Journal für Kulturpflanzen, 66 (1). S. 81–89, 2014, ISSN 1867-0911, DOI: 10.5073/JfK.2014.03.01 Verlag Eugen Ulmer KG, Stuttgart

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Influence of repeated pyrethroid applications on the sensitivity of pyrethroid-resistant pollen beetles (*Meligethes aeneus* F.) and their offspring

Einfluss wiederholter Pyrethroid-Behandlung auf die Sensitivität des Rapsglanzkäfers (*Meligethes aeneus* F.) und dessen Nachkommen

Abstract

In the past, control of pollen beetles was mainly based on the application of synthetic pyrethroids. The frequent and indiscriminate use of this insecticide class resulted in a high selection pressure, ensuing in the formation of resistance, which has spread over the whole area of Germany and many European countries. Replacement by insecticides with other modes of action is limited as few alternative products are available.

To analyze the relative sensitivity of adult pollen beetles and their offspring to lambda-cyhalothrin, a field trial was conducted in 2012 which included two control plots without insecticide applications and two plots sprayed five times with lambda-cyhalothrin. The Adult-Vial-Test was used to analyze the sensitivity of the beetles when exposed to dosages from 0.015 to 0.375 µg lambda-cyhalothrin cm^{-2}. All samples were classified as resistant or highly resistant. Over the trial period fluctuations of the sensitivity were noticed. Only small differences occurred between the sensitivity of beetles from treated and untreated plots. However, the fluctuation of the sensitivity between successive sampling dates was smaller in treated plots.

New generation pollen beetles were caught by using photoeclectors and the resistance classified relative to their parent generation. In comparison to the sensitivity of the overwintered generation, new generation beetles showed lower LD_{50}-values. The number of larvae migrating to soil for pupation in plots treated with lambda-cyhalothrin, was 18% lower than in untreated plots. Similar differences occurred with the number of emerging adults of the new generation. This indicates a negligible effect of pyrethroid applications on population dynamics of resistant pollen beetles.

Key words: lambda-cyhalothrin, oilseed rape, population dynamic, insecticide resistance, temporal variability

Zusammenfassung

In der Vergangenheit erfolgte die Kontrolle von Rapsglanzkäfern hauptsächlich durch die Anwendung von synthetischen Pyrethroiden. Durch die häufige und einseitige Nutzung dieser Insektizidgruppe entstand ein hoher Selektionsdruck, der die Ausbildung einer Resistenz zur Folge hatte, die sich über ganz Deutschland und viele europäische Länder ausgebreitet hat. Das Ausweichen auf Insektizide mit anderen Wirkmechanismen ist nur bedingt möglich, da nur wenige solcher Produkte verfügbar sind.

Um die Sensitivität von adulten Rapsglanzkäfern und deren Nachkommen gegenüber lambda-cyhalothrin zu untersuchen, wurde 2012 ein Feldversuch mit zwei unbehandelten Kontrollparzellen und zwei fünfmal mit lambda-cyhalothrin behandelten Parzellen durchgeführt. Um die Sensitivität der Käfer, die Dosierungen von 0.015 bis

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Accepted
22 November 2013

0.375 µg lambda-cyhalothrin cm^{-2} ausgesetzt waren, zu überprüfen, wurde der Adult-Vial-Test genutzt. Alle Proben wurden als resistent oder hoch resistent eingestuft. Über den Versuchszeitraum wurden Schwankungen in der Sensitivität festgestellt. Es traten nur geringe Sensitivitätsunterschiede zwischen Käfern aus behandelten und unbehandelten Parzellen auf. Dennoch waren die Sensitivitätsschwankungen zwischen aufeinanderfolgenden Sammelterminen in den behandelten Parzellen geringer.

Die mittels Photoeklektoren gefangenen Jungkäfer wiesen ein ähnliches Resistenzniveau wie die Elterngeneration auf. Im Vergleich zur Sensitivität der überwinterten Generation zeigten die Jungkäfer geringere LD_{50}-Werte. Die Anzahl der zur Verpuppung in den Boden abwandernden Larven war in den mit lambda-cyhalothrin behandelten Parzellen 18% geringer als in den unbehandelten Parzellen. Ähnliche Unterschiede wurden bei der Anzahl der schlüpfenden Jungkäfer festgestellt. Dies deutet auf einen unerheblichen Effekt der Pyrethroid-Applikationen auf die Populationsdynamik von resistenten Rapsglanzkäfern hin.

Stichwörter: Lambda-cyhalothrin, Raps, Populationsdynamik, Insektizidresistenz, zeitliche Variabilität

Introduction

The pollen beetle, *Meligethes aeneus* F. (Col.: Nitidulidae) is a major pest on oilseed rape. Adults emerging from overwintering sites in spring immigrate to oilseed rape crops at temperatures exceeding 15°C (Müller, 1941; Fritzsche, 1957). To get access to pollen, they bite into the sepals and petals of buds, resulting in bud abortion (Burkhardt and von Lengerken, 1920; Friederichs, 1921). Plants are very susceptible to this damage particularly during the green bud stage (Slater et al., 2011). Yield losses up to 80% have been reported (Thieme and Gloyna, 2008).

