, chemical control is used.

Oilseed rape, *Brassica napus* L., (Brassicaceae) (OSR) is one of the most important break crops in cereal dominated crop rotations in Europe (Lundin 2021; Zheng et al. 2020). Its seeds are processed into versatile oil for human nutrition, industry and biodiesel, and its by-products are used as animal feed (Friedt and Snowdon 2009). A major challenge in the cultivation of OSR is the control of various pest insects, that cause average annual yield losses of 15% across Europe (Zheng et al. 2020). Substantial efforts have been made to develop IPM strategies in oilseed rape production and extensive knowledge on pest insects has been published (Alford et al. 2003; Nilsson et al. 2015; Williams 2010a). Nevertheless, there is still no IPM strategy for the brassica pod midge, *Dasineura brassicae* Winnertz (Diptera: Cecidomyiidae) and there has been little research on this insect during the last two decades. *Dasineura brassicae* is a major pest insect of OSR and widely spread throughout all important growing areas in Europe and in parts of China (Alford et al. 2003; Zheng et al. 2020). During the ripening period of seeds, the larval instars of *D. brassicae* feed on the inner pod walls and secrete enzymes, causing the pods to turn yellowish, swell and deform (Meakin and Roberts 1991). Significant yield losses occur due to a decrease in seed weight by up to 80% (Williams 2010b) and a premature splitting of the pods, resulting in the loss of seeds (Meakin and Roberts 1991). In traditional OSR growing areas, *D. brassicae* spawns two generations in winter OSR and a third generation develops in spring OSR (Williams et al. 1987a, 1987b). To prevent damage from *D. brassicae*, farmers regularly spray insecticides during the bloom of OSR, which is critical for several reasons. Insecticides are often added to fungicide treatments for *Sclerotinia sclerotiorum* (Lib.) de Bary and thus usually miss the optimal timing aligned with the flight of the first generation of *D. brassicae*.

Additionally, insecticide applications during the bloom of OSR have a particularly high risk to harm non-target organisms, such as pollinators (Karise et al. 2017; Mänd et al. 2010) and many important parasitoids of OSR pest insects (Ulber et al. 2010a). In line with the IPM objectives, pesticides should be applied only after economic thresholds have been exceeded and in a targeted manner. In case of *D. brassicae*, the size of single generations is highly fluctuating and cumulated larval densities of individual years can range from a few hundred to over 30.000 larvae m⁻² (Felsmann 2007; Hausmann et al. 2019). Most studies found the second generation to be the largest (Buhl 1960; Erichsen 1982; Fröhlich 1956a). However, applications against the second generation are not sensible, because OSR branches out during pod set and late applications result in major plant damage along the driving alleys, among other problems. Consequently, the second generation develops undisturbed and can only be controlled indirectly by a reduction of the previous generation. Thus, a thorough understanding of the factors driving *D. brassicae* population dynamics is absolutely necessary for the successful management of this multivoltine pest insect, with only one application against the first generation early in the season. For targeted control, it is important to precisely determine the time point at which a pest insect emerges and immigrates into the crop. Species-specific migration behaviour has a major impact, on which fields, or areas within, will be infested. Knowledge about the maximum reproduction capacity of a single female is important for estimating the maximum possible population increase over several generations at a given pest insect density. These and other aspects are linked to biological traits in the life cycle of *D. brassicae*, which are addressed in the first part of this review. To predict the occurrence of a pest insect and the related damage, the most important factors influencing its population dynamics have to be known. This is analysed in the second part of this review. Together, this information can provide the basis for the development of economic thresholds and decision support systems. The obstacles that currently prevent the implementation of an effective IPM programme against *D. brassicae* and how to overcome those shortcomings, are described in third part of the review.

This review elucidates that the management of *D. brassicae* in agricultural praxis is difficult, because monitoring systems are not yet available and knowledge gaps in the biology allow only vague predictions of pest insect populations and damage, which also prevents the establishment of sustainable thresholds. It is intended to provide a

comprehensive overview of the state of knowledge on *D. brassicae* and a basis for further research activities.

Current knowledge on the biology of *Dasineura brassicae*

Emergence of the first generation

Dasineura brassicae hibernates as larva in a cocoon in fields where OSR was grown in previous years (Williams 2010b). After diapause, the larvae pupates inside the cocoons (Buhl and Schütte 1964) and the pupa moves up to the soil surface (Buhl 1960). The emergence of the first generation is mainly driven by soil temperatures (Axelsen 1992b; Hansen 1994; Kirchner 1966). For the development of the cocoons in the soil, 141 day degrees above 8.1 °C after the first of January, are needed (Axelsen 1992b). The first generation of midges emerges when the soil temperature in 5 cm depth exceeds 15 °C and air temperature is around 19 °C (Kirchner 1966), which coincidences with the early flowering stage of OSR in most years (Fröhlich 1956a; Williams et al. 1987a). Pupation itself is not influenced by soil moisture (Erichsen 1982; Hughes 1998; Kirchner 1966). However, soil moisture affects the warming of the soil and the mechanical resistance for the movement of pupae to the soil surface (Blume et al. 2016). Consequently, the emergence of midges within one field can vary due to small-scale differences in soil moisture and soil type and depends also on the depths at which the cocoons were located in the soil. The flight period of the first generation lasts about 3-6 weeks (Williams et al. 1987a). Shifting temperatures can delay emergence and often there is no constant flight, but several waves of migration.



Figure 1 Female of *Dasineura brassicae*. The adults of *D. brassicae* are delicate with body length of 0.7–1.5 mm (♂) and 0.9–2.2 mm (♀), respectively (Kirk 1992). Males have stalked antennae with annular sensory hairs. Distinctive features of the female are the non-stalked antennae and the ovipositor. The three larval instars live gregariously within the pods and grow up to 2 mm. The first larval instar is almost translucent, and older larvae are white colored with yellow fat bodies

The emergence of *D. brassicae* occurs mainly in the morning (Buhl 1960). At the emergence site, virgin females show a “calling behavior” and release a sex pheromone (Williams and Martin 1986) that is produced by glandular tissue in the epidermis of an intersegmental membrane in the ovipositor (Isidoro et al. 1992). After mating, the females immediately search for host plants, which is illustrated by the curve of the flight activity at the emergence sites (Murchie et al. 2001). It was observed that the curve is unimodal with a peak for

males at 9:00 (searching for virgin females) and time shifted at 11:00 for females (mated females search for suitable host plants). There is no flight activity at night time (Murchie et al. 2001).

Finding and accepting host plants

In general, the range of dissemination of *D. brassicae* is rather low. In search of new host plants, the females mainly fly downwind (Sylvén 1970). Just as they deflect the wind, hedges and other landscape elements can act as barriers or sluices for the dispersal of *D. brassicae*. Distances of up to 500 m upwind were observed only at low wind speeds of max. 4.5 m s⁻¹ (Schütte 1964), which indicates an olfactory-mediated upwind anemotaxis. This was confirmed by olfactometer bioassays in which mated females (but no males) were attracted to leaves and pods of OSR (Pettersson 1976) and crushed OSR leaves (Williams and Martin 1986). At a shorter distance, males and females of *D. brassicae* respond positively to the yellow colour of the flowers (Williams and Cook 2010).

Host plants of *D. brassicae* are OSR, close relatives such as turnip rape (*Brassica rapa subsp. oleifera*), cabbage (*Brassica oleracea*), and some cruciferous weeds. In general, the survival on *Brassica* spp. is higher than on other cruciferous plants, which only serve as hosts to a limited extent (Åhman 1988; Speyer). In choice tests between OSR and other crucifers, females did land more frequently on OSR and the number of batches per ovipositing female was higher (Åhman 1988). The initial host plant adaption behaviour is probably elicited by the wax composition of the cuticula (Åhman 1986). On a potential host plant, females can be observed walking along pods. Tactile and sensory stimuli are perceived by the tarsi and antennae (Coutin 1964). In most cases, females lay their eggs in pods that were previously damaged (Åhman 1987). Potential oviposition sites are externally palpated with the maxillary palps and finally investigated by the insertion of the ovipositor, which is equipped with different types of sensory hairs at its tip (Hallberg and Åhman 1987).

Reproduction of *Dasineura brassicae*

Virgin females produce up to 140 eggs in their ovaries (Sylvén (1949) cited by Axelsen 1992a), however the average number of eggs laid per female is estimated to be around 60 (Buhl and Schütte 1971). Adult midges were observed licking on plant sap (Fröhlich 1960), but no feeding behaviour of the adults was observed so far. Whether egg maturation in *D. brassicae* is proovigenic, or whether additional eggs also mature after eclosion, is not yet known. However, fecundity is correlated with the body length of a female (Sylvén (1949), cited by Åhman 1985), which indicates that all eggs mature before eclosion. Environmental stimuli affect oviposition as well. According to Fröhlich (1956a) temperatures above 19 °C are preferred for oviposition. Additionally, the quality of a host plant influences the egg loads and at a later stage, larval weights which are higher on suitable hosts, compared to low-quality hosts (Åhman 1988). Females lay their eggs in several batches and there is evidence for monogeny of broods of a single midge (Murchie and Hume 2003). Regarding the overall sex ratio in *D. brassicae*, several authors described a slight bias towards females (Buhl 1960; Fröhlich 1956a; Murchie and Hume 2003).

The development of the eggs and larvae depends on temperature (Axelsen 1992b; Hughes 1998). Eggs hatch after 3–4 days and the egg-larval stage of the first generation needs 134 °DD above a developmental threshold of 6.7 °C, which lasts about 3–4 weeks

in the field (Axelsen 1992b). At maturity, larvae leave the pods and drop down to the soil where they burrow in 0–5 cm depth (Buhl 1960; Fröhlich 1956a).

According to Buhl (1960) about 5% of the first generation larvae, but almost 80% of the second generation larvae enter winter diapause. In contrast, Axelsen et al. (1997) found that the proportion of larvae that enter diapause varies within a season and between years, and is not constantly increasing in every generation. The authors suggested that the cumulative solar radiation perceived by the growing larvae triggers diapause. Still the specific factors that control diapause induction remain unknown. Additionally, not all diapausing larvae pupate in the following year; e.g. Schütte (1979) investigated that 13% of the overwintering larvae did not emerge in spring following diapause. Diapausing larvae of *D. brassicae* can survive in the soil for up to 5 years (Buhl 1960; Nilsson et al. 2004), but there is a negative correlation between the duration of the cocoon stage in the soil and the average lifespan of adults (Schütte 1979).

