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Effects of different insecticide applications on population development of pollen beetle (*Meligethes aeneus* (Fabricius)) on oilseed rape (*Brassica napus* L.)

Dissertationen aus dem Julius Kühn-Institut

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Bundesforschungsinstitut für Kulturpflanzen

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Effects of different insecticide applications on population development of pollen beetle (*Meligethes aeneus* (Fabricius)) on oilseed rape (*Brassica napus* L.)

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Summary

The pollen beetle, *Meligethes aeneus* (Fabricius) (syn. *Brassicogethes aeneus* (Fabricius)) is a major pest in the production of oilseed rape, *Brassica napus* L., in Europe. At high levels of crop infestation, adult beetles can cause severe economic damage by destroying the buds. Chemical control of this pest is biased due to the widely distributed pyrethroid resistance and a decreasing availability of insecticides with different modes of action. Effective insecticides are required not only to avoid yield losses by overwintered pollen beetles but also to minimize the reproduction rate of the beetle, thereby reducing the infestation pressure and the frequency of insecticide treatments in following years.

The objectives of the present study were to determine the effects of the systemic neonicotinoid Biscaya (a.i. thiacloprid, 72 g ha^{-1}) and the two contact pyrethroids Mavrik (tau-fluvalinate, 48 g ha^{-1}) and Karate Zeon (lambda-cyhalothrin, 7.5 g ha^{-1}) on population development of pollen beetle. In the years 2013-2015, field trials were established on crops of oilseed rape in the region of Braunschweig (northern Germany) and further locations in Germany. Recommended dose rates of the insecticides were applied at the bud stage, at the beginning of flowering or at full flowering of the crop. The effect of insecticide applications on overwintered pollen beetles was assessed by counting pollen beetles on plants before and up to two weeks after application. Furthermore, the impact of insecticide application at the bud stage on infestation of buds with eggs and larvae was investigated in field trials as well as in greenhouse experiments using plants and pollen beetles collected from untreated and Biscaya- or Mavrik-treated field plots. The effects of insecticides on larval instars and emergence of new generations of pollen beetles were studied in field trials. In addition, the impact of the insecticide treatments on parasitism rates of pollen beetle larvae by *Tersilochus heterocerus* and *Phradis* spp. was analysed by collecting samples of larvae from different field trials distributed over Germany.

Application of Biscaya and Mavrik during the bud stage significantly reduced the abundance of the overwintered pollen beetles up to seven days after application. In contrast, application of Karate Zeon did not affect beetle numbers on plants. Consequently, the number of buds infested with eggs and larvae was reduced at least for 14 days after application of Biscaya and Mavrik compared to Karate Zeon-treated and untreated plots. Biscaya had a stronger effect on infestation of buds with eggs and larvae than Mavrik. Results of greenhouse experiments indicated that the lower number of infested buds found in Mavrik-treated plots resulted from a reduced number of overwintered pollen beetles, whereas in Biscaya-treated plots in addition to effects on overwintered pollen beetles a further reduction of bud infestation occurred. In all three experimental years the application of Biscaya at the beginning of flowering or full flowering resulted in significantly higher numbers of premature L1-larvae dropping down from the plants to the ground. This effect was only observed in Biscaya-treated plots, with up to 425% more L1-larvae dropping within one week compared to the control. Numbers of mature L2-larvae dropping down for pupation was reduced in plots treated with Mavrik and especially Biscaya. In Karate Zeon-treated plots the number of dropping L2-larvae was even higher than in control plots. In accordance with lower numbers of L2-larvae in

Biscaya- and Mavrik-treated plots the number of emerging new generation beetles was reduced.

No significant effect of insecticide application on parasitism rates of pollen beetle larvae by *T. heterocerus* and *Phradis* spp. was found. The parasitoid *T. heterocerus* was predominant with parasitized pollen beetle larvae occurring in all field trials and all experimental years, usually not earlier than in BBCH 65. In contrast *Phradis* spp. larvae were not detected in all field trials in 2015 and not before BBCH 67.

Altogether, the results demonstrate that applications of Biscaya and Mavrik cause reductions of pollen beetle population growth. Especially the neonicotinoid Biscaya could form an important part of a resistance management program for controlling populations of pyrethroid resistant pollen beetles. Applications during the bud stage did not only reduce the overwintered pollen beetles, but in addition had sublethal effects on infestation of buds with eggs and larvae. Particularly Biscaya targeted for control of cabbage seedpod weevil or brassica pod midge during flowering has shown long-lasting effects on pollen beetle populations without affecting parasitization of pollen beetle larvae.

Keywords: *Meligethes aeneus*, thiacloprid, tau-fluvalinate, lambda-cyhalothrin, bud infestation, larvae, *Tersilochus heterocerus* and *Phradis* spp.

Zusammenfassung

Der Rapsglanzkäfer, *Meligethes aeneus* (Fabricius) (syn. *Brassicogethes aeneus* (Fabricius)) ist ein bedeutender Schädling im Raps (*Brassica napus* L.). Ein hoher Befall durch überwinterte Altkäfer kann durch Knospenschädigung zu deutlichen wirtschaftlichen Schäden führen. Die Kontrolle dieses Schädlings mit Insektiziden ist aufgrund weit verbreiteter Pyrethroidresistenz und einer abnehmenden Verfügbarkeit von Insektiziden mit unterschiedlichen Wirkungsweisen nur einseitig möglich. Insektizide, die nicht nur Ertragsverluste durch überwinterte Altkäfer verhindern, sondern auch die Reproduktion der Käfer begrenzen, werden benötigt, um den Befallsdruck und damit die Häufigkeit von Insektizidbehandlungen in Folgejahren zu reduzieren.

Im Rahmen der vorliegenden Arbeit sollen die Effekte des systemisch wirkenden Neonicotinoids Biscaya (a.i. Thiacloprid, 72 g ha^{-1}) sowie der beiden Pyrethroide Mavrik (tau-Fluvalinat, 48 g ha^{-1}) und Karate Zeon (lambda-Cyhalothrin, 7,5 g ha^{-1}) auf die Populationsentwicklung des Rapsglanzkäfers untersucht werden. In den Jahren 2013 bis 2015 wurden Feldversuche mit Winterraps in der Umgebung von Braunschweig (Norddeutschland) und in weiteren Regionen Deutschlands etabliert. Die empfohlenen Aufwandmengen der Insektizide wurden im Knospenstadium, zu Beginn der Blüte oder in der Vollblüte appliziert. Der Einfluss der Insektizidapplikationen auf überwinterte Altkäfer wurde durch Auszählen der Käfer auf den Haupttrieben direkt vor und bis zu zwei Wochen nach der Applikation erfasst. Außerdem wurden die Effekte von Insektizidapplikationen im Knospenstadium auf die Knospenbelegung mit Eiern und Larven in Feld- und Gewächshausversuchen untersucht. Für die Gewächshausversuche wurden Pflanzen und Rapsglanzkäfer aus unbehandelten und Biscaya oder Mavrik behandelten Parzellen der Feldversuche genutzt. Der Einfluss der Insektizide auf die Larvenstadien und den Schlupf der neuen Rapsglanzkäfergeneration wurde ebenfalls in Feldversuchen geprüft. Desweiteren wurde untersucht, ob die Insektizidbehandlungen die Parasitierungsrate der Rapsglanzkäferlarven durch *Tersilochus heterocerus* und *Phradis* spp. beeinflussen. Dafür wurden Larvenproben aus deutschlandweiten Feldversuchen gesammelt und untersucht.

Die Applikation von Biscaya und Mavrik im Knospenstadium reduzierte bis zu sieben Tage nach Applikation signifikant die Anzahl überwinterter Rapsglanzkäfer. Karate Zeon hingegen beeinflusste die Käferzahlen auf den Pflanzen nicht. Folglich war der Anteil mit Eiern und Larven befallener Knospen im Vergleich zu Karate Zeon behandelten und unbehandelten Parzellen für mindestens 14 Tage nach der Applikation von Biscaya und Mavrik reduziert. Dabei zeigte Biscaya stärkere Effekte auf die Knospenbelegung mit Eiern und Larven als Mavrik. Die Ergebnisse der Gewächshausversuche deuten darauf hin, dass der geringere Anteil belegter Knospen in den Mavrik behandelten Parzellen durch die reduzierten Käferzahlen begründet ist, während in Biscaya behandelten Parzellen zusätzlich zu der Altkäferwirkung weitere Effekte zur Reduktion der Knospenbelegung beitrugen. In allen drei Versuchsjahren resultierte die Applikation von Biscaya zu Beginn der Blüte oder in der Vollblüte in signifikant höheren Zahlen zu Boden fallender unausgewachsener L1-Larven. Dieser Effekt wurde nur in Biscaya behandelten Parzellen beobachtet. Hier fielen innerhalb einer Woche im Vergleich zur Kontrolle bis zu 425% mehr L1-Larven zu Boden. Die Anzahl ausgewachsener L2-Larven, die zur

Verpuppung zu Boden fielen, war in Mavrik und insbesondere in Biscaya behandelten Parzellen reduziert. In Karate Zeon behandelten Parzellen hingegen war die Zahl abwandernder L2-Larven sogar noch höher als in unbehandelten Kontrollparzellen. Der geringere Anteil an L2-Larven in Biscaya und Mavrik behandelten Parzellen spiegelte sich auch in einer reduzierten Zahl schlüpfender Jungkäfer wider.

Die Parasitierungsraten der Rapsglanzkäferlarven wurden durch die Insektizidapplikationen nicht signifikant beeinflusst. In allen Versuchsjahren und in allen Feldversuchen wurden die Rapsglanzkäferlarven vor allem durch *T. heterocerus* parasitiert, allerdings nicht vor BBCH 65. Larven von *Phradis* spp. wurden 2015 nicht in allen Feldversuchen und nicht vor BBCH 67 nachgewiesen.

Die Ergebnisse dieser Arbeit zeigen, dass Applikationen von Biscaya und Mavrik das Populationswachstum des Rapsglanzkäfers reduzieren. Besonders das Neonicotinoid Biscaya kann einen wichtigen Teil eines Resistenzmanagementprogramms ausmachen, um pyrethroidresistente Rapsglanzkäfer zu kontrollieren. Applikationen im Knospenstadium reduzieren nicht nur die Anzahl überwinterter Altkäfer, sondern durch sublethale Effekte zusätzlich auch die Knospenbelegung mit Eiern und Larven. Wird Biscaya zur Bekämpfung des Kohlschotenrüsslers oder der Kohlschotenmücke während der Blüte eingesetzt, wird als Nebeneffekt auch Wirkung auf die Rapsglanzkäferpopulation erzielt, ohne die Parasitierung der Rapsglanzkäferlarven zu beeinflussen.

Stichwörter: *Meligethes aeneus*, Thiacloprid, tau-Fluvalinat, lambda-Cyhalothrin, Knospenbelegung, Larven, *Tersilochus heterocerus* und *Phradis* spp.

Chapter I

General introduction

Oilseed rape and its pests

Oilseed rape (*Brassica napus* L.) is a very important crop in northern and central Europe. The largest areas grown with oilseed rape are located in France (1.5 million hectares in 2015) (Agreste 2015) and Germany (1.3 million hectares) (DESTATIS 2016). In Germany oilseed rape is the fourth biggest arable crop behind wheat, maize and barley. In 2015 about 5 million tons of oilseed rape were produced in Germany (DESTATIS 2016). In central Europe, winter oilseed rape is predominating while spring oilseed rape is more commonly grown in northern Europe, where winter conditions are more extreme (Alford 2003, Williams 2010). The extracted oil is used for human nutrition or for industrial purposes as for production of soaps or biodiesel. After the pressing process the seed-cake is used as animal feed (Alford 2003).

Oilseed rape crop is known to host a large variety of herbivores. The most widespread insect pests in oilseed rape in Europe are cabbage stem flea beetle (*Psylliodes chrysocephala* (L.)), rape stem weevil (*Ceutorhynchus napi* Gyllenhal), cabbage stem weevil (*C. pallidactylus* (Marsham)), pollen beetle (*Meligethes aeneus* (Fabricius), syn. *Brassicogethes aeneus* (Fabricius)), cabbage seedpod weevil (*C. obstrictus* (Marsham)) and brassica pod midge (*Dasineura brassicae* Winnertz) (Alford et al. 2003, Williams 2010). At high levels of infestation each of these pests can cause severe plant damage and high yield losses (Williams 2010, Slater et al. 2011). Insect pests of minor importance that can be relevant only temporally and regionally, are the cabbage root fly (*Delia radicum* L.), flea beetles (*Phyllotreta* spp.), and the rape winter stem weevil (*C. picitarsis* Gyllenhal) as well as the turnip sawfly (*Athalia rosae* L.) (Alford et al. 2003, Williams 2010). Further pests are the cabbage aphid (*Brevicoryne brassicae* (L.)) and the peach/potato aphid (*Myzus persicae* (Sulzer)), which are vectors of the Turnip yellows virus (Schliephake et al. 2000).

In 2009 the genus *Meligethes* was revised and *M. aeneus*, as well as, inter alia, *M. coracinus* and *M. viridescens* have been transferred to the genus *Brassicogethes* (Audisio et al. 2009). Because actually the new name is still not enforced, in the present study still the genus name *Meligethes* is used.

Pyrethroid resistance

To control pests in oilseed rape a mean of about three insecticide applications is used in Germany (Freier et al. 2015, Roßberg 2016). The main insecticide class used for more than 20 years as foliar sprays in oilseed rape against pests were pyrethroids (Nauen 2005, Heimbach et al. 2006, Müller et al. 2008, Thieme et al. 2010a). Products of this mode of action group are cheap and farmers often mix these insecticides with fungicides prophylactically without considering the pest threshold values. Because of this frequent and indiscriminate use of pyrethroids and the overlapping appearance of different pest species some of these pests such as pollen beetle, cabbage seed pod weevil and cabbage stem flea beetle have developed resistance against pyrethroids (Heimbach & Müller 2013). In France pyrethroid resistance is also known for the rape winter stem weevil (Robert et al. 2015). In Germany first resistant rape winter stem weevils were found in Baden-Wuerttemberg in 2015 (Elias, personal communication).

Pyrethroid resistance of the pollen beetle is especially pronounced. In recent years *M. aeneus* is the dominant species of pollen beetles in Germany and the only species with economic importance and documented pyrethroid resistance (Thieme et al. 2010b). According to Nilsson (1988a) and Williams (2010) *M. aeneus* is dominating throughout Europe. In contrast to *M. aeneus*, the species *M. viridescens* is still sensitive to pyrethroids (Derron 2007, Thieme et al. 2010b). Reduced pyrethroid susceptibility of pollen beetles was first recorded in eastern France in 1999 (Thieme et al. 2010a, Slater et al. 2011). Since then pyrethroid resistance has been found all over Europe (Hansen 2003, Nauen 2005, Wegorek 2005, Heimbach et al. 2006, Tiilikainen & Hokkanen 2008, Slater et al. 2011, Zimmer & Nauen 2011a). Slater et al. (2011) analysed sensitivity of pollen beetles over a period of three years and found resistant beetles in 20 of 21 European countries monitored.

Since 2005 the development of resistance in Germany is surveyed by a monitoring at the Julius Kühn-Institut using the Adult-Vial-Test according to IRAC Method No. 11 (IRAC 2009). In 2005 more than 50% of pollen beetle populations were classified as highly sensitive or sensitive. The number of sensitive beetles decreased continuously over the years and vanished since 2010 completely. The percentage of highly resistant pollen beetles increased from 7% in 2005 to 92% in 2015 (Heimbach & Müller 2013, Brandes & Heimbach 2016). This is probably the result of pyrethroids still dominating the insecticide market with sufficient products with active substances with alternative modes of action lacking. For European pollen beetle populations two resistance mechanisms are known: metabolic resistance, mediated by enhanced expression of cytochrome P450 monooxygenases (Zimmer & Nauen 2011b) and target-site resistance caused by a mutation in voltage-gated sodium channels (Nauen et al. 2012).

Biology of the pollen beetle

Pollen beetles (Coleoptera: Nitidulidae) are generally abundant on winter and spring oilseed rape (Williams 2010). They hibernate in the upper soil layers of hedgerows and forest areas in a depth of 2-5 cm (Fritzsche 1957). The beetles leave their hibernation sites in spring at temperatures exceeding 9°C. If the temperatures increase further the beetles colonize spring flowers of different families to feed on pollen (Müller 1941b). Maturation feeding is important for the females because they leave the hibernation sites with undeveloped ovaries, whereas at the same time the males are sexual mature (Müller 1941b, Fritzsche 1957). At temperatures around 15°C pollen beetles colonize fields with oilseed rape crop (Fritzsche 1957). To get access to pollen the beetles bite into the sepals and petals of buds. The plant is very susceptible to severe bud damages caused by the beetles during the green bud stage resulting in bud abortion and high yield losses (Slater et al. 2011). When the plants begin to flower the pollen beetles prefer pollen in open flowers to pollen in buds which causes no damage (Fritzsche 1957).

For oviposition females prefer buds of 2-3 mm length (Fritzsche 1957, Nilsson 1988b, Ekbom & Borg 1996). The number of eggs laid depends according to Fritzsche (1957) on temperature, humidity and nutrition of the beetles. During the most active part of oviposition an average of 5.3 eggs per female and day was observed by Nilsson (1988b), who concluded, that at favourable weather conditions a female can produce 200-300 eggs during her lifetime. This is in accordance with Fritzsche (1957). At 20-22°C and a relative humidity of > 95% the larvae hatch four days after oviposition (Friederichs 1921, Fritzsche 1957, Bromand 1983). In field trials eggs hatched within four to seven days in spring oilseed rape (Nielsen & Axelsen 1988, Nilsson 1988c) and within ten days in winter oilseed rape (Nilsson 1988c). Bromand (1983) observed in laboratory studies an egg hatching rate of 90%.

The two larval instars of the pollen beetle feed on pollen (Osborne 1964, Nilsson 1988d). The first instars have a length of 0.8-2.3 mm (Osborne 1964) and usually develop within the buds (Williams & Free 1978). The development of the first instar is completed after five to ten days (Burkhardt & von Lengerken 1920, Nilsson 1988c). The second instars have a length of 2.3-4.4 mm (Osborne 1964), are very mobile and move every few days into younger flowers and feed in several flowers during development (Williams & Free 1978). 20-25 days after oviposition mature L2-larvae drop down from the plants (Nilsson 1988c) and dig into the soil for pupation in 0.5-2 cm depth (Fritzsche 1957). The pupal period lasts, depending on temperature and humidity, about ten to 18 days (Fritzsche 1957, Bromand 1983). The emerging new generation beetles start feeding pollen of different plant families before seeking their hibernation sites at the turn of July to August (Müller 1941a). The complete development from egg to adult takes 40 to 50 days, depending on temperature (Friederichs 1921, Nilsson 1988c).

Factors influencing population development of the pollen beetle

Population development of pollen beetle is influenced by many factors such as mortality during migration to and from hibernation sites and during hibernation (Nilsson 1994). Average mortality of beetles in hibernation sites was 5.7% in four consecutive years in trials of Kdimati (1990). Vietinghoff (1985) observed winter mortality of up to 25%. According to Hokkanen (1993) overwintering survival is significantly influenced by mean body weight of pollen beetle. Only beetles with high fat reserves are able to survive the winter. Winter mortality can also be influenced by entomopathogenic fungi as *Beauveria bassiana* (Kdimati 1990, Hokkanen 1993). With fungi infected beetles consume more energy to defend fungal attack at the expense of fitness (Hokkanen 1993). Warm and moist conditions during winter enhance mortality during hibernation (Kdimati 1990). Pyrethroid resistance of pollen beetle is not linked with a higher overwintering mortality (Gloyna & Thieme 2013).

Another important factor influencing population development is the coincidence between time of migration of the pollen beetles in oilseed rape and the time period of the bud stage, as females of pollen beetle prefer buds of a size of 2-3 mm for oviposition (Fritzsche 1957, Nilsson 1988b, Ekbom & Borg 1996). At a very high population density also small buds (< 2 mm) were used for egg laying which resulted very often in bud abortion caused by the hole chewing for deposition of eggs (Nilsson 1988b). Eggs and larvae in aborted buds die (Nilsson 1988c). If the period providing suitable buds is short, the reproduction can be reduced (Nilsson 1994). This can be caused by higher temperatures promoting fast plant development or by early flowering winter oilseed rape cultivars (Tölle 2014).

Furthermore the population density of the pollen beetle can be substantially reduced by natural enemies as predators such as carabid and staphylinid beetles and spiders (Büchs & Nuss 2000, Büchs & Alford 2003, Piper & Williams 2004). In addition several hymenopteran endoparasitoids attack the egg and larval stage of pollen beetle (Nilsson & Andreasson 1987). The most abundant larval parasitoids of the pollen beetle are the ichneumonid parasitoids *Phradis interstitialis* (Thomson), *P. morionellus* (Holmgren) and *Tersilochus heterocerus* (Thomson). *T. heterocerus* often predominates in winter oilseed rape (Nilsson 2003, Nitzsche 1998). These univoltine species are widely distributed throughout Europe (Nilsson 2003). Parasitization levels of more than 50% are reported (Nitzsche 1998, Nilsson 2003, Ulber et al. 2010). In Germany *T. heterocerus* and *P. interstitialis* predominate (Ulber et al. 2010). *Diospilus capito*, a braconid, is also widely distributed throughout Europe, but it is more common in spring rape in northern Europe (Nilsson 2003, Veromann et al. 2006). Several other parasitoids of the pollen beetle are of minor importance (Nilsson 2003).

P. interstitialis colonizes oilseed rape crops in Germany from mid of April onwards. *T. heterocerus* and *P. morionellus* occur one to two weeks later at the end of April or the beginning of May (Nilsson 1985, Nitzsche 1998, Johnen et al. 2010, Neumann 2010). Parasitoid females emerging from overwintering sites are sexually mature (Nilsson 2003). The females of *P. interstitialis* lay their eggs through the bud walls into the eggs and first instar larvae of pollen beetle (Osborne 1960), whereas the other two species prefer large L2-larvae for oviposition (Nilsson & Andreasson 1987). The coincidence between pollen beetles and parasitoid migration is not precise, resulting in an escape of a part of the host larval population from parasitization in some years (Nilsson 2003).

The first instar of *Phradis* spp. larvae hatch shortly after oviposition (Nilsson 2003). But *T. heterocerus* larvae emerge from the egg not until the host larvae are fully developed and ready to drop down to the soil for pupation (Osborne 1960, Nitzsche 1998). In the soil the parasitoid larvae finish development and kill their hosts in the hosts' cocoon in the upper soil layer shortly before the hosts pupate. Approximately one month after pupation the parasitoids develop into adults, but stay in diapause until the next spring (Nilsson 2003, Ulber et al. 2010). In the soil parasitoid population density is strongly affected by soil tillage (Nilsson 1985, Nitzsche 1998). Nitzsche (1998) observed a significant reduced number of *T. heterocerus* and *P. interstitialis* emerging in the following year after ploughing and use of rotary harrow, whereas effects of soil tillage with a cultivator did not differ significantly from zero tillage.

Population development of pollen beetles can be influenced by further factors. Mortality of larvae and pupae under the soil surface is also an important, but poorly investigated factor (Nilsson 1988c). Kdimati (1990) reported mortality rates during pupation between 15 to 65%. This might be caused by ground beetles and rove beetles preying upon larvae and pupae of pollen beetles in the soil (Büchs & Nuss 2000, Büchs 2003).

Application of insecticides usually causes direct mortality of overwintered beetles and it is possible that insecticides affect beetles indirectly by influencing behaviour. This is described in literature for different insects for example for the parasitic wasp *Aphidius ervi*, which showed impaired orientation and oviposition behaviour after contact to low doses of lambda-cyhalothrin (Desneux et al. 2004). Neumann (2010) reported uncoordinated, slow movements of parasitoids of pollen beetle in contact with thiacloprid, tau-fluvalinate and lambda-cyhalothrin. Furthermore insecticide treatments may result in reduced egg production by females. Different synthetic pyrethroids caused a reduced number of eggs laid by *Pectinophora gossypiella* (Bariola 1984). Hajjar & Ford (1989) observed abnormal oviposition behaviour and reduced egg laying of the mustard leaf beetle (*Phaedon cochleariae*) on radish plants treated with sublethal doses of the pyrethroid active substance cypermethrin. Ako et al. (2004, 2006) observed a decrease in oviposition of the two-spotted spider mite (*Tetranychus urticae*) caused by field-relevant dose rates of different neonicotinoids. In addition Shi et al. (2011) reported a reduced fecundity of the cotton aphid, *Aphis gossypii* after treatment with different neonicotinoids.

