

**Factors regulating the population dynamics and
damage potential of pollen beetle (*Meligethes
aeneus* F.) on crops of oilseed rape**

Dissertation
zur Erlangung des Doktorgrades
der Fakultät für Agrarwissenschaften
der Georg-August-Universität Göttingen

vorgelegt von
Marie-Luise Tölle
geboren in Gifhorn

Göttingen, Mai 2014

D 7

1. Referentin/Referent: Prof. Dr. Stefan Vidal

2. Korreferentin/Korreferent: Prof. Dr. Andreas von Tiedemann

Tag der mündlichen Prüfung: 12.05.2011

Table of contents

	page
Chapter I	
General introduction	1
The pest: <i>Meligethes aeneus</i>	2
Factors influencing the population dynamics of pollen beetle	3
Possible effects of insecticides on population growth and damage of pollen beetle	4
Parasitoids and parasitisation of pollen beetle	5
Trap cropping in oilseed rape	6
References	7
Chapter II	
Cultivar and phenology of winter oilseed rape affect the abundance and reproduction of <i>Meligethes aeneus</i> (Fabricius)	11
Abstract	11
Introduction.....	11
Materials and methods	12
Results	15
Discussion	21
Acknowledgements	24
References	25
Chapter III	
Effects of insecticides on abundance of pollen beetles (<i>Meligethes aeneus</i> Fabricius) and yields in oilseed rape	27
Abstract	27
Introduction.....	27
Materials and methods	28
Results	30
Discussion	32
Acknowledgements	34
References	34
Chapter IV	
Effects of site-specific factors and insecticide application on parasitisation of <i>Meligethes aeneus</i> (Fabricius)	37
Abstract	37
Introduction.....	37
Materials and methods	38
Results	39
Discussion	44
Acknowledgements	46
References	47

Table of contents

	page
Chapter V	
Effect of trap cropping and spatial targeting of insecticide application on the abundance and spatio-temporal distribution of pollen beetle (<i>Meligethes aeneus</i> Fabricius) on oilseed rape	49
Abstract	49
Introduction.....	49
Materials and methods	50
Results	52
Discussion.....	57
Acknowledgements	59
References.....	59
Chapter IV	
General discussion	61
Factors regulating the population dynamics and population growth	61
Effects of insecticide application on population growth and damage.....	65
Effects of site-specific factors and insecticide application on parasitisation.....	66
Effect of trap crops to control pollen beetle	67
Approaches of integrating pest management strategies in oilseed rape	68
References.....	69
Summary.....	73
Acknowledgements.....	75
Curriculum vitae.....	76
Declarations/ Erklärungen	77

General introduction

Oilseed rape (*Brassica napus* L. var. *oleifera* Metzg.) (*Brassicaceae*) was the fourth biggest crop harvested in Europe in 2009 (FAO, 2011). In 1973, fewer erucic acid cultivars were established for the market and caused an increase in the cultivation area of oilseed rape in Germany (Brauer, 2007). The breeding of cultivars with low glucosinolate content advanced the production of oilseed rape for animal food. In addition, it is grown for oil, human consumption and renewable energy resources (Brauer, 2007). In 2010, 1.46 Million ha of oilseed rape were grown in Germany (UFOP, 2011). With an increasing area cultivated with oilseed rape, the reproductive success of oilseed rape pests, including the pollen beetle *Meligethes aeneus* (Fabricius) (Col.: Nitidulidae), have increased as well (Hokkanen, 2000; Alford, 2003). The pollen beetle causes high yield losses in oilseed rape, occasionally exceeding 80% (Ahuja et al., 2010). From 2007 until 2011 pesticide use has steadily increased in oilseed rape crops in Germany, particularly due to insecticide applications (Freier et al., 2012). The extensive use of pyrethroids for more than 20 years and the overlapping exposure of different oil seed rape pests caused resistance of *M. aeneus* against pyrethroid compounds (Zlof, 2008). Pyrethroid resistance has been recorded in several countries in Europe, including Germany (Heimbach et al., 2006), Denmark (Hansen, 2003), Sweden (Kazachkova et al., 2007), France (Délès, 2008), Finland (Tiilikainen & Hokkanen, 2008) and Poland (Wegorek & Zamoyska, 2008) in recent years.

To reduce the application of synthetic insecticides in oilseed rape integrated pest management strategies are needed (Cook & Denholm, 2008). Knowledge on trap cropping and conservation biocontrol in oilseed rape has been collected for years (Hokkanen, 1991; Cook et al., 2006; Ulber et al., 2010b), but further information including the biology and behaviour of the pollen beetle are necessary. Particularly, the influence of local climate conditions, site-specific factors and agricultural methods have to be included for a successful pest management strategy (Evans & Scarisbrick, 1994). For this, information about the mortality factors including the biology, population development that reduce the damage of the pollen beetle will be helpful. Little is known about the influence of different winter oilseed rape cultivars on the population growth of the pollen beetle.

The main objectives of this study are:

- (1) to investigate the effect of inflorescence stages and phenology of four winter oilseed rape (*B. napus*) cultivars and parasitism by hymenopterous parasitoids on the population growth of *M. aeneus*
- (2) to investigate the influence of insecticides on population growth and damage of the pollen beetle in field experiments
- (3) to analyse site-specific factors and insecticide treatment on parasitism by parasitoids as a mortality factor on population growth of the pollen beetle
- (4) to study the effect of trap cropping and insecticide application on spatial-within field distribution of the pollen beetle

The pest: *Meligethes aeneus*

M. aeneus occurs on many plants of different families, but mainly on brassicaceous crops (Free & Williams, 1978). When the air temperature reaches 12°C the pollen beetles fly short distances after hibernation and feed on pollen and nectar from a large variety of plants (Fritzsche, 1957b). They fly long distances when temperatures exceed 15°C. The females start maturation feeding on plants of different family or immediately on oilseed rape in spring. It takes about 10-14 days at 15°C or 6-10 days at over 20°C for the ovaries of female pollen beetles to mature (Fritzsche, 1957b). The adult beetles damage the buds by eating anthers and the ovary (Nilsson, 1988a). This can cause blind stalks due to bud abortion resulting in yield loss (Winfield, 1961).