Life expectancy of *Dasineura brassicae*

In the field a life expectancy of 1–3 days is assumed for adult *D. brassicae* (Williams 2010b), though conclusive reports on the lifespan of *D. brassicae* are missing. Hughes (1998) demonstrated that the lifespan of *D. brassicae* is temperature dependent, however, experiments were biased because midges had no access to water and did not survive longer than 48 hours in any of the temperature regimes.

To shed more light on this question, I have conducted an experiment in which larvae of the second generation were collected in the field in 2019 and were held for almost one year in moist sand at 5 °C in the climate chamber, before they were reared to adult midges. The midges used for this experiment, emerged from the same sample in the morning of the 16th June 2020. Groups of five females were transferred into transparent plastic cups (500 ml, height 16.5 cm), which had three openings (each 4 cm²), covered with gauze to allow the circulation of air. A water saturated cube (3.5 cm³) of floral foam in each cup supplied moisture to the midges. The foam cubes were resupplied with drops of tap water every 12 hours through the top gauze net. Each six cups (replicates) were placed in climate chambers (RUMED®, P 210) at constant temperature levels of 10 °C, 15 °C, 20 °C and 25 °C. The experiments started at 12 a.m. and the mortality of the midges was

recorded every six hours for three days. Thereafter, assessments were made every 12 hours.

Data analysis was performed using R (version 3.6.1) (R Core Team 2019). A generalized linear model (glm) explaining the number of dead midges per cup by temperature treatments and time was fitted. A binomial error distribution was assumed. The effects of different variables were tested via an analysis of deviance using the “Chi-test”. For model diagnostics, the residuals were plotted against the predicted values and the explanatory variables and were checked visually. The LT_{50} values were calculated using the function *dose.p* from the MASS package (Venables and Ripley 2007). The effect of different temperatures on the mortality of midges over time was also analysed using Kaplan-Meier survival analysis.

As a result, 50% of female *D. brassicae* were alive for 35 h ($SE \pm 1.2$) at 25 °C (max. 66 h) (Fig. 2). At a constant temperature of 10 °C the LT_{50} was 224 h ($SE \pm 6.8$) and single females lived up to 17 days. Adult lifespan was significantly dependent from the temperature treatments (Kaplan-Meier survival analysis, $p < 0.001$). This outcome confirmed that under humid conditions adult lifespan is inversely related to temperature.

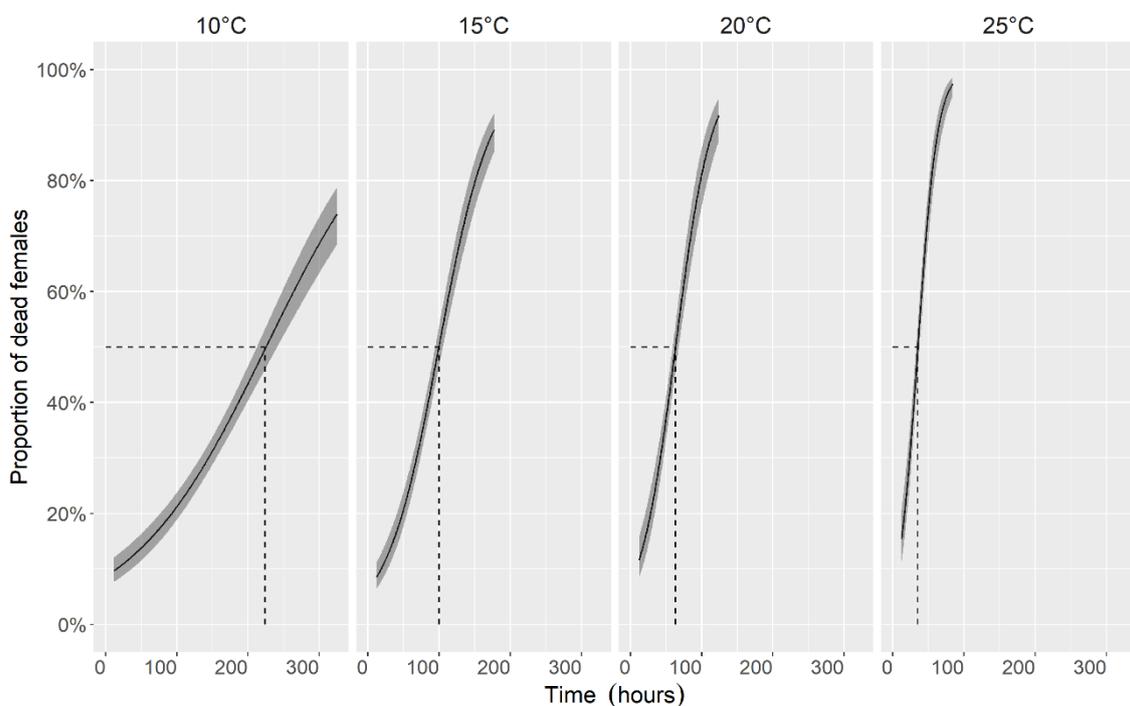


Figure 2 Calculated mortality with 95% confidence limits of *Dasineura brassicae* females over the time (hours). Midges were kept in plastic cups with a moist cube of floral foam at constant temperatures. Dashed lines indicate the time after which 50% of the midges have died (LT_{50}). $N = 30$

Drivers of population dynamics

A distinction can be made between factors that have the potential to promote population dynamics of *D. brassicae* and others that have negative effects (Fig. 3).

Regarding the life cycle, Axelsen (1992c) identified two critical life stages, in which the population of *D. brassicae* suffers larger losses, namely the pre-cocoon stage and the diapause during winter. The author calculated a generation survival of about 2–4% (Axelsen 1992c). During the pre-cocoon stage, which is the time period between the dropping of larvae from the pods until they spin a cocoon in the soil, losses may occur because of predation by carabids (Warner et al. 2000) and spiders (Felsmann 2007). Additionally, dry weather conditions that lead to desiccation are being discussed (Axelsen 1992c). The losses during diapause can be attributed to parasitism (Ferguson et al. 2004; Murchie 1996), pathogens (Hokkanen et al. 2003), and adverse effects of tillage e.g. ploughing (Axelsen 1992c). There is still a need for research on all of these points.

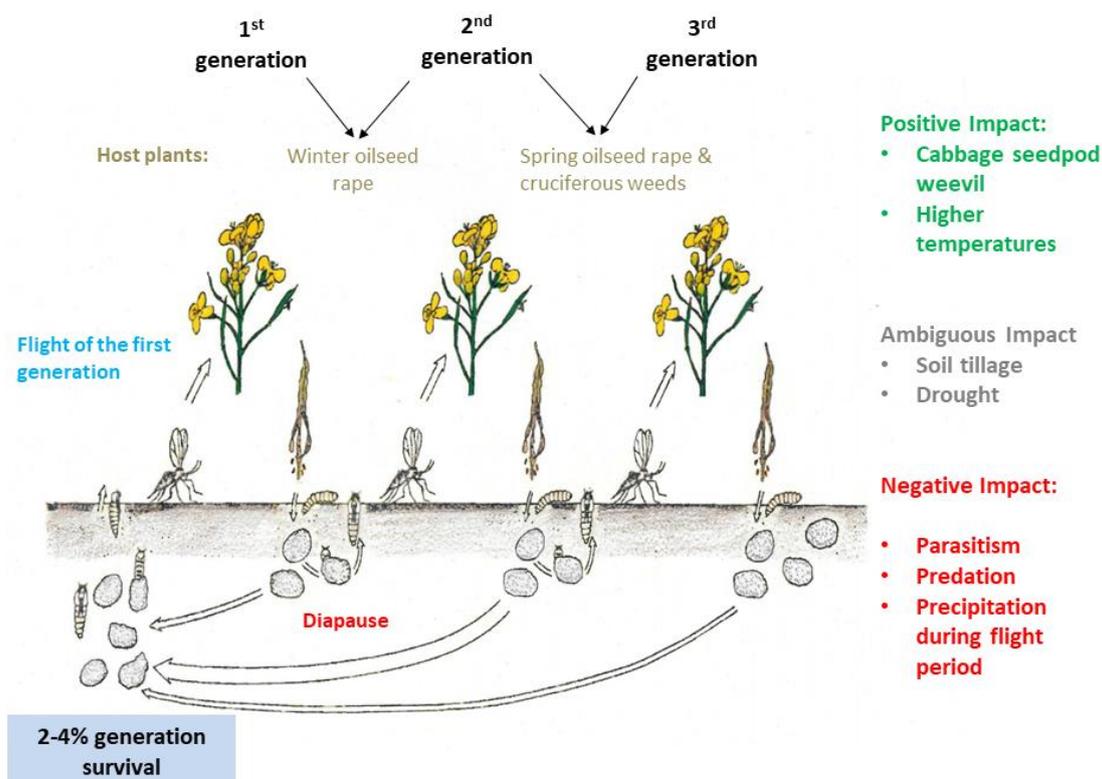


Figure 3 The life cycle of *Dasineura brassicae*, modified after Buhl 1960

Weather conditions can affect the population dynamics of *D. brassicae* in different ways. As described, many physiological processes, like the emergence of the first

generation, egg-laying, larval and pupal development, and lifespan, are temperature dependent. Warmer temperatures reduce the developmental time of eggs, larvae and pupae, resulting in a faster generation turnover. Whether diapause is induced by environmental conditions remains to be investigated. On the other hand, it seems likely that quickly changing weather conditions during the flight period of the first and second generation, especially precipitation, may kill adults and prevent successful oviposition. Strong winds may disperse the first generation adults and partly prevent colonization of suitable crops.