Insecticide applications can not only affect the pests, but also influence natural enemies and thereby influence population development of pollen beetles. Predators as spiders or carabid species, attacking pollen beetle larvae dropping down for pupation on the soil surface can be affected by different insecticides. Several papers reported negative effects of lambda-cyhalothrin and other insecticides on these predators, significantly reducing both the number of predatory species and individuals (Wehling & Heimbach 1991, Devotto et al. 2007, Liu et al. 2013, Rodrigues et al. 2013).

Also parasitoids can be affected by insecticide applications. Treatments in wheat or barley following oilseed rape to control cereal aphids can affect in spring emerging adult parasitoids of pollen beetle (Nilsson & Andreasson 1987, Jansen & San Martin y Gomez 2014). Applications of insecticides in oilseed rape to control pollen beetle can also affect parasitoids searching for hosts. In the period shortly before flowering to full flowering the main activity of tersilochine parasitoids was observed (Ulber & Nitzsche 2006). Consequently application of insecticides in this activity phase may have negative effects on parasitoids searching for hosts (Johnen & Ulber 2004). Exposition of parasitoids to insecticides can occur through direct contact with the spray mist, through contact to insecticidal residuals on the leaves while searching for hosts or through uptake of contaminated material by feeding (Croft & Brown 1975, Longley & Jepson 1996). In addition to direct effects pesticides may affect natural enemies indirectly, for example by influencing the host searching behaviour, fertility or oviposition, or the insecticides may have a repellent effect (Wright & Verkerk 1995).

Because of widely distributed pyrethroid resistance and the lack of alternative insecticides with different modes of action, control of pollen beetles is difficult. To slow down resistance development for available insecticidal actives, insecticides have to be used targeted, considering pest thresholds. For effective control of pollen beetles in oilseed rape, insecticides are required not just for the effective direct control of the pest to avoid yield losses by bud damage but simultaneously to reduce the reproduction of pollen beetle and thereby influencing population growth. This reduces the probability of a high infestation pressure in following crops. If insecticide application is not targeting pollen beetles but brassica pod midge or cabbage seedpod weevil during flowering, side-effects on larvae of pollen beetles help reduce population density of the pest. In addition predators and parasitoids should not be influenced by insecticides to integrate natural enemies into pest control.

Objectives of the study

The pollen beetle is threatening oilseed rape production in many places. Control of this pest is mainly based on insecticide applications, as other means are lacking. The aim of the present study is to analyse the effects of the neonicotinoid Biscaya (a.i. thiacloprid, 72 g ha^{-1}) and the pyrethroids Mavrik (tau-fluvalinate, 48 g ha^{-1}) and Karate Zeon (lambda-cyhalothrin, 7.5 g ha^{-1}) on population development of pollen beetle. The main objectives are:

- Investigation of the effects of the three insecticides, applied at different growth stages of winter oilseed rape, on overwintered pollen beetles in field trials.
  It was expected that the three insecticides had different effects on overwintered pollen beetle mortality. Because of pyrethroid resistance, a low efficacy of Karate Zeon was expected as described in several papers (Schröder et al. 2009, Smatas et al. 2012, Brandes et al. 2014). For Biscaya and Mavrik significant effects on pollen beetle reduction in the field are known (Vaitelyte et al. 2011, Smatas et al. 2012, Jansen & San Martin y Gomez 2014, Tölle 2014).
- Determination of the effects of applications of these insecticides at the bud stage of winter oilseed rape on bud infestation with eggs and larvae of pollen beetles in field trials and greenhouse experiments.
  It is known that bud infestation with eggs and larvae of pollen beetle is reduced by Biscaya (Tölle 2014), but the exact reasons for the reduced bud infestation are not known, as well as the effects of Mavrik and Karate Zeon. It was expected that the three insecticides have different effects on bud infestation with eggs and larvae.
- Study of the impact of applications of these insecticides at different growth stages of winter oilseed rape on first and second larval stages and the new generation of pollen beetles.
  It was expected that the insecticides might have different effects on the two larval stages and consequently the number of new generation of pollen beetles might be influenced. A significant effect of Biscaya on the number of dropping L2-larvae and emerging new generation beetles is reported by Tölle (2014), but no details are known, as well as effects of Mavrik and Karate Zeon. Any information on the effects of the insecticides on L1-larvae is missing.

- Analysis of the impact of the three insecticides, applied at different growth stages of winter oilseed rape, on parasitism rates of pollen beetle larvae by the parasitoids *T. heterocerus* and *Phradis* spp.
  Effects on parasitism rates might be different depending on the growth stage of the crop and the products. Several papers reported negative effects of insecticides on parasitoids (Veromann et al. 2006, Neumann 2010, Jansen & San Martin y Gomez 2014) but no clear and consistent effects of lambda-cyhalothrin, tau-fluvalinate and thiacloprid on parasitization of pollen beetle are reported in literature. Effects of individual insecticides differ between authors and between years within one paper. Some effects on parasitization would be expected in situations when parasitoid activity and insecticide application coincidate, because lethal effects of lambda-cyhalothrin, tau-fluvalinate and thiacloprid on adult parasitoids in glass-vial tests have been reported (Neumann 2010).

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Chapter II

Effects of the neonicotinoid Biscaya and the pyrethroids Mavrik and Karate Zeon on overwintered pollen beetles (*Meligethes aeneus* (Fabricius)) and their offspring in oilseed rape

Abstract

To prevent damage by pollen beetles (*Meligethes aeneus* (Fabricius), syn. *Brassicogethes aeneus* (Fabricius)) often applications of insecticides are necessary. It would be an advantage if spraying of pollen beetle would not only avoid yield losses by bud damage by overwintered pollen beetles but also minimize reproduction of the pest and also have side effects on the population development of other pests present in the crop. The effects of the neonicotinoid Biscaya (a.i. thiacloprid, 72 g ha^{-1}) and the pyrethroids Mavrik (tau-fluvalinate, 48 g ha^{-1}) and Karate Zeon (lambda-cyhalothrin, 7.5 g ha^{-1}) applied at different times at the bud stage and at the beginning of flowering to full flowering of winter oilseed rape on the abundance of overwintered pollen beetles were determined in field trials in 2013-2015. In addition effects on the two larval instars and new generation beetles were studied.

Biscaya and Mavrik significantly reduced the number of overwintered pollen beetles up to seven days after application whereas Karate Zeon had no effect on pollen beetle density. Application of Biscaya at the beginning of flowering resulted in a high mortality of L1-larvae in all years. The number of premature L1-larvae dropping down from the plants during the first week after application increased up to 425% compared to the control. The number of L2-larvae dropping down to the ground for pupation and the number of emerging new generation beetles was significantly reduced by insecticide applications at different growth stages except for Karate Zeon application. Depending on the time of application the efficacy against L2-larvae in field trials near Braunschweig varied between 25% and 64% for Biscaya and between 13% and 53% for Mavrik. In contrast in Karate Zeon-treated plots the number of L2-larvae dropping to the ground increased by 10% compared to the control. In accordance with the number of L2-larvae dropping down for pupation, less new generation pollen beetles emerged, efficacy varying between 57% and 76% in Biscaya-treated plots and 32% and 57% in Mavrik-treated plots in 2014 and 2015. The results show that Mavrik and especially Biscaya are effective in controlling pollen beetles with a sustainable effect, reducing infestation pressure and insecticide treatment frequency in following years.

Introduction

The pollen beetle, *Meligethes aeneus* (Fabricius), syn. *Brassicogethes aeneus* (Fabricius) (Coleoptera: Nitidulidae) is a major pest on winter and spring oilseed rape across Europe (Williams 2010). In spring the overwintered beetles leave their hibernation sites and often feed on pollen of different spring flowers before colonizing oilseed rape crops where they feed on the buds to get access to pollen (Müller 1941b). High numbers of pollen beetles during the green bud stage can result in bud abortion and substantial loss in yield (Slater et al. 2011). After maturation females lay their eggs into the buds (Fritzsche 1957, Nilsson 1988a, Ekbom & Borg 1996). The developmental time for eggs depends on temperature and humidity (Fritzsche 1957). In field trials eggs hatched within ten days in winter oilseed rape (Nilsson 1988b). The two larval instars feed on pollen (Osborne 1964, Nilsson 1988c). According to Williams & Free (1978) the first immature larval instars develop within the buds whereas the second larval instars are very active and consume pollen from several flowers during their development. The majority of mature L2-larvae drop down to the soil for pupation during the petal fall. Depending on the temperature the total development from egg to adult takes 40 to 50 days (Friederichs 1921, Nilsson 1988b). After emerging new generation pollen beetles feed on pollen of different plant families before seeking their hibernation sites at the turn of July to August (Müller 1941a).

To minimize damage by various insect pests oilseed rape is sprayed relatively often with insecticides. In the years 2007 to 2013 the mean number of annual insecticide treatments in winter oilseed rape in Germany was 2.7 (Freier et al. 2015). The highest intensity was targeted on cabbage seedpod weevils (*Ceutorhynchus obstrictus* (Marsham)) and pollen beetles (Freier et al. 2015). For more than 20 years the main insecticide class extensively used against pests in oilseed rape are pyrethroids (Nauen 2005, Heimbach et al. 2006, Müller et al. 2008, Thieme et al. 2010). Frequent applications of this insecticide class and the overlapping exposure of different oilseed rape pests resulted in a high selection pressure and finally some of these species as pollen beetle, cabbage seedpod weevil and cabbage stem flea beetle (*Psylliodes chrysocephala* (L.)) developed resistance against pyrethroids (Heimbach & Müller 2013). Pyrethroid resistance is also known for the rape winter stem weevil (*C. picitarsis* Gyllenhal) in France (Robert et al. 2015).

Decreasing pyrethroid sensitivity in pollen beetles was first detected in 1999 in France (Thieme et al. 2010, Slater et al. 2011). Since then pyrethroid resistance has been found all over Europe (Hansen 2003, Nauen 2005, Wegorek 2005, Heimbach et al. 2006, Tiilikainen & Hokkanen 2008, Slater et al. 2011, Zimmer & Nauen 2011a). To control pyrethroid resistant pollen beetles other insecticides with different modes of action are necessary. In addition these insecticides should not only prevent bud damage and yield losses by overwintered pollen beetles but also diminish the reproduction rate of the targeted pest thereby reducing the probability of a high infestation pressure and insecticide treatment frequency in following years. An effect of insecticide application on population development of pyrethroid sensitive pollen beetles was reported by Kdimati (1990). After application of Decis (a.i. deltamethrin) Kdimati observed a reduced bud infestation with eggs and a reduced number of dropping larvae resulting in a reduced number of new generation pollen beetles.

The systemic neonicotinoid Biscaya (a.i. thiacloprid, 72 g ha^{-1}) and the non-systemic pyrethroids Mavrik (type I pyrethroid, tau-fluvalinate 48 g ha^{-1}) and Karate Zeon (type II pyrethroid, lambda-cyhalothrin 7.5 g ha^{-1}) are registered for control of pollen beetles. Type I and II pyrethroids are distinguished by the absence (type I) or presence (type II) of an α-cyano group in their structure (Dong 2007, He et al. 2008). Tau-fluvalinate and lambda-cyhalothrin are sodium channel modulators and interfere with nerve conduction (Roberts et al. 1999). The active substance thiacloprid acts as agonist on the insect nicotinic acetylcholine receptor (Elbert et al. 2008). All named insecticides are classified as contact and stomach poisons.

In contrast to *C. obstrictus* which showed similar sensitivity to different active substances of pyrethroids (Heimbach & Müller 2013), differences in the susceptibility of pollen beetles are known. Type I pyrethroids are known to have a higher efficacy against resistant pollen beetles in the field than type II pyrethroids as Karate Zeon (Schröder et al. 2009, Smatas et al. 2012). This was confirmed in laboratory tests by Wegorek et al. (2009). The authors recorded in laboratory tests with oilseed rape inflorescences and leaves dipped in test solutions of different insecticides better effects on pollen beetles for type I pyrethroids (in the study tau-fluvalinate and bifenthrin) than for type II pyrethroids (beta-cyfluthrin, zeta-cypermethrin and esfenvalerate). However, in standard biotests applied for resistance monitoring at the Julius Kühn-Institut pollen beetles have shown a decreasing sensitivity to type I pyrethroids as well (Heimbach & Müller 2013). This is in accordance with Makunas et al. (2011). The authors tested Lithuanian pollen beetle populations in biotests in three consecutive years and observed lower effects of type II pyrethroids than of tau-fluvalinate with decreasing susceptibility to all active substances over the experimental years.

The objective of the present study was to determine effects of the insecticides Biscaya, Mavrik and Karate Zeon applied at different dates between the bud and full flowering stage of winter oilseed rape on the abundance of overwintered pollen beetles, the two larval instars and the new generation of pollen beetles.

Materials and Methods

The effects of insecticides on the abundance and population growth of pollen beetles were studied from 2013 to 2015 in field trials at the trial sites of the Julius Kühn-Institut in the region of Braunschweig (52°32'80.29''N, 10°63'16.74''E (Wendhausen 2013), 52°21'72.27''N, 10°62'72.30''E (Sickte 2014), 52°21'00.81''N, 10°67'36.03''E (Lucklum 2015); according to WGS 84). The winter oilseed rape cultivar “Visby” was used for the experiments in 2013 and 2014; in 2015 the cultivar “Avatar” was established. For characterizing the growth stages of winter oilseed rape the BBCH code of Lancashire et al. (1991) was used. The field trials were established in a randomized block design. The plots had a size of 720 to 1080 m^2 each and the treatments were replicated four times. Insecticide application was carried out at the bud stage or at the flowering stage, using recommended product application rates in 300 l water ha^{-1} (Tab. 1). In 2013 the neonicotinoid Biscaya (a.i. thiacloprid, 72 g ha^{-1}) and the pyrethroid Karate Zeon (lambda-cyhalothrin, 7.5 g ha^{-1}) were used. In 2014 and 2015 the pyrethroid Mavrik (tau-fluvalinate, 48 g ha^{-1}) was applied instead of Karate Zeon. For the application of Biscaya and Mavrik at different growth stages different plots were used whereas the three applications of Karate Zeon in 2013 were carried out in a sequence to the same plots.

Tab. 1: Dates of insecticide application, respective growth stages (BBCH), product dose rates and active substances in field trials near Braunschweig 2013 to 2015

Year	Date of application	BBCH	Insecticide product	Product dose rate	Active substances and rate
2013	23 April	53	Biscaya	300 ml ha^{-1}	72 g ha^{-1} thiacloprid
			Karate Zeon	75 ml ha^{-1}	7.5 g ha^{-1} lambda-cyhalothrin
	2 May	60	Biscaya	300 ml ha^{-1}	72 g ha^{-1} thiacloprid
			Karate Zeon	75 ml ha^{-1}	7.5 g ha^{-1} lambda-cyhalothrin
	15 May	65	Biscaya	300 ml ha^{-1}	72 g ha^{-1} thiacloprid
			Karate Zeon	75 ml ha^{-1}	7.5 g ha^{-1} lambda-cyhalothrin
2014	31 March	55	Biscaya	300 ml ha^{-1}	72 g ha^{-1} thiacloprid
			Mavrik	200 ml ha^{-1}	48 g ha^{-1} tau-fluvalinate
	16 April	62	Biscaya	300 ml ha^{-1}	72 g ha^{-1} thiacloprid
			Mavrik	200 ml ha^{-1}	48 g ha^{-1} tau-fluvalinate
2015	16 April	55	Biscaya	300 ml ha^{-1}	72 g ha^{-1} thiacloprid
			Mavrik	200 ml ha^{-1}	48 g ha^{-1} tau-fluvalinate
	29 April	62	Biscaya	300 ml ha^{-1}	72 g ha^{-1} thiacloprid
			Mavrik	200 ml ha^{-1}	48 g ha^{-1} tau-fluvalinate

In addition field trials located in Lower Saxony, Mecklenburg-Western Pomerania and Bavaria with diverse cultivars and different plot sizes of 36 to 480 m^2 each were carried out in 2013 to 2015 (Tab. 2). The field trials were established in a randomized block design. Equal to the field trials carried out in the region of Braunschweig the insecticides Biscaya, Mavrik and Karate Zeon with recommended product dose rates (Tab. 1) were used. Applications of the insecticides were carried out between the green bud stage (BBCH 53) and the yellow bud stage (BBCH 59). Plots of different treatments were replicated four times.

Tab. 2: Dates of insecticide application, respective growth stages (BBCH), insecticide products, plot size and cultivar used in field trials in Lower Saxony, Mecklenburg-Western Pomerania and Bavaria 2013 to 2015

Year	Location	Date of application	BBCH	Insecticide product	Plot size (m^2)	Cultivar
2013	Puch Bavaria	23 May	55-57	Biscaya Karate Zeon	480	Genie
	Cramonshagen Mecklenburg-Western Pomerania	8 May	59	Biscaya Karate Zeon	72	Avatar
	Stöckendrebber Lower Saxony	28 April	55-57	Biscaya Mavrik Karate Zeon	120	Visby
2014	Puch Bavaria	31 March	55	Biscaya Karate Zeon	300	Genie
	Kleefeld Mecklenburg-Western Pomerania	31 March	55	Biscaya Mavrik	72	Avatar
	Stöckendrebber Lower Saxony	31 March	53	Biscaya	120	Sherpa
2015	Puch Bavaria	16 April	57	Biscaya Mavrik Karate Zeon	122.5	Avatar
	Kleefeld Mecklenburg-Western Pomerania	23 April	57-59	Biscaya Mavrik	36	Mercedes
	Niedernstöcken Lower Saxony	16 April	56	Biscaya	120	Sherpa

The abundance of overwintered pollen beetles in the field trials near Braunschweig was assessed according to EPPO Standard PP 1/178 (3) (EPPO 2005) directly before and one, three to four, six to eight and 13 to 15 days after application between 9 and 11 a.m. The number of pollen beetles was recorded by beating 50 randomly selected main stems per plot over a plastic tray (31.5 cm x 25.5 cm). The abundance of overwintered pollen beetles in the additional field trials distributed over Germany was recorded in a similar way but the results will not be presented in this chapter.

To collect larvae dropping from inflorescences to the ground in the field trial in the region of Braunschweig, ten plastic bowls (17 cm x 12.1 cm) per plot filled with a 10% sodium benzoate water solution for conservation were placed in the centre of each plot near the tramline (approximately 1 m distance) on the ground before the first application at BBCH growth stage 53 in 2013 and at BBCH 55 in 2014 and 2015. The plastic bowls were controlled regularly and after the first larvae dropped down from BBCH 62 until BBCH 76-78 emptied each week. The larvae were stored in 70% Ethanol, later separated by their development stage according to Osborne (1964) and counted under the binocular microscope (eight- to tenfold magnification). The number of larvae was extrapolated from 10 x 205.7 cm² to 1 m^2.

At the other locations four plastic bowls (18.3 cm x 13.6 cm) per plot were used and also placed in the field. In 2013 the plastic bowls were placed at BBCH 61 (Puch), BBCH 63 (Stöckendrebber) and BBCH 70 (Cramonshagen). In 2014 and 2015 the plastic bowls were placed at all locations at the bud stage and emptied weekly. These larvae were also stored in 70% Ethanol and counted and separated by their development stage as described above. As a consequence of the different number and size of the bowls the number of larvae was extrapolated from 4 x 248.9 cm² to 1 m^2. For these external field trials only the results of the L2-larvae will be presented in this chapter.

New generation pollen beetles were collected in the field trials near Braunschweig using three soil-photoeclectors (0.25 m^2, ecoTech GmbH) per plot, established near the tramline (approximately 1 m distance). The soil rings of the photoeclectors were dug into the soil in early spring before beginning of stem elongation of plants, not to disturb the development of the oilseed rape plants within and outside the photoeclector area. At BBCH 76-78 the photoeclectors were closed by a fabric tent, before emergence of new generation pollen beetles started. A perforated plastic bag was fixed to the opening on top of the photoeclectors to catch the beetles alive. The plastic bags were checked regularly and emptied twice a week when beetle emergence started at BBCH 80 until the end of emergence at BBCH 84.

Statistical analyses

Statistical analyses were carried out using the software R, version 3.1.2 (R Core Team 2014; packages: *lme4* (Bates et al. 2015)*, multcomp* (Hothorn et al. 2008)*, effects* (Fox 2003)*, MASS* (Venables & Ripley 2002), *glmmADMB* (Fournier et al. 2012), *coin* (Hothorn et al. 2006)). Data of each year were analysed separately, because of high variability in infestation levels, weather conditions and field trial locations. To analyse the abundance of overwintered pollen beetles per main stem in the different treatments Generalized Linear Mixed Models (GLMM, poisson for count data) were used. The treatment and the date were included into the model as main effects and their interaction was tested. The replicates were integrated as random effect. The optimal model was selected by using Akaike Information Criteria (AIC) described in Zuur et al. (2009). The model was checked for dispersion. The treatments were compared pairwise for each day of assessment with the package *lsmeans* (Lenth 2015) for post-hoc testing. P values were adjusted with the Hochberg method (Blakesley et al. 2009). To test for unidirectional immigration of pollen beetles into the field trial in spring the model fit was compared with and without autocorrelation function via AIC.

Differences between numbers of larvae in different treatments were analysed using Generalized Linear Models (GLM). Because the poisson model revealed overdispersion, a negative binomial model was used. The treatment and the date were included into the model as main effects and their interaction was tested. To account for a different number of plastic bowls (sometimes plastic bowls were tipped over or destroyed by animals) an offset was included into the model. Pairwise comparison of the treatments for each collection period was conducted as described for the overwintered pollen beetles. To compare the accumulated number of L2-larvae dropping down for pupation in the different treatments over the total collection periods an Analysis of Variance (ANOVA) was performed. Assumptions on variance homogeneity and normality of residuals were visually inspected. The differences between the means were evaluated by the TukeyHSD test. To analyse differences in the abundance of new generation pollen beetles in the different treatments GLMM (poisson) were used. The sums of new generation beetles over the complete collection periods were analysed as described for L2-lavae. The emergence rate of new generation beetles was calculated as the percentage of new generation beetles in proportion to L2-larvae dropped to the soil for pupation. The efficacy of insecticide treatments was calculated according to the formula of Abbott (1925).

Results

Effects on overwintered pollen beetles:

In each experimental year of the field trials in the region of Braunschweig, immigration of pollen beetles into the field trial in spring was not unidirectional and no significant spatial trend was observed. The first assessment of pollen beetles was carried out just before the first insecticide application in each respective year. In 2013 on average 2.0 pollen beetles per main stem were recorded at BBCH 53 in the untreated plots. One day after treatment the number of pollen beetles per main stem was significantly reduced in the plots treated with Biscaya compared to the control (GLMM, $p = 0.0032$) and the plots treated with Karate Zeon (GLMM, $p = 0.0308$) (Tab. 3). In the Biscaya-treated plots pollen beetle numbers were reduced by 47%. Three days after treatment the pollen beetle density increased caused by migration when maximum daily temperature increased to > 20°C the days before. The lowest number of pollen beetles occurred again in plots treated with Biscaya (efficacy 33%).

Seven days after treatment pollen beetle numbers did not differ between the treatments (GLMM, $p > 0.05$), while 13 days after treatment the number of pollen beetles significantly increased in Biscaya-treated plots in comparison to the control (GLMM, $p = 0.0014$). Compared to Karate Zeon-treated plots number of pollen beetles in Biscaya-treated plots did not differ significantly (GLMM, $p > 0.05$). Effects of Biscaya on pollen beetle densities in the period after the application at BBCH 60 in 2013 were similar: including four days after treatment the lowest pollen beetle density was assessed in Biscaya-treated plots whereas Karate Zeon had no effects on overwintered pollen beetles. At the following assessments six and 14 days after treatment no significant differences occurred between the treatments (GLMM, $p > 0.05$). In the period after the insecticide application at BBCH 65 infestation rate was too low (on average 0.3 pollen beetles per main stem) to supply valid data.