For oviposition the pollen beetles prefer buds of 2 to 3 mm length (Nilsson, 1988a). After hatching the larvae undergo two stages of development in the buds and the open flowers before they fall to the ground and pupate in the soil (Burkhardt and von Lengerken, 1920). Beetles of the new generation emerging from the soil feed on different flowering plants before they seek for overwintering sites at the end of July (Müller, 1941).

In the past 20 years synthetic pyrethroids have been used extensively for control of pollen beetles and other oilseed rape pests in Europe. Because of the high value of harvested seeds and low application costs, insecticide application in a tank-mix with fungicides became very common. In addition prophylactic applications were supported by the low price of pyrethroids (Thieme et al., 2010). Pyrethroids are contact insecticides with broad-spectrum activity against sucking and chewing insects. They have a low to medium persistence but low toxicity for mammals.

The extensive and indiscriminate application of pyrethroids has resulted in a high selection pressure on pollen beetles and finally in resistance. Reduced sensitivity of pollen beetles to pyrethroids was first reported from northeastern France in 1999 (Nauen, 2005). Since this time the resistance to pyrethroids has spread all over Europe (Slater et al., 2011). In Germany, reduced effectiveness of pyrethroids against pollen beetles was recognized for the first time in 2001 in Rhineland-Palatinate (Thieme et al., 2010) and Schleswig-Holstein (Slater and McCaffery, 2006), and it has subsequently spread all over Germany (Thieme et al., 2010; Heimbach and Müller, 2013). While in 2005 more than 50% of the tested beetle populations were classified as very sensitive or sensitive, this percentage has decreased constantly over the last years and has declined to 0% in Germany since 2010. The percentage of beetles showing a high level of resistance increased from 6.7% in 2005 to 77.8% in 2012 (Müller and Heimbach, 2013). Switching to insecticides with different modes of action is only partly possible as only few effective products are available (Slater et al., 2011; Heimbach and Müller, 2013).

Due to the extensive use of pyrethroids and the extended overlapping occurrence of different insect pests in oilseed rape which often require two to four insecticide applications, a high selection pressure arises on other pests, too (Müller et al., 2008; Heimbach and Müller, 2011). The area-wide resistance monitoring which is being carried out by the Julius Kühn-Institut since 2005 has revealed first indications of resistance to pyrethroids in Northern Germany in the cabbage seedpod weevil (*Ceutorhynchus obstrictus*) attacking the pods and cabbage stem flea beetle (*Psylliodes chrysocephala*) attacking young plants (Heimbach and Müller, 2011; Müller and Heimbach, 2013).

Little is known if there is still a resistance selection on highly resistant populations and how the resistance status of pollen beetle varies during a season on a field. To evaluate the spatial-temporal within-field variability of sensitivity of pollen beetles to pyrethroids, resistance development was followed during the season on treated and control plots of a field.

In previous studies only short term effects of pyrethroids on beetle numbers up to one or two weeks after application or on the damage have been assessed (EPPO, 2004). In contrast, only little information is available on the influence of pyrethroid applications on the population dynamics of pollen beetles. In this study the effect of pyrethroid application to resistant pollen beetle populations on reproduction and abundance of new generation beetles was analyzed.

Materials and Methods

Field trial

A field trial was conducted in 2012 in the southeast of Lower Saxony near Hötzum (district of Wolfenbüttel). The previous crop on the selected field was spring barley and in the immediate vicinity winter wheat and some field with sugar beet were located. Within a radius of 2 km no further oilseed rape existed. A forest was in a distance of 1.5 km in northern direction. The field of

6.3 ha was divided in four plots of approximately 1.5 ha each (Fig. 1) with directly bordering plots. The plots B1 and B2 were sprayed five times with the pyrethroid insecticide Karate® Zeon (a.s. 100 g/l lambda-cyhalothrin) in about weekly intervals to provoke high resistance selection. The application rate was 75 ml Karate® Zeon in 300 l water ha^{-1} each time. This corresponds to 7.5 g a.s. ha^{-1}. No other insecticides were applied during spring and summer 2012. The plots K1 and K2 served as controls and were not treated with insecticides. Drift of the pesticide application might have reached only the outer few meters of control plots. The headlands, which were approximately 12 m wide, were treated according to the adjacent plot. No rainfall was recorded within 24 h after each of the pyrethroid application.

Living pollen beetles were sampled regularly for the sensitivity tests in the laboratory. They were collected along two tramlines in the plot centre until a sufficient high number (at least 300 beetles per plot) for the resistance tests was sampled by beating inflorescences into a funnel with a perforated plastic bag attached. By this it was ensured that sampling areas in treated and control plots were in at least 14 m distance to differently treated plots which is more than 2 m used in EPPO Standards (EPPO, 2004). Beetles of the overwintered generation were collected on eight occasions in treated and untreated plots a few days before and after each insecticide application (Tab. 1). Before testing the beetles were kept in perforated plastic bags in a climatic chamber at 10°C and 16:8-hours L:D for a maximum of one week. They were provided with water and oilseed rape inflorescences from the untreated headlands as food.

To catch the emerging new beetle generation eight photoeclectors (each covering 0.25 m^2 of ground) were established on 23 March in the sampling area of each plot (four near to each tramline). The digging of the soil rings of the photoeclectors into the soil was carried out very carefully not to disturb the oilseed rape plants within and outside the photoeclector area. They were set up at a distance of 35 m to each other and approximately 0.5 to 1 m apart from the tramline.