As mentioned earlier, *D. brassicae* is reliant on damaged pods for oviposition because the ovipositor is not suitable for piercing or drilling of mature pod walls, as demonstrated by morphological studies (Hallberg and Åhman 1987; Stechmann and Schütte 1978). For this reason, the cabbage seedpod weevil, *Ceutorhynchus obstrictus* Marsham (Coleoptera: Curculionidae), by injuring on the pods through its feeding and oviposition activity, is considered the most important factor for a severe infestation of an OSR crop with *D. brassicae* (Ferguson et al. 1995; Free et al. 1983; Speyer 1921). Strong increases in the seed weevil population lead to a time shifted growth of the *D. brassicae* population (Schütte 1979). In many cage trials on OSR, no oviposition of *D. brassicae* could be observed without artificial injury of the pods or the addition of *C. obstrictus* (Ankersmit 1955; Buhl 1957; Doberitz 1973; Fröhlich 1956b; Hughes and Evans 2003). In contrast, several authors described that *D. brassicae* can also independently oviposit in small and young pods up to a length of 40 mm (Axelsen 1992c; Fröhlich 1956b; Hoßfeld 1963; Mühle 1951; Nietzke 1976). Thus, *C. obstrictus* is probably more important for the development of the second than the first generation. Also, at low pest insect densities, the abundance of *C. obstrictus* is not limiting the population size of *D. brassicae* (Axelsen 1992a; Fröhlich 1956b). This can be explained by the larvae of *D. brassicae* living gregariously and more than one female using a suitable pod for oviposition. For this reason, the average number of larvae within one pod can fluctuate considerably and is dependent on the availability of damaged pods for female midges. In addition, other phytophagous insects, such as lygid bugs (*Lygus* spp. (Heteroptera: Miridae)) (Hughes and Evans 2003) or abiotic damages such as hail and wind (Winfield 1992), can provide suitable oviposition sites. Concluding, *C. obstrictus* is important for the economic damage potential of *D. brassicae* within one year, but not a mandatory prerequisite.

Challenges for the implementation of integrated pest management

Defining economic thresholds

Dasineura brassicae is a typical *r*-strategist, with short generation time and high reproductive capacity. The short lifespan of the adults is particularly challenging for farmers, because the midges start egg-laying immediately after their emergence in OSR, leaving little time for control measures. As stated above, it was shown that the abundance of the weevil *C. obstrictus* at full flowering promotes pod damage by *D. brassicae* later in the season. However, predation, parasitism and weather conditions (especially temperature) seem to be additional important drivers of the midge's population dynamics. Main losses during the life cycle occur during the winter diapause and the overall generation survival is low. Nevertheless, small numbers of individuals seem to be able to build up a strong population over the season, if conditions are favourable. Hence, the shift from the first to the second generation should be the focal point for the overall reproduction capabilities and population size within one season. Altogether, it is difficult to make a prediction about the population dynamics of two generations, at the time when the decision for or against an active control has to be made. This challenge should be met by an economic threshold, the creation of which is the first step of the IPM approach. Therefore, knowledge about the potential damage of a pest insect and the tolerance of a host plant towards a pest insect is needed, as well as a reliable monitoring system (Ramsden et al. 2017).

The overall damage by *D. brassicae* is usually determined by counting infested pods per plant during the ripening stage of OSR (see EPPO Standard PP 1/220). The proportion of damaged pods can exceed 50% and high infestation rates with yield losses were reported regularly in the past hundred years (Buhl 1960; Döring and Ulber 2012; Fröhlich 1956b; Kirchner 1966; Nilsson et al. 2015; Schütte 1979; Speyer; Thiem 1970). However, the damage is often concentrated at field margins and the head lands (Ferguson et al. 2004; Thiem 1970; Warner et al. 2000). In addition, the proportion of damaged pods cannot be equated with yield losses, since OSR compensates early pod losses up to 10% by increasing grain weight (Diepenbrock 2000; Erichsen 1982; Williams and Free 1979), and also compensates early pod losses at the main inflorescence on lateral shoots (Pinet et al. 2015). The plant's ability to compensate, however, is less pronounced for damage caused by the second generation of the midge.

Knowing the important driving factors of an insect's population dynamics and their relation to potential damage, an economic action threshold can be derived. Presently, threshold values for direct control of *D. brassicae* do not exist in most European countries. Most commonly, the economic threshold for *C. obstrictus*, the alleged pioneer of the midge, is lowered from one weevil per plant to one weevil per two plants, if both pest insect species occur together (Heimbach 2017; Ramsden et al. 2017). Older thresholds for *D. brassicae* ranged between 0.25–1 midges per plant (Heimbach 2017; Lauenstein 1993). Other approaches try to predict the damage potential from the overall population size of *D. brassicae* estimated from the number of *D. brassicae* cocoons that are washed out of soil samples. Buhl and Schütte (1964) recommended to treat OSR fields in a region if the number of cocoons with living larvae in the soil exceeds the threshold of 40 per 100 cm². A similar method is used in several federal states in northeast Germany. Here, the threshold for insecticide applications in the following year against *C. obstrictus* is lowered if the average number of 25 cocoons per 100 cm² (assessed after the harvest of OSR) is exceeded (Hahn, M pers. communication). However, it should be stated that a scientifically based and peer reviewed economic threshold does not exist.

Pest monitoring

The establishment of an economic threshold requires monitoring of the pest insect and represents the second stage in the implementation of an IPM strategy. In case of



Figure 4 The veining of *Dasineura brassicae*, modified after Kirk 1992. Wings have three veins reaching the wing margin. Typically, the long vein from the base ends just before the tip of the wing

D. brassicae, the monitoring is challenging for several reasons, not least because of the insect's small size (Fig. 1). Adults of *D. brassicae* can be reliably differentiated from other gall midge species by their specific wing veining (Fig. 4), but this feature is difficult to identify with the naked eye. Consequently, monitoring of *D. brassicae* midges by farmers did never catch on in praxis.

The emergence and flight of the first generation can be monitored by using photoelectors that are placed at the OSR fields of the previous year (Waede 1960). In addition, there are commercial prediction models that indicate which days are suitable for the first generation

of *D. brassicae* to emerge and that also calculate what percentage of the flight period has passed (Johnen et al. 2010). Based on regional meteorological data, these prediction models can help farmers to determine the critical time when immigration of *D. brassicae* in their region is probable. Field specific forecasts are not possible though, since the migration of females to the new crop is affected by factors like wind and the distance from the previous year's OSR fields. Information on which specific fields or parts of a field are colonized is crucial for the implementation of IPM. Yellow water traps are potentially suitable for this purpose, although they create the risk of misidentification, as several similar gall midge species can be found in the OSR fields. For this reason, attempts were made to selectively catch *D. brassicae* using baited traps. The addition of an extract of OSR seeds with a high content of glucosinolates to water traps increased the capture efficiency at the emergence sites (Erichsen and Daebeler 1987). Traps baited with allyl isothiocyanate caught more male and female *D. brassicae* than traps baited with 2-phenylethyl isothiocyanate or unbaited traps (Murchie et al. 1997). However, mostly males were caught in both studies. Traps baited with the sex pheromone of virgin females (Williams and Martin 1986) also selectively caught *D. brassicae*, but also predominately males (Williams 1990). Since males usually remain at the emergence sites after mating, their catch is not suitable to predict the colonization of the OSR fields by females. The development of a trap attracting specifically female midges would be a great step forward in the monitoring of *D. brassicae*.

In recent years, agriculture has experienced a trend towards the use of new, innovative technologies. Artificial intelligence and camera traps enable the development of real-time monitoring systems. For example, lidar technology allows insects to be identified *in situ* by their wing beat, colour and the proportions of wing width to body size. Technology offers promising possibilities for the monitoring of flying insects (Brydegaard and Svanberg 2018) and first tests with pest insects in OSR have been carried out (Kirkeby et al. 2021).

Preventive measures

In IPM, preventive measures that reduce pest insect densities are preferable to direct control measures. Such indirect and preventive measures can include cultural measures, crop rotation, resistant cultivars, landscape management, and the enhancement of natural enemies (Hokkanen 2015).

Dasineura brassicae can use spring sown OSR to develop a strong third generation (Axelsen 1992c). For this reason, the growing of winter and spring sown OSR in the same region has been considered a key factor for the development of large *D. brassicae* populations and heavy infestations for a long time (Doberitz 1973; Speyer 1921). To what extent a third generation is limited by the lack of suitable host plants or by the induction of diapause of second generation larvae, needs to be further investigated. The control of cruciferous weeds, especially on non-crop habitats, was recommended by Speyer (1921). However, it was shown that *D. brassicae* survives only in small numbers on such weeds, which limits the potential of this measure (Åhman 1988). As the dispersal capabilities of *D. brassicae* are limited, the cultivation area of OSR at landscape scale affects the damage potential of the midge as well. The risk of infestation is reduced to less than half, if a distance of more than 3 km of this year's OSR is maintained from the previous year's cropland (Erichsen 1982). To interfere with the build-up of high pest populations, the interruption of OSR cultivation at a landscape scale is suggested as a possibility (Zheng et al. 2020). This was successfully demonstrated for *C. obstrictus* and *D. brassicae* in a one-season trial in northern Germany (Schütte 1979).

Soil tillage affects the vertical distribution of cocoons in the soil (Buhl 1960; Froese 1992). Ploughing did neither reduce the number of *D. brassicae* cocoons in the soil (Nielsen et al. 1994) nor the total emergence of adults in spring (Axelsen 1995), though the temporal distribution of the emergence was expanded. Still, some authors assume that ploughing is an important mortality factor for *D. brassicae* (Axelsen 1992c; Ferguson et al. 2004; Williams 2010b).

Dasineura brassicae can discriminate between low and good quality hosts (Åhman 1988). The differences in alighting frequency between different *Brassica* spp. indicate the possibility of antixenosis (Åhman 1988). The lower rates of survival and larval weights on low quality hosts are probably related to lower nutritional values or substances that are harmful for *D. brassicae* (Åhman 1985), which could be seen as a kind of antibiosis. In summary, these are different starting points for breeding resistant cultivars. However, successful resistance breeding against *D. brassicae* is not foreseeable yet based on the limited knowledge of variability in resistance of OSR and related species for this pest.