In the field trial of 2014 the pollen beetle density was clearly lower than in 2013 but similar effects of Biscaya were found. On average 1.5 pollen beetles per main stem were recorded at BBCH 55 in the untreated plots. After application at BBCH 55 the beetle density decreased continuously in all plots (Tab. 3). One day after treatment and three days after treatment the number of pollen beetles was significantly reduced by the application of Biscaya and Mavrik compared to the control (GLMM, Biscaya: $p < 0.001$/ $p = 0.0171$; Mavrik: $p < 0.001$/ $p = 0.0076$). One day after treatment the efficacy of Biscaya and Mavrik was 68% of 82%, respectively. Three days after treatment the pollen beetle density in Biscaya- and Mavrik-treated plots was reduced by ca. 50%, respectively. At the following assessments seven and 15 days after treatment no significant differences between the treatments in pollen beetle density occurred (GLMM, $p > 0.05$). Because of a very low infestation rate (on average 0.08 pollen beetles per main stem) in the period following insecticide application at BBCH 62 the data are not valid.

In 2015 on average 2.1 pollen beetles per main stem were recorded at BBCH 55 in the untreated plots. As in 2014 pollen beetle density decreased in all plots after application and again Biscaya and Mavrik showed similar effects on pollen beetle density (Tab. 3). One day after treatment pollen beetle density decreased by 70% in Biscaya-treated plots and by 96% in Mavrik-treated plots. In insecticide-treated plots the number of pollen beetles was significantly reduced up to seven days after treatment, with Mavrik having stronger effects. At 14 days after treatment the pollen beetle density significantly increased in Biscaya-treated plots in comparison to the control (GLMM, $p = 0.0002$) and Mavrik-treated plots (GLMM, $p = 0.0110$) and as observed in 2013. At the treatment of the insecticides at BBCH 62 pollen beetle density was low but the same trend was observed. The number of pollen beetles was significantly reduced in Biscaya- and Mavrik-treated plots including three days after treatment. Eight days after treatment no difference between the treatments existed.

Tab. 3: Mean number (± SE) of pollen beetles per main stem in untreated and insecticide-treated plots before and several days after application (DAA) in field trials in Wendhausen 2013, Sickte 2014 and Lucklum 2015. Different letters within each column and date of application indicate significant differences between treatments, GLMM, p ≤ 0.05; n.a. = data not assessed

		Mean number of pollen beetles per main stem				
Date of application	Treat-ment	0 DAA ± SE	1 DAA ± SE	3-4 DAA ± SE	6-8 DAA ± SE	13-15 DAA ± SE
23 April 2013 BBCH 53	Control	**2.25** ± 0.15 A	**2.71** ± 0.18 A	**9.52** ± 0.47 A	**5.63** ± 0.25 A	**3.84** ± 0.20 A
	Biscaya	**2.05** ± 0.13 A	**1.45** ± 0.10 B	**6.43** ± 0.39 A	**4.97** ± 0.30 A	**6.93** ± 0.31 B
	Karate Zeon	**1.84** ± 0.12 A	**2.80** ± 0.18 A	**9.00** ± 0.39 A	**5.25** ± 0.23 A	**4.24** ± 0.21 AB
2 May 2013 BBCH 60	Control	**3.80** ± 0.20 A	**2.11** ± 0.14 A	**3.84** ± 0.20 A	**2.07** ± 0.16 A	**0.22** ± 0.03 A
	Biscaya	**3.87** ± 0.19 A	**1.03** ± 0.08 B	**2.60** ± 0.20 A	**2.24** ± 0.16 A	**0.23** ± 0.03 A
	Karate Zeon	**3.52** ± 0.17 A	**2.11** ± 0.14 A	**4.24** ± 0.21 A	**1.67** ± 0.12 A	**0.12** ± 0.02 A
15 May 2013 BBCH 65	Control	**0.25** ± 0.04 AB	**0.22** ± 0.03 AB	**0.14** ± 0.03 AB	**0.13** ± 0.03 AB	n.a.
	Biscaya	**0.33** ± 0.04 B	**0.31** ± 0.04 B	**0.35** ± 0.05 B	**0.32** ± 0.04 B	n.a.
	Karate Zeon	**0.18** ± 0.03 A	**0.12** ± 0.02 A	**0.10** ± 0.02 A	**0.09** ± 0.02 A	n.a.
31 March 2014 BBCH 55	Control	**1.90** ± 0.26 A	**1.33** ± 0.11 A	**0.85** ± 0.14 A	**0.50** ± 0.06 A	**0.07** ± 0.02 A
	Biscaya	**1.57** ± 0.21 AB	**0.42** ± 0.05 B	**0.40** ± 0.08 B	**0.40** ± 0.07 A	**0.14** ± 0.03 A
	Mavrik	**1.17** ± 0.20 B	**0.24** ± 0.04 B	**0.42** ± 0.04 B	**0.47** ± 0.12 A	**0.11** ± 0.04 A
16 April 2014 BBCH 62	Control	**0.07** ± 0.03 A	**0.05** ± 0.01 A	**0.06** ±0.01 A	**0.14** ± 0.03 A	**0.08** ± 0.01 A
	Biscaya	**0.09** ± 0.03 A	**0.02** ± 0.01 AB	**0.06** ± 0.03 A	**0.14** ± 0.03 A	**0.11** ± 0.04 A
	Mavrik	**0.08** ± 0.04 A	**0.02** ± 0.01 B	**0** ± 0 A	**0.12** ± 0.03 A	**0.06** ± 0.02 A
16 April 2015 BBCH 55	Control	**2.43** ± 0.14 A	**1.52** ± 0.09 A	**1.29** ± 0.13 A	**1.06** ± 0.06 A	**0.43** ± 0.03 A
	Biscaya	**1.96** ± 0,10 B	**0.46** ± 0.09 B	**0.29** ± 0.04 B	**0.25** ± 0.06 B	**0.76** ± 0.15 B
	Mavrik	**1.82** ± 0.14 B	**0.06** ± 0.03 C	**0.07** ± 0.03 C	**0.46** ± 0.03 C	**0.51** ± 0.07 A
29 April 2015 BBCH 62	Control	**0.53** ± 0.06 A	**0.43** ± 0.03 A	**0.22** ± 0.06 A	**0.11** ± 0.01 A	**0.03** ± 0.01 A
	Biscaya	**0.44** ± 0.04 A	**0.10** ± 0.03 B	**0.04** ± 0.02 B	**0.10** ± 0.03 A	**0.08** ± 0.04 A
	Mavrik	**0.37** ± 0.05 A	**0.10** ± 0.03 B	**0.05** ± 0.02 B	**0.08** ± 0.02 A	**0.02** ± 0.01 A

Effects on larvae:

Dropping of premature L1-larvae started at BBCH 62 to 65 in all three years in the field trials in the region of Braunschweig. At the first check of the plastic bowls in 2013 (collection period 2 May to 15 May, BBCH 60-65) the highest number of L1-larvae was counted in the plots treated with Biscaya in BBCH 60 (Tab. 4). In this treatment 706 L1-larvae m^{-2} dropped down to the ground. This number differed significantly from all other treatments. Compared to the untreated plots number of L1-larvae increased up to 388%. The application of Biscaya at BBCH 62 in 2014 and 2015 resulted in similar effects. In both years high numbers of L1-larvae were observed after the treatment of Biscaya at BBCH 62 at the first emptying of the plastic bowls (collection period 2014: 16 April to 23 April, BBCH 62-65; 2015: 29 April to 4 May, BBCH 62-65). In 2014 and 2015 in the Biscaya-treated plots the number of L1-larvae increased up to 425% and 200%, respectively, compared to untreated plots. In both years the lowest number of dropping L1-larvae was found in plots treated with Mavrik at BBCH 55.

In the second period of collecting larvae in 2013 (15 May to 23 May, BBCH 65-69) the highest number of L1-larvae dropped down in the plots treated with Biscaya at BBCH 60 and 65. The Biscaya treatment at BBCH 60 resulted in an increase of L1-larvae up to 60% in comparison to the untreated plots. In the plots treated with Biscaya at BBCH 65 1122 L1-larvae m^{-2} were counted (number of L1-larvae increased up to 283% compared to the control). In 2014 in the second period of collection (23 April to 30 April, BBCH 65-67) only a low number of L1-larvae dropped down from the plants. There was no significant difference between treatments (GLM, $p > 0.05$). In 2015 (collection period 4 May to 11 May, BBCH 65-67) number of L1-larvae in the plastic bowls in the plots treated with Biscaya at BBCH 62 increased up to 112% compared to the control. The significantly lowest number of L1-larvae was found in the plots treated with Biscaya and Mavrik in BBCH 55. In each case in these plots L1-larvae were reduced by 63% compared to the control.

In all experimental years the number of L1-larvae captured in the following weekly collecting periods decreased continuously. In 2013 there was no significant difference between the treatments during the third emptying period (BBCH 69-71) (GLM, $p > 0.05$). In the fourth emptying period in 2013 (BBCH 71-74) significantly more larvae were found in the control compared to Biscaya treatments at BBCH 53 (GLM, $p = 0.001$) and BBCH 60 (GLM, $p = 0.0162$), respectively. In plots treated with Biscaya at BBCH 53 number of L1-larvae was reduced by 82% and by 70% in plots treated at BBCH 60. In 2014 and 2015 only few larvae were found in the plastic bowls in the third (2014: BBCH 67-69; 2015: 67-69) and fourth emptying period (2014: BBCH 69-72; 2015: 69-71) without significant differences between treatments (GLM, $p > 0.05$).

The number of mature L2-larvae dropping down to the ground for pupation was also recorded using the same plastic bowls as for L1-larvae. In all experimental years in the field trials in the region of Braunschweig, the peak of L2-larvae migration was observed at BBCH 65-69 at petal fall. The mean sum of L2-larvae of the complete period of migration resulted in a lower number of L2-larvae in plots treated with Biscaya (Tab. 4). In 2013 the highest efficacy (58%) was achieved by the application of Biscaya at BBCH 60. The efficacy of the Biscaya application at the bud stage and at full flowering against L2-larvae was 25% and 38%, respectively. The repeated application of Karate Zeon in three different growth stages finally ended in a higher number of L2-Larvae dropping to the ground. The number of L2-larvae increased up to 10% compared to the control. Higher numbers of L2-larvae dropping down in Karate Zeon-treated plots were also observed in the other field trials distributed over Germany and different years with increased numbers up to 42% dropping down in Karate Zeon-treated plots compared to the control (Tab. 5).

In 2014 insecticide applications reduced the number of L2-larvae. Applications of Biscaya at BBCH 55 and BBCH 62 in the field trials in the region of Braunschweig resulted in a significant reduction compared to the control (TukeyHSD, Biscaya BBCH 55: $p = 0.0332$; Biscaya BBCH 62: $p = 0.0004$) with efficacies of 34% and 59% whereas Mavrik reached efficacies of 30% by the application at BBCH 55 and 17% at BBCH 62 (TukeyHSD, $p > 0.05$). In 2015 a similar tendency was observed. The number of L2-larvae was significantly reduced by the applications of Biscaya at BBCH 55 and 62 and Mavrik at BBCH 55 compared to the control (TukeyHSD, Biscaya BBCH 55: $p = 0.0003$; Biscaya BBCH 62: $p = 0.0008$; Mavrik BBCH 55: $p = 0.0025$). In the plots treated with Biscaya at BBCH 55 the number of L2-larvae was reduced by 64% and in Mavrik-treated plots by 53%. The treatment with Biscaya at BBCH 62 resulted in an efficacy of 58% whereas the application of Mavrik at this growth stage reduced the number of L2-larvae only by 13%. Also in the other field trials distributed over Germany in three consecutive years Biscaya reduced the number of second instars, in most cases significantly compared to the control (Tab. 5). Mavrik also reduced number of L2-larvae, but in most cases less effective than Biscaya.

Effects on new generation pollen beetles:

In all experimental years emerging of new generation pollen beetles started at BBCH 80. In 2013 deep cracks in the soil caused by extended drought periods reduced the efficiency of the catching method and not all emerging new generation beetles were caught. In 2014 and 2015 the number of emerging new generation pollen beetles was significantly reduced in all insecticide-treated plots with Biscaya showing a higher efficacy at both application dates in each year (Tab. 4). In 2014 the application of Biscaya at BBCH 55 and 62 resulted in a reduction of new generation beetles by 57% and by 59% whereas the treatment with Mavrik reduced number of new generation beetles by 47% at BBCH 55 and by 42% at BBCH 62. In 2015 the lowest number of new generation pollen beetles was recorded in the plots treated with Biscaya at BBCH 55 (efficacy 76%), followed by the Biscaya treatment at BBCH 62 (72%) and the application of Mavrik at BBCH 55 (57%). The application of Mavrik at BBCH 62 resulted in a reduction of beetles by 32% compared to the control. Average emergence rate of all treatments was 22.9% in 2014 and 40.4% in 2015. No trend that pupation in soil and development of new generation pollen beetles was affected by insecticide treatment was obvious.

Tab. 4: Mean number (± SE) of L1-larvae, cumulated mean number of L2-larvae and of new generation pollen beetles (NG PB) m^{-2} in different treatments in field trials in Wendhausen 2013, Sickte 2014 and Lucklum 2015; collection period 1 = 2 – 15 May 2013 (BBCH 60 – 65)/ 16 – 23 April 2014 (BBCH 62 – 65)/ 29 April – 4 May 2015 (BBCH 62 – 65); collection period 2 = 15 – 23 May 2013 (BBCH 65 – 69)/ 23 – 30 April 2014 (BBCH 65 – 67)/ 4 – 11 May 2015 (BBCH 65 – 67). Different letters within each column and year indicate significant differences between treatments, L1-larvae: GLM, $p \leq 0.05$, L2-larvae and NG PB: Tukey HSD-Test, $p \leq 0.05$

Year	Treatment/ BBCH	L1-larvae m^{-2} ± SE Period 1	L1-larvae m^{-2} ± SE Period 2	L2-larvae m^{-2} ± SE	NG PB m^{-2} ± SE
2013	Control	**144.6** ± 12.1 A	**292.9** ± 45.9 A	**4831.7** ± 370.3 AC	**124.7** ± 19.6 AB
	Biscaya 53	**108.2** ± 21.5 A	**260.1** ± 70.2 A	**3624.8** ± 375.2 AD	**171.0** ± 25.1 A
	Biscaya 60	**706.1** ± 52.7 B	**469.1** ± 91.4 AB	**2051.5** ± 235.3 B	**78.3** ± 14.5 B
	Biscaya 65	**143.4** ± 12.3 A	**1121.8** ± 433.2 B	**3003.2** ± 223.6 BD	**82.2** ± 15.0 B
	Karate 53, 60, 65	**76.6** ± 22.3 A	**393.8** ± 71.8 A	**5310.7** ± 344.6 C	**186.0** ± 18.9 A
2014	Control	**24.3** ± 9.5 AC	**14.6** ± 3.4 A	**809.4** ± 56.7 A	**223.2** ± 18.8 A
	Biscaya 55	**23.1** ± 15.0 AC	**13.4** ± 4.2 A	**532.3** ± 36.2 BC	**95.3** ± 4.4 B
	Biscaya 62	**127.6** ± 40.5 B	**23.1** ± 9.4 A	**334.2** ± 25.1 B	**92.3** ± 7.5 B
	Mavrik 55	**3.6** ± 2.3 C	**7.3** ± 2.4 A	**570.0** ± 69.0 AB	**119.0** ± 17.2 B
	Mavrik 62	**65.6** ± 24.2 AB	**8.5** ± 1.2 A	**673.3** ± 87.4 AC	**129.3** ± 13.3 B
2015	Control	**19.4** ± 6.0 AB	**160.2** ± 25.3 AC	**1716.1** ± 179.3 A	**862.7** ± 50.1 A
	Biscaya 55	**20.7** ± 7.0 AB	**59.6** ± 6.4 B	**620.9** ± 70.7 B	**207.7** ± 7.3 B
	Biscaya 62	**58.3** ± 18.9 A	**340.3** ± 65.7 C	**723.1** ± 77.6 B	**238.0** ± 36.7 BC
	Mavrik 55	**15.8** ± 8.0 B	**58.9** ± 4.0 B	**810.4** ± 107.0 B	**372.3** ± 58.6 C
	Mavrik 62	**19.4** ± 6.9 AB	**87.5** ± 22.2 AB	**1485.2** ± 189.2 A	**586.0** ± 62.6 D

Tab. 5: Cumulated mean number (± SE) of L2-larvae m^{-2} dropping down for pupation in different treatments in field trials 2013-2015. Different letters within each location and year indicate significant differences between treatments, Tukey HSD-Test, $p \leq 0.05$, efficacy (%) calculated according to Abbott (1925)

Year	Location/ Application at BBCH	Treatment	L2-larvae m^{-2} ± SE		Efficacy (%)
2013	Puch, Bavaria BBCH 55-57	Control	**3545.9** ± 381.5	A	
		Biscaya	**2574.0** ± 122.9	B	27.4
		Karate Zeon	**4841.7** ± 121.6	C	-36.5
	Cramonshagen, Mecklenburg-Western Pomerania BBCH 59	Control	**582.6** ± 88.6	A	
		Biscaya	**482.2** ± 126.3	A	17.2
		Karate Zeon	**750.9** ± 65.0	A	-28.9
	Stöckendrebber, Lower Saxony BBCH 55-57	Control	**3283.9** ± 103.4	A	
		Biscaya	**1597.2** ± 173.9	B	51.4
		Karate Zeon	**3337.5** ± 274.6	A	-1.6
		Mavrik	**2604.2** ± 257.7	A	20.7
2014	Puch, Bavaria BBCH 55	Control	**1152.7** ± 56.4	A	
		Biscaya	**647.9** ± 84.9	B	43.8
		Karate Zeon	**1511.8** ± 151.9	A	-31.2
	Kleefeld, Mecklenburg-Western Pomerania BBCH 55	Control	**2350.5** ± 238.2	A	
		Biscaya	**2235.0** ± 228.1	A	4.9
		Mavrik	**2245.1** ± 279.4	A	4.5
	Stöckendrebber, Lower Saxony BBCH 53	Control	**1805.6** ± 119.7	A	
		Biscaya	**1218.0** ± 121.4	B	32.5
2015	Puch, Bavaria BBCH 57	Control	**1750.3** ± 139.5	A	
		Biscaya	**1737.8** ± 78.8	A	0.7
		Karate Zeon	**2486.1** ± 96.1	B	-42.0
		Mavrik	**1212.9** ± 111.7	C	30.7
	Kleefeld, Mecklenburg-Western Pomerania BBCH 57-59	Control	**2599.1** ± 274.6	A	
		Biscaya	**1700.1** ± 153.7	B	34.6
		Mavrik	**1665.0** ± 190.7	B	35.9
	Niedernstöcken, Lower Saxony BBCH 56	Control	**1044.7** ± 80.9	A	
		Biscaya	**482.2** ± 26.3	B	53.8

Discussion

In the present field trials the density of overwintered pollen beetles was significantly reduced up to seven days after application of Biscaya and Mavrik whereas Karate Zeon had no effect. Type I pyrethroids (as tau-fluvalinate in Mavrik) are known to have still a higher efficacy against pollen beetles in the field than type II pyrethroids as lambda-cyhalothrin in Karate Zeon (Schröder et al. 2009, Smatas et al. 2012). However, in standardized laboratory biotests (IRAC 2009) pollen beetle populations have shown a decreasing sensitivity to type I pyrethroids (Makunas et al. 2011, Heimbach & Müller 2013). But sensitivity changes in biotests may not always be reflected in field data. In biotests the active substance is used, whereas in the field the formulated product is applied, this may explain different responses of beetles (Thieme et al. 2008). In addition in the coated vials in the biotests beetles stay in permanent contact to the active substance while in the field beetles can visit also untreated parts of the plants.

No signs of resistance to thiacloprid in European *M. aeneus* populations are known (Nauen et al. 2012, Zimmer et al. 2014). Effects seen in the present field trials confirm the laboratory findings with Mavrik and Biscaya still controlling pollen beetles. Significant reduction of overwintered pollen beetles in the field by Biscaya and Mavrik was also reported by Smatas et al. (2012). Jansen & San Martin y Gomez (2014) observed significant effects on pollen beetle reduction by Biscaya for at least up to eight days after application. Significant effects of Mavrik for at least up to seven days after application are described by Vaitelyte et al. (2011).

The low pollen beetle density after application of Biscaya and Mavrik at the bud stage in treated plots may be caused by direct mortality or sublethal effects, i.e. that the individuals that survive an exposure to an insecticide can be affected in their physiology or behaviour (Desneux et al. 2007). Sublethal effects may influence coordination of body movements of the beetles or result in increased activity (Desneux et al. 2007). These effects may be the reason for the phenomenon described by Gödeke (2016): In field trials more pollen beetles dropped down from plants treated with Biscaya at least up to seven days after application compared to the untreated plots, whereas application of Mavrik did not result in higher numbers of dropping beetles.

Furthermore repellent effects of Biscaya and Mavrik may have contributed to the reduced beetle number in the presented field trials. A reduced residence time of pollen beetle parasitoids on buds treated with thiacloprid and tau-fluvalinate, indicating a repellent effect, was observed by Neumann (2010) whereas for lambda-cyhalothrin no repellency was reported. A repellent effect of the pyrethroid deltamethrin on aphid parasitoids of the genus *Aphidius* was reported by Longley & Jepson (1996). The pyrethroids permethrin and cypermethrin had a repellent effect on honey bees (Rieth & Levin 1988). Easton & Goulson (2013) reported repellent effects of the neonicotinoid active substance imidacloprid on different Diptera and Coleoptera species.

The increase of pollen beetle density 14 days after treatment at the bud stage in Biscaya treated plots in 2013 and 2015 compared to the control may be explained by a higher attractiveness of these plants after insecticidal residuals vanished. These plants were less colonized for several days after application and accordingly less used for feeding and oviposition (chapter III). Recolonization of treated plots after decline of insecticidal activity was also reported by Tölle (2014). It is uncertain whether larvae hatched from eggs laid by the increased number of beetles in Biscaya-treated plots 14 days after treatment can complete their development. Without sufficient food the larvae can be forced to pupate before completion of their development, resulting in high mortality (Nielsen & Axelsen 1988). According to Nilsson (1994) the larvae should be ready to pupate at petal fall, otherwise they have to move more frequently between flowers or finally feed on stems or pods to finish their development. This increases the risk of predation and parasitization.

The reduced pollen beetle density in plots treated with Biscaya and Mavrik at the bud stage contributed to a reduced infestation of buds with eggs (chapter III) which finally resulted in a lower number of larvae and new generation pollen beetles. But the application of Biscaya at BBCH 60-65 had additional effects. Applications of Biscaya in the flowering stage resulted in enhanced dropping of premature L1-larvae. The enhanced dropping of L1-larvae may have been caused by lethal effects of thiacloprid or by sublethal effects impairing larval coordination; however, this could not be tested in detail. Uncoordinated or stumbling movements of third instar larvae of the Cabbage Looper (*Trichoplusia ni*) after topical application of pyrethroids was reported by Toth & Sparks (1990). Zimmer et al. (2014) observed that L2-larvae of pollen beetle were highly sensitive to thiacloprid in a larval dip laboratory assay while they showed insensitivity to lambda-cyhalothrin. It is likely that also L1-larvae are highly sensitive to thiacloprid resulting in an enhanced dropping of larvae after application. Mechanical effects of the spraying activity on enhanced larval dropping could be excluded because no such effects were seen in Mavrik- or Karate Zeon-treated plots. L1-larvae are not fully developed and not ready for pupation. Once dropped to the ground, larvae are likely to fall prey to predators as ground beetles (Carabidae), rove beetles (Staphylinidae) and spiders (Araneae) (Büchs & Alford 2003, Piper & Williams 2004, Öberg et al. 2011) or they may desiccate or die by the lack of food.

The number of mature L2-larvae dropping down for pupation is an important factor determining the effects of insecticides on population growth of pollen beetle. Higher numbers of L2-larvae dropping down in Karate Zeon-treated plots in the field trial in Wendhausen in 2013 (number of L2-larvae increased up to 10% compared to the control) were also observed in other field trials distributed over Germany and different years. An increased number of up to 42% dropped down in Karate Zeon-treated plots compared to the control. Reason for the enhanced number of L2-larvae dropping down in Karate Zeon-treated plots might be an elimination of antagonists such as spiders by the active substance lambda-cyhalothrin resulting in an undisturbed development of pollen beetle larvae. Negative effects on the abundance of spiders by lambda-cyhalothrin were observed by Wehling & Heimbach (1991), Devotto et al. (2007) and Liu et al. (2013). Also staphylinid larvae can influence density of pollen beetle larvae and are affected by lambda-cyhalothrin (Felsmann 2008).