Additionally eight plastic containers (17 cm × 12.5 cm) filled with a 10% sodium benzoate water solution for conservation were placed on 24 April close to each photoeclector in all plots to collect the larvae dropping off the plants. The containers were emptied weekly from 3 May to 23 May and the larvae were counted. The mortality of larvae and pupae in the soil was inferred by relating the number of emerged beetles to the number of captured larvae.

The photoeclectors were closed by a fabric tent on 16 May (BBCH 69 (Weber and Bleiholder, 1990; Lancashire et al., 1991)), before emergence of the new beetle generation started. To catch the beetles alive, a perforated plastic bag was fixed to the opening on top of the photoeclectors. The plastic bags were checked once a week in May and from the beginning of June every three to four days. First new generation beetles were collected on 14 June in the bags of the photoeclectors. After this the bags were emptied every two to three days. The beetles were maintained alive in a climatic chamber, similar to the overwintered adults. They were supplied with pollen as food. Beetles trapped during the first sampling period (14 June until 18 June) and the last sampling period (18 June until 25 June) were pooled within each variant to obtain sufficient beetles for the sensitivity test.

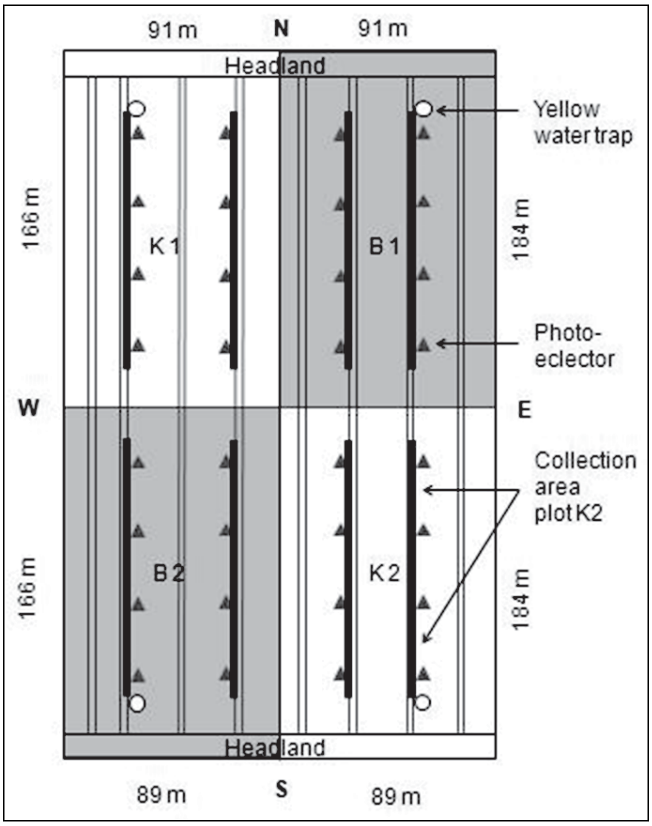

Fig. 1. Layout of the field trial in Hötzum; B1 and B2 = treated with Karate® Zeon, K1 and K2 = untreated.
Skizze des Versuchsschlages in Hötzum; B1 und B2 = behandelt mit Karate® Zeon, K1 und K2 = unbehandelt.

Laboratory tests

All laboratory experiments focused on lambda-cyhalothrin, the active substance of the field-applied pyrethroid Karate® Zeon. The Adult-Vial-Test according to IRAC-Method No. 11 for pollen beetles (IRAC, 2009) was used to determine the sensitivity of the overwintered beetles and the new generation of beetles in the laboratory. In this test the inner walls of glass vials are coated with increasing rates of pyrethroids. In each vial, 10 beetles are exposed at 20°C and constant lighting. Contrary to the IRAC-Method, only the number of affected beetles obtained from the 5-hour assessment was used for the evaluation. Because pyrethroids show a fast effect, the assessment after 5 hours seems to be appropriate and has been used frequently (e.g. Heimbach and Müller, 2013). A control and five different dosages were used in the Adult-Vial-Test, ranged from 0.015 µg lambda-cyhalothrin cm^{-2}, which corresponds to 20% of the registered field ap-

Tab. 1. Sampling plan of pollen beetles in the field trial in Hötzum, dates of insecticide applications in plot B1 and B2 (application rate 75 ml ha^{-1}) and respective growth stages (BBCH)
Übersicht der durchgeführten Rapsglanzkäfersammlungen in Hötzum, Termine der Insektizidapplikation in den Parzellen B1 und B2 (Aufwandmenge 75 ml ha^{-1}) sowie Entwicklungsstadien (BBCH)

Date	Measure	BBCH
20 March 2012	1st application of Karate® Zeon	51
27 March 2012	1st collection of overwintered pollen beetles	53
28 March 2012	2nd application of Karate® Zeon	53
02 April 2012	2nd collection of overwintered pollen beetles	53–55
10 April 2012	3rd collection of overwintered pollen beetles	55–57
16 April 2012	3rd application of Karate® Zeon	60
18 April 2012	4th collection of overwintered pollen beetles	60
24 April 2012	5th collection of overwintered pollen beetles	60–62
27 April 2012	4th application of Karate® Zeon	63
30 April 2012	6th collection of overwintered pollen beetles	65
09 May 2012	7th collection of overwintered pollen beetles	67
14 May 2012	5th application of Karate® Zeon	67
16 May 2012	8th collection of overwintered pollen beetles	69
14–25 June 2012	Collection of new pollen beetle generation	80–81

plication rate in Germany, to 0.375 µg lambda-cyhalothrin cm^{-2} (corresponding to 500% of the field rate) (Tab. 2).