Natural enemies contribute to the control of *D. brassicae* populations by predation and parasitisation. At least 31 parasitoid species parasitize eggs and larval stages of *D. brassicae* (Ulber et al. 2010b). The key parasitoid species are *Platygaster subuliformis* (Hymenoptera: Platygasteridae) and *Omphale clypealis* (Hymenoptera: Eulophidae), both widely distributed in Central and Northern Europe (Ulber et al. 2010b). However, the multivoltine life history of the host and its parasitoids complicate investigations and extensive studies on parasitism in *D. brassicae* are scarce. Existing studies indicate substantial variability in parasitism rates (Murchie 1996). Generally, parasitism rates post-diapause seem to be higher and can exceed 50% (Ferguson et al. 2004; Murchie 1996). Predation of larvae might also affect *D. brassicae* populations. Larvae are especially vulnerable to predation by spiders or ground dwelling predators, when they drop down from the pods to pupate into the soil. Altogether, 11 carabid species fed on pod midge larvae in laboratory studies, however in choice tests, species differed in their preference for *D. brassicae* and in the amount of consumed larvae (Williams et al. 2010). Only *Amara similata* (Coleoptera: Carabidae) was proven to feed on larvae in field studies (Schlein and Büchs 2006). Spiders may prey on larvae and emerging midges of the first generation. Studies of Felsmann (2007) revealed a temporal coincidence between web densities of linyphiid spiders and both, the dropping of larvae, and the emergence of new generation midges in OSR.

In conclusion, preventive measures can contribute to a reduction of *D. brassicae* infestation to a limited extent. In particular, the potential of natural antagonists, such as predators and parasitoids, should be further explored and promoted through adherence to good agricultural practice (i.e. IPM, use of economic thresholds). The distance to OSR fields of the previous year, and the avoidance of winter and spring sown oilseed rape in the crop rotation, are effective measures to reduce pest pressure.

Control measures

The brassica pod midge has been a subject of research since 1850 (Winnertz 1853) and different control options have been under discussion so far. Early on, the control of *D. brassicae* was rather indirect, while direct measures aimed for control of the cabbage seedpod weevil *C. obstrictus* (Speyer 1921). In general, insecticide spray treatments of field margins and headlands are considered adequate for moderate pest insect pressure (Ferguson et al. 2004; Thiem 1970). Today, control of the cabbage seedpod weevil and

the brassica pod midge pest insect complex relies mainly on the use of synthetic chemical insecticides. All direct measures target *C. obstrictus* and the first generation of *D. brassicae*. Insecticides with non-systemic properties have to be applied before the main flight, to hit the midges before they start laying eggs. Since OSR is rapidly growing during the flowering period and temperatures can be quite warm, the residual activity of the insecticides will often decline rapidly in the field. Hence, it is a challenge to protect the crop with a single insecticide application, which requires a precise timing of the application. Therefore, reliable and precise monitoring data would be a prerequisite.

Until now, *D. brassicae* is sensitive to insecticides and there is no evidence of insecticide resistance. However, in Germany increasing pyrethroid resistance in populations of *C. obstrictus* can be observed (Brandes and Heimbach 2019).

Insecticide applications below the flowering canopy of the crop with dropleg technique can reduce side effects on pollinators, but tend to have reduced efficacy against *D. brassicae* (Hausmann et al. 2019). Regarding alternative control measures, only few studies have investigated botanicals such as extracts from neem tree (*Azadirachta indica*) (Pavela et al. 2009) or the application of nitrophenoles (Kazda et al. 2015), which have shown some efficacy in field trials.

Conclusions

This literature review shows that so far IPM of *D. brassicae* in OSR is rather a collection of promising ideas than state of the art. Most preventive control measures are limited in terms of their efficiencies. Resistance breeding or a large-scale interruption of OSR cultivation in individual years is difficult to establish in the short term and more knowledge about the effectiveness of all these measures is needed. De facto, direct control with insecticides remains the most effective control measure and it is therefore essential that it is practiced within the framework of IPM. So far, the most practicable option to predict *D. brassicae* damage appears to be monitoring of *C. obstrictus*, which provides access to pods and so for a mass propagation. However, a clear relation between the two species only exists at high pest insect densities. At present, insecticide sprays in flowering OSR are often retrospectively motivated and tend to have the character of an insurance spray rather than being targeted and economically justified from an integrated pest management perspective. To improve this, the development of an economic threshold for

D. brassicae is a necessary requirement. So far, it is poorly understood what number of individuals constitutes the critical threshold for a potential mass propagation within the season. Therefore, the mortality rate of the first generation of *D. brassicae* and other factors favouring the development of a large second generation need to be studied in detail. To be reliable, prediction models of potential crop damages should further include temperature as a variable, as this affects many aspects of population development. However, the absence of long-term weather forecast has a limiting effect. Furthermore, there is great need for an improved method that allows easy monitoring of *D. brassicae*. Currently, the identification of midges in the field is not practicable and it is therefore impossible to determine which fields or specific areas within a given field are infested and are likely to suffer crop damage. Promising avenues of research are the development of selective attractants and real time monitoring of insects, using remote sensing technology.

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Chapter VI

General discussion

The pest complex of cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham), and brassica pod midge, *Dasineura brassicae* (Winnertz), can damage oilseed rape, *Brassica napus* L., (OSR) crops, resulting in significant yield losses in some years. Farmers can apply insecticides during the flower to protect the crop from pod damaging pest insects. However, as the previous chapters have shown, insecticide treatment of flowering OSR is a controversial topic. On the one hand, the safeguarding of yields with agrochemicals is essential for farmers and for the efficient use of the resources allocated to plant production. On the other hand, European farmers are supposed to follow the principles of integrated pest management (IPM), which prescribe pesticide applications only as the ultima ratio. Additionally, there are social and political demands to reduce the use of chemical plant protection products, mainly due concerns about declines in biodiversity. In this context, the European Commission announced the target to reduce the use of pesticides by about 50% by 2030.

The application of pesticides with the dropleg technique could be suitable for a more efficient use of agrochemicals in flowering OSR. In the present thesis, dropleg technique was evaluated from an entomological perspective. On one hand, the efficiency of insecticides applied with dropleg technique to control insect pests in OSR was investigated. In addition, its potential to protect natural antagonists of pest insects such as parasitoids from side effects of insecticides was examined. For this, field trials were conducted comparing dropleg and conventional application technique to assess the effects of different insecticides on *C. obstrictus* and *D. brassicae* as well as associated parasitoid species. In a separate chapter, the state of integrated pest management of *D. brassicae* was reviewed and challenges for an improved and more targeted control were identified.

In the following general discussion, the main findings of the individual chapters are summarized to conclusively discuss the potential of droplegs as a more sustainable method of application in the control of pod damaging pest insects. Furthermore, the control of pod damaging insects in its current form is questioned. Promising approaches

to be considered to further an even more targeted application of insecticides in flowering OSR and to avoid unnecessary treatments will be discussed.

Effectiveness of dropleg technique against pest insects in flowering oilseed rape

The results of chapter II suggest that the use of the dropleg application technique results in a slight loss of efficacy with respect to the control of pest insects. At the same time, the technique can help reduce negative side effects of insecticides on certain parasitoids of pest insects (chapter III). It was shown that parasitoids had a significant contribution on the population dynamics of the pollen beetles and the cabbage seedpod weevil at the trial sites.

In general, the effectiveness of insecticides depends on their intrinsic toxicities (Casida 2009; Jeschke and Nauen 2019) and the exposure of the targeted pests to the active ingredients. The application of pesticides with droplegs differs from the conventional application due to a different spatial distribution of the spray liquid. The flower canopy of the crop remains largely free of active ingredients (Brandes 2021; Rosenkranz et al. 2019; Wallner 2019).

Results from three years of field trials (2015–2017) on the effectiveness of the dropleg technique on the control of *D. brassicae* revealed slightly reduced efficiency compared to conventional application (chapter II). However, only moderate infestations of pod-damaging insects were observed during the study period. The field trials were continued in 2018 and 2019, when pest pressure was much higher. Information on the trials of these years including the results on pod infestation rates and yields can be found in Appendix B. Since sufficient protection of the crop by plant protection measures is particularly important in situations with heavy pest infestation, the results from the five years of field trials will be summarized at this point. **An interaction between the effectiveness of the selected insecticides and the choice of application technique was observed with respect to the control of *D. brassicae*.** The application of the neonicotinoid product Biscaya (a.i. thiacloprid) consistently and significantly reduced pod infestation rates of the first generation *D. brassicae* compared to the control. In each year, a second insecticide was tested as well. In 2015, this was the pyrethroid Mavrik Vita (a.i. tau-fluvalinate) and in 2016/17 and 2018/19, respectively, formulations of the neonicotinoid Mospilan (SG / SL; a.i. acetamiprid). These products were generally less

effective compared to Biscaya. The three insecticides studied all act as contact and feeding poisons. In addition, significant ovicidal activity against codling moth (*Cydia pomonella* L.) has been described for the active ingredient thiacloprid (Jeschke and Nauen 2019). The superior efficacy of Biscaya against *D. brassicae* can be explained by its ovicidal and larvicidal activity.

Nevertheless, the tested products also differ in their mobility in plant tissues. Mospilan is translaminar and acropetally active and also Biscaya is translocated acropetally in plant tissues (Jeschke and Nauen 2019). Mavrik Vitae, however, does not translocate and acts only locally. As mentioned, the exposure of the pests to the active ingredients is critical to their effectiveness. In general, one would expect primarily (or even only) systemic insecticides to be effective after dropleg application. However, this is opposed by the results from the field trials as there were significant differences between the application techniques in four out of five years regarding the effects of Biscaya on the first generation of *D. brassicae*. Concerning the insecticide Mospilan, application technique differed significantly only in 2018. There was no difference between application techniques with Marvrik Vita. With respect to the weevil *C. obstrictus*, no effect of application technique on the effectiveness of insecticides was found either (chapters II, IV). It can be inferred that, with the exception of Biscaya, **contact action was preeminently important to insecticide efficacy.**

Therefore, it is important to know where the target organisms are located if, as with dropleg application, parts of the crop are not sprayed with active ingredients. Investigations on the vertical distribution of pod damaging insects (chapter III) revealed that these pests were mostly caught in the layer of pods and open flowers at daytime and in dry crops. This will be the main reason for reduced efficacy of insecticides on *D. brassicae*, when dropleg technique is used. It remains unclear whether the pest insects seek shelter in lower crop layers during the night or during unfavourable weather conditions, as suggested by some older literature (Edner 1983; Fröhlich 1956; Williams et al. 1987).