Another reason for an enhanced number of L2-larvae might be insecticide hormoligosis. Luckey (1968) described the phenomenon that sublethal quantities of stress agents, as insecticides, can be stimulatory to insects and an increase of reproduction or other parameters can be expected at low concentrations of insecticides. The fast degradation of pyrethroids by monoxygenase enzymes, mainly responsible for pyrethroid resistance of pollen beetles (Zimmer & Nauen 2011b) may be responsible for a stimulation of the females resulting in increased oviposition and consequently high numbers of larvae.

As a consequence of the reduction of overwintered pollen beetles, effects on egg laying (chapter III) and additional direct effects on L1-larvae in Biscaya-treated plots, the number of second instars was (in most field trials significantly) reduced compared to the control. A significant reduced number of L2-larvae after two applications of Biscaya in field trials in three consecutive years was also reported by Tölle (2014). In addition Jansen & San Martin y Gomez (2014) observed a larval reduction by 84% in plots treated with Biscaya at the end of the bud stage. In the present field trials Mavrik also reduced number of L2-larvae, but in most cases less effective than Biscaya. It was observed that Mavrik did not contribute to a reduced infestation of buds with eggs (chapter III), so it can be concluded that effects of Mavrik on the number of L2-larvae dropping down for pupation were mainly caused by reduction of overwintered pollen beetles after application at the bud stage. Applications of Mavrik at the flowering stage were less effective because effects on L1-larvae were missing.

L2-larvae dropping down to the soil in order to pupate can be consumed by ground beetles, rove beetles (Büchs 2003) or spiders (Öberg et al. 2011). Also the pupae in the soil are likely to be preyed upon by ground beetles and rove beetles (Büchs & Nuss 2000, Büchs 2003). It is known that these natural enemies can be affected by insecticides (Pfiffner & Luka 2003, Felsmann 2008). In the present study the insecticide applications were carried out during an oilseed rape growth stage with a very tight canopy. It is known that spraying of oilseed rape during the flowering growth stage (in the EFSA study defined as BBCH 40-89) with conventional spraying techniques results in an interception value of 80, which means that only low quantities of insecticides get to the soil (EFSA 2014). So it can be assumed that the predators in the present field trials were exposed to low amounts of insecticides with limited effects on larvae and pupae of pollen beetle on and in the soil, but this could not be tested in detail.

The number of L2-larvae dropping down for pupation in different treatments was reflected in the number of emerging new generation pollen beetles in all years. The treatments with the lowest number of L2-larvae showed also the lowest number of emerging beetles. The application of Biscaya and Mavrik resulted in significantly lower numbers of new generation beetles, with Mavrik being less effective than Biscaya, as described before for the number of L2-larvae dropping down for pupation. No clear trend that the emergence rate of new generation pollen beetles was influenced by insecticides was obvious. Reduced numbers of new generation pollen beetles after application of Biscaya were also described by Tölle (2014).

In the presented field trials Biscaya and partly also Mavrik showed effects on pollen beetle population not assessed in usual efficacy evaluation of insecticides according to EPPO Standard 1/178 (3) (EPPO 2005), where only effects on overwintered pollen beetles are recorded. Biscaya not only showed effects on overwintered beetles, but also on infestation of buds with eggs and larvae (chapter III). In addition applications of Biscaya in flowering oilseed rape are highly effective in elimination of L1-larvae. Despite effects on overwintered pollen beetles, effects of Mavrik on infestation of buds with eggs and larvae were less compared to Biscaya (chapter III) and effects on L1-larvae were missing. In conclusion Biscaya is effective in controlling pollen beetles in the bud stage with a sustainable effect and may reduce infestation pressure and insecticide treatment frequency in following years. Once the plants begin to flower the pollen beetles prefer pollen in open flowers to pollen in buds without causing damage (Fritzsche 1957). Applications at the flowering stage against pollen beetle are not common. But if insect pests as *Ceutorhynchus obstrictus* or *Dasineura brassicae* have to be controlled by insecticides at the flowering stage, Biscaya has positive side effects on the reproduction of simultaneously occurring pollen beetles.

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Chapter III

Impact of the insecticides Biscaya, Mavrik and Karate Zeon on infestation of buds with eggs and larvae of pollen beetle (*Meligethes aeneus* (Fabricius))

Abstract

Pollen beetle (*Meligethes aeneus* (Fabricius), syn. *Brassicogethes aeneus* (Fabricius) is one of the main insect pests in oilseed rape. At high levels of infestation adult pollen beetles can cause severe plant damage and high yield losses. Insecticides are required that not just prevent yield losses by bud feeding of overwintered pollen beetles but simultaneously minimize the reproduction of the pest, thereby reducing the size of the following beetle generation infesting next years oilseed rape. The effects of the neonicotinoid Biscaya (a.i. thiacloprid, 72 g ha^{-1}) and the pyrethroids Mavrik (tau-fluvalinate, 48 g ha^{-1}) and Karate Zeon (lambda-cyhalothrin, 7.5 g ha^{-1}), applied at the bud stage on infestation of buds with eggs and larvae of pollen beetles were tested in field trials in 2013-2015. In field trials of 2014 and 2015, individual plants were covered during the insecticide application to investigate whether effects on bud infestation were caused by direct lethal effects on pollen beetle or by insecticidal residuals on plants causing repellency or sublethal effects. This was studied also in additional greenhouse experiments under controlled conditions using untreated and Biscaya- or Mavrik-treated plants and pollen beetles, both collected in the field trials.

In the field trials, application of Biscaya and Mavrik significantly reduced the percentage of buds containing eggs and L1-larvae while Karate Zeon had no effect. A significant effect of Biscaya on bud infestation was observed up to 14 days after application. In 2014 and 2015, 14 days after treatment, bud infestation on main stems was reduced by 86% and 82% respectively in Biscaya-treated plots and by 51% and 71% respectively in Mavrik-treated plots compared to the untreated plots. The covered plants in treated plots showed a higher infestation of buds with eggs and larvae compared to adjacent uncovered plants treated with insecticides. In all experimental years the number of eggs and L1-larvae per infested bud on main stems increased from sampling date to sampling date but did not differ significantly between treatments in contrast to percentage of infested buds. In all years, similar effects of insecticides on bud infestation were observed on buds from main stems and side shoots. In the greenhouse experiments, no significant differences were observed in percentage of bud infestation with eggs between the control (untreated pollen beetles caged on untreated plants) and the combinations of untreated plants and insecticide-treated pollen beetles. In all greenhouse experiments the lowest percentage of bud infestation with eggs and L1-larvae was recorded on Biscaya-treated plants whereas on Mavrik-treated plants no significant difference to the control was recorded, independent on pollen beetle sampling from untreated or Mavrik-treated plots.

The results of the present field trials show that application of Biscaya and Mavrik reduced bud infestation with eggs and larvae of pollen beetles primarily by lethal effects on overwintered pollen beetles or repellency. Biscaya had additional effects on egg laying

which was supported by the greenhouse experiments in which only on Biscaya treated plants additional sublethal effects contribute to reduced bud infestation.

Introduction

Meligethes aeneus (Fabricius), syn. *Brassicogethes aeneus* (Fabricius) (Coleoptera: Nitidulidae) is the most common species of pollen beetles in Europe (Nilsson 1988b, Williams 2010) and the only species of economic importance (Thieme et al. 2010). After leaving the hibernation sites in spring pollen beetles immigrate into crops of oilseed rape during the bud stage. They bite into buds to get access to pollen, thereby causing injuries and abortion of buds, resulting in podless stalks (Nilsson 1987) and substantial yield loss (Slater et al. 2011). Following maturation feeding females start egg-laying into buds. The timing and extend of oviposition in winter oilseed rape depends on temperature, time of ovarial maturity and availability of medium sized buds (Nilsson 1988c).

For oviposition females prefer buds of 2-3 mm length (Fritzsche 1957, Nilsson 1988c, Ekbom & Borg 1996, Ferguson et al. 2015). This size allows the L1-larvae to develop the longest possible period in a single bud (Nilsson 1994). No eggs were found in buds < 1.5 mm and > 5 mm or in open flowers (Fritzsche 1957, Nilsson 1988c). Small buds (≥ 0.5 mm, < 2 mm) are mostly used by adults for feeding (Ferguson et al. 2015). Nilsson (1988c) observed that small buds (< 2 mm) contained eggs only at very high beetle densities. Oviposition into these small buds very often resulted in bud abortion. Damage caused by oviposition into buds of 2-3 mm length usually had no effect on development of these buds into flowers as larvae develop inside the buds until they start flowering. Damage by oviposition may occur when a high number of eggs are laid per bud and the hatching larvae destroy the anthers (Nilsson 1988a, Hervé 2014).

The number of eggs laid depends on temperature, humidity and nutrition of beetles (Fritzsche 1957). The amount of food consumed by females is more important than the nutritional quality of the pollen (Hervé 2014). Hopkins & Ekbom (1999) showed that females of *M. aeneus* can adapt oviposition rate to available oviposition resources. If there is no favourable plant found, eggs are retained and production of new eggs is reduced or stopped. During the most active period of oviposition females laid on average 4.5-6.5 eggs per day (Nilsson 1988c). Under favourable weather conditions females can produce 200-300 eggs during their lifetime (Nilsson 1988c). Multiple oviposition by several females into one bud is not uncommon (Burkhardt & von Lengerken 1920, Nilsson 1988c). First instar larvae feed on pollen within buds while second instar larvae feed in several open flowers. Mature larvae drop down to the soil for pupation. Emerging new generation beetles feed on pollen of different plant families before they seek their hibernation sites (Müller 1941).

A high intensity of insecticide application is used to prevent damage by pollen beetles in Germany (Freier et al. 2015, Roßberg 2016). However, chemical control is biased because of pyrethroid resistance all over Europe and a limited choice of registered insecticides with different modes of action (Slater et al. 2011). Among other insecticides the systemic neonicotinoid Biscaya (a.i. thiacloprid, 72 g ha^{-1}) and the non-systemic pyrethroids Mavrik (type I pyrethroid, tau-fluvalinate, 48 g ha^{-1}) and Karate Zeon (type II pyrethroid, lambda-cyhalothrin, 7.5 g ha^{-1}) are available for control of pollen beetles. These insecticides are classified as contact and stomach poison. Thiacloprid acts as agonist on the nicotinic acetylcholine receptor of insects (Elbert et al. 2008) whereas tau-fluvalinate and lambda-cyhalothrin are modulators of sodium channels and interfere with nerve conduction (Roberts et al. 1999). To achieve a long-lasting reduction of pollen beetle populations, insecticides are required that not only have direct lethal effects on overwintered pollen beetles to avoid yield losses by bud feeding, but also reduce the reproduction of the pest resulting in a lower infestation pressure in following years.

In the present study, effects of the insecticides Biscaya, Mavrik and Karate Zeon applied at the bud stage on infestation of buds with eggs and larvae of pollen beetles were investigated in winter oilseed rape field trials. In additional greenhouse experiments using untreated and Biscaya- or Mavrik-treated plants and pollen beetles, all collected from insecticide-treated and untreated plots of the field trials, effects on bud infestation were studied in more detail. Under controlled conditions it was investigated whether bud infestation was influenced not only by reduction of overwintered pollen beetles but also by insecticidal residuals on treated plants causing repellency or direct sublethal effects on females.

Materials and Methods

Field trials:

The effects of different insecticides on bud infestation by pollen beetles were studied from 2013 to 2015 in field trials, established in a randomized block design at the trial sites of the Julius Kühn-Institut in the region of Braunschweig (52°32'80.29''N, 10°63'16.74''E (Wendhausen 2013), 52°21'72.27''N, 10°62'72.30''E (Sickte 2014), 52°21'00.81''N, 10°67'36.03''E (Lucklum 2015); according to WGS 84). In 2013 and 2014 the winter oilseed rape cultivar “Visby” was used for the experiments, “Avatar” was established in 2015. To characterize the growth stages of winter oilseed rape the BBCH code of Lancashire et al. (1991) was used. Insecticide applications were carried out at the green bud stage at BBCH 53 on 23 April 2013 and at BBCH 55 on 31 March 2014 and on 16 April 2015 using recommended product dose rates in 300 l water ha^{-1}. The neonicotinoid Biscaya (a.i. thiacloprid, 72 g ha^{-1}) was tested in all experimental years while the pyrethroid Karate Zeon (lambda-cyhalothrin, 7.5 g ha^{-1}) was tested only in 2013 and replaced by the pyrethroid Mavrik (tau-fluvalinate, 48 g ha^{-1}) in 2014 and 2015. Each treatment was established with four replicated plots measuring 720 to 1080 m^2.

According to EPPO Standard PP 1/178 (3) (EPPO 2005) the abundance of overwintered pollen beetles in the field trials was assessed directly before and one, three, seven and 13 to 15 days after application, between 9 and 11 a.m. The number of pollen beetles was recorded by beating 50 randomly selected main stems per plot over a plastic tray (31.5 cm x 25.5 cm).

In all experimental years subsamples of ten plants per plot were randomly collected from untreated and from insecticide-treated plots one to two days after application, seven to nine days after application and 14 days after application in 2014 and 2015 (in 2014 at 14 days after treatment only five plants per plot were collected) (Tab. 1). Furthermore in 2014 and 2015 in all insecticide-treated plots 15 randomly selected plants per plot were marked with a small tape and stick for a later recovery. The plants were enclosed by using a plastic bag shortly before application to protect them from contamination by the insecticide spray. This was done to investigate whether effects on bud infestation were caused by direct lethal effects on overwintered pollen beetles or by insecticidal residuals on plants causing repellency or sublethal effects. The plastic bag was removed immediately after application, leaving a covering period of about three hours.

At one to two, seven to nine and 14 days after application, five plants per plot previously covered during the application were collected in Biscaya- and Mavrik-treated plots, respectively. These plant samples were stored for further investigations in a dark climate chamber at 4°C to prevent further development of eggs and larvae. All buds of the main stem and two side shoots of these plants having a bud size larger than 2 mm were counted and the infested buds assessed. Buds showing characteristic oviposition holes (Börner & Blunck 1919, Burkhardt & von Lengerken 1920, Ekbom & Borg 1996) were dissected individually under the binocular microscope (ten- to twelvefold magnification) to record numbers of eggs and first instar larvae per bud in the different treatments.

Tab. 1: Date and number of plant sample collected at different growth stages (BBCH) and days after application (DAA) in field trials in Wendhausen 2013, Sickte 2014 and Lucklum 2015

Year and date of application	Date of sampling	BBCH	DAA	Number of plants analysed per treatment	
				uncovered plants	plants covered during application
2013	25 April	53-55	2	40	-
23 April	2 May	60	9	40	-
2014	2 April	55	2	40	20
31 March	9 April	58	9	40	20
	14 April	60-62	14	20	20
2015	17 April	55	1	40	20
16 April	23 April	58-60	7	40	20
	30 April	62	14	40	20

Greenhouse experiments:

To investigate whether effects of insecticides on bud infestation with eggs and larvae of pollen beetles are caused by lethal effects on overwintered pollen beetles or by repellent or sublethal effects of insecticides also greenhouse experiments were carried out in all experimental years. Pollen beetles and plants used in these experiments were collected from untreated and insecticide-treated plots of the field trials. Pollen beetles were randomly collected approximately 30 minutes after application in the plots treated with Biscaya in 2013 and with Biscaya and Mavrik, respectively, in 2014 and 2015 by beating randomly selected plants over a plastic funnel ending in a perforated plastic bag. Untreated pollen beetles were collected in untreated areas of the field. The beetles were stored for one to three days in perforated plastic bags in a climate chamber at 10°C and 16:8-hours L:D. They were provided with untreated oilseed rape inflorescences and water. Immediately before the beginning of the experiments the beetles were released in a plastic box in the climate chamber and healthy beetles were selected with a mini vacuum cleaner and caged in glass vials. In each vial 10 beetles were caged. Beetles that were not able of co-ordinated movements were not selected for the experiments.

One to two days after treatment untreated plants (BBCH 55) were randomly selected in the control plots as well as insecticide-treated plants from plots treated with the respective insecticide. The plants were cut at the basis and bundled per plot. Until the start of the experiments they were stored in the dark in a climate chamber at 4°C in buckets with water. Immediately before the beginning of the experiments superfluous side shoots were removed; only the main stem and two side shoots with inflorescences were left. The plants were placed in Erlenmeyer flasks of 500 ml content filled with water. One to three days after the plants were treated with insecticides in the field trials ten pollen beetles were caged per plant using perforated plastic bags (Tab. 2). Based on randomly taken samples of beetles and using the method of Ruther & Thiemann (1997), the sex ratio was estimated to be 1:1. This sex ratio was also found by Free & Willams (1979) and Nilsson (1988b). Different combinations of insecticide-treated and untreated pollen beetles and insecticide-treated and untreated plants were tested with ten replicates each (Tab. 3). The plants were randomly mixed and placed in the greenhouse chamber at a temperature regime of 14 hours at 15°C and 10 hours at 12°C over night, without artificial lighting. After three to four days of exposure pollen beetles were removed from all cages, the vitality was assessed and classified as healthy or affected/ dead. Buds were dissected as described above.

Tab. 2: Date of sampling pollen beetles and plants in field trials in Wendhausen 2013, Sickte 2014 and Lucklum 2015 after treatment, release of pollen beetles into cages fixed on the plants in the greenhouse and end of exposure; DAA= days after application

Year	Date of sampling		Pollen beetle	End of
	pollen beetles	plants	release to cages	exposure
2013	23 April (= date of application)	25 April	25 April (2 DAA)	29 April
2014	31 March (= date of application)	2 April	3 April (3 DAA)	7 April
2015	16 April (= date of application)	17 April	17 April (1 DAA)	20 April

Tab. 3: Combinations of insecticide-treated and untreated pollen beetles and plants sampled in the field trials with 10 replicates each in greenhouse experiments in 2013 to 2015

Treatments
Untreated plants without pollen beetles (pre-infestation in the field)
Untreated plants with untreated pollen beetles (control)
Untreated plants with Mavrik-treated pollen beetles
Untreated plants with Biscaya-treated pollen beetles
Biscaya-treated plants with untreated pollen beetles
Biscaya-treated plants with Biscaya-treated pollen beetles
Mavrik-treated plants with untreated pollen beetles
Mavrik-treated plants with Mavrik-treated pollen beetles

Statistical analyses

For statistical analyses the software R, version 3.1.2 (R Core Team 2014; packages: *lme4* (Bates et al. 2015), *multcomp* (Hothorn et al. 2008), *effects* (Fox 2003), *glmmADMB* (Fournier et al. 2012)) was used. Data of each year were analysed separately, because of high variability in infestation levels, weather conditions and field trial locations. Selection of the optimal model was made by using Akaike Information Criteria (AIC) described in Zuur et al. (2009). To analyse differences in the percentage of buds containing eggs and L1-larvae Generalized Linear Mixed Models (GLMM, binomial for proportion data) were used for field trials and greenhouse experiments. As main effects the treatment and the date were included into the model and their interaction was tested. The replicates were integrated as random effect. The model was checked for dispersion. Because of overdispersion an observation-level random intercept (i.e. a unique ID for each plant) was included in the model. The treatments were compared pairwise for each day of assessment with the package *lsmeans* (Lenth 2015) for post-hoc testing. P values were adjusted with the Hochberg method (Blakesley et al. 2009).

The effects of insecticide application on the number of eggs and L1-larvae per infested bud were analysed by using GLMM (truncnbinom for count data without zero values) and treatments compared pairwise as described above. The given average number of eggs and L1-larvae per infested bud on the main stem in the different treatments in the greenhouse experiments did not include the pre-infestation of the plants. To analyse whether treatment of plants or treatment of pollen beetles with insecticides affected mortality of the beetles in the greenhouse experiments also GLMM (poisson for count data) were used. The mortality of untreated pollen beetles caged on untreated plants was compared with the mortality of Biscaya-/ Mavrik-treated beetles also caged on untreated plants. In addition the mortality of untreated and insecticide-treated beetles each caged on insecticide-treated plants was compared. The pairwise comparisons were conducted as described above.

To compare the number of overwintered pollen beetles per m^2 in insecticide-treated and untreated plots of the field trials cumulative insect-days were calculated up to 15 days after treatment at BBCH 53/55 according to Ruppel (1983). Differences were analysed by using the non-parametric Kruskal-Wallis test with following Post-hoc Wilcoxon-Mann-Whitney test with Benjamini and Hochberg correction.

Results

Field trials:

Overwintered pollen beetles:

In 2013 the cumulative insect-days of Biscaya-treated plots were lower compared to the control and the Karate Zeon-treated plots but the treatments did not differ significantly (Kruskal-Wallis test, $p > 0.05$) (Tab. 4). But the assessment of the abundance of overwintered pollen beetles in the field trial of 2013 showed that the density of beetles was significantly reduced by 47% in Biscaya-treated plots at one day after application at BBCH 53 compared to untreated plots (chapter II). Treatment of Karate Zeon did not differ to untreated plots. On the following days increasing temperatures favoured migration of new pollen beetles into the field. At three and seven days after application the lowest number of pollen beetles was found again in Biscaya-treated plots but there was no significant difference between all treatments.

The results of 2014 and 2015 showed that treatments with Biscaya and Mavrik at BBCH 55 significantly reduced cumulative insect-days of pollen beetle compared to the control, with Mavrik showing a slightly higher effectiveness than Biscaya (Tab. 4). In 2014 and 2015 abundance of overwintered pollen beetles was lower than in 2013. In 2014 application of Biscaya and Mavrik at BBCH 55 resulted in a significantly reduced pollen beetle density for at least three days (chapter II). At one day after application number of pollen beetles was reduced by application of Biscaya and Mavrik by 68% and 82%, respectively. At three days after application the beetle density was reduced by ca. 50%, respectively. At the following assessments at seven and 15 days after application no significant differences between the treatments in the number of pollen beetles were observed. In the field trials of 2015 similar results were found. At one day after application pollen beetle density was reduced in Biscaya- and Mavrik-treated plots by 70% and 96%, respectively. Beetle density was significantly reduced in Biscaya- and Mavrik-treated plots for at least seven days after application, when numbers of beetles were reduced by 76% and 57%, respectively.

Tab. 4: Cumulative insect-days (± SE) of overwintered pollen beetles in insecticide-treated and untreated plots in field trials in Wendhausen 2013, Sickte 2014 and Lucklum 2015 up to 15 days after application at BBCH 53/55. Different letters within each year indicate significant differences between treatments, Kruskal-Wallis test with following Wilcoxon-Mann-Whitney test, p ≤ 0.05

Year	Days after application	Treatment	Cumulative insect-days ± SE
2013	0-13	Control	1929.3 ± 218.7 A
		Biscaya	1729.2 ± 219.3 A
		Karate Zeon	1931.1 ± 131.4 A
2014	0-15	Control	421.5 ± 43.6 A
		Biscaya	276.4 ± 28.4 B
		Mavrik	266.7 ± 28.9 B
2015	0-14	Control	392.8 ± 6.4 A
		Biscaya	171.0 ± 20.8 B
		Mavrik	153.7 ± 7.8 B

Bud infestation with eggs and larvae of pollen beetle:

The percentage of buds containing eggs and L1-larvae on the main stem varied between experimental years depending on the level of infestation by overwintered pollen beetles. In 2013, two days after treatment, 21.2% of the buds on main stems in the untreated plots were infested with eggs (Fig. 1) whereas two days after treatment in 2014 and one day after treatment in 2015 0.7% and 8.2% of buds were infested, respectively (Fig. 2, 3). In all experimental years the percentage of infested buds did not differ significantly between all treatments one to two days after treatment (GLMM, $p > 0.05$); the only exception was a significant difference in 2013 between Biscaya and Karate Zeon-treated plots (GLMM, $p = 0.0406$).

In 2013 and 2014, nine days after insecticide treatment the lowest percentage of infested buds was counted in Biscaya-treated plots. Bud infestation in these plots was significantly reduced by 57% and 80% compared to the untreated plots (GLMM, 2013: $p < 0.001$; 2014: $p = 0.0004$). In 2013 Karate Zeon had no significant effect on bud infestation compared to the untreated plots (GLMM, $p > 0.05$). In 2014, nine days after application of Mavrik bud infestation was significantly reduced by 65% compared to the control (GLMM, $p = 0.0433$). In 2015, seven days after treatment no significant differences between all treatments were observed (GLMM, $p > 0.05$). In 2014 and 2015, a significant effect of Biscaya on bud infestation was observed up to 14 days after treatment compared to the control (GLMM, 2014: $p = 0.0436$; 2015: $p < 0.0001$). Dissection of buds 14 days after treatment showed that bud infestation with eggs and larvae in Biscaya-treated plots was reduced by 86% and 82% and in Mavrik-treated plots by 51% and 71%, respectively, compared to the untreated plots (for Mavrik: GLMM, 2014: $p = 0.5694$; 2015: $p < 0.0001$).