Statistics
The calculation of dose-response relationships and LD_{50}-values was performed by applying probit analysis, using the program POLO PLUS 2.0 (LeOra Software). Because the LD_{50}-value is usually more exact and less variable than LD_{90}-values, in this study only LD_{50}-values are reported. Control mortality in all biotests was below 4%; therefore no control mortality correction was done.

To compare calculated curve progressions of the dose-response relationships of two populations, the hypotheses of equality and parallelism of the curves were tested by using the program POLO PLUS 2.0. If the hypotheses are rejected, a significant difference between the sensitivity of the two populations compared exists. Further, if the confidence intervals do not overlap, the populations differ significantly.

Tab. 2. Dosages used in the Adult-Vial-Test corresponding to the field application rate of Karate® Zeon registered in Germany
Im Adult-Vial-Test verwendete Dosierungen im Verhältnis zur in Deutschland zugelassenen Feldaufwandmenge

lambda-cyhalothrin (µg cm^{-2})	Corresponding to field application rate (%)
0.0150	20
0.0375	50
0.0750	100
0.1875	250
0.3750	500

Results

The first insecticide application of lambda-cyhalothrin on 20 March was targeted primarily to control stem weevils *Ceutorhynchus napi* and *C. pallidactylus*. At this time only low numbers of pollen beetles were present in the oilseed rape plots. The first sample of pollen beetles was collected in the plots on 27 March when beetle numbers increased. Additional samples of overwintered pollen beetles were collected at weekly intervals until end of flowering in BBCH 69 (Tab. 1). Over the whole trial period an estimated average of two to three beetles per main stem was recorded in untreated as well as in treated plots. Oviposition of pollen beetles was first detected on 11 April during the sensitivity test in the laboratory when females caught on 10 April laid their eggs to the inner surface of the test glasses.

Based on the mortality of overwintered pollen beetles in the Adult-Vial-Test, 81.25% of samples were classified as highly resistant (according to Heimbach and Müller, 2013). This classification means that more than 50% of beetles survived at 0.075 µg lambda-cyhalothrin cm^{-2} or at higher doses. The remaining 18.75% of the samples were classified as resistant (11–50% survival at 0.075 µg lambda-cyhalothrin cm^{-2} or at higher dose). Similarly, 75% of the new beetle generation was classified as highly resistant and 25% as resistant. These results were confirmed by the LD_{50}-values (Tab. 3).

Temporal variability of pollen beetle sensitivity
To examine the temporal variability of the sensitivity of overwintered pollen beetles during the trial period, the LD_{50}-values of beetles collected on eight sampling dates (Tab. 1) were compared in each plot. The LD_{50}-values varied widely over time, with conspicuous fluctuations especially in the untreated plots K1 and K2 (Fig. 2).

The highest LD_{50}-values of beetles were found in three of the four plots at the first sampling date. At this time favourable weather conditions with a maximum daily temperature of around 20°C encouraged the main immigration of beetles. This was confirmed by yellow water trap samples. The lowest LD_{50}-values were determined in the plots K1 and K2 on the second sampling date (Fig. 2). In plot K1, the sensitivity of beetles differed significantly between the second and the third sampling date, between the fifth and the sixth sampling date and between the sixth and the seventh sampling date. Similar results were obtained from plot K2. As an example, the dose-response relationships of pollen beetles collected on the fifth (24 April) and the sixth sampling date (30 April) were compared in plots K1 and K2 (Fig. 3). Based on the analysis of equality and parallelism of the curves the hypothesis of equality was rejected. Consequently, a significant difference between the sensitivity of the two populations compared exists (Tab. 4).

Tab. 3. Classification of all overwintered pollen beetle samples and of the new generation in the resistance classes resistant and highly resistant as well as LD_{50}-values (µg lambda-cyhalothrin cm^{-2})
Einteilung aller überwinterten Rapsglanzkäfer- und Jungkäferproben in die Resistenzklassen resistent und hoch resistent sowie LD_{50}-Werte (µg lambda-cyhalothrin cm^{-2})

Resistance class	Overwintered pollen beetles (n = 8 sampling dates × 4 plots)	New pollen beetle generation (n = 2 sampling dates × 4 plots)
Resistant	18.75%	25.00%
LD_{50} (µg cm^{-2})	0.044 to 0.096 (n = 31)	0.062 to 0.076
Highly resistant	81.25%	75.00%
LD_{50} (µg cm^{-2})	0.088 to 0.247 (n = 31)	0.078 to 0.111

In contrast to the untreated plots K1 and K2, analyses of the LD_{50}-values of treated plots B1 and B2 showed no significant differences between individual sampling dates (Fig. 2).