The sex ratio of male to female is mostly 1:1 in fields of winter and spring oilseed rape (Free & Williams, 1979). Eggs are laid in the buds of Brassica plants preferably 2-3mm in size (Fritzsche, 1957b; Ekblom & Borg, 1996). Total egg production per female of the pollen beetle depends on temperature and relative humidity (Fritzsche, 1957b). According to a relative humidity of 95% the egg production increases from 78 eggs/female at 15-16°C to 211 eggs/female at 20-22°C and decreases at 27°C to 206 eggs/female. Nilsson (1988c) concluded that during her lifetime a female can produce 200-300 eggs at favourable weather conditions. Depending on temperature and relative humidity different development times for eggs were reported varying from 2 to 12 days (Scherney, 1953; Fritzsche, 1957b; Bromand, 1983; Nielsen & Axelsen, 1988).

Two larval instars occur during *M. aeneus* development (Osborne, 1965; Nilsson, 1988b). The first instar larvae feed on pollen in the bud, and the older larvae feed also on open buds and flowers (Williams & Free, 1978). Mobility of older larvae is enhanced by moving from flower to flower on one plant. Only at high densities they cause damage by ovary feeding of buds. In laboratory experiments, the developmental times for first instar larvae and second instar larvae are 2-10 and 3-20 days, respectively, depending on temperature and humidity regimes (Scherney, 1953; Fritzsche, 1957b; Bromand, 1983; Nielsen & Axelsen, 1988). By the end of the second instar the larvae fall to the ground to pupate in the upper soil layer (Fritzsche, 1957b). Development of pupation of pollen beetle takes about 10-18 days (Nolte, 1954; Fritzsche, 1957b). The optimal conditions for development of egg maturity, oviposition and larval development of the pollen beetle lie within a temperature range of 20-27°C and at a relative humidity of 95%, as the adult beetles showed the highest activity at these conditions as well.

The newly emerged beetles feed on pollen from brassicaceous and other plant families (Müller, 1941a). They start to locate their hibernation sites in August. The pollen beetles overwinter in moist mould in the upper 5cm layer under a moderately thick layer of leaves in deciduous forests (Müller, 1941b). The overwintering sites are exposed on a hill-site up to 400m into the forest. The spermatogenesis of male beetles lasts from August until November, in contrast to the females, whose ovaries mature after leaving the hibernation sites (Müller, 1941b). There is just one generation per year (Fritzsche, 1957b).

Factors influencing the population dynamics of pollen beetle

The population dynamics of the pollen beetle depends on several factors: Pollen beetle location on host plant, synchronisation of pest and host, available buds for oviposition, and abiotic conditions, among many other examples (Nilsson, 1994; Ekbom & Borg, 1996; Williams & Cook, 2010).

Oilseed rape is grown mostly as an annual break crop in a cereal rotation and is sown in a different field in each successive autumn or spring (Williams & Cook, 2010). So it is essential for the pollen beetle to search for the host plants after emerging from the hibernation sites in spring (Fritzsche, 1957b). The pollen beetles are strong flyers and cover distances from 200-300m in 2 hours and up to 13.5km in 10 days (Taimr et al., 1967). They locate their host plants by using upwind-anemotaxis (Williams et al., 2007b). The adult beetles are strongly attracted by the colour yellow, for example of yellow water traps, but when the crops begin to flower they prefer the flowering plants (Nolte, 1959; Hiisaar et al., 2003). Petals of flowers marked with a small black dot to simulate the presence of adult beetles, stimulate pollen beetles to land (Free & Williams, 1978). Plant growth stage influences the spatial distribution of pollen beetles within a field (Frearson et al., 2005). In two-choice-tests conducted in a polytunnel arena, they prefer plants in early-flower to those in bud stage (Cook et al., 2006). In addition to colour stimuli, the adult beetles respond to olfactory cues (Williams & Cook, 2010). They respond to volatiles of rape leaves, stems and buds, as well as to odours of the flower. Many components of the odour of flowering oilseed rape attract the pollen beetle, including isoprenoids and derivatives of amino acids, such as the isothiocyanates and nitriles, and fatty acids (Williams & Cook, 2010). Cook et al. (2002) reported that in a linear track olfactometer the males and females were significantly more attracted to the whole oilseed rape flowers, anthers and pollen compared with a blank air control, indicating that the beetles can locate their host over short distances. Over a longer distance of 20 m, pollen beetles were sampled in yellow water traps, baited with extracts of oilseed rape flowers (Evans & Allen-Williams, 1994). It seems that they use oilseed rape floral odours as cues in resource location over various distances (Cook et al., 2002).

Another way to influence the population development may be the use of oilseed rape cultivars with an early flowering. The phenological stages of the host and insect must synchronize (Singh & Singh, 2005). For example, early flowering could provide the best way to evade the attack by a pest. It has been shown that the plant development stage manipulate the behaviour and the migration of the adult pollen beetle in oilseed rape crops (Frearson et al., 2005). The number of buds available oviposition is reduced in an advanced development stage of the oilseed rape plant (Nolte, 1954; Ekbom & Borg, 1996), indicating a potential impact of the phenology stage of host plant on the population growth of the pollen beetle (Nilsson, 1994). Nielsen and Axelsen (1988) assumed that eggs laid late in the oviposition period will result in larvae that cannot complete their development until the end of flowering.