In 2014 plants in Biscaya-treated plots, covered during insecticide application, showed nine and 14 days after treatment a significant higher infestation of buds on the main stem compared to Biscaya exposed plants (GLMM, 9 days after application: p = 0.0160; 14 days after application: p = 0.0215). Bud infestation of the covered plants did not differ significantly to the untreated control (GLMM, p > 0.05) (Fig. 2). The covered plants in Mavrik-treated plots showed nine and 14 days after treatment also a higher but not significantly different infestation rate compared to Mavrik-treated plants and to the control (GLMM, p > 0.05). In 2015 14 days after treatment the covered plants showed also a higher percentage of buds containing eggs and L1-larvae compared to the treated plants for both insecticides but differences between treated and covered plants were not significant (GLMM, p > 0.05), respectively (Fig. 3).

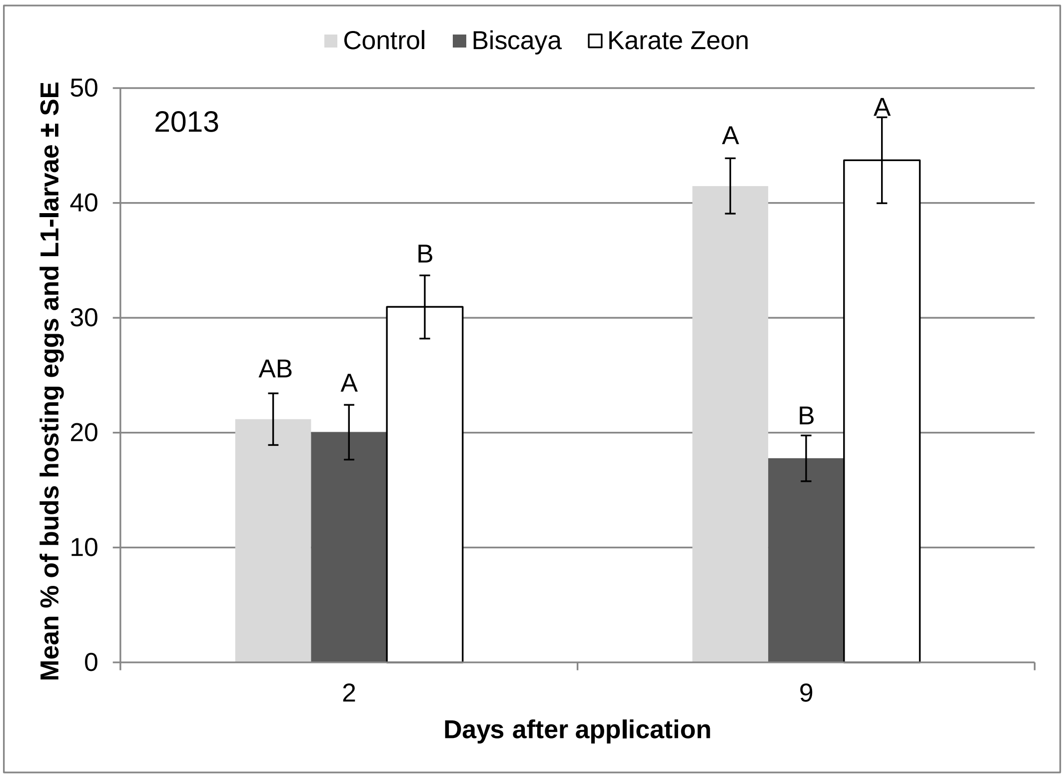

Fig. 1: Mean percentage (± SE) of buds on the main stem hosting eggs and L1-larvae in different treatments 2 days after application (25 April) at BBCH 53 and 9 days after application (2 May) in a field trial in Wendhausen 2013; 40 plants per treatment and sampling date. Different letters within each day indicate significant differences between treatments, GLMM, p ≤ 0.05

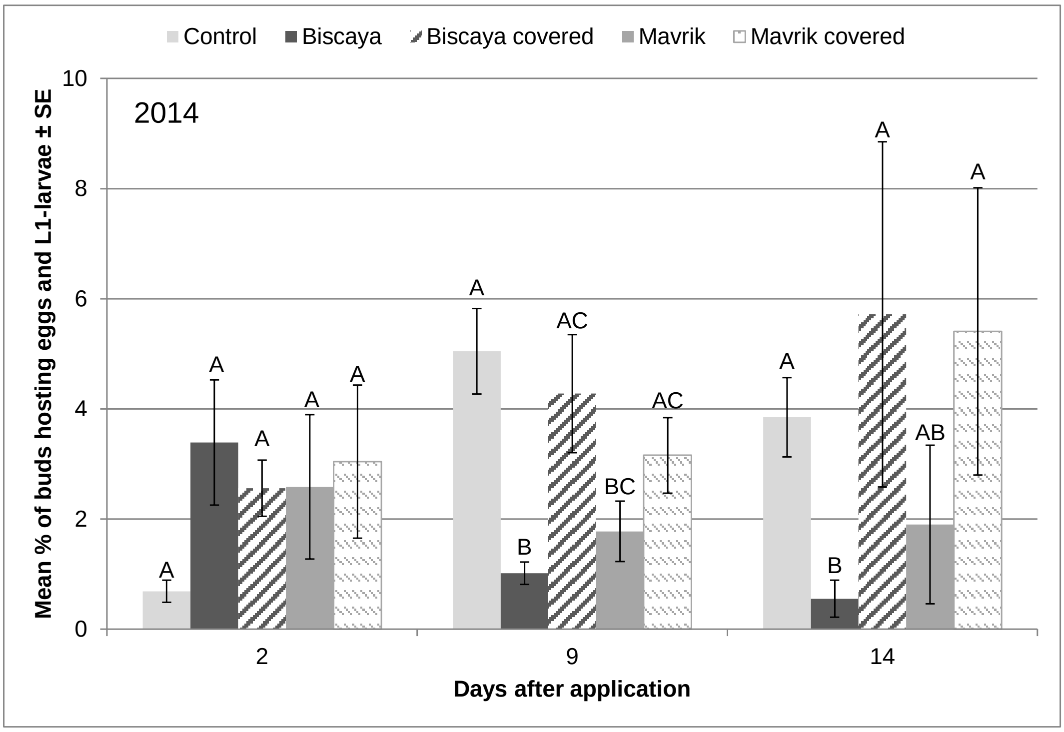

Fig. 2: Mean percentage (± SE) of buds on the main stem hosting eggs and L1-larvae in different treatments 2 days after application (2 April) at BBCH 55, 9 days after application (9 April) and 14 days after application (14 April) in a field trial in Sickte 2014; 2 and 9 days after application 40 plants per treatment and sampling date, 14 days after application 20 plants, 2-14 days after application 20 plants covered during application. Different letters within each day indicate significant differences between treatments, GLMM, $p \le 0.05$

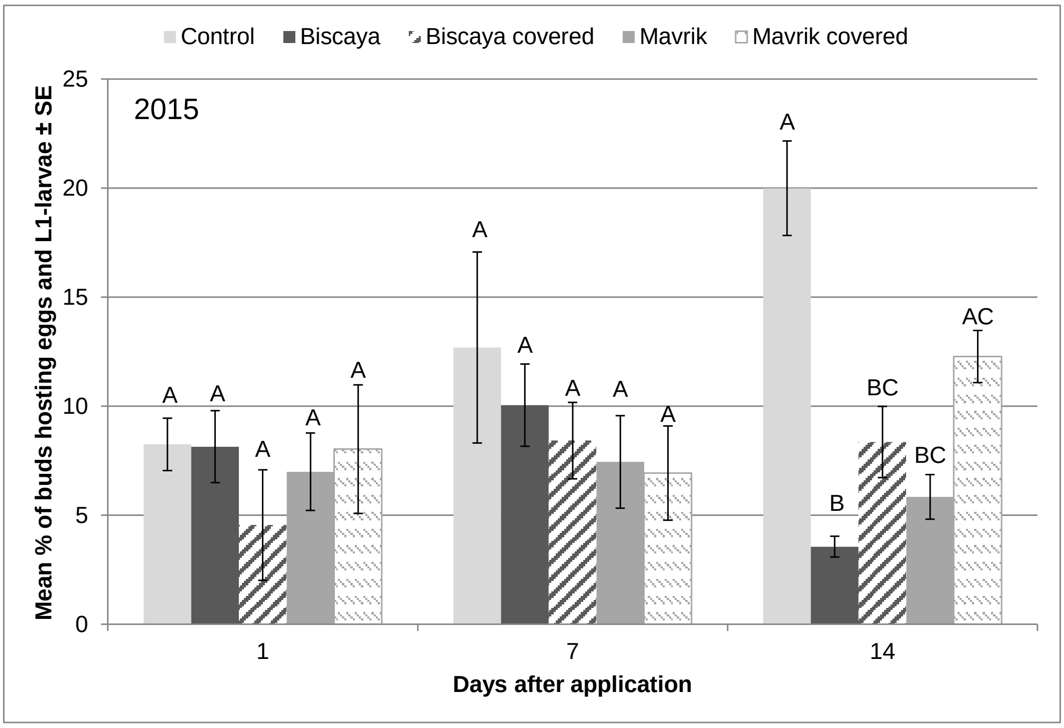

Fig. 3: Mean percentage (± SE) of buds on the main stem hosting eggs and L1-larvae in different treatments 1 day after application (17 April) at BBCH 55, 7 days after application (23 April) and 14 days after application (30 April) in a field trial in Lucklum 2015; 40 plants per treatment and sampling date, 20 plants covered during application. Different letters within each day indicate significant differences between treatments, GLMM, p ≤ 0.05

In all experimental years the number of buds containing eggs and L1-larvae was also recorded on two side shoots. The differences of infested buds on the main stems between different treatments were also obvious on the side shoots. Reduction of percentage of infested buds of insecticide-treated plants was almost in all cases higher compared to the main stem. In 2013 and 2014, nine days after treatment bud infestation in Biscaya-treated plots was significantly reduced by 82% and 87% compared to the untreated plots (Tab. 5). In 2015, seven days after treatment bud infestation in Biscaya-treated plots was reduced by 44% but no significant difference to the untreated plots was noticed. In 2014 and 2015, nine and seven days after treatment bud infestation in Mavrik-treated plots was reduced by 37% and 54%, respectively, compared to the control. In 2014 and 2015, analysis of buds 14 days after treatment resulted in a significant reduction by 93% and 90% in Biscaya-treated plots and 94% and 77% in Mavrik-treated plots.

In 2014, nine and 14 days after treatment the covered plants in Biscaya-treated plots showed a higher infestation rate on the side shoots compared to Biscaya exposed plants as seen for the main stem. Nine days after treatment the infestation rate of covered plants did not differ significantly to Biscaya-treated plants and the control (GLMM, p > 0.05), whereas 14 days after treatment significantly more buds were infested compared to Biscaya exposed plants (GLMM, p = 0.0446) (Tab. 5). In 2014 the covered plants in Mavrik-treated plots showed nine and 14 days after treatment also a higher but not significantly different infestation rate compared to Mavrik-treated plants and to the control

(GLMM, p > 0.05). In 2015 seven days after treatment covered plants did not differ significantly from insecticide-treated plants (GLMM, p > 0.05). In 2015 14 days after treatment the covered plants showed a significantly higher percentage of buds containing eggs and L1-larvae compared to the treated plants for both insecticides(GLMM, Biscaya: p = 0.0220; Mavrik: p = 0.0056).

Tab. 5: Mean percentage (± SE) of buds on two side shoots hosting eggs and L1-larvae in different treatments 1-2 days after application (DAA) at BBCH 53 in field trials in Wendhausen 2013 and at BBCH 55 in Sickte 2014 and Lucklum 2015, 7-9 DAA and 14 DAA; 40 plants per treatment and sampling date (exception 14 DAA in 2014: 20 plants), 20 plants covered during application. Different letters within each column and year indicate significant differences between treatments, GLMM, p ≤ 0.05

Year/ BBCH	Treatment	1-2 DAA mean % infested buds ± SE		7-9 DAA mean % infested buds ± SE		14 DAA mean % infested buds ± SE	
2013	Control	**6.59** ± 0.85	AB	**39.94** ± 3.60	A		
53	Biscaya	**3.21** ± 0.54	A	**7.24** ± 1.70	B		
	Karate Zeon	**11.10** ± 1.52	B	**28.63** ± 3.53	A		
2014	Control	**0.76** ± 0.53	AB	**1.53** ± 0.47	A	**1.58** ± 0.78	AB
55	Biscaya	**0.60** ± 0.36	AB	**0.21** ± 0.07	B	**0.12** ± 0.11	B
	Biscaya-covered	**0.57** ± 0.20	AB	**1.28** ± 0.49	AB	**3.17** ± 0.92	A
	Mavrik	**0.18** ± 0.11	A	**0.96** ± 0.51	AB	**0.10** ± 0.09	B
	Mavrik-covered	**1.95** ± 0.91	B	**1.09** ± 0.15	AB	**2.32** ± 0.68	AB
2015	Control	**9.50** ± 1.16	A	**12.16** ± 2.90	A	**14.48** ± 2.67	A
55	Biscaya	**7.08** ± 1.76	AB	**6.86** ± 2.16	AB	**1.51** ± 0.13	B
	Biscaya-covered	**2.86** ± 1.34	B	**4.07** ± 0.49	B	**5.06** ± 0.92	CD
	Mavrik	**5.16** ± 0.78	AB	**5.60** ± 1.90	B	**3.30** ± 0.94	BC
	Mavrik-covered	**7.48** ± 3.75	AB	**5.80** ± 0.49	AB	**10.12** ± 2.42	AD

In all experimental years the number of eggs and L1-larvae per infested bud on the main stem increased from sampling date to sampling date but did not differ significantly between treatments. In all three years one to two days after treatment only eggs were found in buds. The mean number of eggs per infested bud was 1.7 in 2013, 1.6 in 2014 and 1.5 in 2015. Seven to nine days after treatment number of eggs and L1-larvae per infested bud increased to 1.9 in 2013 and 2014 and 1.6 in 2015. 14 days after treatment number of eggs and L1-larvae per infested bud was 1.9 in 2014 and 1.7 in 2015. The mean average of eggs and L1-larvae per infested bud on two side shoots increased in 2013 from 1.5 eggs two days after treatment to 1.9 eggs and L1-larvae per infested bud nine days after treatment. In 2014 the number of eggs and L1-larvae per infested bud remained stable at 2.1. In the field trials of 2015 the mean average of eggs and L1-larvae per infested bud increased from 1.5 two days after treatment to 1.8 14 days after treatment.

Greenhouse experiments:

In all experimental years the mean percentage of buds containing eggs and L1-larvae on the main stem was not significantly different between the control (untreated plants with untreated pollen beetles) and untreated plants and treated pollen beetles for both insecticides in all years (GLMM, $p > 0.05$) (Fig. 4-6). The lowest percentage of infested buds was recorded on Biscaya-treated plants. Especially the combination of untreated pollen beetles and Biscaya-treated plants resulted in a significantly reduced bud infestation compared to the control. This combination differed not significantly from the pre-infestation in all years. Mean percentage of buds containing eggs and L1-larvae on Mavrik-treated plants differed not significantly from the control independent of pollen beetles were Mavrik-treated or not. The differences of infested buds on the main stems between different treatments were also obvious on the side shoots in all years (data not presented).

In all years the mean number of eggs and L1-larvae per infested bud on the main stem was 2.1 and did not differ significantly between treatments. Treatment of pollen beetles with Mavrik or Biscaya in the field had no significant effect on beetle mortality in the greenhouse experiments in all experimental years similar to the treatment of plants with Mavrik. In contrast the treatment of plants with Biscaya caused a significant reduction of pollen beetle vitality compared to the control when beetles were caged on plants already one or two days after plant treatment. In 2013 for pollen beetles caged on untreated plants mortality was up to 3% whereas beetles caged on Biscaya-treated plants two days after plant treatment in the field showed mortality up to 14%. In 2015 pollen beetles were caged on the plants already one day after plant treatment. Mortality on untreated plants was 1% whereas treatment of the plants with Biscaya the day before resulted in up to 41% beetle mortality. In 2014, three days after plant treatment in the field no significant effect of plant treatment with Biscaya on mortality of beetles was observed.

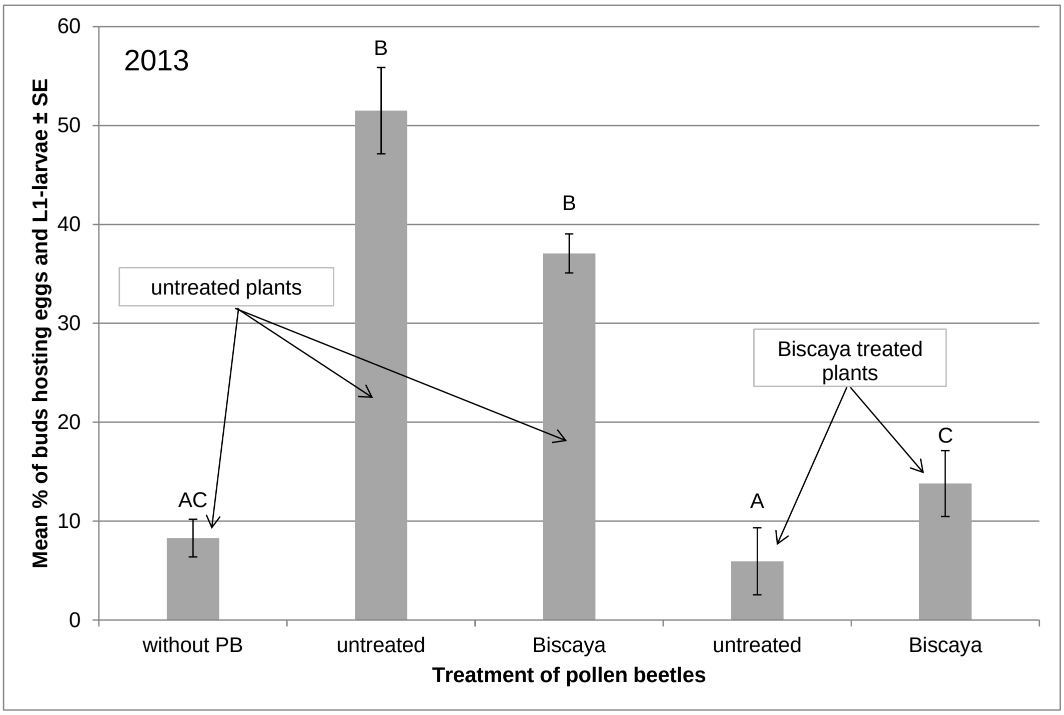

Fig. 4: Mean percentage (± SE) of buds on the main stem hosting eggs and L1-larvae in different combinations of field collected insecticide-treated and untreated plants and pollen beetles exposed in greenhouse experiments in 2013; begin of experiment 2 days after application at BBCH 53, exposure time 4 days; 10 replicates per combination. Different letters indicate significant differences between treatments, GLMM, $p \leq 0.05$

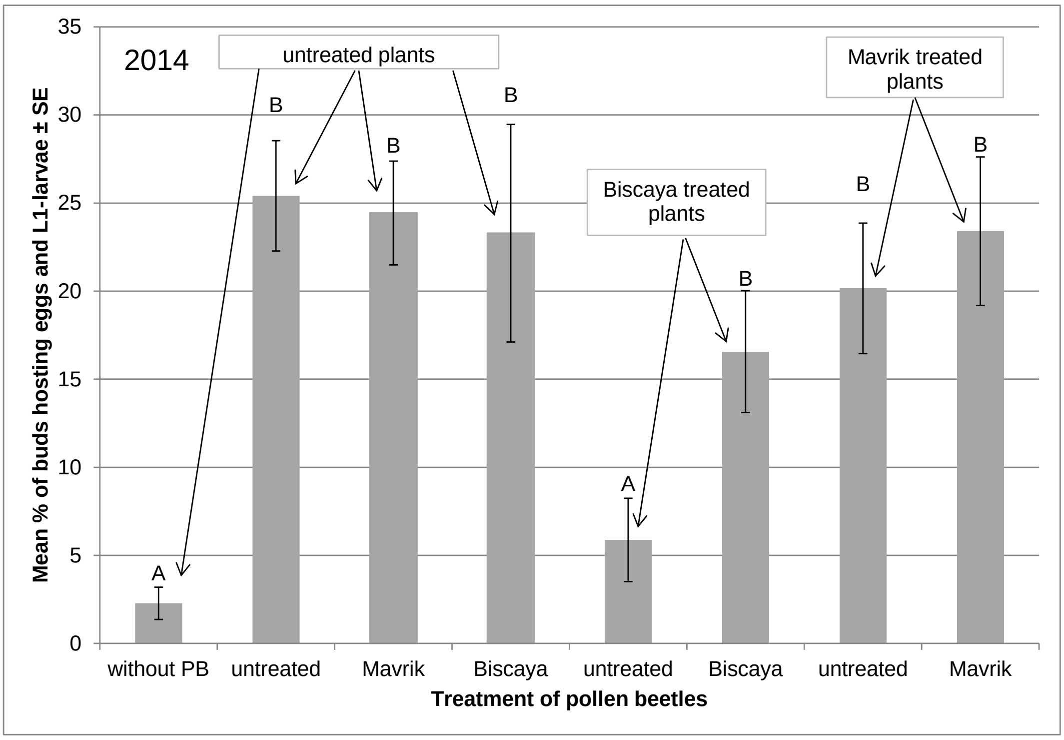

Fig. 5: Mean percentage (± SE) of buds on the main stem hosting eggs and L1-larvae in different combinations of field collected insecticide-treated and untreated plants and pollen beetles exposed in greenhouse experiments in 2014; begin of experiment 3 days after application at BBCH 55, exposure time 4 days; 10 replicates per combination. Different letters indicate significant differences between treatments, GLMM, $p \leq 0.05$

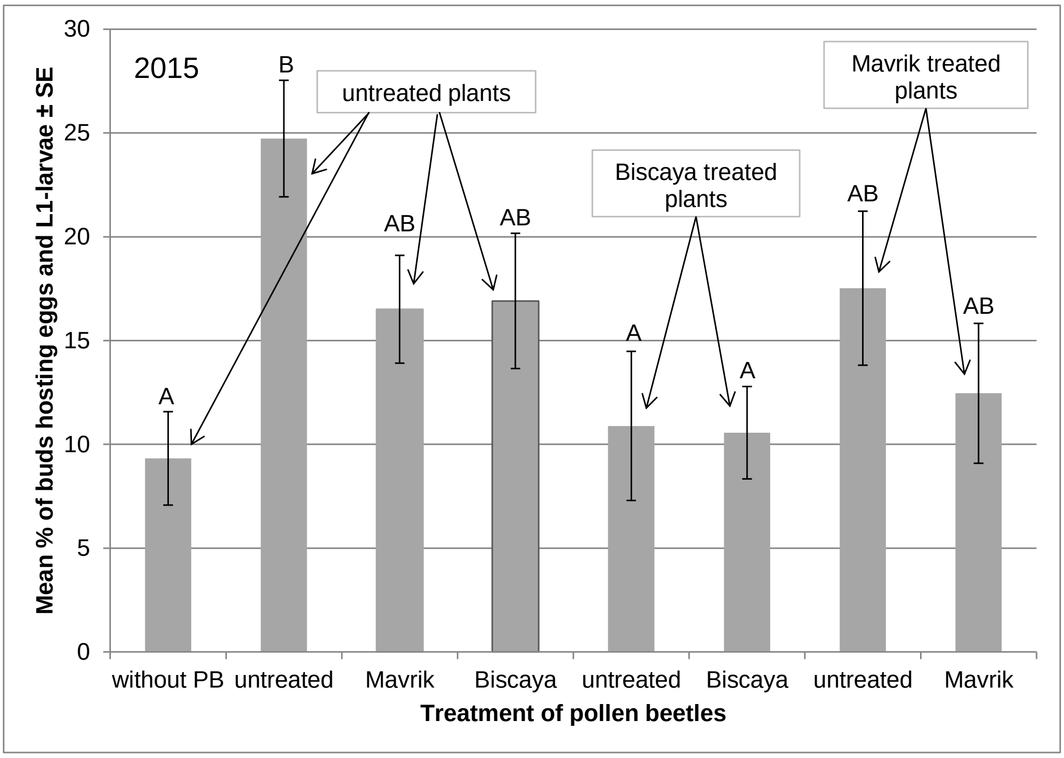

Fig. 6: Mean percentage (± SE) of buds on the main stem hosting eggs and L1-larvae in different combinations of field collected insecticide-treated and untreated plants and pollen beetles exposed in greenhouse experiments in 2015; begin of experiment 1 day after application at BBCH 55, exposure time 3 days; 10 replicates per combination. Different letters indicate significant differences between treatments, GLMM, $p \leq 0.05$

Discussion

In the field trials the treatments with Biscaya and Mavrik resulted in most cases significantly lower percentages of infested buds with eggs and larvae up to 14 days after treatment, whereas Karate Zeon had no effect. While in 2013 and 2014 significant effects of Biscaya and Mavrik (in 2014) were already visible nine days after treatment, significant effects of insecticides in 2015 were only found at 14 days after treatment. This may be explained by lower temperatures prevailing after application in 2015. Mean temperature during the first seven days after application in 2013, 2014 and 2015 was 13.0°C, 14.0°C and 10.5°C, respectively. According to Fritzsche (1957) oviposition depends among other things on temperature and occurs only from 15°C upwards. Favourable conditions for egg laying are temperatures exceeding 20°C (Fritzsche 1957). Also Ferguson et al. (2015) found that number of buds containing eggs and the total number of eggs laid increased with temperature. Temperatures exceeding 15°C were recorded during the first seven days after application in the field trials of 2013, 2014 and 2015 over 60, 64 and 33 hours.