Sensitivity of the overwintered and new pollen beetle generation
For the comparison of the sensitivity of the overwintered and new pollen beetle generation, the LD_{50}-values calculated from overwintered beetles which were collected on 24 April and of the new beetle generation emerging ap-

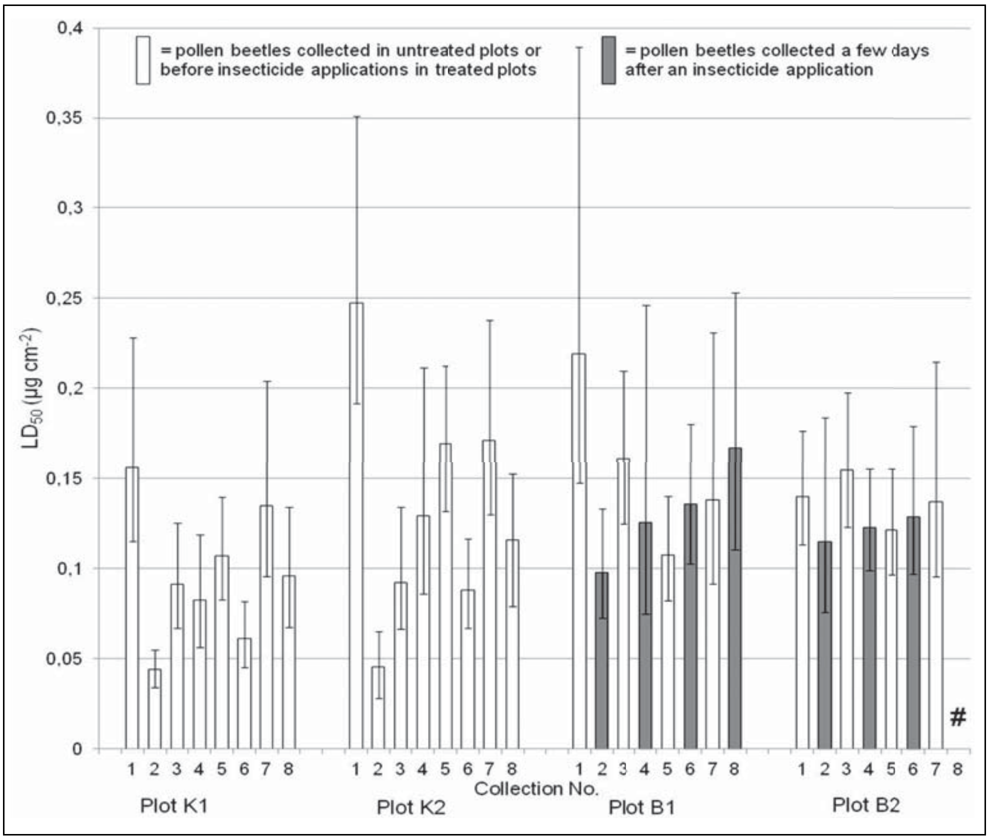

Fig. 2. LD_{50}-values (µg lambda-cyhalothrin cm^{-2}) and 95% confidence intervals of overwintered pollen beetles collected at eight sampling dates in untreated plots K1 and K2 and lambda-cyhalothrin treated plots B1 and B2, assessment after 5 hours; # = calculation of LD_{50} not possible.
LD_{50}-Werte (µg lambda-cyhalothrin cm^{-2}) und 95% Konfidenzintervalle der überwinterten Rapsglanzkäfer, die an acht Terminen in den unbehandelten Kontrollparzellen K1 und K2 und den mit lambda-cyhalothrin behandelten Parzellen B1 und B2 gesammelt wurden, Bonitur nach 5 Stunden; # = Berechnung des LD_{50}-Wertes nicht möglich.