Little is known about the effect of different oilseed rape cultivars on the acceptance as a host plant for oviposition by *M. aeneus*. It has been reported that pollen beetles had, in contrast to oviposition, no feeding preference for different cruciferous plant species (Fritzsche, 1957b;

Williams & Free, 1978; Ekbom & Borg, 1996) . *Sinapis alba* is poorly accepted as a host for oviposition (Ekbom & Borg, 1996). The crucifers *Barbarea verna* and *Barbarea vulgaris* were also not preferred for reproduction (Börjesdotter, 2000). Egg production by gravid females of pollen beetles varies in relationship to host plant quality (Hopkins & Ekbom, 1996). Hopkins and Ekbom (1996) suggested that the beetles are adapted to host plant quality to save resources for future egg production or that the oogenesis is arrested or incomplete on a “low-quality” host plant. Other influences of individual oviposition characteristics, for example isothiocyanates, may be responsible for oviposition preference in *Brassica* spp.. In field experiments, more eggs were found in buds of *Brassica napus* (L.) than in *B. juncea* and *B. campestris* (Ekbom & Borg, 1996).

Possible effects of insecticides on population growth and damage of pollen beetle

The exposure of insects to insecticide spraying is composed by direct topical, residual and dietary uptake. Some insecticides were included from plant parts after systemic uptake of the sprayed crop (Hallmann et al., 2009). In addition, the effectiveness of insecticide exposure is affected i. e. by a biotic environment, the behaviour of the pest and the specific toxicity of the insecticide to the different developmental stages of the pest (Winfield, 1961; Sedivy, 1993; Charmillot et al., 2007). Insecticides with a wide range of mode of actions are available and the neurotoxic insecticides with the targets acetylcholinesterase (organophosphate, carbamate), volta-gated sodium channel (pyrethroids) and acetylcholine receptor (neonicotinode) are still dominated the world market (Nauen & Bretschneider, 2002).

The active ingredients pymetroxin, indoxocarb and thiacloprid achieved an insecticide effectiveness up to 80% against adult adult pollen beetles in field experiments in Germany (Schroeder et al., 2009). Also repellent effects of insecticides were observed in field experiments (Sedivy et al., 1970). The numbers of adult pollen beetles decreased 3 days following insecticide spraying, but decreased on an adjacent unsprayed field. However, there is little knowledge about the effect of insecticide application on the population growth of the pollen beetle. Direct effects of insecticides on adult mortality were found to decrease oviposition into buds resulting in a reduced larvae abundance (Winfield, 1961).

Injury by feeding to small buds cause them to abort, leaving podless stalks (Winfield, 1961). Seed yield in insecticide untreated plots in spring oilseed rape was up to 70% less than in treated plots (Nilsson, 1994). However, between 1 and 10 treatments of methoxychlor, azinphosmethyl, fenvalerate or permethrin were needed to reduce pollen beetle infestation. The high compensatory reactions of oilseed rape due to feeding may affect the damage potential of pollen beetles (Vietinghoff, 1985, Nilsson, 1994). Further, the damage is depended on the temporal occurrence of the adult beetles in oilseed rape, in addition to the number of pollen beetles, the effectiveness of insecticide treatment and the weather conditions (Fritzsche, 1957b).

Parasitoids and parasitisation of pollen beetle

The larvae of the pollen beetle are attractive to several endoparasitoids (Jourdeuil, 1960). In Europe, including United Kingdom, Sweden, Estonia, Poland and Germany (Ulber et al., 2010b), the key parasitoids of the pollen beetle in oilseed rape are the univoltine species *Phradis interstitialis* (Thomson), *P. morionellus* (Holmgren) and *Tersilochus heterocerus* (Holmgren) (Hym.: Ichneumonidae) (Nilsson, 2003). The multivoltine parasitoid species *Diospilus capito* (Haliday) (Hym.: Braconidae) has also been recorded in northern and central Europe. Nilsson & Andreasson (1987) reported that *D. capito* is more abundant in spring oilseed rape, in contrast to the univoltine parasitoids which occur more in winter oilseed rape (Ulber et al., 2010b).

The tersilochine parasitoids of the pollen beetle are koinobionts, i. e. their host larva continues to develop with the parasitoid developing inside (Jourdeuil, 1960). The parasitoid larva of *Phradis* spp. emerges from the host larva at temperatures from 18-20°C. It starts feeding on the haemolymph immediately. In the following 3-4 days the tersilochine larva orientates towards the gut of host larva and stop feeding (Jourdeuil, 1960). The larva of *T. heterocerus* emerges when host larva drop down for pupation (Osborne, 1960). After migration of the host larva for pupation in the soil, the histolysis of the host begins (Jourdeuil, 1960). Most of the larval growth of the parasitoids takes places in the host pupal chamber. The parasitoids have 5 larval instar stages. Pupation and larval development depends on temperature and the pupation stage takes 15 to 20 days. The adult parasitoid stays in diapause in the cocoon in the pupal chamber until the following spring on the old rape field. The complete development from migration of host larvae into the soil to imago of the tersilochine parasitoid takes 35 days at 25°C, 50 days at 20°C and 80 days at 15°C (Jourdeuil, 1960).