In the present field trials lower bud infestation with eggs and larvae of pollen beetle in Biscaya- and Mavrik-treated plots was observed. This might primarily result from a lower density of overwintered pollen beetles after insecticide treatment. Calculation of cumulative insect-days showed that treatments of Biscaya and Mavrik in 2014 and 2015 significantly reduced pollen beetle density, with Mavrik showing a slightly higher effectiveness than Biscaya. In 2014 and 2015 number of pollen beetles was significantly reduced in insecticide treated plots for at least three and seven days after application, respectively (chapter II). Although the effectiveness of Mavrik in pollen beetle reduction was higher, the bud infestation with eggs and larvae was more reduced in Biscaya-treated plots.

In the trial of 2013 the cumulative insect-days of Biscaya- and Karate Zeon-treated plots did not differ to the control but at nine days after application bud infestation was significantly reduced by 57% in Biscaya-treated plots compared to the control in contrast to Karate Zeon-treated plots. The lower efficacy of Biscaya on overwintered pollen beetles in 2013 might be explained by migration of new pollen beetles into the field two to three days after application (chapter II). Beetles that migrated after application were not exposed to insecticides by direct contact with the spray mist and might not be killed immediately. But contact to insecticidal residuals on Biscaya-treated plants might have had indirect effects on beetles resulting in reduced bud infestation even though the number of beetles was only reduced for a short time.

The fact that Biscaya showed a high effectiveness in reducing bud infestation despite moderate reduction of pollen beetles in 2013 and lower effectiveness than Mavrik in 2014 and 2015 indicates that not only direct lethal effects on overwintered pollen beetles contribute to reduced bud infestation in Biscaya-treated plots. A reduced infestation of buds with pollen beetle eggs and L1-larvae following application of Biscaya was also observed by Tölle (2014). Application of Karate Zeon in the field trials of 2013 had no effects neither on pollen beetle density nor on bud infestation. Low efficacy of Karate Zeon on overwintered pollen beetles was also observed in field trials of Schröder et al. (2009) and can be explained by widely distributed pyrethroid resistance of pollen beetles (Slater et al. 2011). Differences in the susceptibility of pollen beetles to different active substances of pyrethroids (Heimbach & Müller 2013) may explain the different results of Karate Zeon and Mavrik.

The low pollen beetle density in Biscaya- and Mavrik-treated plots may be explained by direct lethal effects on beetles or repellent effects of insecticides. The residence time of parasitoids of pollen beetle was reduced on buds treated with thiacloprid and tau-fluvalinate, indicating a repellent effect, whereas for lambda-cyhalothrin no repellent effect was observed (Neumann 2010). Longley & Jepson (1996) recorded a repellent effect of the pyrethroid deltamethrin on aphid parasitoids of the genus *Aphidius*. Repellency on adults of *Bactericera cockerelli* caused by different insecticides including the active substances thiamethoxam, bifenthrin, cyfluthrin + imidacloprid, dinotefuran and lambda-cyhalothrin was reported by Gharalari et al. (2009). Coudriet et al. (1985) observed reduced oviposition of *Bemisia tabaci* on foliage treated with solutions of neem-seed extract caused by adult repellency. A repellent effect of plants treated with insecticides in the present field trials would explain the higher infestation rate of buds with pollen beetle eggs and larvae covered during application compared to adjacent exposed plants. If only the reduced pollen beetle density was responsible for the low infestation of buds in the treated plots also the covered plants not exposed to the insecticides would have shown reduced bud infestation.

In addition to repellent effects, beetles may be influenced by sublethal effects after contact to insecticidal residuals on treated plants resulting in reduced bud infestation compared to untreated plants. This may explain the differences in bud infestation between Biscaya and Mavrik also seen in the greenhouse experiments where only Biscaya had a strong effect on infestation of buds. Excluding the Biscaya treatment in the greenhouse experiments 2015 out of analysis because of significantly increased beetle mortality, a trend of reduced infestation of buds on plants treated with Biscaya was obvious. In contrast, on Mavrik-treated plants bud infestation was only slightly reduced and differed not significantly to the control. The differences might be caused by additional sublethal effects of thiacloprid on oviposition behaviour. It could be possible, that females in contact to thiacloprid are not able to fully coordinated body movements and are incapable of controlled mouth part activity to bite a hole into the bud and insert their ovipositor for egg laying precisely.

Effects of insecticides on behaviour of insects are known in literature. Desneux et al. (2004) observed impaired orientation and oviposition behaviour of the parasitic wasp *Aphidius ervi* by low doses of lambda-cyhalothrin. Uncoordinated, slow movements of two parasitoid species of pollen beetle (*Phradis interstitialis* and *Tersilochus heterocerus*) in contact with thiacloprid, tau-fluvalinate and lambda-cyhalothrin were described by Neumann (2010). Pollen beetles surviving treatment with acetamiprid showed slow reactions (Wegorek et al. 2009). An influence of thiacloprid on coordination of body movements of pollen beetles might explain the higher number of beetles dropped down from plants treated with Biscaya up to one week after application compared to untreated plots and Mavrik-treated plots in field trials of Gödeke (2016). Teeters et al. (2012) recorded stimulatory effects on locomotor activity of honey bees at low levels of imidacloprid exposure and at high concentrations a reduced activity. After treatment with tau-fluvalinate bees moved significantly less than control bees. The widely distributed pyrethroid resistance of pollen beetles caused mainly by fast degradation of pyrethroids by monoxygenase enzymes (Slater & Nauen 2007, Wegorek et al. 2011) may reduce more or less such effects of pyrethroids on behaviour described for non resistant insects.

Furthermore insecticide treatments may result in reduced egg production by females. Abnormal oviposition behaviour and reduced egg laying of the mustard leaf beetle (*Phaedon cochleariae*) on radish plants treated with sublethal doses of the pyrethroid cypermethrin was reported by Hajjar & Ford (1989). A reduced number of eggs laid by pink bollworms (*Pectinophora gossypiella*) was caused by different synthetic pyrethroids (Bariola 1984). Field-relevant dose rates of different neonicotinoids resulted in a decrease in oviposition of the two-spotted spider mite (*Tetranychus urticae*) (Ako et al. 2004, 2006). Shi et al. (2011) observed a reduced fecundity of the cotton aphid, *Aphis gossypii* after treatment with thiacloprid and other neonicotinoids.

Toxic effects of Biscaya on eggs and larvae of pollen beetle were assumed by Tölle (2014) and might be possible by the systemic transport of thiacloprid in plants reported by Zimmer et al. (2014). In the own experiments toxic effects of thiacloprid taken up into the plant tissue on eggs or larvae are unlikely, because no dead L1-larvae were found during dissection of buds. This is in accordance with Ako et al. (2004) who did not find significant differences in the hatching rate of the eggs of *T. urticae* after applications of different neonicotinoids.

Effects of Biscaya on infestation of buds with eggs are not caused by chemical additives used for the formulation of the product, but by the active substance thiacloprid. This was tested in additional greenhouse experiments with a product formulation of Biscaya containing not the active substance thiacloprid (data not presented). In summary, the reduced infestation of buds with eggs and larvae in Biscaya- and Mavrik-treated plots is not relevant for the damage in the current year. But it is a prerequisite for a significantly lower number of larvae and emerging new generation pollen beetles and may reduce infestation pressure in following crops of oilseed rape.

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Chapter IV

Effects of neonicotinoid and pyrethroid insecticide applications on the parasitism rates of pollen beetle larvae (*Meligethes aeneus* (Fabricius)) by tersilochine parasitoids

Abstract

Larval parasitoids can substantially reduce the population density of the pollen beetle. The most abundant tersilochine parasitoids of pollen beetle (*Meligethes aeneus* (Fabricius), syn. *Brassicogethes aeneus* (Fabricius)) are *Tersilochus heterocerus*, *Phradis interstitialis* and *P. morionellus*. The main activity of these parasitoids was observed in the period shortly before flowering to full flowering. Insecticide applications during this activity phase may have negative effects on parasitoids. In the present study the effects of the insecticides Biscaya (a.i. thiacloprid, 72 g ha^{-1}), Mavrik (tau-fluvalinate, 48 g ha^{-1}) and Karate Zeon (lambda-cyhalothrin, 7.5 g ha^{-1}) applied at different development stages of winter oilseed rape (between green bud stage to full flowering) on parasitization of pollen beetle larvae by *T. heterocerus* were studied in field trials at different locations in Germany in 2013 to 2015.

Parasitism of pollen beetle larvae by *T. heterocerus* was found in all field trials in all experimental years, but mostly not before full flowering. Maximum percentage of parasitized larvae at the different locations ranged between 3.4% to 16.8% in 2013, 8.3% to 22.4% in 2014 and from 11.1% to 29.1% in 2015. Levels of parasitism were not significantly different between the untreated control and insecticide treatments within each location. Applications of Biscaya and Mavrik at the beginning of flowering to full flowering resulted in slightly but not significantly lower parasitism rates of larvae in comparison to applications of these insecticides in the bud stage. The effects of the insecticides on parasitism of pollen beetle larvae by *Phradis* spp. were assessed in field trials of 2015. In contrast to *T. heterocerus*, *Phradis* spp. was not detected at all locations and not before flowering declining. In field trials at Lucklum and Puch the maximum level of parasitism by *Phradis* spp. was 9.4% and 18.3%, respectively. No significant effect of insecticide application on parasitism by *Phradis* spp. was observed between the treatments. The results of this study showed that the insecticides used in the field trials did not affect parasitization of pollen beetle larvae by *T. heterocerus* and *Phradis* spp., regardless whether applied at the bud stage, at the beginning of flowering or full flowering.

Introduction

Several hymenopteran endoparasitoids can attack the egg and larval stages of pollen beetle (*Meligethes aeneus* (Fabricius), syn. *Brassicogethes aeneus* (Fabricius)) and may have significant impact on the population density (Nilsson & Andreasson 1987). Parasitism levels of more than 50% have been reported by Nitzsche (1998), Nilsson (2003) and Ulber et al. (2010a). The most abundant larval parasitoids of pollen beetle are the ichneumonid parasitoids *Phradis interstitialis* (Thomson), *P. morionellus* (Holmgren) and *Tersilochus heterocerus* (Thomson), the latter being often predominant in crops of winter oilseed rape (Nitzsche 1998, Nilsson 2003) and *P. morionellus* in spring oilseed rape (Nilsson 2003). These univoltine species are widely distributed throughout Europe (Nilsson 2003), whereas *T. heterocerus* and *P. interstitialis* predominate in Germany (Ulber et al. 2010a). Also widely distributed throughout northern Europe is the braconid *Diospilus capito*, but it is more common in spring oilseed rape (Nilsson 2003, Veromann et al. 2006).

In Germany *P. interstitialis* colonizes oilseed rape crops from mid of April onwards, whereas *T. heterocerus* and *P. morionellus* commonly occur one to two weeks later at the end of April or beginning of May (Nitzsche 1998, Johnen et al. 2010, Neumann 2010). Females are sexually mature when they emerge from their overwintering sites (Nilsson 2003). The females of *P. interstitialis* lay their eggs through the walls of the bud into the eggs and first instar larvae of pollen beetle (Osborne 1960). The other two species prefer L2-larvae for oviposition, which are numerous in open flowers (Nilsson & Andreasson 1987). The first larval instar of *Phradis* spp. hatches shortly after oviposition (Nilsson 2003), whereas larvae of *T. heterocerus* hatch from the egg only when the host larva is fully developed and ready to drop down to the soil for pupation (Osborne 1960, Nitzsche 1998). The parasitoid larvae complete their development in the soil and kill their hosts before they pupate in the cocoon of their hosts. Approximately one month after pupation the parasitoids develop into adults, which stay in diapause until the next spring (Nilsson 2003, Ulber et al. 2010a).

An important factor influencing parasitoid populations may be the application of insecticides. Adult parasitoids emerging in spring from crops of wheat or barley following oilseed rape can be affected by treatments targeted to control cereal aphids (Nilsson & Andreasson 1987, Jansen & San Martin y Gomez 2014). The main activity of adult tersilochine parasitoids was observed in the period shortly before flowering to full flowering of oilseed rape (Ulber & Nitzsche 2006). In consequence applications of insecticides in this activity phase may have negative effects on parasitoids searching for hosts (Johnen & Ulber 2004). Parasitoids can be exposed to insecticides by direct contact with the spray mist, by contact to insecticidal residues on the leaves while seeking for food or hosts, or by uptake of contaminated food material (Croft & Brown 1975, Longley & Jepson 1996). In addition to lethal effects pesticides may have sublethal effects on natural enemies as for example by influencing host searching behaviour, oviposition or fertility. Further they may have repellent effects (Wright & Verkerk 1995). The aim of the present study was to analyse effects of the insecticides Biscaya (a.i. thiacloprid, 72 g ha^{-1}), Mavrik (tau-fluvalinate, 48 g ha^{-1}) and Karate Zeon (lambda-cyhalothrin, 7.5 g ha^{-1}) applied at different growth stages between the bud stage and full flowering of winter oilseed rape on the parasitism rates of pollen beetle larvae by *T. heterocerus* and *Phradis* spp. in field trials in 2013 to 2015.

Materials and Methods

Effects of the neonicotinoid Biscaya (a.i. thiacloprid, 72 g ha^{-1}) and the pyrethroids Mavrik (tau-fluvalinate, 48 g ha^{-1}) and Karate Zeon (lambda-cyhalothrin, 7.5 g ha^{-1}) on parasitism rates of pollen beetle larvae were studied at various locations in Lower Saxony, Mecklenburg-Western Pomerania and Bavaria in 2013 to 2015 (Tab. 1). The experiments were established in randomized block designs, with four replicated plots of each treatment measuring between 36 to 1080 m^2 each. Different winter oilseed rape cultivars were used for the experiments. To characterize the growth stages of winter oilseed rape the BBCH code of Lancashire et al. (1991) was used. Insecticide applications were conducted at the bud stage or at the flowering stage with recommended product dose rates.

To collect pollen beetle larvae dropping from plants for pupation in soil ten plastic bowls (17 cm x 12.1 cm) per plot filled with a 10% sodium benzoate water solution for conservation were placed in the bud stage in the centre of each plot near the tramline (approximately 1 m distance) on the ground in the field trials in Wendhausen (2013), Sickte (2014) and Lucklum (2015). The plastic bowls were checked regularly and emptied weekly from BBCH 62 until BBCH 80. At all other locations four plastic bowls (18.3 cm x 13.6 cm) per plot were used and also placed in the field. In 2013 the plastic bowls were placed at BBCH 61 (Puch), BBCH 63 (Stöckendrebber) and BBCH 70 (Cramonshagen). In 2014 and 2015 the plastic bowls were placed at all trial sites at the bud stage.

The collected larvae were stored in 70% Ethanol, later separated by their development stage according to Osborne (1964) and counted under the binocular microscope (eight- to tenfold magnification). For determination of parasitism rates by *T. heterocerus* all collected larvae were carefully inspected under the binocular microscope to count the dark brown to black pigmented eggs, which can be easily seen through the cuticle of the host larvae (Osborn 1960). Superparasitism and multiparasitism of larvae were not recorded separately.

In addition, in the field trial in Lucklum 2015 a random subsample of 60 L2-larvae per treatment and date (as long as sufficient larvae were available) were dissected. The larvae were collected during four periods between BBCH 65 to 76. Larvae were fixed with a needle on dark coloured wax within a petri dish and subsequently covered with water containing a detergent. The abdomen and thorax of the larvae were cut off longitudinal by using a needle under a binocular microscope (ten- to twelvefold magnification) to identify the eggs of *T. heterocerus* within the host body. The dissection showed slightly higher parasitism rates by *T. heterocerus* compared to determination of parasitism rates by external inspection. The presented data for parasitism rates of larvae by *T. heterocerus* result on the much easier method by external inspection.

To examine the parasitism rates of pollen beetle larvae by *Phradis* spp. in field trials 2015 the L2-larvae were dissected as described above. The eggs of *Phradis* spp. are colourless and verification of the eggs is difficult (Osborne 1960). Because of this difficulties only the larvae of *Phradis* spp., which are creamy-white with a slightly sclerotized but unpigmented head (Osborn 1960) were recorded. Random samples of 60 L2-larvae per treatment and date (depending on availability) were dissected on two to four sampling periods per location between BBCH 65 to 76. Parasitoid larvae were summarized as *Phradis* spp. because a morphological separation of *P. interstitialis* and *P. morionellus* is only possible by using adults (Ulber, personal communication).

Tab. 1: Locations of field trials in 2013, 2014 and 2015 with insecticide products, product dose rates and respective growth stages (BBCH) at the day of application

Year	Location	Insecticide product (dose rate)	BBCH at application
2013	Wendhausen,	Biscaya (300 ml ha^{-1})	53/60/65$^{\#}$
	Lower Saxony	Karate Zeon (75 ml ha^{-1})	53+60+65$^{\#\#}$
	Stöckendrebber,	Biscaya (300 ml ha^{-1})	55-57
	Lower Saxony	Mavrik (200 ml ha^{-1})	
		Karate Zeon (75 ml ha^{-1})	
	Cramonshagen,	Biscaya (300 ml ha^{-1})	59
	Mecklenburg-Western Pomerania	Karate Zeon (75 ml ha^{-1})	
	Puch,	Biscaya (300 ml ha^{-1})	55-57
	Bavaria	Karate Zeon (75 ml ha^{-1})	
2014	Sickte,	Biscaya (300 ml ha^{-1})	55/62$^{\#}$
	Lower Saxony	Mavrik (200 ml ha^{-1})	
	Stöckendrebber,	Biscaya (300 ml ha^{-1})	53
	Lower Saxony		
	Kleefeld,	Biscaya (300 ml ha^{-1})	55
	Mecklenburg-Western Pomerania	Mavrik (200 ml ha^{-1})	
	Puch,	Biscaya (300 ml ha^{-1})	55
	Bavaria	Karate Zeon (75 ml ha^{-1})	
2015	Lucklum,	Biscaya (300 ml ha^{-1})	55/62$^{\#}$
	Lower Saxony	Mavrik (200 ml ha^{-1})	
	Niedernstöcken,	Biscaya (300 ml ha^{-1})	56
	Lower Saxony		
	Kleefeld,	Biscaya (300 ml ha^{-1})	57-59
	Mecklenburg-Western Pomerania	Mavrik (200 ml ha^{-1})	
	Puch,	Biscaya (300 ml ha^{-1})	57
	Bavaria	Mavrik (200 ml ha^{-1})	
		Karate Zeon (75 ml ha^{-1})	

$^{\#}$ = applications at different growth stages were carried out in different plots,
$^{\#\#}$ = applications at different growth stages were carried out in a sequence to the same plots

Statistical analyses

In the field trials of 2013 parasitized pollen beetle larvae were not separated by their larval stage and data on parasitism of L1- and L2-lavae were pooled. Results are presented for both development stages together. In the following years parasitized larvae were separated into L1- and L2-larvae. Statistical analyses were carried out using the software R, version 3.1.2 (R Core Team 2014; packages: *lme4* (Bates et al. 2015)*, multcomp* (Hothorn et al. 2008)*, effects* (Fox 2003)*, MASS* (Venables & Ripley 2002), *lsmeans* (Lenth 2015)). Data of each year and field trial location were analysed separately, because of a high variability in infestation levels and weather conditions. A Generalized Linear Model (GLM) was used to analyse the differences in the parasitism level of pollen beetle larvae by *T. heterocerus* in the different treatments. A negative binomial model was used, because the poisson model revealed overdispersion. The treatment and date were included into the model as main effects and their interaction was tested. To compare the sum of parasitized larvae in the different treatments over the total collection periods a linear model was used and the confidence intervals obtained from model estimates were used for interpretation of effects.

Parasitism rates of larvae by *Phradis* spp. in the field trials of 2015 were analysed by using GLM (binomial for proportion data). The treatment and the date were included into the model as main effects and their interaction was tested. The treatments were compared pairwise for each day of assessment with the package *lsmeans* for post-hoc testing. P values were adjusted with the Hochberg method (Blakesley et al. 2009).

Results

In 2013, parasitism of larvae by *T. heterocerus* was detected in all field trials (Fig. 1). The lowest level of parasitism was recorded in Stöckendrebber with a maximum percentage of parasitized larvae of 3.4% in Karate Zeon-treated plots. In Wendhausen parasitized larvae were recorded from BBCH 63 onwards. For the total collection period a maximum of 8.0% of the larvae in the control were parasitized. In Puch first parasitized larvae were detected during BBCH 61-65, but parasitism rate was low at this time. For the total collection period maximum percentage of parasitized larvae was 13.2% in Karate Zeon-treated plots in Puch. Unfortunately the plastic bowls were removed too early (BBCH 75) and the end of larval migration was missed in Puch. In the field trial in Cramonshagen 2013 the plastic bowls were placed too late (ca. at BBCH 70) to record the start of larval dropping and the end of larval migration was also missed because the bowls were retracted too early (BBCH 78). In Cramonshagen maximum percentage of parasitized larvae for the total collection period was 16.8% in Karate Zeon-treated plots. Within each location no significant differences in parasitism rates of larvae were observed between different insecticide treatments and the untreated control (GLM, $p > 0.05$). Application of Biscaya in the field trial in Wendhausen at the beginning of flowering at BBCH 60 and at full flowering resulted in minimal reduced parasitism level of larvae compared to application of Biscaya at the bud stage at BBCH 53. These differences were not significant (GLM, $p > 0.05$).