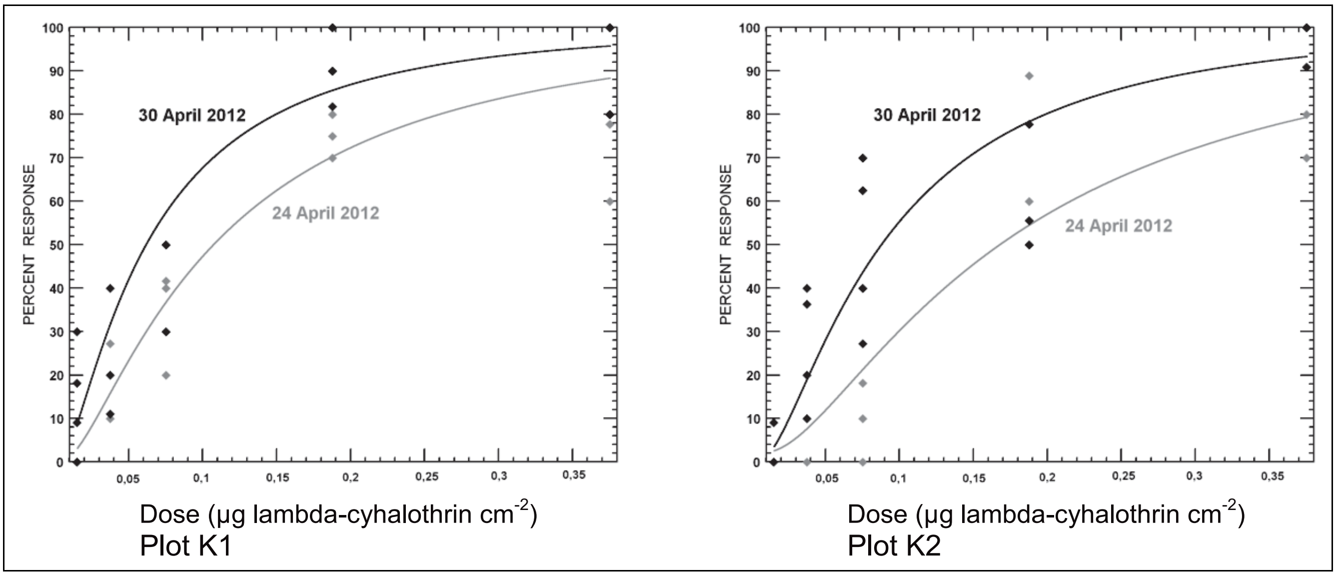

Fig. 3. Dose-response relationships of lambda-cyhalothrin for overwintered pollen beetles collected on 24 April and 30 April in plots K1 and K2 in Hötzum, assessment after 5 hours.
Dosis-Wirkungsbeziehung von lambda-cyhalothrin von überwinterten Rapsglanzkäfer, die am 24. April und 30. April in den Parzellen K1 und K2 in Hötzum gesammelt wurden, Bonitur nach 5 Stunden.

Tab. 4. LD_{50}**-values (µg lambda-cyhalothrin cm**$^{-2}$**), lower and upper 95% confidence interval (CI), Chi**^{2}**, degrees of freedom and heterogeneity as well as the hypothesis of equality and parallelism for overwintered pollen beetle samples collected in plot K1 and K2 on 24 April and 30 April 2012**
LD_{50}-Werte (µg lambda-cyhalothrin cm^{-2}), untere und obere Konfidenzintervalle (CI), Chi^2, Freiheitsgrade und Heterogenität sowie Hypothesen der Gleichheit und Parallelität für überwinterte Rapsglanzkäferproben, die am 24. April und 30. April 2012 in den Parzellen K1 und K2 gesammelt wurden

Date	Plot	LD_{50} (µg cm^{-2})	Lower CI	Upper CI	Chi^2	Degrees of freedom	Heterogeneity
24 April 2012	K1	0.10697	0.08270	0.13958	20.976	18	1.1653
	K2	0.16921	0.13208	0.21232	15.404	17	0.906
30 April 2012	K1	0.06139	0.04553	0.08168	24.483	18	1.3602
	K2	0.08799	0.06718	0.11654	21.520	18	1.1956
Hypothesis of equality	Rejected						
Hypothesis of parallelism	Not rejected						

proximately eight weeks later between 21 to 25 June were used. The development period from oviposition of the overwintered beetle generation to the hatching of the new generation out of the soil depends on the weather conditions and usually lasts seven to eight weeks (Friederichs, 1921); thus the overwintered beetles might have been the parents of the new generation. In all plots the LD_{50}-values of the new beetle generation were lower than LD_{50}-values of overwintered beetles (Fig. 4). The LD_{50}-values of the overwintered and new beetle generation differed significantly for the untreated plots K1 and K2. No significant difference was found between the overwintered and new beetle generation in plot B1 and B2.

Population growth of pollen beetle in insecticide-treated and untreated plots

In the untreated plots the cumulative number of larvae dropping to the ground was higher than in the treated plots (Tab. 5). In total, 3269 larvae per m^2 and 3747 larvae per m^2 were counted in plot K1 and in plot K2, respectively. The density of larvae in the treated plots was lower.

The first pollen beetles of the new generation were trapped in photoeclectors on 14 June. In plot K2 the higher number of larvae dropping was parallel to a higher number of emerging beetles (Fig. 5, Tab. 5). During the first sampling period from 14 to 18 June approximately 380 beetles per m^2 emerged from plot K2, while numbers

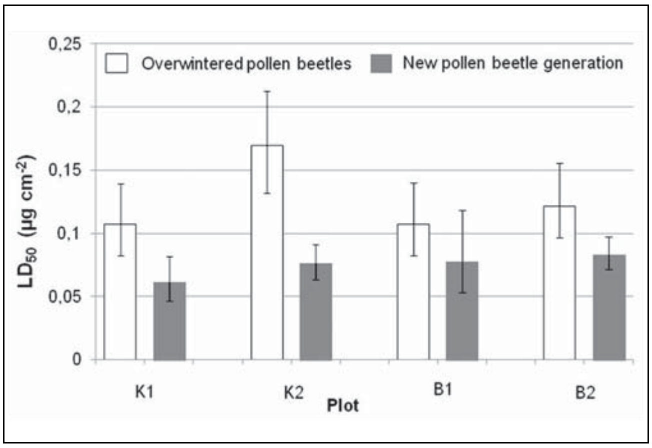

Fig. 4. LD_{50}-values (µg lambda-cyhalothrin cm^{-2}) and 95% confidence intervals of overwintered pollen beetle samples collected on 24 April and of the new pollen beetle generation caught in photo-eclectors from 21–25 June in a field trial in Hötzum.
LD_{50}-Werte (µg lambda-cyhalothrin cm^{-2}) und 95% Konfidenzintervalle von überwinterten Rapsglanzkäfer, die am 24. April gesammelt wurden sowie der Jungkäfer, die vom 21.–25. Juni mit Photoeklektoren im Feldversuch in Hötzum gefangen wurden.