Parasitoids locate their host-habitat probably by using upwind anemotaxis (Williams et al., 2007a). They start host searching during the late bud and flowering stages of oilseed rape crops (Jönsson et al., 2005; Jönsson et al., 2007). Nilsson and Andreasson (1987) reported that *T. heterocerus* parasitised the larvae of the pollen beetle mostly in the flower and prefers the second instar larvae. The parasitoids are more attracted by odours from infested oilseed rape plants with host larvae compared to uninfested plants (Jönsson et al., 2005; Neumann, 2010). In two-choice tests, *Phradis* spp. preferred the odour of oilseed rape buds and *T. heterocerus* also the odour in the flower stage (Jönsson & Anderson, 2008). In experiments, where the odour stimuli were combined with visual stimuli, different behaviours between the three key parasitoids of pollen beetle were recorded. When odours of flowering rape were combined with the colour yellow, and odours of the bud stage were combined with the colour green, *P. interstitialis* was equally attracted to both combinations, and *T. heterocerus* showed an increased preference for flower odours, while *P. morionellus* were not affected by the visual stimuli (Jönsson & Anderson, 2008). The spatial distribution within the field differs between parasitoid species: *Phradis interstitialis* has, like its host, a patchy distribution, while *T. heterocerus* is evenly spread across the field (Ferguson et al., 2003).

Parasitism of pollen beetle larvae can be a decisive factor for the population dynamics and population growth of this pest (Ulber et al., 2010b). Depending on year, country and

meteorological factors, total parasitism rates from 0% to more than 90% were observed in Europe (Nilsson & Andreasson, 1987; Nielsen & Axelsen, 1988; Hokkanen, 2000; Büchi, 2002; Williams, 2006; Thies et al., 2008). The relative abundance of the key parasitoids of *M. aeneus* varies between countries (Ulber et al., 2010b). In Germany, Poland and United Kingdom, *T. heterocerus* and *P. interstitialis* are predominant, while *P. morionellus* and *D. capito* were more common in Estonia and in Sweden. An efficient biocontrol agent requires the coincidence between the parasitoid and its host (Barari et al., 2005). Adults of *T. heterocerus* emerge later in the season than adults of *P. interstitialis* (Klingenberg & Ulber, 1994, Ferguson et al., 2003). Fritzsche (1957a) recorded an increasing parasitism rate from 30-40% of up to 94% by *Phradis* spp. and *T. heterocerus* with an increasing occurrence of parasitoid species during plant development.

Landscape structure has been shown to directly affect antagonists of pollen beetles in field experiments where a complex structure with conservation strips and fallows enhanced the parasitisation (Thies & Tschardtke, 1999; Büchi, 2002; Thies et al., 2008; Zaller et al., 2009). Levels of rape crop areas in a region show contradictory impact on parasitism (Thies et al., 2008; Zaller et al., 2008).

Studies on the behaviour and temporal occurrence of parasitoids of pollen beetle indicate that insecticide treatment during late bud and flowering stage of oilseed rape crops can be very harmful to natural enemies (Jönsson et al., 2005; Jönsson & Anderson, 2008). Mortality of parasitoids can occur by direct contact with spray droplets and chemicals residues on a plant surface (Ulber et al., 2010a). They can also be affected during their development within the host. In addition to lethal effects, sublethal effects of insecticides may have an impact on life span, fertility, fecundity and changes in behaviour like feeding, host searching and oviposition (Stark & Banks, 2003).

Trap cropping in oilseed rape

The knowledge of host location and manipulation of behaviour and migration of the pollen beetle in oilseed rape have been used in investigation for trap cropping systems (Hokkanen, 1991). Trap crops are used to attract the pest population and protect the target crop plants from infestation, with the possibility to control the pest on the trap crop with insecticides (Hokkanen, 1991; Shelton & Badenes-Perez, 2006). Turnip rape (*Brassica rapa* L.) crop borders reduced *Psylliodes chrysocephala* infestation of oilseed rape plots (Barari et al., 2005). The adults of *P. chrysocephala* showed a feeding preference for turnip rape leaves over oilseed rape leaves. In field studies in Finland calabrese, chinese cabbage and oilseed rape were used successfully as trap crops, however, chinese cabbage needs to be sown early enough to induce flowering (Hokkanen et al., 1986). Mixtures of different plant species in combination with insecticide spraying were very effective to protect cauliflower from damage by the pollen beetle. Trap cropping in spring oilseed rape was also effective. About a 50% reduction in the number of beetles and insecticide use was recorded, when the trap crop flowers about 2 weeks earlier than the main crop spring oilseed rape. It was very important, that the trap crop was grown in the direction from which the adult beetles had

been observed to immigrate into the field (Hokkanen et al., 1986). *B. rapa* is preferred to *B. napus* by *M. aeneus* and shows good potential as a trap crop (Cook et al., 2006, 2007). To guarantee that the direction of immigration of pollen beetles into the field does not have an effect, the turnip rape was grown on each site of the field (Cook et al., 2004). By surrounding the main crop spring oilseed rape with a border strip of turnip rape, the spatial distribution of the adult beetles within the field was manipulated. In spring oilseed rape the beetles were retained in the turnip rape border and the number of beetles in the centre was reduced compared to control plot totally grown with spring oilseed rape. The pollen beetle is attracted by the earlier growth development (Cook et al., 2004) and by volatile cues (Cook et al., 2007). However, *B. rapa* is also preferred over *B. napus* when both species were in the bud stage. Phenylacetaldehyde, indole and (*E,E*)- α -farnesene were found to be present in air entrainment samples of both plant species at the flowering stage, but only in those of *B. rapa* at the bud stage (Cook et al., 2007). It might be possible that the successful use of trap cropping in spring oilseed rape crops may show the same results for winter oilseed rape crops.