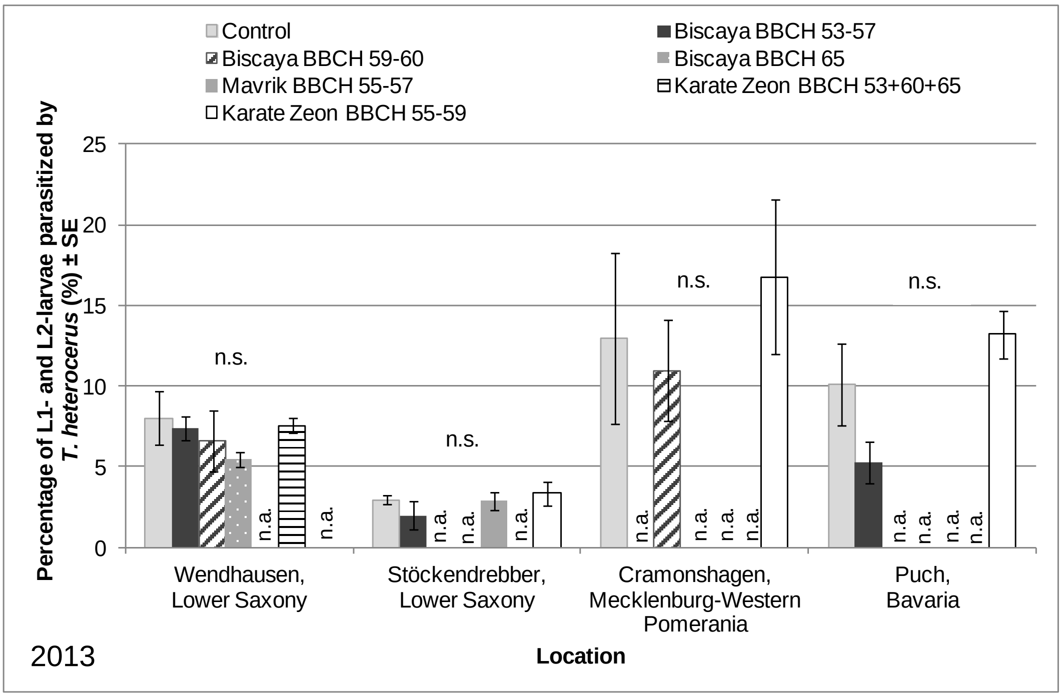

Fig. 1: Level of parasitism (%) of L1- and L2-larvae by *T. heterocerus* (± SE) following application of insecticides in field trials of 2013 at Wendhausen (collection period 2 May – 21 June, BBCH 60 – 80), Stöckendrebber (14 May – 12 June, BBCH 63 – 79), Cramonshagen (28 May – 18 June, BBCH ca. 70 – 78) and Puch (6 May – 5 June, BBCH 61 – 75). GLM, $p \leq 0.05$; n.s. = no significant difference between treatments within each location, n.a. = treatment not tested

In 2014 and 2015, the larval stages of parasitized pollen beetle larvae were differentiated. In 2014, parasitism of L1-larvae by *T. heterocerus* was detected in field trials in Bavaria and Mecklenburg-Western Pomerania, but not in Lower Saxony. The maximum level of parasitism of L1-larvae was 5.4% in Biscaya-treated plots in Puch and 2.6% in Mavrik-treated plots in Kleefeld. No significant differences between the treatments within each location were recorded (GLM, $p > 0.05$). Parasitized L1-larvae were not detected before BBCH 65.

Parasitism of L2-larvae by *T. heterocerus* was observed in all field trials in 2014. Maximum percentage of parasitized L2-larvae was 8.3% in untreated plots in Stöckendrebber, 9.4% in untreated plots in Kleefeld, 17.4% in untreated plots in Sickte and 22.4% in Karate Zeon-treated plots in Puch (Fig. 2). At all locations parasitized L2-larvae were first recorded from BBCH 65 onwards but not in earlier collection periods. Parasitism rates of L2-larvae were not significantly different between insecticide-treated and untreated plots within each location (GLM, $p > 0.05$). Application of Biscaya and Mavrik at early flowering (BBCH 62) in the field trial in Sickte resulted in slightly lower parasitism rates of larvae compared to an application of these insecticides at the bud stage at BBCH 55. Parasitism rates in plots treated with Biscaya at BBCH 62 were reduced by 1.6% compared to treatment at the bud stage. Treatment with Mavrik at BBCH 62 resulted in 5.2% lower parasitism level compared to the treatment at BBCH 55. All observed differences were not significant (GLM, $p > 0.05$).

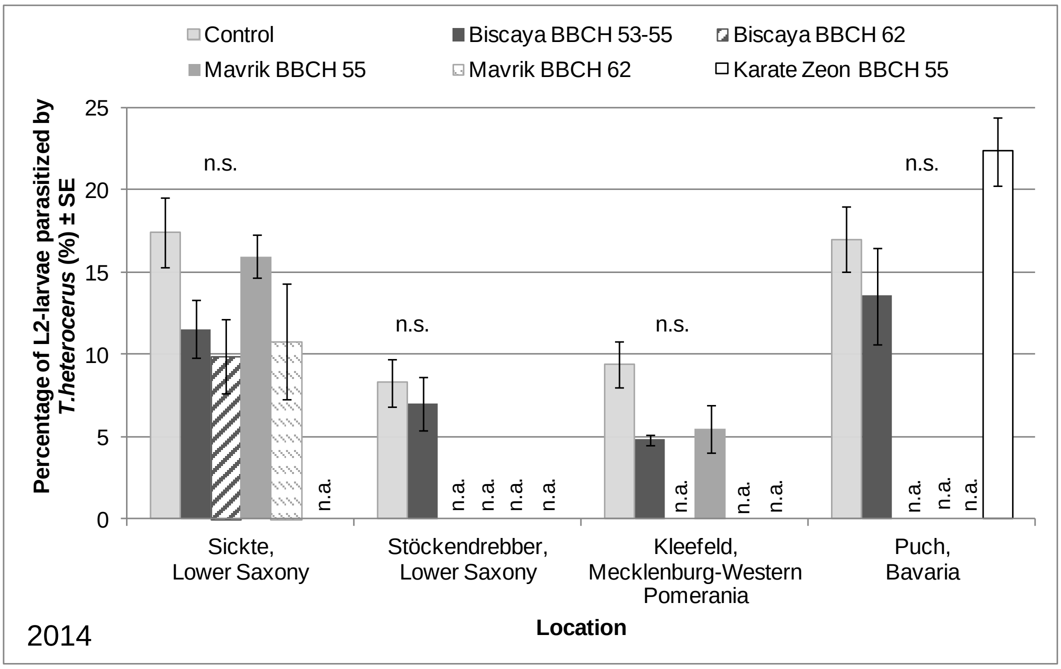

Fig. 2: Level of parasitism (%) of L2-larvae by *T. heterocerus* (± SE) following application of insecticides in field trials of 2014 at Sickte (collection period 16 April – 21 May, BBCH 62 – 76), Stöckendrebber (15 April – 19 May, BBCH 62 – 79), Kleefeld (22 April – 26 May, BBCH 64 - 80) and Puch (23 April – 14 May, BBCH 65 – 69). GLM, $p \leq 0.05$; n.s. = no significant difference between treatments within each location, n.a. = treatment not tested

In 2015 parasitism of L1-larvae by *T. heterocerus* was detected in all field trials, except Niedernstöcken. The maximum level of parasitism of L1-larvae was 2.1% in plots treated with Mavrik at BBCH 62 in Lucklum, 10.6% in untreated plots in Kleefeld and 4.7% in Biscaya-treated plots in Puch. No significant differences between the treatments within each location were recorded (GLM, $p > 0.05$). As in 2014 no parasitized L1-larvae were detected before BBCH 65.

Parasitism of L2-larvae by *T. heterocerus* was detected in all field trials in 2015, but again not before BBCH 65. Maximum percentage of L2-larvae parasitized by *T. heterocerus* was 11.1% in Biscaya-treated plots in Niedernstöcken, 14.1% in untreated plots in Puch, 15.2% in plots treated with Biscaya at BBCH 55 in Lucklum and 29.1% in Biscaya-treated plots in Kleefeld (Fig. 3). As in 2014 no significant differences were observed between different insecticide treatments and the control within each location (GLM, $p > 0.05$). Again insecticide applications of Biscaya and Mavrik at early flowering (BBCH 62) in the field trial in Lucklum resulted in slightly lower parasitism rates of L2-larvae compared to applications of the insecticides at the bud stage at BBCH 55. Parasitism rates in plots treated with Biscaya at BBCH 62 were reduced by 6.6% compared to treatment of Biscaya at the bud stage. Treatment with Mavrik at BBCH 62 resulted in 5.2% lower parasitism rate compared to the treatment at BBCH 55. All differences were not significant (GLM, $p > 0.05$).

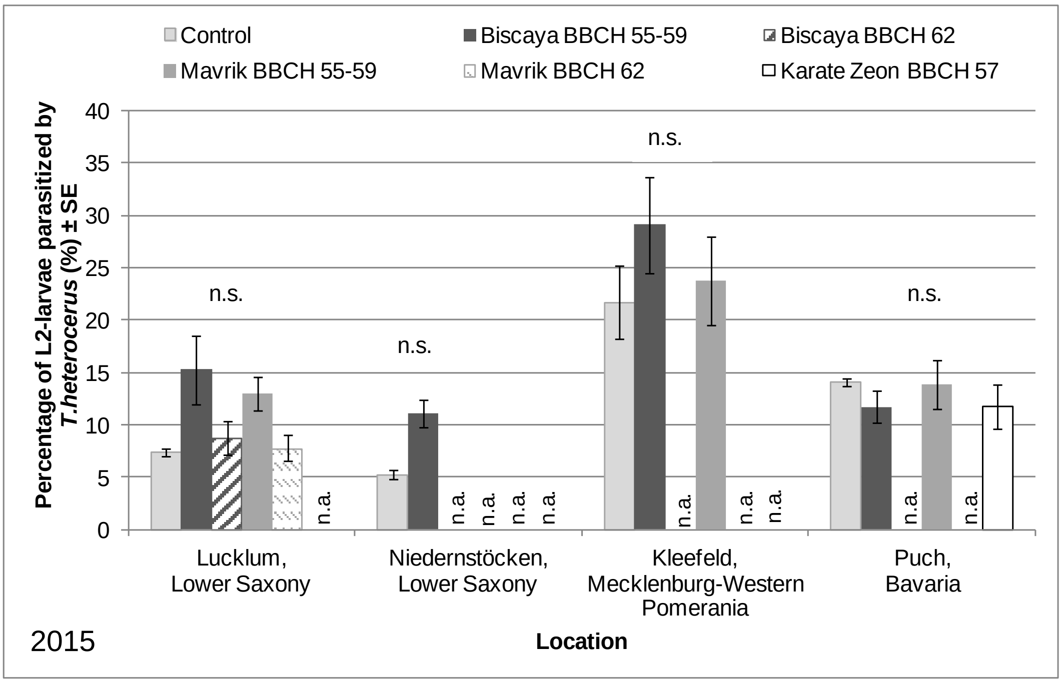

Fig. 3: Level of parasitism (%) of L2-larvae by *T. heterocerus* (± SE) following application of insecticides in field trials of 2015 at Lucklum (collection period 4 May – 9 June, BBCH 65 – 78), Niedernstöcken (29 April – 10 June, BBCH 62 – 79), Kleefeld (11 May – 22 June, BBCH 63 - 80) and Puch (29 April – 10 June, BBCH 65 – 79). GLM, p ≤ 0.05; n.s. = no significant difference between treatments within each location, n.a. = treatment not tested

In 2015, the parasitoids *Phradis* spp. were not detected in all field trials in contrast to *T. heterocerus*. By dissection of L2-larvae from the field trial in Mecklenburg-Western Pomerania collected between BBCH 65 to 67 and between BBCH 67 to 69 no larvae parasitized by Phradis spp. were found. Also in Niedernstöcken between BBCH 65 to 67 and BBCH 67 to 70 no parasitism of larvae by *Phradis* spp. was recorded. The subsequent dissection of larvae collected between BBCH 70 to 75 showed a parasitism rate of 4.2% for larvae from the untreated plots and 1.7% for larvae from plots treated with Biscaya, with no significant difference between the treatments (GLM, p > 0.05).

In Lucklum also no *Phradis* spp. were detected before BBCH 69. In the collection period between BBCH 69 to 71 in all treatments 3.3% of the larvae were parasitized, except larvae from the plots treated with Biscaya at BBCH 62, where no parasitism was found (Fig. 4). One week later the dissection of the larvae collected between BBCH 71 to 76 showed that parasitism of larvae collected in untreated plots was 9.4%. In Biscaya treated plots, separately treated at BBCH 55 or 62 parasitism of larvae was 1.7%. Larvae from plots treated with Mavrik (regardless of the application date) showed a parasitism rate of 3.3%. Between all treatments no significant difference was observed (GLM, p > 0.05).

In addition, a random sample of L2-larvae from the field trial in Bavaria collected between BBCH 67 to 69 was dissected. In all treatments parasitism rates were 3.3%, except larvae from plots treated with Mavrik at BBCH 57 with 8.3% being parasitized, but differences were not significant (GLM, p > 0.05) (Fig. 4). One week later the dissection of the larvae collected between BBCH 69 to 75 showed that the parasitism rate of the larvae from untreated plots was 8.3%. The highest parasitism rate was recorded from plots treated with Biscaya at BBCH 57 (18.3%), followed by plots treated with Mavrik at BBCH 57 (13.3%). Dissection of larvae from Karate Zeon-treated plots resulted in a parasitism rate of 8.3%, similar to the control. Observed differences between all treatments did not differ significantly (GLM, p > 0.05).

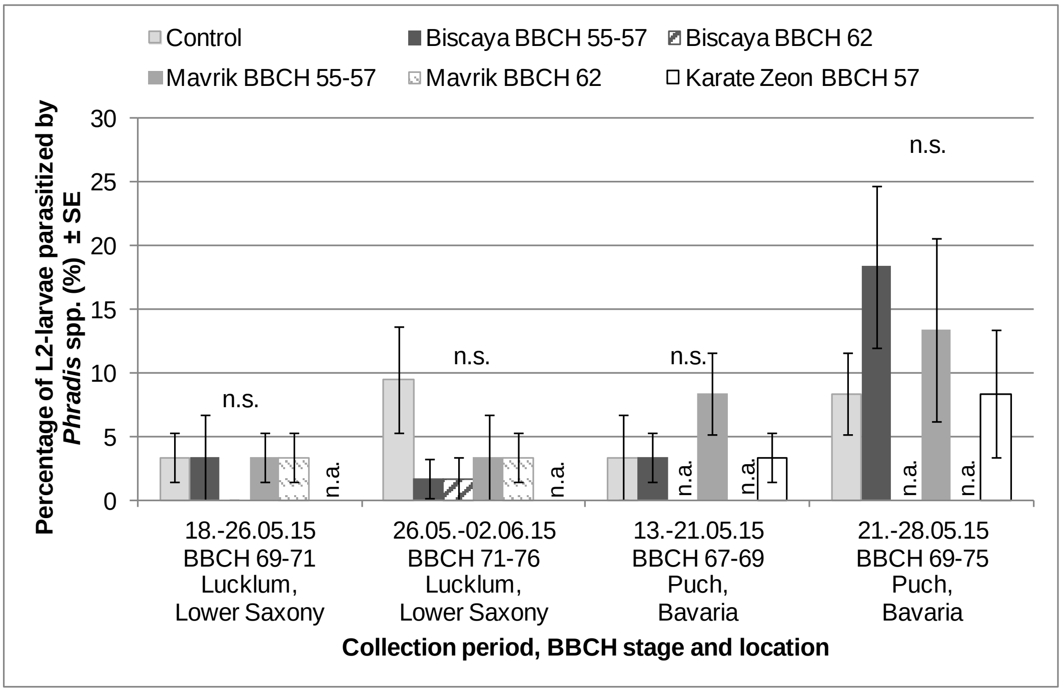

Fig. 4: Level of parasitism (%) of L2-larvae by *Phradis* spp. (± SE) following application of insecticides in field trials of 2015 at Lucklum and Puch at different collection periods. GLM, p ≤ 0.05; n.s. = no significant difference between treatments within each location, n.a. = treatment not tested

Discussion

In the present study effects of the insecticides Biscaya, Mavrik and Karate Zeon applied at different growth stages between the green bud stage and full flowering of winter oilseed rape on the parasitism rates of pollen beetle larvae by *T. heterocerus* and *Phradis* spp. in field trials in 2013 to 2015 were analysed. Parasitization of L1-larvae of pollen beetle by *T. heterocerus* was either not present or only low in experimental years in 2014 and 2015 in all field trials. This corresponds with observations of Nilsson & Andreasson (1987), that large L2-larvae were preferred by *T. heterocerus* for oviposition.

For the determination of parasitism rates of L2-larae by *T. heterocerus* two different methods were used. The dissection of L2-larvae showed a slightly higher parasitism rate by *T. heterocerus* compared to determination of dark pigmented eggs by external inspection. This was seen only for the larvae of the last two collection periods from BBCH 69 onwards. But according to Nilsson & Andreasson (1987) this error is negligible.

In this study the parasitism rates of pollen beetle larvae in Biscaya- and Mavrik-treated plots were lower in some cases compared to the control, but without significant differences. In all field trials, the application of Biscaya, regardless whether applied at the bud stage or at flowering, reduced the number of L2-pollen beetle larvae compared to untreated plots. For example, in plots treated with Biscaya at BBCH 55 in Lucklum 2015, the number of L2-larvae dropping to the ground was reduced by 64%. In most experiments these effects of Biscaya were significant (data presented in chapter II). In plots treated with Mavrik, the number of L2-larvae was also reduced, but less than in Biscaya-treated plots. In contrast, application of Karate Zeon in 2013 in all field trials resulted in a higher number of L2-larvae compared to the control. For example, number of larvae increased up to 29% in Cramonshagen or up to 37% in Puch. In 2014 and 2015 Karate Zeon was tested only at Puch in Bavaria where the number of L2-larvae increased up to 31% and 42%, respectively, compared to untreated plots. In consequence in Biscaya- and Mavrik-treated plots a reduced number of larvae were available for parasitoids than in the untreated plots, whereas in Karate Zeon-treated plots the highest numbers occurred.

Natural enemies may show a density-dependent reaction to pest populations. Contrasting results on density-dependence on parasitism have been reported in literature: Longley & Jepson (1996) observed that plants which were infested by low numbers of *Sitobion avenae*, were less favoured or avoided by female parasitoids of *Aphidius* spp. than plants with higher aphid infestation. Also parasitoids of pollen beetle might concentrate on plots with a higher host density as reported by Zaller et al. (2009). A positive correlation between the density of the host population of pollen beetle larvae and the parasitism rate was detected by Neumann (2010) in one of three experimental years. But Nilsson & Andreasson (1987) did not find a correlation between the number of pollen beetle larvae and parasitism.

Volatiles of oilseed rape which are released after insect feeding have an effect on the searching behaviour of *T. heterocerus* and *Phradis* spp. (Jönsson et al. 2005). These synomones can attract natural enemies to infested plants. For example *Cotesia glomerata*, a larval parasitoid of *Pieris* species was attracted by synomones released from Brussels sprout after feeding damage by *Pieris brassicae* (Mattiacci et al. 1994). As described by Jönsson et al. (2005) the ichneumonid parasitoids were attracted by plant synomones, which were released after feeding of pollen beetle and larvae. Because of a lower infestation of Biscaya- and Mavrik-treated plots with beetles and larvae it can be assumed that less synomones were released in these plots and parasitoids visited these plots less frequently.

In 2014 and 2015 parasitization of L1- and L2-larvae by *T. heterocerus* was not observed before BBCH 65. This is in accordance with Nitzsche (1998) and Johnen et al. (2010), who observed that in Germany the parasitoid does not occur before the beginning of flowering, at the end of April or beginning of May, depending on weather conditions. In all experimental years no significant effects of insecticides applied in the bud stage on *T.*

heterocerus were observed. For *T. heterocerus* and *P. morionellus* this is in accordance with Nilsson (1985), who assumed that pesticide applications during the bud stage will hardly affect these parasitoids, but could have an effect on *P. interstitialis*. It can be assumed that at the time of application at the bud stage *T. heterocerus* was not present in the fields and immigration occurred not until the beginning of flowering, which was the case for example in Wendhausen (2013), Sickte (2014) and Lucklum (2015) at least 10 days after application. Therefore the insecticides used seem to have a low persistent efficacy against these beneficials.

In the region of Goettingen, the main activity of tersilochine parasitoids was observed in the period shortly before flowering until full flowering (Ulber & Nitzsche 2006). In consequence application of insecticides in this activity phase, as occurred in the field trial in Wendhausen, Sickte and Lucklum at BBCH 60 to 65, might have negative effects on parasitoids. Insecticide applications of Biscaya at BBCH 60, 62 or 65 and of Mavrik at BBCH 62 resulted in slightly lower parasitism rates of larvae by *T. heterocerus* in all years compared to applications of these insecticides at the bud stage (BBCH 53-55), but these differences were not significant and again larvae occurred less frequent in treated variants.

In 2015, the dissection of L2-larvae to determine parasitism rates by *Phradis* spp. showed that parasitism did not occur in all field trials (no detection in Mecklenburg-Western Pomerania) and were not detected before BBCH 67. In Niedernstöcken and Lucklum larvae parasitized by *Phradis* spp. were only detected from BBCH 70-75 (Niedernstöcken) and BBCH 69-71 (Lucklum) onwards, but not at earlier periods. The earliest parasitization was recorded in the field trial in Bavaria where larvae were parasitized from BBCH 67 onwards. According to Ulber et al. (2010a) *P. interstitialis* predominates in winter oilseed rape in Germany, whereas *P. morionellus* is more abundant in spring oilseed rape in northern Europe (Nilsson 2003, Ulber et al. 2010a), but was found in Germany by Ulber et al. (2010a) in a small proportion only. So it is likely that most of the *Phradis* larvae found in the field trials in 2015 were *P. interstitialis*. For discrimination between *P. interstitialis* and *P. morionellus* rearing out of adults from the larvae would have been necessary (Ulber, personal communication) which was not possible in the own trials.

Nitzsche (1998) and Johnen et al. (2010) described that immigration into oilseed rape fields of *P. interstitialis* starts from mid of April onwards. Applications at the bud stage in mid-April as in the present field trials (BBCH 53 to 57-59) might have an effect on *P. interstitialis*. The females are sexually mature at this time (Nilsson 2003) and oviposit through the wall of the buds into the eggs and first instar larvae of pollen beetle (Osborne 1960). Taking into account the development time of pollen beetle it takes approximately ten days in winter oilseed rape, depending on temperature from egg laying to hatch of L1-larvae (Nilsson 1988). The first larval instar period lasts approximately five to ten days (Burkhardt & von Lengerken 1920, Nilsson 1988). The first *Phradis* spp. larvae were found in L2-pollen beetle larvae not before mid of May 2015. Subtracting the development time of the pest larvae of about 15 to 20 days it can be assumed that *P. interstitialis* did not migrate into the fields before end of April or beginning of May 2015. This means that the parasitoid occurred two to three weeks after the application at each field trial and did not get into contact with the insecticides. Consequently no significant effects of insecticides on

Phradis spp. were observed. Unfortunately the start of migration of the parasitoids was not assessed by yellow water traps in the field trials as described by Nitzsche (1998) or Williams et al. (2003). Also applications of Biscaya and Mavrik at BBCH 62 as conducted in Lucklum did not have significant effects on *P. interstitialis*.

The insecticides used in the present field trials in 2013 to 2015 did not affect parasitization of pollen beetle larvae by *T. heterocerus* and *Phradis* spp., regardless whether applied at the bud stage, at the beginning of flowering or full flowering. In contrast examples of negative effects of insecticides on parasitoids can be found in literature. Veromann et al. (2006) described reduced parasitism rates of pollen beetle larvae by *D. capito* and *Phradis* spp. after application of the pyrethroids Fastac (a.i. alpha-cypermethrin) and Karate (lambda-cyhalothrin) at BBCH 51 and 67 in one year field trials in Estonia. It has to be taken into account that parasitism rate in these field trials was very low (maximum 7.4%). In addition application of the insecticides reduced number of overwintered pollen beetles in winter oilseed rape significantly. Consequently number of eggs and finally number of larvae are expected to be lower in treated plots. But plots with reduced egg and larval density may be unattractive to the parasitoids.

Another study on the impact of insecticides on parasitization of pollen beetle larvae has been reported by Jansen & San Martin y Gomez (2014). In a one year trial the authors tested several insecticides including Mavrik 2F (tau-fluvalinate), Biscaya (thiacloprid), Boravi WG (Phosmet), Plenum (Pymetrozine) and Pyrinex (Chlorpyriphos-ethyl) applied at the end of the bud stage. All insecticide treatments had a significant impact on the adult population of parasitic hymenoptera. The parasitism rate of pollen beetle larvae was also reduced by all treatments. The strongest reduction was achieved by Biscaya and Pyrinex, but only Biscaya reduced larval parasitism significantly. The other products had no significant effects.

The described effect of Biscaya in the field trial of Jansen & San Martin y Gomez (2014) is in contrast to the results of the present study. It has to be considered, that Jansen & San Martin y Gomez (2014) as well as Veromann et al. (2006) did not take into account the significant reduction of overwintered pollen beetles due to the insecticide treatments and consequently a lower larval density in the treated plots. In the field trial of Jansen & San Martin y Gomez (2014) in Biscaya- and Pyrinex-treated plots 84% and 93% less larvae were recorded. This is in accordance with the present field trials, where application of Biscaya, regardless whether treated at the bud stage or at beginning of flowering, resulted in a reduction of L2-larvae. Consequently as described above a density-dependent reaction of parasitoids is possible and plots with low larval density might be visited less frequently by parasitoids. Further it is important to note that Jansen & San Martin y Gomez (2014) did not separate between the different parasitoids, which occur in spring at different times (Nitzsche 1998, Johnen et al. 2010) and prefer different development stages of pollen beetle (Osborne 1960). In addition Jansen & San Martin y Gomez (2014) as well as Veromann et al. (2006) collected larvae from only 20/25 plants per plot. A higher number of plants (at least 50 plants), as recommended in EPPO Standard PP 1/178 (3) (EPPO 2005) for assessment of the abundance of overwintered pollen beetles to evaluate efficacy of insecticides, would have given a more precise overview.