An additional exposure pathway was assumed for weevils, which tend to fall from the plants because of the crop shaking during the dropleg application; though, this effect was never demonstrated in the field trials. Anyway, a significant reduction of pod

infestation rates by weevil larvae was only observed in 2015 and 2019, and only when using the insecticide Biscaya (chapters II, IV).

Generally, a blurring of effects was observed regarding the second generation of *D. brassicae*. Only in 2015 and 2018, conventional application of insecticides significantly reduced pod infestation rates of the second generation compared to the untreated control. This could be due to the experimental design. Plot sizes were between 240 and 540 m², which is a size that pests can quickly recolonise. **Therefore, the overall effects might be slightly better when the entire crop is treated.**

In analogy to the effects on pest insects, exposure to active ingredients can also be reason for undesirable side effects of pesticides on non-target organisms (Desneux et al. 2007; Ulber et al. 2010). Thus, if the upper plant layer remains largely free of active ingredients due to the use of dropleg technique, this can also reduce non-target effects on parasitoids that seek for their hosts in the horizon of open flowers and pods. So far, there were many studies focusing on the spatial distribution of pest insects and its antagonists within OSR fields (Felsmann 2007; Ferguson et al. 2003a; Ferguson et al. 2003b; Ferguson et al. 2004) or within landscapes (Berger et al. 2018; Thies et al. 2003). On the other hand, very few studies have investigated the vertical patterns of insects in the crop layer (Neumann 2010; Nitzsche 1998). For this reason, the vertical distribution of parasitoids was studied using a portable aspirator during the flowering period of OSR in two years. **The results indicate species-specific residence preferences for the flower and below flower canopy, respectively** (chapter III). It is obvious that the parasitoids mostly stay where they find their host larvae. For example, the larger tersilochine species, predominantly parasitoids of stem weevil larvae, were found in more than 80% of all catches below the flower canopy. In contrast, it was surprising that species of both, the larval parasitoids of the pollen beetle *Brassicogethes aeneus* Fabricius and of *C. obstrictus* showed some kind of spatial separation. With respect to the pollen beetle parasitoids *Phradis interstitialis* Thomson and *Tersilochus heterocerus* Thomson, the results support findings of Berger et al. (2015), who found evidence for niche separation of these parasitoid species. As *T. heterocerus* was predominately caught in the flower canopy, this species was expected to benefit from the use of dropleg application technique. Assessments of pollen beetle larvae in the field trials confirmed that the use of dropleg technique reduced parasitism rates only in one case in 2019. Instead, conventional

applications significantly reduced parasitism rates by *T. heterocerus* in three out of four years. **Regarding the larval parasitoids of *C. obstrictus*, it can be concluded that temporal targeting of applications is more important than spatial targeting.** There were no clear benefits of dropleg application technique on parasitoids of *C. obstrictus* compared to conventional application.

The perspective of dropleg technique in OSR

The results on the use of dropleg application technique in flowering OSR are contradictory, as reduced efficacy of insecticides against pest insects is counterbalanced by possible advantages in terms of pollen beetle parasitoids. So how likely is the further spread of dropleg technology in oilseed rape cultivation? Looking at yield effects, significant increases were observed in three out of five years in the field trials after conventional spraying compared to the untreated control. **Yields in dropleg treatments were intermediate between the control and conventional treatments, but not significantly different from either.** Recent studies regarding the control of the fungal pathogen *Sclerotinia sclerotiorum* with fungicides using dropleg application technique, revealed no differences in efficacy and yields were equal to conventional application technique (Dicke 2018; Haberlah-Korr 2016; Haberlah-Korr et al. 2018).

For farmers, the purchase of droplegs initially means an investment in money and labour. Direct benefits, e.g. an improved pest control do not exist for the use in OSR. Generally, the adaption of novel or innovative techniques by farmers in praxis is influenced in various ways by a number of factors (Shang et al. 2021). The latter identified farm as well as operator characteristics, interactions with institutions, attributes of the technology and psychological factors as relevant influencing factors for the adoption of digital farming techniques. However, some of these points will apply just as much to a rather simple purchase like droplegs (Fig.1). The development of the dropleg technique originally intended to increase the efficiency of fungicide measures in vegetable production, for example in *Phaseolus* beans or potatoes (Brandes 2021). Additionally, dropleg technique can be suitable for late herbicide applications in sugar beet or maize or for the spreading of liquid fertilizers (Brandes 2021; Rüegg and Total 2013). For this reason, farm characteristics as farm size and land use (Shang et al. 2021) will influence the adaption of dropleg application technique. It can be assumed that droplegs are currently of particular interest to specialised vegetable growers, large-scale farms with

mixed crop rotations and agricultural contractors who offer crop protection services to many customers.

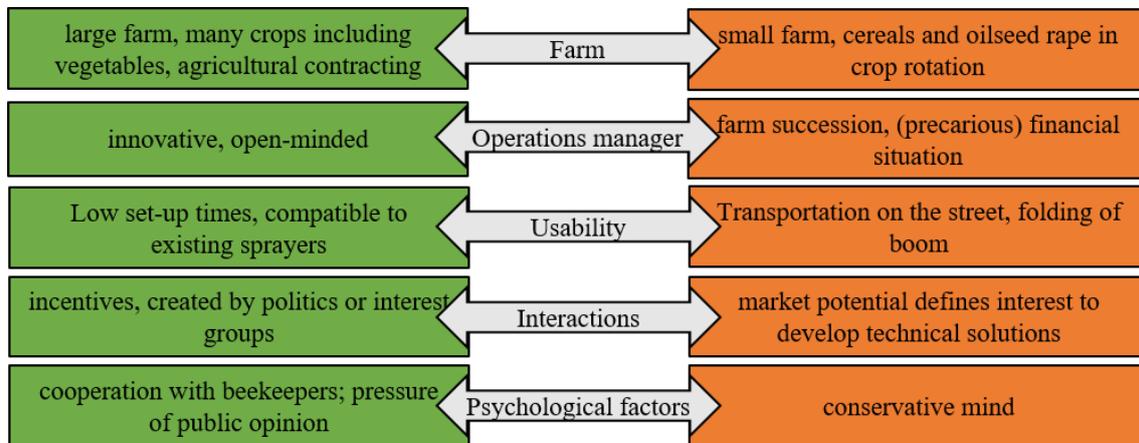


Figure 1 Arguments for (left) and against (right) the purchase of dropleg technique in oilseed rape

Just the same, operator characteristics e.g. age, income, farming experience or knowledge influence the adaption of new technologies (Shang et al. 2021). It is probable that farm operators, who do not have a settled farm succession or are in a precarious financial situation, will not make an investment in a technology without a direct monetary advantage.

Furthermore, new technologies can be promoted through interactions with consultants and the providers of sprayers. Basically, all sprayers can be equipped with droplegs. However, some type of sprayers have no technical solution for the transportation of droplegs on the street (Brandes 2021), which hinders widespread use of the technology. Additionally, the set-up time to equip the sprayer with droplegs within the season should be short. Of course, the design of appropriately equipped sprayers interacts with the economic returns that the engineering companies hope to achieve. At this point, institutions and politics could contribute significantly to a further spread of the dropleg technique by creating incentives. This is exactly what some stakeholders demand (dpa 2021; UFOP 2019) and in Switzerland, there was a state promotional programme in the years 2014–2019 including subsidies for dropleg technology (Imhof 2013). However, there is nothing comparable in Germany. Ultimately, here mostly psychological factors or personal convictions motivate farmers to equip their sprayers with dropleg technique. For example, it may be a personal concern for a farmer that dropleg technique can

possibly lead to a more sympathetic cooperation between agriculture, beekeeping and society.

Concluding, the technology could certainly be considered a contribution to environmentally friendly farming practices, at least as a standard for fungicide applications during the flower of OSR. **However, economic support is needed to enforce the spread of the technology in praxis.**

Effectiveness of insecticide sprays against pod damaging insects in oilseed rape

In the field trials of the present study, mainly neonicotinoid insecticides were used. Since 2021, the neonicotinoid active ingredient thiacloprid is no longer approved by the EU (European Union 2020). Currently, only insecticides from the class of pyrethroids are authorised for use in flowering OSR in Germany (BVL 2021). Pyrethroids have been used extensively in OSR in the last decades and there is increasing resistance in this class of insecticides (Heimbach and Müller 2013; Willis et al. 2020; Zimmer et al. 2014). Knockdown resistance (kdr) has been reported in populations of *C. obstrictus*, significantly reducing the efficacy of pyrethroid insecticides to control this pest species (Brandes and Heimbach 2018; Robert et al. 2019). The question must therefore be raised, whether and to what extent the cabbage seedpod weevil can and needs to be controlled in the future.

Farmers generally spray insecticides to prevent yield losses. Significant yield increases due to insecticide applications were observed in the field trials conducted for this thesis in 2016, 2018 and 2019 (compare chapter II and Annex B). However, in 2016, all treatments except from the control were treated with a fungicide. Therefore, the significant increase in yield in the conventional Biscaya treatment might be partly caused by the applied fungicide as well. In the years 2018 and 2019, the increases in yield were remarkable and definitely justified the use of insecticides. In both years, crop stands were stressed due to weather conditions, which probably reduced their ability to compensate damage by herbivorous insects (Pinet et al. 2015; Williams and Free 1979). For example, in 2018, there was a dense colonisation of single flowers with pollen beetle larvae that occurred because many buds had been aborted by the plants due to physiological stress response. In the conventionally treated plots, there was a noticeable compensation and increased flowering one week after the application, which is likely because of the

insecticide effects on pollen beetle larvae. Therefore, it cannot be ruled out that, on the one hand, the larvae of *B. aeneus* had an impact on yields this year. On the other hand, such plots with more flowers might then have attracted *C. obstrictus* and somehow masked effects of insecticides regarding this species.