References

- Ahuja, I., Rohloff, J. & Bones, A. (2010). Defence mechanisms of Brassicaceae: implications for plant-insect interactions and potential for integrated pest management. A review. *Agronomy for Sustainable Development* 30, 311-348.
- Alford, D. V. (2003). Insect pest of oilseed rape crops. In: Alford D.V. (ed.) *Biocontrol of oilseed rape pests*. Oxford, UK: Blackwell Publishing, 9-41.
- Barari, H., Cook, C. M., Clark, S. J. & Williams, I. H. (2005). Effect of turnip rape (*Brassica rapa*) trap crop on stem-mining pest and their parasitoids in winter oilseed rape (*Brassica napus*). *BioControl* 50, 69-86.
- Börjesdotter, D. (2000). *Barbarea verna* and *Barbarea vulgaris* as host plants for the pollen beetle (*Meligethes aeneus*). *Journal of Agricultural Science* 134, 213-220.
- Brauer, D. (2007). Geschichte des Rapsanbaus. In: Christen O, Friedt W. (Ed.) *Winterraps-Das Handbuch für Profis*, Frankfurt am Main, Germany: DLG Verlag, 9-16.
- Bromand, B. (1983). Possibility of continuous rearing of *Meligethes aeneus* Fabr. (Col.). *Zeitschrift für angewandte Entomologie* 96, 419-422.
- Büchi, R. (2002). Mortality of pollen beetle (*Meligethes* spp.) larvae due to predators and parasitoids in rape fields and the effect of conservation strips. *Agriculture, Ecosystems and Environment* 90, 255-263.
- Charmillot, P., Pasquier, D., Salamin, C. & Ter-Hovannesyanyan, A. (2007). Ovicidal and larval effectiveness of insecticides applied by dipping apples on the small fruit tortrix *Grapholita lobarzewskii*. *Pest Management Science* 63, 677-681.
- Cook, S. M., Bartlett, E., Murray, D. A. & Williams, I. H. (2002). The role of pollen odour in the attraction of pollen beetles to oilseed rape flowers. *Entomologia Experimentalis et Applicata* 104, 43-50.
- Cook, S. M. & Denholm, I. (2008). Ecological approaches to the control of pollen beetles in oilseed rape. *IOBC/wprs Bulletin* 38, 110-113.
- Cook, S. M., Rasmussen, H. B., Birkett, M. A., Murray, D. A., Pye, B. J., Watts, N. P. & Williams, I. H. (2007). Behavioural and chemical ecology underlying the success of turnip rape (*Brassica rapa*) trap crops in protecting oilseed rape (*Brassica napus*) from the pollen beetle (*Meligethes aeneus*). *Arthropod-Plant Interactions* 1, 57-67.
- Cook, S. M., Smart, L. E., Martin, J. L., Murray, D. A., Watts, N. P. & Williams, I. H. (2006). Exploitation of host plant preferences in pest management strategies for oilseed rape (*Brassica napus*). *Entomologia Experimentalis et Applicata* 119, 221-229.
- Cook, S. M., Watts, N. P., Hunter, F., Smart, L. E. & Williams, I. H. (2004). Effects of a turnip rape trap crop on the spatial distribution of *Meligethes aeneus* and *Ceutorhynchus assimilis* in oilseed rape. *Bulletin IOBC/wprs: Integrated Control in oilseed crops* 27, 199-206.