The effects of lambda-cyhalothrin and tau-fluvalinate on key parasitoids of oilseed rape pests were studied in the EU MASTER project in Poland in two years (Ulber et al. 2010b). The insecticides were applied at BBCH 54/56 and 65/68. Applications of insecticides resulted in mortality of parasitoids. Lambda-cyhalothrin showed a more persistent effect compared to tau-fluvalinate, which reduced parasitism level of host larvae less. Unfortunately the total number of pollen beetle larvae available for the parasitoids in different treatments was not mentioned in the reference. Treatment of insecticides may have reduced larval density. In the period of the field trial (2002 and 2004) the pyrethroid resistance of pollen beetles was not as far developed as nowadays, so it has to be expected that Karate reduced pollen beetle density as well as Mavrik. Consequently a lower number of larvae might have been available for the parasitoids which may respond density-dependent.

The results obtained within the EU MASTER project are in accordance with laboratory tests with adult parasitoids of Jackowski et al. (2008). Effects of lambda-cyhalothrin and tau-fluvalinate with full and reduced application rates were observed on *Phradis* spp. in a tarsal plate test, with Mavrik being less toxic than Karate. A 50% reduced application rate of Mavrik had no effect on mortality of *Phradis* spp. compared to effects of the 100% rate. In contrast the reduction of the application rate of 50% of Karate resulted in parasitoid mortality but less compared to 100% dose rate of this insecticide.

In three years field trials of Neumann (2010) the effects of lambda-cyhalothrin, tau-fluvalinate and thiacloprid on the parasitoids *T. heterocerus* and *P. interstitialis* were analysed. Insecticides were applied with full and half recommended dose rate at BBCH 61/62 and BBCH 65/69. The results of Neumann (2010) showed in contrast to the studies of Jackowski et al. (2008) and Ulber et al. (2010b) consistent over three years no significant effects of lambda-cyhalothrin on parasitism, regardless of applications of 50% or 100% dose rate and of applications at early flowering or full flowering. Applications of 50% and 100% dose rate of tau-fluvalinate at early flowering resulted in significant reduced parasitism although the application was conducted nine days before parasitoid immigration. Application of 50% dose rate of tau-fluvalinate at full flowering, simultaneously to the activity peak of the parasitoids, also resulted in significant reduced parasitism, whereas the application of the full dose rate of tau-fluvalinate at full flowering did not significantly reduce parasitism.

Unfortunately the active substance tau-fluvalinate was tested only in the first year by Neumann (2010) and was replaced by thiacloprid in the second and third year. For thiacloprid no clear trend was detectable. In one year thiacloprid significantly reduced the number of pollen beetle larvae when applied with full dose rate at the beginning of flowering and when applied at full flowering with 50% and 100% dose rate. No significant differences were observed concerning parasitism. This is in accordance with own results of the present field trials. In the following year Neumann (2010) observed that Biscaya had a significant effect on parasitoids if applied with full dose rate at the beginning of flowering and with both application rates at full flowering. But it has to be considered that the number of larvae per plant was very low in this year (2 larvae per plant).

Finally it can be concluded that no clear and consistent effects of lambda-cyhalothrin, tau-fluvalinate and thiacloprid on parasitism rates of pollen beetle larvae are reported in literature. Effects of individual insecticides differ between authors and between years within one paper. More information on effects of insecticides on parasitoids is urgently needed to protect parasitoids in the crop and support natural control of pests. Therefore trials over several years with different locations are necessary to test such side effects of insecticides more detailed. It would be important to monitor the immigration and the activity peak of the different parasitoids, to determine whether there may be long term effects of insecticides applied before immigration of parasitoids or whether the application was coincident with the activity peak of the parasitoids. To examine parasitized larvae it has to be considered that pollen beetle larvae should be collected over a longer period and not only during one or two collection dates, as own data show that eggs or larvae of parasitoids occur in most cases not before BBCH 65 (*T. heterocerus*) or BBCH 67 (*Phradis* spp.) in higher numbers and still occur in larvae collected after the end of flowering. In addition the total number of adult pollen beetle and host larvae needs to be monitored to separate between direct toxic and indirect effects.

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Chapter V

General discussion

Oilseed rape is known to host a large variety of insect pests (Alford et al. 2003). Some of these pests are present in the crop for a long time, simultaneously with other pests. Control of these pests is dominated by insecticide applications. Compared to other crops oilseed rape is a crop with a high treatment index for insecticides (Freier et al. 2015). Mainly the insecticide class of pyrethroids was extensively used in the past decades to control oilseed rape pests (Nauen 2005, Heimbach et al. 2006, Müller et al. 2008, Thieme et al. 2010), resulting in high selection pressure and finally resistance to pyrethroids. Pyrethroid resistance of pollen beetle (*Meligethes aeneus* (Fabricius), syn. *Brassicogethes aeneus* (Fabricius)) is especially pronounced and spread all over Europe (Hansen 2003, Nauen 2005, Wegorek 2005, Heimbach et al. 2006, Tiilikainen & Hokkanen 2008, Slater et al. 2011, Zimmer & Nauen 2011a). But also other insect pests of oilseed rape are affected by pyrethroid resistance (Heimbach & Müller 2013, Robert et al. 2015).

Because of widely distributed pyrethroid resistance of pollen beetles and the lack of alternative insecticides with different modes of action the control of the pest is difficult. To save the efficiency of available insecticides, they have to be used targeted, considering pest thresholds. When threshold values are exceeded insecticides are required that not only have a direct lethal effect on bud damaging overwintered pollen beetles to avoid yield losses, but in addition reduce reproduction of the pest, to achieve a sustainable reduction of pollen beetles. This would result in a lower probability of high infestation pressure and insecticide treatment frequency in following years. It would also be an advantage if spraying to controll a specific pest would also have side effects on the population development of other pests present in the crop.

Population development of pollen beetles is influenced by numerous factors. One of them is insecticide use. In the present study the effects of the neonicotinoid Biscaya (a.i. thiacloprid, 72 g ha^{-1}) and the pyrethroids Mavrik (tau-fluvalinate, 48 g ha^{-1}) and Karate Zeon (lambda-cyhalothrin, 7.5 g ha^{-1}) applied at BBCH 53/55 or 60-65 on population development of pollen beetle were analysed. The results show that Mavrik and especially Biscaya can reduce the population growth of pollen beetle, whereas the application of Karate Zeon resulted in a higher number of offspring. The reduction of population growth by Biscaya and Mavrik was caused by different underlying mechanisms:

Effects on overwintered pollen beetles

Because of widely distributed pyrethroid resistance of pollen beetles (Slater et al. 2011) and low efficacy of Karate Zeon in different field trials (Schröder et al. 2009, Smatas et al. 2012, Brandes et al. 2014) it was expected that the application of Karate Zeon has no effect on overwintered pollen beetle mortality. In contrast application of Biscaya and

Mavrik was expected to result in a reduction of the number of overwintered pollen beetles. These expectations were fulfilled although in 2013 Biscaya showed only a moderate efficacy. Only at one day after application of Biscaya at BBCH 53 a significant difference in the number of beetles was observed and population density was reduced by 47%, whereas Karate Zeon had no effect (chapter II). At the following assessments starting three days after application no significant difference in beetle density was recorded between the treatments. Consequently the calculated cumulative insect-days for 13 days after application of Biscaya and Karate Zeon did not differ to the control (chapter III). The reduced efficacy of Biscaya on pollen beetles in the field trials of 2013 might be explained by migration of pollen beetles into the field after application (chapter II). These beetles were not exposed directly to the insecticide and seemed to be not affected.

In the field trials of 2014 and 2015 the number of overwintered pollen beetles was significantly reduced in Biscaya- and Mavrik-treated plots for three and seven days after application, respectively (chapter II). The calculation of the cumulative insect-days of overwintered pollen beetles (Ruppel 1983) for 14-15 days after application at BBCH 55 showed that infestation levels in Biscaya- and Mavrik-treated plots were significantly reduced compared to the control (chapter III). Significant effects of Biscaya on overwintered pollen beetles for several days after application are described also for other field trials (Jansen & San Martin y Gomez 2014, Tölle 2014), as well as significant effects of Mavrik (Vaitelyte et al. 2011).

The exact reasons for the reduction of the number of overwintered pollen beetles in Biscaya- and Mavrik-treated plots were not studied in detail. The low density of beetles might be explained by direct mortality of the beetles. But if the reduced infestation of buds with eggs and larvae observed in treated plots was only caused by direct mortality, also the plants covered during insecticide application would have shown a low percentage of bud infestation, similar to treated plants (chapter III). The reduced beetle density and the reduced infestation of buds with eggs and larvae may be the combined effect of direct lethal, repellent or sublethal effects. Repellent effects of insecticides are known (Longley & Jepson 1996, Neumann 2010) and may contribute to a lower density of pollen beetles and consequently result in a lower number of buds infested with eggs and larvae in Biscaya- and Mavrik-treated plots. In addition to repellent effects sublethal effects of insecticides may influence behaviour of insects as described by Desneux et al. (2004) and Neumann (2010). Sublethal effects influencing behaviour of the beetles might result in dropping of the beetles from the plants and/or effects on oviposition behaviour.

Effects on bud infestation with eggs and larvae

In this study different effects of the three insecticides on bud infestation with eggs and larvae were expected. Especially for Biscaya reduced bud infestation with eggs and L1-larvae were expected because of results reported by Tölle (2014). The high effectiveness of Biscaya in reducing bud infestation in all years despite moderate reduction of pollen beetle density in 2013 and lower direct effects on beetles than Mavrik in 2014 and 2015 indicates that the insecticides affected pollen beetles in different ways. Insecticidal residuals on Biscaya-treated plants might have an effect on beetle egg laying resulting in reduced bud infestation even though the number of beetles was reduced only for a short time. This was confirmed by greenhouse experiments where only Biscaya-treated plants showed reduced infestation of buds whereas on Mavrik-treated plants bud infestation was only slightly reduced and differed not significantly to the control. It can be concluded that application of Biscaya and Mavrik at the bud stage resulted in a reduced number of pollen beetles and infested buds with eggs and larvae caused by direct lethal and repellent effects. In addition for Biscaya sublethal effects were observed. Consequently a lower number of mature larvae dropping down for pupation and a reduced number of emerging new generation beetles were recorded especially for Biscaya-treated plots (chapter II).

In contrast to Biscaya and Mavrik the insecticide Karate Zeon does not seem to have effects on pollen beetles because in the field trial in 2013 no effect on overwintered pollen beetles and bud infestation was detected in plots treated with Karate Zeon. The lack of efficacy of Karate Zeon on overwintered pollen beetles observed in the presented field trials can be explained as a consequence of the widely distributed pyrethroid resistance of pollen beetles (Slater et al. 2011). Different results obtained with Karate Zeon and Mavrik may be explained by different susceptibility of pollen beetles to active substances of pyrethroids (Makunas et al. 2011, Heimbach & Müller 2013).

In addition to insecticide applications in the bud stage the effects of applications at the beginning of flowering to full flowering (Biscaya in 2013) on population development of pollen beetle were studied. In the plots treated at the beginning of flowering or at full flowering beetles could oviposit undisturbed as in the control until the day of application. In most cases number of pollen beetles per main stem was very low before and several days after application at the flowering stage and consequently with a high variability. But it has to be considered that the infestation level of beetles was underestimated after the plants had built side shoots. With progressive development of the plants beetles concentrate on the side shoots which provide suitable buds for oviposition while the main stem with open flowers is used for feeding or has already finished flowering and is no longer attractive for the beetles. Beetle density on side shoots was not assessed because usually efficacy evaluation of insecticides is done according to EPPO (2005) on the main stem. It has to be assumed that the application of Biscaya and Mavrik at the beginning of flowering or at full flowering had an effect on beetles concentrated on the side shoots and reduced beetle density (as observed for the applications at the bud stage) and consequently buds were less infested with eggs and larvae after application, but this was not analysed.

Effects on larval stages and new generation pollen beetles

In addition to different effects on overwintered pollen beetles and on bud infestation with eggs and larvae by insecticide applications, the three insecticides might have different effects on the two larval stages and consequently the number of new generation pollen beetles. It was observed that the L1-larvae hatching from eggs laid before insecticide application at the beginning of flowering or full flowering were affected by Biscaya. An enhanced dropping of premature L1-larvae after insecticide treatment during the flowering stage was only observed in Biscaya-treated plots, not in Mavrik- or Karate Zeon-treated plots. Mechanical effects of the spraying activity on enhanced larval dropping can be excluded because such effects would occur in all treated plots.

L1-larvae might get into direct contact with the active substance or by feeding contaminated plant parts when they are located in buds open during application or in buds opening shortly afterwards. It was not analysed whether the enhanced dropping of larvae was caused by lethal effects of thiacloprid or by sublethal effects impairing larval coordination. It can be assumed that thiacloprid is highly toxic to L1-larvae explaining the high number of dropping larvae several days after application of Biscaya. For L2-larvae high sensitivity to thiacloprid is known (Zimmer et al. 2014). A systemic transport of thiacloprid in plants is reported by Zimmer et al. (2014), but effects on L1-larvae in closed buds in the present study can be excluded, because no dead larvae were found during the dissection of buds. Dropped premature larvae on the ground are likely to fall prey to predators as carabid and staphylinid beetles and spiders (Büchs & Nuss 2000, Büchs & Alford 2003, Piper & Williams 2004, Öberg et al. 2011).

An important factor determining effects of insecticides on population growth of pollen beetle is the number of L2-larvae dropping down for pupation. Application of Biscaya in different field trials distributed over Germany resulted in most cases in a significant reduction of dropping L2-larvae compared to the control. Mavrik also reduced the number of second instars, but in most cases less effective than Biscaya. Significant reduction of L2-larvae after application of Biscaya in field trials is also described by Jansen & San Martin y Gomez (2014) and Tölle (2014). In contrast Karate Zeon increased number of dropping L2-larvae in presented field trials. L2-larvae dropping down in Karate Zeon-treated plots increased up to 42% compared to the control. The elimination of antagonists such as spiders or staphylinids by lambda-cyhalothrin is known (Wehling & Heimbach 1991, Devotto et al. 2007, Felsmann 2008, Liu et al. 2013) and might have encouraged undisturbed development of larvae in the flowers resulting in enhanced dropping of L2-larvae in Karate Zeon-treated plots. In addition it might be possible that only low concentrations of lambda-cyhalothrin reach the target site in the pest because of the fast degradation of pyrethroids by monoxygenase enzymes responsible for pyrethroid resistance of pollen beetles (Zimmer & Nauen 2011b). These low concentrations of the insecticide might stimulate oviposition of females and consequently result in high numbers of larvae. The phenomenon of stimulatory effects of stress agents, such as insecticides, is described as insecticide hormoligosis (Luckey 1968).

The effects of Biscaya and Mavrik resulted finally in a reduced number of emerging new generation beetles in the present study. In the treatments with low numbers of dropping L2-larvae consequently significantly lower numbers of beetles emerged. Mavrik was less effective than Biscaya, as seen before for the number of L2-larvae dropping down for pupation. Similar effects of Biscaya on reduced new beetle emergence were described by Tölle (2014).

Effects on parasitism rates of pollen beetle larvae

In addition to insecticide applications larval parasitoids as *Tersilochus heterocerus* and *Phradis* spp. can reduce the population density of pollen beetle substantially (Nitzsche 1998, Nilsson 2003, Ulber et al. 2010). The main activity of tersilochine parasitoids was observed in the period shortly before flowering to full flowering (Ulber & Nitzsche 2006). In consequence applications of insecticides in this activity phase may have negative effects on parasitoids searching for hosts (Johnen & Ulber 2004). In the present study the effects of the insecticides Biscaya, Mavrik and Karate Zeon applied at different development stages of winter oilseed rape on the parasitism rates of pollen beetle larvae by *T. heterocerus* were observed. In addition effects of the insecticides on pollen beetle larvae parasitized by *Phradis* spp. were studied in 2015. In all experimental years no significant effects of insecticides on the parasitism level of pollen beetle larvae were observed, regardless whether insecticides were applied at the bud stage, at the beginning of flowering or full flowering (chapter IV).

In some cases the level of parasitism of larvae was lower in Biscaya- and Mavrik-treated plots compared to the control but without significant differences. It is known that natural enemies may show a density-dependent reaction to pest populations (Zaller et al. 2009). As described before, less larvae were recorded in Biscaya- and Mavrik-treated plots, consequently less larvae were available for parasitoids than in the control plots, making the plots less attractive for the parasitoids, whereas in Karate Zeon-treated plots the highest numbers of pollen beetle larvae occurred. In addition volatiles of oilseed rape can have an effect on the searching behaviour of *T. heterocerus* and *Phradis* spp. (Jönsson et al. 2005). Plants release synomones after insect feeding, which can attract natural enemies (Mattiacci et al. 1994). *T. heterocerus* and *Phradis* spp. were attracted by plant synomones that were released after feeding of pollen beetle and larvae (Jönsson et al. 2005). It can be assumed that less synomones were released in Biscaya- and Mavrik-treated plots, attracting less parasitoids, because less beetles and larvae caused damages.

In contrast to the present results several studies describe negative effects of insecticide applications on parasitoids of pollen beetle (Veromann et al. 2006, Neumann 2010, Jansen & San Martin y Gomez 2014, Tölle 2014). But it can be concluded, that no clear and consistent effects of thiacloprid, tau-fluvalinate and lambda-cyhalothrin on parasitism level of pollen beetle larvae are reported in literature. Effects of individual insecticides differ between authors and between years within one paper. Often only a one year trial was reported. In addition it is often not considered that the application of the insecticides reduced the number of overwintered pollen beetles and larvae significantly. Plots with reduced egg and larval density may be visited less by parasitoids because of a possible density-dependent reaction of parasitoids.

Alternative control strategies

The presented results demonstrate that applications of Biscaya and Mavrik cause a reduction of the population development of pollen beetle. But although population densities will be overall reduced, damage on oilseed rape by pollen beetle is still possible. In some regions with low availability of oilseed rape crops high numbers of beetles might concentrate on individual fields. To reduce reliance on insecticide treatments which are limited because of pyrethroid resistance and the lack of insecticides with alternative modes of action, alternative control strategies have to be involved. Different mechanical techniques to control pests in oilseed rape are known for a long time. Already in 1897 Frank described a cart to catch pollen beetles. Blunck & Hähne (1929), Buhl & Meyer (1940) and von Weiß (1940) presented an overview over different further developed instruments. All these instruments were carried or pulled through the crop by man or cattle. With different trapping solutions as for example tar, glue or soapy water the beetles, dropping down from the plants, were caught. Although these techniques were effective, they did not assert oneself in agriculture, but could be used now as a first idea to develop a more efficient technique with today´s possibilities.

Another alternative strategy to control pollen beetle is the use of trap crops. By use of these trap crops pollen beetles can be attracted and retained or targeted killed in the trap crop, preventing damage of oilseed rape (von Weiß 1940, Hokkanen 1991, Cook et al. 2004). Turnip rape (*Brassica rapa*) has suitability as trap crop for insect pests in oilseed rape (Barari et al. 2005, Cook et al. 2006a, Döring et al. 2013, Gotlin Culjak et al. 2016). It is important that the trap crop starts flowering before the oilseed rape plants to be attractive to the pest (Frearson et al. 2005, Tölle 2014). An ideal trap crop plant should provide buds and flowers for a long period to retain the pest in the trap crop (Frearson et al. 2005). In addition it is important that the crop is accepted for oviposition. *Sinapis alba* for example is accepted for feeding, but is a poor host plant for oviposition (Ekbom & Borg 1996). Treatment of insecticides to infested trap crops is often necessary to control the pest before they migrate into the main crop (Cook et al. 2004), but the total use of insecticides will be reduced (Hokkanen 1991).

Also other non-chemical mechanisms to control pollen beetles are reported. Mauchline et al. (2013) showed that the number of pollen beetles was significantly reduced by repellent effects during the critical period from the bud stage to the beginning of flowering under field conditions by lavender odour released from sachets. In contrast to the pollen beetle their parasitoids *Phradis interstitialis* and *P. morionellus* were not repelled by lavender (Cook et al. 2007). Repellent effects of different essential oils on pollen beetles in laboratory experiments are reported by Pavela (2011) and Daniel (2014). But these control methods are not very common and they need to be tested in practical farming.

Furthermore oilseed rape resistance to insect pests may provide an important part of integrated pest management. Actually no resistance of oilseed rape cultivars to infestation by pollen beetles is known. The manipulation of the petal colour through plant breeding may reduce the attractiveness of oilseed rape to the pollen beetle during flowering. The beetles are attracted to the yellow colour of the flowers of oilseed rape (Cook et al. 2006b, Döring et al. 2012). Cook et al. (2006b, 2013) demonstrated that red and blue-coloured flowers were significantly less attractive to pollen beetles compared to yellow- and white-coloured flowers.

In addition to visual cues, pollen beetles use olfactory cues, particularly isothiocyanates (glucosinolate catabolites), to locate their host plant (Blight & Smart 1999). Bartlet et al. (1999) suggested that by manipulating the glucosinolate content of oilseed rape plants these plants may be less attractive for oilseed rape pests. Hervé (2014) found differences in the attractiveness of six genotypes of oilseed rape to pollen beetle in an olfactometer bioassay. Furthermore Cook et al. (2006a) tested in field trials a cultivar with low emissions of isothiocyanates in comparison to a cultivar with a conventional glucosinolate profile and turnip rape as a trap crop. The cultivar with low isothiocyanate emissions was less infested than the conventional cultivar, which in turn was less attractive than turnip rape. In combination with adjacent trap crops beetles may be pulled away from the visually and/or olfactory less attractive main crop and can be targeted killed in the trap crop. But breeding of oilseed rape is difficult, time consuming and expensive and effects on several oilseed rape insect pests and diseases as well as effects on natural enemies have to be considered.

It can be concluded that there are new approaches to control pollen beetles. Most of the methods need to be tested in real farm situations and the cost of using the methods need to be minimized to make them competitive to insecticide use.

Conclusion

In the presented field trials Biscaya showed effects on population development of pollen beetle that are not assessed during the usual efficacy evaluation of insecticides according to EPPO Standard PP 1/178 (3) (EPPO 2005). Biscaya affects population growth of pollen beetle not only by direct lethal effects on beetles but by additional effects on bud infestation with eggs and larvae. In addition application of Biscaya at the flowering stage of oilseed rape is highly effective in eliminating L1-larvae. Despite direct effects on overwintered pollen beetles, effects of Mavrik on infestation of buds were lower compared to Biscaya and effects on L1-larvae were missing. Application of Karate Zeon did not affect beetle numbers on plants at all and resulted in an increasing number of offspring.

In conclusion Biscaya and Mavrik cause reduction of pollen beetle population growth without affecting parasitism rates of pollen beetle larvae. Especially Biscaya applied at the bud stage is effective in controlling pollen beetles with an additional sustainable effect, reducing infestation pressure and insecticide treatment frequency in following oilseed rape crops. Applications at the flowering stage to control pollen beetle as conducted in the present study are not common because once the plants start to flower, the pollen beetles prefer pollen in open flowers to pollen in buds without causing damage any more (Fritzsche 1957). But if insect pests such as *Ceutorhynchus obstrictus* and *Dasineura brassicae* have to be controlled by insecticides, Biscaya has positive side effects on the reproduction of simultaneously occurring pollen beetles. Thus Biscaya could form an important part of a resistance management program for controlling populations of pyrethroid resistant pollen beetles by reducing overall pollen beetle densities resulting in an overall lower need for insecticide treatments.

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Erklärungen

1. Hiermit erkläre ich, dass diese Arbeit weder in gleicher noch in ähnlicher Form bereits anderen Prüfungsbehörden vorgelegen hat.

   Weiter erkläre ich, dass ich mich an keiner anderen Hochschule um einen Doktorgrad beworben habe.

   Göttingen, den 15. September 2016

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2. Hiermit erkläre ich eidesstattlich, dass diese Dissertation selbständig und ohne unerlaubte Hilfe angefertigt wurde.

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