Previous studies agree that yield losses due to *C. obstrictus* itself only occur if pod infestation rates by the weevil exceed approximately 25% (Buntin 1999; Lerin 1984), because OSR plants can compensate damage by increasing grain weights and the formation of new pods (Williams and Free 1979). A pod infestation rate greater than 25% is probable, if about one weevil per plant is found at full flowering (Free et al. 1983), which is the valid damage threshold in many European countries (Heimbach 2017; Ramsden et al. 2017). In the field trials conducted for this thesis, the threshold of one weevil per plant was reached only in 2019. Pod infestation rates in the untreated control were about 18% in this year (chapter IV). The application of Biscaya tended to reduce pod infestation rates in 2019, however, reduction was only significant in comparison to the conventional Mospilan treatment. When weevil densities were lower, insecticide applications significantly lowered pod infestation rates by *C. obstrictus* larvae only in 2015 (Chapters II, IV). From this perspective, the use of insecticides to control *C. obstrictus* does not seem appropriate or justified. Treatments in practice indeed target *C. obstrictus*, because the weevil allows *D. brassicae* to access pods through its feeding and egg-laying activities. Whether insecticide applications in the presented field trials have really prevented secondary infestation by *D. brassicae* by reducing pod damages by *C. obstrictus* needs some discussion.

The abundance of both pest insects, *C. obstrictus* and *D. brassicae*, can significantly increase yield losses (Free et al. 1983). For the midge itself, there is no practical damage threshold (chapter V). Mostly, the threshold for *C. obstrictus* is lowered to one weevil on two plants (Heimbach 2017; Ramsden et al. 2017), because the weevil is regarded as the main precursor for heavy infestation with *D. brassicae*. Yet, it was discussed that **the weevil rather influences the damage potential of *D. brassicae* than the population dynamics of the midge**. In the presented field trials, the date of application was always adjusted to the flight of *D. brassicae*. The significant reduction of pod infestation rates by the first *D. brassicae* generation indicates that the main flight of the midge was hit in all years. This raises the question, whether insecticides against pod damaging insects

should strictly orientate at the abundance of *D. brassicae* and better target the flight of the midge, instead of being applied against the weevil. **Therefore, the bottleneck of monitoring *D. brassicae* in a proper way needs to be overcome to allow farmers to make field specific decisions.** Currently, there is no selective monitoring, and the identification of the midge in the field is difficult due to its small size and possible confusion with other species. To summarize, there is currently no IPM of pod damaging pest insects in OSR, especially with regard to the brassica pod midge.

Another problem, which was discussed in chapter V, is the loss of effectiveness of insecticides within a short period after application in the field. Since the flight of first generation *D. brassicae* often lasts 3–4 weeks, one insecticide application can only reduce oviposition by the midge, but will never prevent damages at all. Similar, Buntin (1999) describes that at least two applications are needed to prevent seed injuries by *C. obstrictus*. The efficiency and persistence of an insecticidal product is defined by its mode of action and the formulation (Jeschke et al. 2019; Sánchez-Bayo 2012). In Germany, currently only pyrethroids are authorized for use in flowering OSR. They act as contact insecticides and must be applied before adult *D. brassicae* migrate into the crop, because the midge immediately starts with oviposition. In opposite, a systemic insecticide will probably also affect eggs or young larvae of *D. brassicae* in the pods, which might be an explanation for the higher efficacy of Biscaya (systemic) compared to Mavrik (not-systemic) in 2015. However, in the field trials also formulations of Mospilan were tested. The active ingredient acetamiprid is also systemic but was significantly less effective against *D. brassicae* compared to Biscaya in the field trials. One explanation could be that the intrinsic toxicity of acetamiprid against the pod damaging insects is lower, which could be the reason why the authorisation holder of Mospilan has not yet applied for use of the product in flowering OSR.

Besides the properties of active ingredients and its formulations, the timing of insecticide sprays influence its efficacy. In agricultural praxis, insecticide application in flowering OSR are often conducted in the evening or during night to reduce both, the direct exposure of pollinators and plant damages. In the trials presented in this thesis, applications were always carried out in the morning until midday, which could be a limitation for the transfer of results to practice conditions.

Despite the uncertainties in effects described above, it can be concluded that **with the remaining insecticidal class of pyrethroids, the applications must be precisely timed regarding *D. brassicae* to achieve sufficient efficacy. Only limited effectiveness can be expected with respect to the control of *C. obstrictus* due to increasing resistance.** Therefore, improved monitoring methods such as selective pheromone traps or the use of novel technologies and field-specific damage prognosis for the midge are necessary.

Consequences for the integrated pest management of pod damaging insects in oilseed rape

It is a general understanding of IPM that pesticide treatments are conducted judiciously and guided by monitoring and damage thresholds (Zalucki et al. 2009). Rather comprehensive approaches of IPM focus on the enhancement of ecosystem services prior to the use of interrupting measures such as the application of insecticides (Lundin et al. 2021; Zalucki et al. 2009). A general problem for IPM strategies is the comparably low cost of insecticides, which is especially true for the pyrethroid insecticide class. Farmers in north-east Germany spent an average of 36.20 € per hectare for insecticides in winter oilseed rape in the last decade (Andert and Ziesemer 2021). If the cost of application is also considered, a yield increase of less than 100 kg ha⁻¹ covers the cost of an insecticide application, given a revenue of 400 € per tonne of rapeseed. The careless use of insecticides during the flower of OSR is particularly critical, because large numbers of pollinators, as well as many antagonists of OSR pests, such as ichneumon wasps, are in the crop at this time. The results on parasitism rates of *B. aeneus* larvae and additionally of *C. obstrictus* larvae in the field trials 2016–2020 demonstrate that insecticide sprays at this time can have non-target effects on parasitoids (chapters III/IV). On the other hand, parasitism rates in the untreated control ranged between 55% and 82% for pollen beetle larvae and between 50% and 68% for seedpod weevil larvae. Thus, **larval parasitism significantly contributed to reduce pest populations** (Hokkanen 2008; Thies et al. 2008) and at least for *C. obstrictus* the effects were stronger compared to effects of the applied insecticides. Generally, farmers often tend to have a poor sense of the possible contributions antagonists of pest insects can have on pest abundance and its population dynamics (Busse et al. 2021; Wyckhuys et al. 2019). This probably has several reasons. Primarily, the parasitoids are not as easy to spot as many pest insects or at least their

damages. Secondly, OSR pest insects are mostly univoltin and high parasitism rates reduce the pest population of the next year. However, pest populations and parasitoids interact at landscape scales (Thies et al. 2003, 2008), so locally high parasitism rates in one year do not give a guarantee for protection of a crop in the next year. In order to sensitise farmers to refrain from insecticide treatment in case of doubt, their focus should be more strongly directed towards an enhancement of natural pest control and possible drawbacks by insecticide treatments. Previous works have shown that parasitism of insect pests in OSR is affected by the abundance of refuges for specialized parasitoids at local (Krimmer et al. 2022) and landscape scale (Bianchi et al. 2006; Shackelford et al. 2013). Additionally, crop management practises such as direct or mulch drilling can have positive impacts on parasitoids (Nilsson 2010; Nitzsche 1998). New cropping systems e.g. strip cropping (Alarcón-Segura et al. 2022; Juventia et al. 2021; Labrie et al. 2016), undersowings (Breitenmoser et al. 2022; Emery et al. 2021) or reduced herbicide intensities could further improve habitats and parasitoid conservation. In the future, more efforts should be made to provide farmers with indicators or forecasting tools that help them to assess the potential of naturally occurring antagonists based on site-specific inputs like cropping systems, field structures or landscape features.

Nevertheless, in situations with high pest pressure effective direct control measures are needed. Sustainable management of such situations requires more than one mode of action of chemical control and additional selective and environmentally friendly measures to control pod damaging pest insects in OSR need to be developed.

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Appendix

A

The effect of insecticide application by dropleg sprayers on pollen beetle parasitism in oilseed rape (Chapter III)

Supplementary Table S1

Table S1 Number of larvae per treatment and sampling date assessed for parasitism by *Tersilochus heterocerus* in the spraying trials from 2016–2019

2016	24.05.2016	31.05.2016			
Control	160	160			
Conventional Biscaya	157	151			
Conventional Mospilan	160	160			
Dropleg Biscaya	160	160			
Dropleg Mospilan	160	151			
2017	18.05.2017	22.05.2017	29.05.2017		
Control	59	133	335		
Conventional Biscaya	63	30	136		
Conventional Mospilan	77	35	164		
Dropleg Biscaya	39	86	205		
Dropleg Mospilan	35	87	263		
2018	08.05.2018	15.05.2018	22.05.2018	29.05.2018	
Control	200	814	907	200	
Conventional Biscaya	200	930	602	180	
Conventional Mospilan	200	832	614	187	
Dropleg Biscaya	200	749	860	201	
Dropleg Mospilan	196	799	794	200	
2019	02.05.2019	09.05.2019	16.05.2019	22.05.2019	29.05.2019
Control	209	754	2421	1517	271
Conventional Biscaya	1652	1398	617	302	189
Conventional Mospilan	1240	1130	722	516	259
Dropleg Biscaya	924	1257	994	573	201
Dropleg Mospilan	445	1025	1834	1041	218

Supplementary Table S2

Appendix

Table S2 Average parasitism of pollen beetle larvae by *Phradis interstitialis* (% \pm SE) in the spraying trials 2016–2019 (NA = not assessed)

Year	2016	2017	2018	2019
Parasitism (% \pm SE)	9.1 \pm 1.5	NA	13.6 \pm 3.0	10.4 \pm 3.6
Multiparasitism (<i>Phradis</i> + <i>Tersilochus</i>) (% \pm SE)	5.9 \pm 0.9	NA	3.1 \pm 1.1	9.8 \pm 3.7
N	1147	NA	330	631