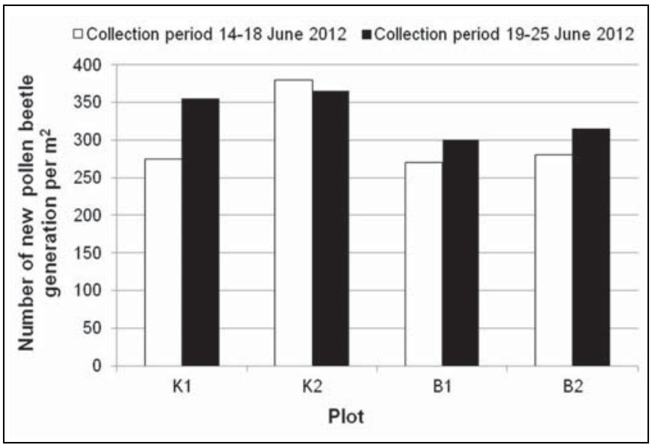

Fig. 5. Number of the new pollen beetle generation per m² emerging from treated and untreated plots from 14–18 June (white) and from 19–25 June (black); no standard deviation because pollen beetles emerging within each plot were pooled.
Anzahl der im Zeitraum vom 14.–18. Juni (weiß) und vom 19.–25. Juni (schwarz) in den behandelten und unbehandelten Parzellen geschlüpften Jungkäfer pro m²; keine Angabe der Standardabweichung, da die in den Parzellen geschlüpften Rapsglanzkäfer gepoolt wurden.

Tab. 5. Total number and standard deviation of larvae per m² dropping to the ground from 3 May to 23 May in all plots, and total number of new pollen beetle generation per m² emerging from 14 June to 25 June and percentage of mortality of larvae and pupae (%); B1 and B2 = treated five times with 75 ml ha^{-1} Karate® Zeon, K1 and K2 = untreated; no standard deviation for the new beetle generation because emerging beetles within each plot were pooled
Anzahl und Standardabweichung der vom 3. Mai bis 23. Mai in allen Parzellen zu Boden gefallenen Larven pro m² sowie Anzahl der vom 14. Juni bis 23. Juni geschlüpften Jungkäfer pro m² und prozentuale Larven- und Puppenmortalität (%); B1 und B2 = fünfmalige Behandlung mit 75 ml ha^{-1} Karate® Zeon, K1 und K2 = unbehandelt; keine Angabe der Standardabweichung für Jungkäfer, da die in den Parzellen geschlüpften Käfer gepoolt wurden

Plot	Total number of larvae per m² ± standard deviation	Total number of new pollen beetle generation per m²	Mortality of larvae and pupae (%)
K1	3268.9 ± 1148.6	630	80.7
K2	3747.1 ± 1052.3	745	80.1
B1	3123.5 ± 639.3	570	81.8
B2	2647.1 ± 800.9	595	77.5

of beetles emerging from the other plots varied between 270 and 280 beetles per m². During the sampling period from 19 to 25 June, the number of beetles caught in untreated plots was higher (355 and 365 beetles per m² in plot K1 and K2, respectively) than in treated plots (300 and 315 beetles per m² in plot B1 and B2, respectively). When the number of adults of the new beetle generation was related to the number of larvae, mean mortality of larvae and pupae in soil was 80.4% and 79.7% in the untreated and treated plots, respectively (Tab. 5). In the plots treated five times with lambda-cyhalothrin, the number of larvae dropping to the soil for pupation was about 18% lower than in the untreated plots. Similar differences were observed for the emerging new beetle generation (15% less in treated plots).

Discussion

In this study the sensitivity of overwintered pyrethroid-resistant pollen beetles to the pyrethroid lambda-cyhalothrin varied widely between successive sampling dates in the course of the trial period from March to May. Surprisingly these changes were more pronounced in the untreated plots and differed significantly between individual sampling dates. In plots treated with Karate® Zeon no significant differences were observed between sampling dates. In all plots the lowest LD_{50} was recorded on the second sampling date on 2 April. The LD_{50}-values were markedly lower than on the third sampling date, but the difference was only significant in the untreated plots.

A high within-season temporal variability in the sensitivity of overwintered pollen beetles to lambda-cyhalothrin was also detected in samples of beetles collected from another field trial located 10 km away from Hötzum in Wendhausen in 2012 (data not reported here). Further, HEIMBACH and MÜLLER (2010) reported a temporal variability in the sensitivity of pollen beetles within one season in fields of oilseed rape near Schwerin. This variability over time might be a result of the continuous migration of beetles between differently treated plots within the experimental field as well as of immigration or emigration of beetles between fields.

In addition, sensitivity of individual pollen beetles may change with age, physiological condition and reproductive status. Egg formation and egg laying might have affected the fitness and sensitivity of females. Thus the low LD_{50}-value observed on the second sampling date in all plots at Hötzum may be connected with the oviposition period. The first eggs were observed in the Adult-Vial-Test from beetles collected on 10 April. Therefore, the initiation of oviposition in beetles collected on 2 April might have increased their sensitivity. Similarly, sensitivity of males might increase because of their mating activity. Some reports indicate a higher susceptibility of male insects to pesticides than of females, e.g. Pielou and Glasser (1952), Respicio and Forgash (1984). Unfortunately, the sensitivity of male and female beetles was not checked separately in our experiments.