- Délos, M. (2008). *Meligethes* du colza: Evolution et gestion de resistances aux pyrethrinoides en France et recommandations pour gerer le risque. *Bulletin OEPP/EPPO, Bulletin* 38, 75-78.
- Ekbom, B. & Borg, A. (1996). Pollen beetle (*Meligethes aeneus*) oviposition and feeding preferences on different host plant species. *Entomologia Experimentalis et Applicata* 78, 291-299.
- Evans, K. A. & Allen-Williams, L. J. (1994). Laboratory and field response of the pollen beetle, *Meligethes aeneus*, to the odour of oilseed rape. *Physiological Entomology* 19, 285-290.
- Evans, K. A. & Scarisbrick, D. (1994). Integrated insect pest-management in oilseed rape crops in Europe. *Crop Protection* 13, 403-412.
- FAO (2011). <http://faostat.fao.org/site/567/default.aspx#ancor>.
- Ferguson, A. W., Campbell, J. M., Warner, D. J., Watts, N. P., Schmitdt, J. E. U. & Williams, I. H. (2003). Spatio-temporal distributions of *Meligethes aeneus* and its parasitoids in an oilseed rape crop and their significance for crop protection. In: *Proceedings of Proc. 11th Int. Rapeseed Congress, Copenhagen, 6-10 July 2003*, 1057-1059.
- Frearson, D. J. T., Ferguson, A. W., Campbell, J. M. & Williams, I. H. (2005). The spatial dynamics of pollen beetles in relation to inflorescence growth stage of oilseed rape: implications for trap crop strategies. *Entomologia Experimentalis et Applicata* 116, 21-29.
- Free, J. B. & Williams, I. H. (1978). The responses of the pollen beetle, *Meligethes aeneus*, and the seed weevil, *Ceuthorrhynchus assimilis*, to oil-seed rape, *Brassica napus*, and other plants. *Journal of Applied Ecology* 15, 761-774.
- Free, J. B. & Williams, I. H. (1979). The infestation of crops of soil-seed rape (*Brassica napus* L.) by insect pests. *Journal for Agricultural Science, Cambridge* 92, 203-218.
- Freier, B., Sellmann, J., Strassemeyer, J., Schwarz, J., Klocke, B., Moll, E., Gutsche, V. & Hornbach, W. (2012). Netz Vergleichsbetriebe Pflanzenschutz Jahresbericht 2011 - Analyse der Ergebnisse der Jahre 2007 bis 2011. *Berichte aus dem Julius Kühn-Institut* 166.
- Fritzsche, R. (1957a). Beeinflussung der Populationsdichte verschiedener *Meligethes*-Arten von gleichen Wirtspflanzen durch Parasiten. (Bericht Hundertjahrfeier Deutscher Entomologen Ges. Berlin).
- Fritzsche, R. (1957b). Zur Biologie und Ökologie der Rapsschädlinge aus der Gattung *Meligethes*. *Sonderdruck aus Zeitschrift für angewandte Entomologie* 40, 222-280.
- Hansen, L. M. (2003). Insecticide-resistant pollen beetles (*Meligethes aeneus* F) found in Danish oilseed rape (*Brassica napus* L) fields. *Pest Management Science* 59, 1057-1059.
- Hallmann, J., Quadt-Hallmann, A. & von Tiedemann, A. (2009). *Phytomedizin*. 2. Aufl. Stuttgart: Verlag Eugen Ulmer.
- Heimbach, U., Müller, A. & Thieme, T. (2006). First steps to analyse pyrethroid resistance of different oil seed rape pests in Germany. *Nachrichtenblatt Deutscher Pflanzenschutzdienst* 58, 1-5.
- Hiisaar, K., Metspalu, L., Lääniste, P., Jogar, K., Kuusik, A. & Joudu, J. (2003). Insect pests on winter oilseed rape studied by different catching methods. *Agronomy Research* 1, 17-29.
- Hokkanen, H., Granlund, H., Husberg, G. & Markkula, M. (1986). Trap crops used successfully to control *Meligehtes aeneus* (Col., Nitidulidae), the rape blossom beetle. *Annales Entomologici Fennici* 52, 115-120.
- Hokkanen, H. M. T. (1991). Trap cropping in pest management. *Annual Review of Entomology* 36, 119-138.
- Hokkanen, H. M. T. (2000). The making of a pest: recruitment of *Meligethes anaeus* onto oilseed Brassicas. *Entomologia Experimentalis et Applicata* 95, 141-149.
- Hopkins, R. J. & Ekbom, B. (1996). Low oviposition stimuli reduce egg production in the pollen beetle *Meligethes aeneus*. *Physiological Entomology* 21, 118-122.
- Jönsson, M., Lindkvist, A. & Anderson, P. (2005). Behavioural responses in three ichneumonid pollen beetle parasitoids to volatiles emitted from different phenological stages of oilseed rape. *Entomologia Experimentalis Et Applicata* 115, 363-369.
- Jönsson, M. & Anderson, P. (2008). Emission of oilseed rape volatiles after pollen beetle infestation; behavioural and electrophysiological responses in the parasitoid *Phradis morionellus*. *Chemoecology* 17, 201-207.
- Jönsson, M., Rosdahl, K. & Anderson, P. (2007). Responses to olfactory and visual cues by overwintered and summer generations of the pollen beetle, *Meligethes aeneus*. *Physiological ecology* 32, 188-193.
- Jourdheuil, P. (1960). Influence de quelques facteurs ecologiques sur les fluctuations de population d'une biocénose parasitaire :Étude relative à quelques Hyménoptères (Ophioninae, Diospilinae, Euphorinae) parasites de divers Coléoptères inféodés aux Crucifères. *Annales des Epiphyties* 11, 445-658.
- Kazachkova, N., Meijer, J. & Ekbom, B. (2007). Genetic diversity in pollen beetles (*Meligethes aeneus*) in Sweden: role of spatial, temporal and insecticide resistance factors. *Agricultural and Forest Entomology* 9, 259-269.