Supplementary Figure S1

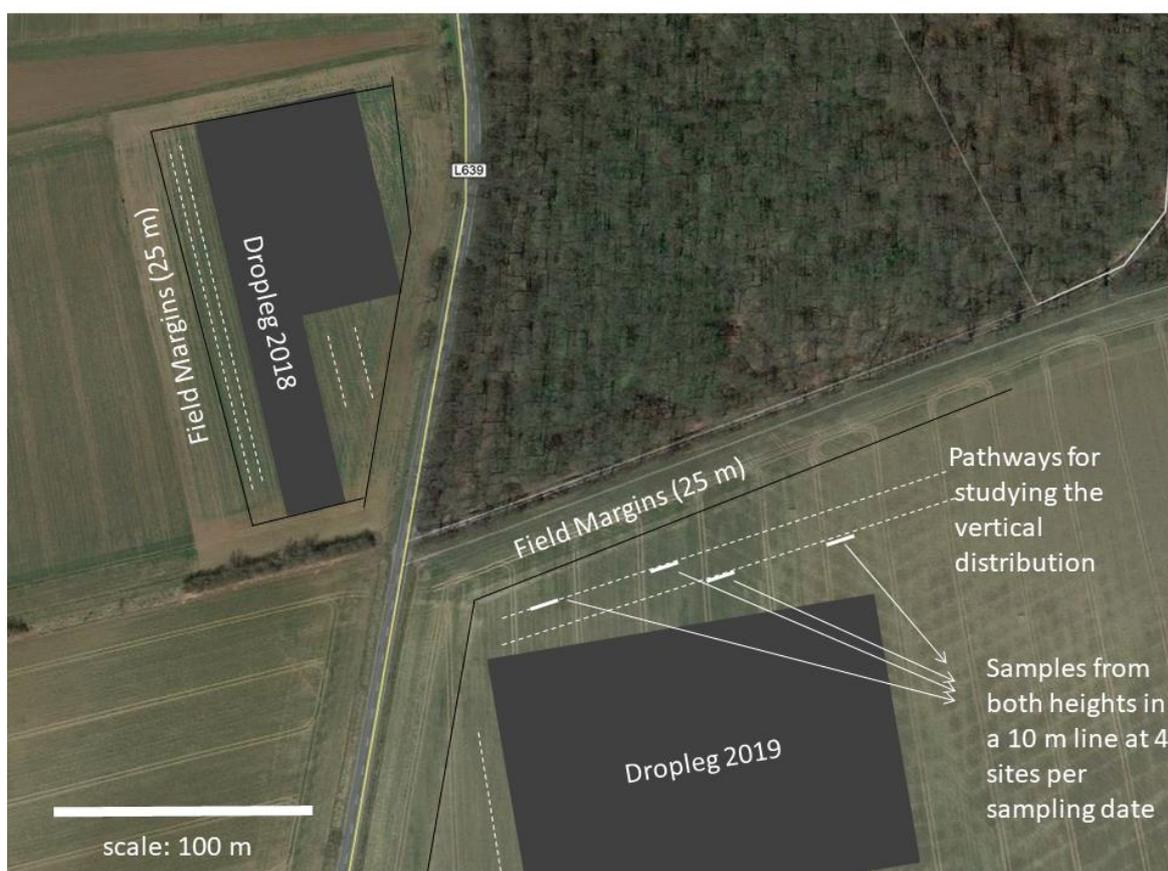


Figure S1 Location of the field trials in the years 2018 and 2019. The vertical distribution of insect pests and their parasitoids was assessed outside the dropleg trial area, with a portable aspirator (pathways indicated by dashed lines). The direction of the tramlines (pathways for machines) in the right field was changed in 2019

Supplementary Figure S2

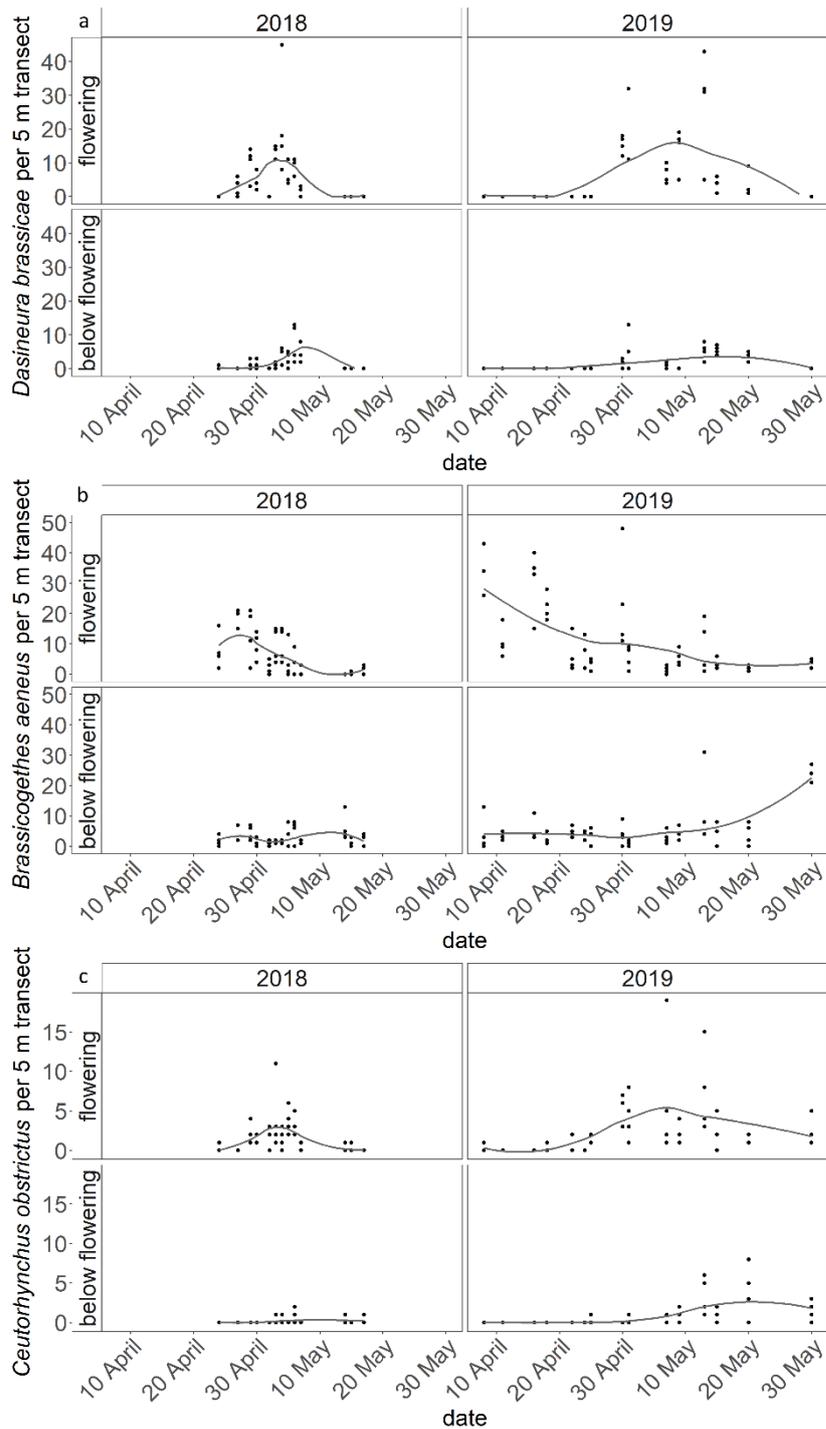


Figure S2 Number of individuals (dots) sampled with an aspiration trap on a 5 m transect of a) *Dasineura brassicae*, b) *Brassicogethes aeneus* and c) *Ceutorhynchus obstrictus* in the flowering canopy and below flowering canopy of OSR at different sampling dates during the period of flowering in 2018 and 2019. Lines = regression lines (method: loess)

B**Field trials using dropleg technique in the years 2018 and 2019****Background**

For the present thesis, field trials using dropleg technique were conducted from 2016 to 2020. Additional, data of a field trial of 2015 (master thesis by Jobst Gödecke) were analysed. Data of the field trials 2015–2017 are published (Hausmann et al. 2019). In this chapter, material and methods as well as the results on pod infestation rates and yields of the field trials 2018 and 2019 are presented.

Material and Methods

The field trials were located near to Braunschweig, Wendhausen (52° 19' 36.89'' N, 10° 38' 2.91''E). The date of application was derived from the emergence of *Dasineura brassicae* adults in photoelectors that were placed on the previous year oilseed rape field (Tab. B1). For conventional application technique IDKN 120-04 nozzles were used. Droplegs were equipped with double tongue nozzles (FT 90°). For further details, see Hausmann et al. 2021.

Table B1 Design of the dropleg field trials in the years 2018 and 2019 with applied insecticides, application dates and crop growth stages

	2018	2019
Trial design	Randomized complete block design (RCBD), 4 replicates	
Treatments	Untreated Control Conventional Biscaya Conventional Mospilan SL Dropleg Biscaya Dropleg Mospilan SL	
Date of application	02.05.2018	30.04.2019
Growth stage	64	65
Plot size (m²)	240 (12 x 20 m)	540 (27 x 20 m)

Insecticides were applied at a driving speed of 7 km h⁻¹ with 300 l ha⁻¹ water. Weather was clear and wind speed was about 1.5–2.2 m s⁻¹ except from 2016 (3.4–3.9 m s⁻¹). All insecticides were applied at recommended field rates.

For pod assessments, 12 plants per plot were cut at BBCH 75 (this when the larvae of the first generation of *D. brassicae* are developed) and the total number of pods and infested pods per plant were counted in the laboratory. A second assessment was conducted at BBCH 85. Yield was recorded with a plot combine harvester (Haldrup C85), which took two samples (each 2 m width x plot length) through the centre of each plot. The grain weight was converted to a standard moisture content of 9% (further details Hausmann et al. 2019).

Statistical analysis

All statistical analyses were performed with R (version 3.6.1) (R Core Team 2019) in the graphical user surface R Studio.

The pod infestation rates by *Dasineura brassicae* were analysed separately for each year. The data had a binomial error distribution. For analysis, a generalized linear mixed model (glmm) with treatment and date of assessment as fixed effects and treatment nested in blocks as random effect was fitted. The interaction and single effects of each variable was compared AIC value. The model with the lowest AIC was chosen as it indicates the best fit (Burnham and Anderson 2002). Additionally, models were compared via an analysis of variance using the “Chi²-test”. For model diagnostics the residuals were plotted against the predicted values and the explanatory variables. If models were significant, a post-hoc analysis was done by performing a Tukey test at an alpha level of 0.05 using the package *emmeans* (Russell 2018). The figures were created using the package *ggplot 2* (Wickham 2016).

Yield data were analysed using a linear model with treatment, year and block as effects.