In addition, the amount and quality of food ingested by the beetles has a strong influence on the physiological condition, and might have affected fitness and sensitivity. A higher sensitivity of worker honey bees to pesticides fed with qualitatively or quantitatively inadequate pollen has been reported by Wahl and Ulm (1983).

The differences between the sensitivity of pollen beetles of treated and untreated plots probably may have been caused by repeated applications of lambda-cyhalothrin in the treated plots B1 and B2. Beetles were selected for low sensitivity to this insecticide in treated plots, whereas in control plots sensitive beetles survived resulting in lower LD_{50}-values. But it should be taken into account that the actively flying pollen beetles could easily move between adjacent treated and untreated plots, even at the large plot size of 1.5 ha used in our experiments. Stechmann and Schütte (1976) showed that pollen beetles are able to fly mean distances of 1 to 3 kilometers per day. Therefore beetles immigrating from surrounding fields might have mixed with beetles present in the plots and affected the sensitivity of the population as well.

Beetles of the new generation emerging in June turned out to be more sensitive to lambda-cyhalothrin than overwintered beetles. The LD_{50}-values of overwintered and new generation beetles differed significantly. The period from the oviposition of overwintered beetles to the hatching of new generation beetles depends on weather conditions and usually lasts seven to eight weeks (Friederichs, 1921); thus the overwintered beetle population tested by the Adult-Vial-Test might have served as parent population of the new generation.

After emergence the new beetle generation was not exposed to resistance selection by pyrethroid applications. Further, unlike the overwintered beetles, they had not been selected for high fitness over a period of several months by environmental toxins or other stress factors such as harsh winter conditions. Different sensitivity of honey bees in the age of two to eight days towards DDT has been reported by Graves and Mackensen (1965). In their experiments the age-dependent LD_{50}-values of young honey bees differed up to three times, with two day old bees being most sensitive. These findings were confirmed by Ladas (1972). In studies comparing the sensitivity of newly emerged adults and more than one year old adults of the Carabid beetle *Harpalus aeneus* to the organophosphorus pesticide thionazin, mature beetles were found to be less susceptible than newly emerged beetles (Critchley, 1972). These findings were explained by the higher migration activity of the younger individuals. As consequence of their high activity they may have taken up a higher dosage of the pesticide.

Another reason for the higher sensitivity of the new generation of pollen beetles might be that they were introduced into the Adult-Vial-Test shortly after emergence, after only one to five days of feeding possibilities before the test. Consequently they could accumulate only small fat reserves which might have increased the sensitivity to the insecticide. A similar effect has been reported by Frey (1950) who found that young pollen beetles were more resistant to the insecticide powder "Kümex" (a.i. rotenone) when they had developed a pronounced fat body. In our experiments the average fresh weight of overwintered beetles and new generation beetles showed only little difference (3% lower weight of the new beetle generation) but their fat bodies might have differed to a larger extend.

The extensive application of five pyrethroid sprays during the developmental period of pollen beetle eggs and larvae showed only a negligible effect on the number of new generation beetles in our experiment. In total the number of both the mature larvae dropping to the soil and the emerging beetles was only slightly higher in untreated plots than in treated plots. The reason for the lower number of new generation beetles in treated plots might be a slightly reduced abundance of overwintered beetles in treated plots due to the insecticide sprays, which was not exactly measured in the experiment, or might be caused by sublethal effects of the insecticide applications on oviposition and larval development. Insecticidal effects on the larvae seem unlikely because they showed a high level of resistance to lambda-cyhalothrin in our laboratory experiments performed with glass fiber filters coated with different dosages of this active. Three populations of larvae from two locations showed LD_{50}-values between 0.22 and 0.44 $\mu g\ cm^{-2}$ (details not reported here). In addition oviposition might have been reduced by a repellent effect of the applied pyrethroid. It is well known that pyrethroids have a repellent effect on aphids and can prevent probing or colonization on plants (Rieckmann and Wüstefeld, 2006; Thieme et al., 2009). Repellency might also have affected the behaviour of pollen beetles by avoiding the treated plots B1 and B2, resulting in fewer eggs and consequently in lower numbers of larvae and new generation beetles.

The mean mortality of larvae and pupae from dropping to the soil to hatching of new generation beetles in treated and untreated plots was on a high level (on average 80%). Parasitism of larvae was observed but not analyzed in detail at Hötzum. Parasitoids such as *Tersilochus heterocerus* (Thomson), *Phradis morionellus* (Holmgren) and *P. interstitialis* (Thomson) are known as important mortality factors of larvae (Nilsson, 1988b; Nilsson and Andreasson, 1994; Ulber et al., 2010). The application of insecticides during flowering of oilseed rape can also affect parasitization (Nitzsche, 1998). Consequently, the insecticide application in treated plots may have reduced parasitoid numbers, leading to a higher number of surviving larvae and new generation beetles. However, in this study the number of larvae and emerging new generation beetles was higher in untreated plots compared to treated plots indicating no negative effects of pyrethroid application on parasitoids, which might similar to pollen beetles have also developed resistance to this insecticidal group. Some studies have shown that some parasitoids can develop resistance to insecticides and subsequently survive field application rates of insecticides (e.g. Baker and Weaver, 1993; Liu et al., 2003).

Acknowledgements

Thanks to the staff of the Julius Kühn-Institut Braunschweig especially to F. Zelmansky, U. Busch and R. Ionascu for technical assistance on the field and in the laboratory.

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