- Klingenberg, A. & Ulber, B. (1994). Investigations on the occurrence of tersilochinae (Hym, Ichneumonidae) as parasitoids of oilseed rape pests in the Göttingen region in 1990 and 1991, and on their emergence following various tillage techniques. *Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie* 117, 287-299.
- Müller, H. J. (1941a). Beiträge zur Biologie des Rapsglanzkäfers, *Meligethes aeneus* F. *Zeitschrift für Pflanzenkrankheiten (Pflanzenpathologie) und Pflanzenschutz* 51, 385-435.
- Müller, H. J. (1941b). Weitere Beiträge zur Biologie des Rapsglanzkäfers, *Meligethes aeneus* F. (Über das Winterlager und die Massenbewegung im Frühjahr.). *Zeitschrift für Pflanzenkrankheiten (Pflanzenpathologie) und Pflanzenschutz* 51, 529-595.
- Nauen, R & Bretschneider T. (2002). New modes of action of insecticides. *Pesticide Outlook* 13, 241-245.
- Neumann, N. (2010). Lethal and sublethal effects of insecticides on mortality, migration and host searching behaviour of tersilochine parasitoids on winter oilseed rape. Diss. Göttingen: Georg-August Universität.
- Nielsen, P. S. & Axelsen, J. (1988). Developmental time and mortality of the immature stages of the pollen beetle (*Meligethes aeneus* F) under natural conditions. *Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie* 105, 198-204.
- Nilsson, C. (1988a). Pollen beetles (*Meligethes aeneus* F.) and flowering in rape. *Swedish J. agric. Res.* 18, 113-118.
- Nilsson, C. (1988b). The number of larval instars of *Meligethes aeneus* (F.) in southern Sweden. *Växtskyddsnotiser* 52, 151-152.
- Nilsson, C. (1988c). The pollen beetle (*Meligethes aeneus* F.) in winter and spring rape at Alnarp 1976-1978. II. Oviposition. *Växtskyddsnotiser* 52, 139-144.
- Nilsson, C. (1994). Pollen beetles (*Meligethes* spp.) in oil seed rape crops (*Brassica napus* L.): Biological interactions and crop losses. Diss. Swedish University of Agricultural Sciences.
- Nilsson, C. (2003). Parasitoids of pollen beetles. In: Alford D. V. (ed.) *Biocontrol of oilseed rape crops*. Oxford, UK: Blackwell Publishing, 73-85.
- Nilsson, C. & Andreasson, B. (1987). Parasitoids and predators attacking pollen beetles (*Meligethes aeneus* F.) in spring and winter rape in Southern Sweden. *IOBC/wprs Bulletin* 10, 64-73.
- Nolte, H. (1954). Käfer bedrohen den Raps. Über die die Biologie, die Bedeutung und die Bekämpfung des Rapsglanzkäfers. Wittenberg: Ziemsen Verlag.
- Nolte, H. (1959). Untersuchungen zum Farbsehen des Rapsglanzkäfers (*Meligethes aeneus* F.). Ergebnisse für die Praxis des Schädlingswarndienstes. *Zeitschrift für angewandte Zoologie* 46, 11-33.
- Osborne, P. (1960). Observations on the natural enemies of *Meligethes aeneus* (F.) and *M. viridescens* (F.) [Coleoptera: Nitidulidae]. *Parasitology* 50, 91-110.
- Osborne, P. (1965). Morphology of the immature stages of *Meligethes aeneus* (F.) and *M. viridescens* (F.) (Coleoptera, Nitidulidae). *Bulletin of Entomological Research* 55, 747-759.
- Scherney, F. (1953). Zur Biologie der an Raps vorkommenden Meligethesarten. *Zeitschrift für Pflanzenbau und Pflanzenschutz* 4, 154-176.
- Schroeder, G., Politz, B., Wolff, C. & Krueger, B. (2009). Alternatives for an effective control of pyrethroid-resistant pollen beetle populations - results of proficiency test of several states. *Gesunde Pflanzen* 61, 19-30.
- Sedivy, J., Taimr, L., Kodys, F., Kudelova, A. & Bermannova, E. (1970). Einfluß der chemischen Behandlung von Raps auf die Populationsdichte des Rapsglanzkäfers (*Meligethes aeneus* F.). *Zeitschrift für angewandte Entomologie* 67, 113-124.
- Sedivy, J. (1993). Variation in the population density of pollen beetles (*Meligethes aeneus* F.) in winter rape. *Ochrana Rostlin* 29, 9-15.
- Shelton, A. M. & Badenes-Perez, E. (2006). Concepts and applications of trap cropping in pest management. *Annual Review of Entomology* 51, 285-308.
- Singh, D. & Singh, A. (2005). *Disease and insect resistance in plants*. Enfield, NH, USA: Science Publishers.
- Stark, J. D. & Banks, J. E. (2003). Population-level effects of pesticides and other toxicants on arthropods. *Annual Review of Entomology* 48, 505-519.
- Taimr, L., Sedivy, J., Bermannova, E. & Hanker, I. (1967). Further experience obtained in studies on dispersal flights of *Meligethes aeneus* F., marked with P³² (Coleoptera). *Acta ent. bohemoslov.* 64, 325-332.
- Thies, C., Steffan-Dewenter, I. & Tschardtke, T. (2008). Interannual landscape changes influence plant-herbivore-parasitoid interactions. *Agriculture, Ecosystems and Environment* 125, 266-268.
- Thies, C. & Tschardtke, T. (1999). Landscape structure and biological control in agroecosystems. *Science* 285, 893-895.

- Tiilikainen, T. M. & Hokkanen, H. M. T. (2008). Pyrethroid resistance in Finnish pollen beetle (*Meligethes aeneus*) populations - is it around the corner? *IOBC/wprs Bulletin* 38, 99-103.
- UFOP (2011). <http://www.ufop.de/index.php>.
- Ulber, B., Klukowski, Z. & Williams, I. (2010a). Impact of insecticides on parasitoids of oilseed rape pests. In: Williams I. H. (ed.) *Biocontrol-based integrated management of oilseed rape pests*. Netherlands: Springer Science+Business Media B. V., 337-335
- Ulber, B., Williams, I., Klukowski, Z., Luik, A. & Nilsson, C. (2010b). Parasitoids of oilseed rape pests in Europe: key species for conservation biocontrol. In: Williams I. H. (ed.) *Biocontrol-based integrated management of oilseed rape pests*. Netherlands: Springer Science+Business Media B. V., 45-76.
- Vietinghoff, J. (1985). Untersuchungen zur Schadwirkung und Befallsprognose des Rapsglanzkäfers (*Meligetes aeneus* F.). Diss. Rostock: Wilhelm-Pieck-Universität Rostock.
- Wegorek, P. & Zamoyska, J. (2008). Current status of resistance in pollen beetle (*Meligethes aeneus* F.) to selected active substances of insecticides in Poland. *IOBC/wprs Bulletin* 38, 91-94.
- Williams, I. H. (2006). Integrating parasitoids into management of pollen beetle on oilseed rape. *Agronomy Research* 4(special issue), 465-470.
- Williams, I. & Cook, S. M. (2010). Crop location by oilseed rape pests and host location by their parasitoids. In: Williams I. H. (ed.) *Biocontrol-based integrated management of oilseed rape pests*, Netherlands: Springer Science+Business Media B. V., 215-244.
- Williams, I. H., Frearson, D. J., Barari, H. & McCartney, A. (2007a). First field evidence that parasitoids use upwind anemotaxis for host-habitat location. *Entomologia Experimentalis et Applicata* 123, 299-307.
- Williams, I. H., Frearson, D., Barari, H. & McCartney, A. (2007b). Migration to and dispersal from oilseed rape by the pollen beetle, *Meligethes aeneus*, in relation to wind direction. *Agricultural and Forest Entomology* 9, 279-286.
- Williams, I. H. & Free, J. B. (1978). The feeding and mating behaviour of pollen beetles (*Meligethes aeneus* Fab.) and seed weevils (*Ceutorhynchus assimilis* Payk.) on oil-seed rape (*Brassica napus* L.). *Journal of Agricultural Science, Cambridge* 91, 453-459.
- Winfield, A. L. (1961). Field observations on the control of blossom beetles (*Meligethes aeneus* F.) and cabbage-seed weevils (*Ceuthorhynchus assimilis* Payk.) on mustard-seed crops in East Anglia. *Annals of Applied Biology* 49, 539-555.
- Zaller, J. G., Moser, D., Drapela, T., Schmöger, C. & Frank, T. (2009). Parasitism of stem weevils and pollen beetles in winter oilseed rape is differentially affected by crop management and landscape characteristics. *Biocontrol* 54, 505-514.
- Zaller, J. G., Moser, D., Drapela, T., Schmöger, C. & Frank, T. (2008). Effect of within-field and landscape factors on insect damage in winter oilseed rape. *Agriculture Ecosystems and Environment* 123, 233-238.
- Zlof, V. (2008). Recommendations and conclusions of the Ad hoc EPPO-Workshop on insecticide resistance of *Meligethes* spp. (pollen beetle) on oilseed rape. *IOBC/wprs Bulletin* 38, 65-67.