Results

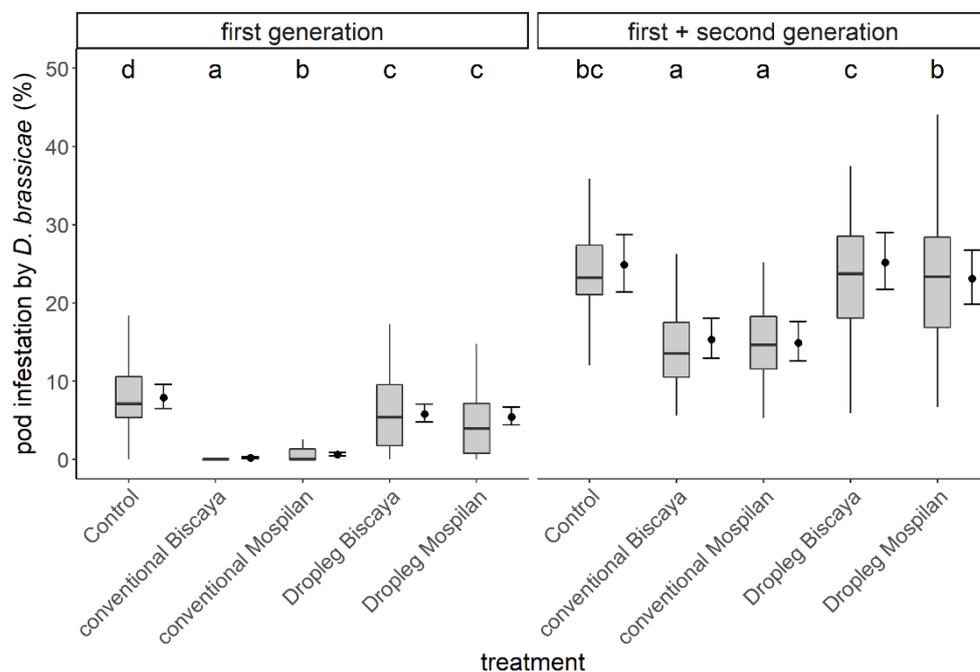


Figure B1 Pod infestation rates (%) by *Dasineura brassicae* in 2018. Boxplots show the mean values per sampling date. Calculated means and 95% confidence limits obtained from the best model are shown (black dots). Treatments with different letters differ significantly from each other. GLMM ($y = \text{treatment} * \text{sampling date} + (1|\text{Block:treatment})$, family=binomial), Tukey-(HSD) $p < 0.05$

In the year 2018, the first generation *D. brassicae* damaged about 7.8% (CI_{95%} 6.5–9.6%) of oilseed rape pods (Fig. B1). Every insecticide treatment significantly reduced pod infestation rates compared to the control. However, reduction of pod infestation after conventional application was significantly stronger compared to treatments with dropleg application technique (conventional Biscaya 0.2%, CI_{95%} 0.1–0.3% compared to Dropleg Biscaya 5.8%, CI_{95%} 4.8–7.1%, $p < 0.001$ and conventional Mospilan 0.6%, CI_{95%} 0.5–0.9% compared to Dropleg Mospilan 5.4%, CI_{95%} 4.4–6.7%, $p < 0.001$). At the second pod assessment, pod infestation rate increased to 24.9% (CI_{95%} 21.4–28.7%) in the untreated control. Only the conventional insecticide treatments resulted in significantly lower pod infestation rates compared to the control (conventional Biscaya 15.3%, CI_{95%} 12.9–18.0%, $p < 0.001$ and conventional Mospilan 14.9% CI_{95%} 12.6–17.6%, $p < 0.001$). Pod infestation in the untreated control was about 7.2% (CI_{95%} 5.8–8.9%) at the first assessment and raised to 24.2% (CI_{95%} 20.4–28.4%) after the second generation larvae have developed in 2019 (Fig. B2). Insecticide treatments reduced the pod infestation by the first generation *D. brassicae* significantly with the exception of the Dropleg Mospilan

treatment (5.6%, CI_{95%} 4.5–7.0). The lowest pod infestation was found in the conventional Biscaya treatment (1.4%, CI_{95%} 1.0–1.8). At the second assessment, treatments no longer differed from each other.

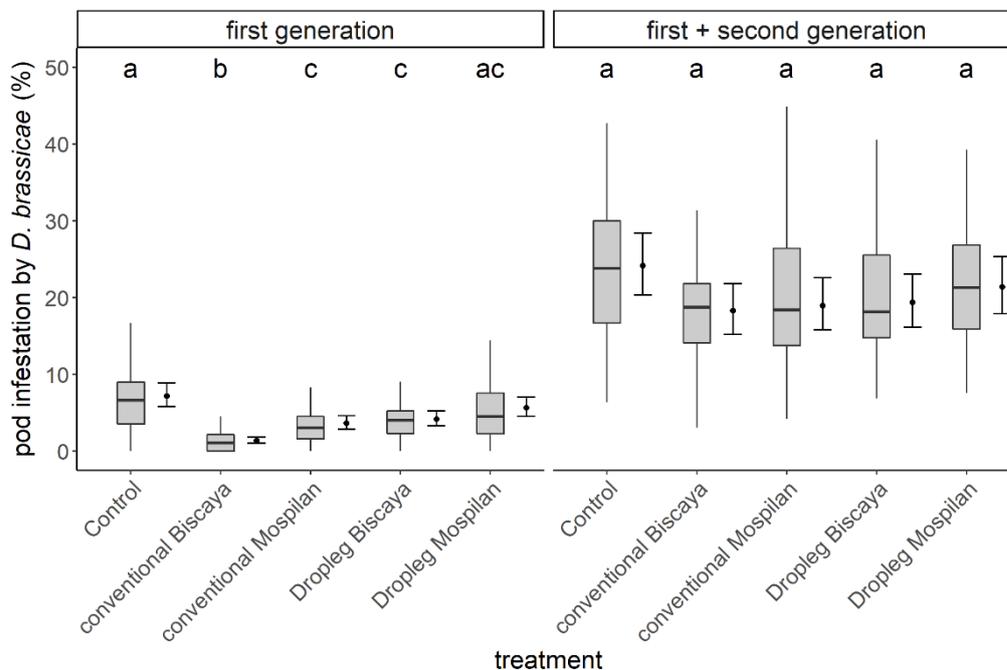


Figure B2 Pod infestation rates (%) by *Dasineura brassicae* in 2019. Boxplots show the mean values per sampling date. Calculated means and 95% confidence limits obtained from the best model are shown (black dots). Treatments with different letters differ significantly from each other. GLMM ($y = \text{treatment} * \text{sampling date} + (1|\text{Block:treatment})$, family=binomial), Tukey-(HSD) $p < 0.05$

Average yields in the untreated control were about 3400 kg ha⁻¹ in 2018 and increased at 3740 kg ha⁻¹ in 2019. Analysed over two years, plots treated with conventional application technique yielded significantly higher (conventional Biscaya 4030 kg ha⁻¹, CI_{95%} 3820–4240 kg ha⁻¹, $p = 0.0255$ and conventional Mospilan 4060 kg ha⁻¹, CI_{95%} 3850–4270 kg ha⁻¹, $p = 0.0095$) compared to the control (3580 kg ha⁻¹, CI_{95%} 3370–3790 kg ha⁻¹) (Fig. B3). Dropleg treatments were intermediate between the control and conventional treatments, but not significantly different from either.

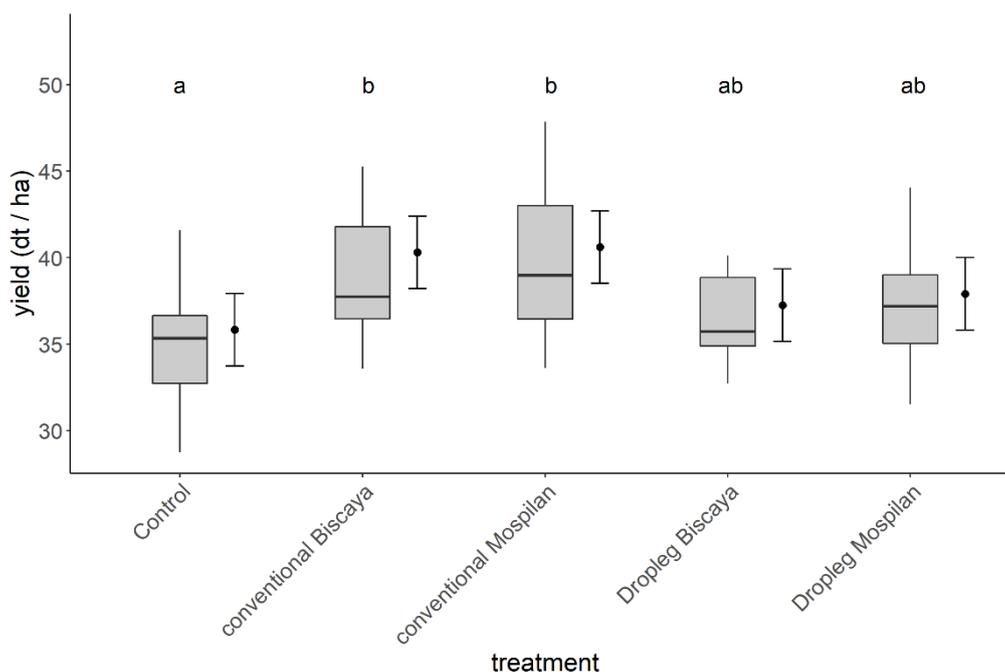


Figure B3 Yields (kg / ha) of the different treatments in the field trials in 2018 and 2019. Boxplots show the average yield per treatment and block for the two years. Calculated means and 95% confidence limits are shown (black dots). Treatments with different letters differ significantly from each other. LM ($y = \text{treatment} + \text{year} + \text{block}$), Tukey-(HSD) $p < 0.05$

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Curriculum Vitae

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Ich erkläre hiermit, dass ich die vorliegende Arbeit selbstständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe. Ich versichere, dass anderweitig keine Promotion beantragt wurde und die vorliegende Arbeit weder in gleicher noch in ähnlicher Form anderweitig eingereicht worden ist.

Braunschweig, 06. Mai 2022

Johannes Hausmann