Cultivar and phenology of winter oilseed rape affect the abundance and reproduction of *Meligethes aeneus* (Fabricius)

Abstract

Host plant phenology and resource availability for oviposition and larval development can have important consequences on the abundance of offspring and the new generation of herbivorous insects. Pollen beetle, *Meligethes aeneus* (F.) (Col.: Nitidulidae), causes high yield losses in European oilseed rape crops. Integrated pest management strategies including the usage of less susceptible cultivars are needed to reduce the extensive application of synthetic insecticides. In three years' field experiments we compared the abundance and population growth of pollen beetle on four cultivars of winter oilseed rape, *Brassica napus* (L.): 'NK Passion' (open-pollinated, early flowering), 'Elektra' (hybrid, early flowering), 'Favorite' (open-pollinated, late flowering) and 'Titan' (hybrid, late flowering). The abundance of adult pollen beetles and eggs was determined in March/April, while numbers of larvae and new-generation-beetles was assessed in May to July. We hypothesized that the availability of buds and flowers for oviposition and larval development is curtailed on early flowering cultivars, leading to a decreased reproduction and development of new-generation beetles. Annual weather conditions in spring strongly affected the coincidence between pollen beetle immigration and the time period of bud to flowering stage. In 2008, the abundance of overwintered pollen beetles on the late flowering cv. 'Favorite' was significantly lower, but the population growth rate was significantly higher than on cv. 'NK Passion' flowering 6 days earlier. In 2009 and 2010, when the phenological stages of all cultivars showed little difference, there was no significant effect of cultivars on population growth. In both years, overwintered pollen beetles significantly preferred buds of the hybrid cultivars for oviposition, however, this did not increase the abundance of new-generation adults compared with open-pollinated cultivars. Results of the field experiment were confirmed by results of a semi-field experiment where pollen beetles were released on caged plants of cv. 'NK Passion' and cv. 'Favorite' at two different plant growth stages. Our results suggest that flowering phenology of host plants and the limitation of resources for oviposition and larval development may be influential for reproduction and establishment of new-generation pollen beetles on different cultivars of oilseed rape. However, this effect is dependent on the annual weather conditions.

Introduction

The pollen beetle, *Meligethes aeneus* (Col.: Nitidulidae), is a major pest of oilseed Brassicas in Europe (Alford, 2003). In spring, following hibernation, adult beetles feed on pollen from various plant families, but oviposition is restricted to brassicaceous plants (Fritzsche, 1957; Free & Williams, 1978). Feeding on the anthers and ovaries causes abortion of buds, resulting in podless stalks (Winfield, 1961; Nilsson, 1988a). After maturation feeding, females

deposit their eggs into medium-sized buds. First instar larvae feed on pollen within buds while second instar larvae continue feeding on pollen in open flowers (Fritzsche, 1957; Williams & Free, 1978). In May/June the full-grown second instar larvae drop to the ground for pupation in the upper soil layer. New-generation beetles emerge in June/July. Before entering winter diapause, they feed on pollen from a large variety of plant families (Fritzsche, 1957).

Ovipositing females discriminate between different brassicaceous plant species (Ekbom & Borg, 1996). *Sinapis alba* and *Barbarea spp.* were poorly accepted as hosts for egg-laying (Ekbom & Borg, 1996, Börjesdotter, 2000). In field experiments, more eggs were laid into buds of *Brassica napus* (L.) than into buds of *B. juncea* and *B. campestris* (Ekbom & Borg, 1996). So far, the reproduction of *M. aeneus* on different cultivars of *B. napus* have not been studied.

M. aeneus adults locate their host plants using both visual and chemical cues (Williams & Cook, 2010). The behavioural response of the beetles to plant odour, particularly to isothiocyanates, the breakdown products of glucosinolates, is modified by the yellow colour of the flowers (Mithen, 1992; Giamoustaris & Mithen, 1996; Cook et al., 2002). In polytunnel and field experiments, the oilseed rape cv. 'Starlight', which show low emissions of isothiocyanates, was less attractive to beetles than the cultivar 'Canyon'. But when these cultivars were offered in different growth stages pollen beetles were more abundant on the cultivar in flower than on the cultivar in the bud stage (Cook et al., 2006b).

Synchronisation of the phenology of adult beetles with suitable host stages is important for the reproductive performance of herbivores (Singh & Singh, 2005). As the number of buds available for feeding and oviposition decreases in the advanced developmental stages of oilseed rape plants (Ekbom & Borg, 1996) the phenological stage of the host plant may strongly impact the population growth of pollen beetle (Nilsson, 1994). Nielsen and Axelsen (1988a) assumed that the eggs deposited late in the oviposition period will result in larvae which are not able to complete their development until the end of flowering stage. This study aimed to determine the effect of plant phenology of winter oilseed rape on reproduction and population growth of pollen beetle. In field experiments, the early flowering cultivars 'NK Passion' and 'Elektra' were compared with the late flowering cultivars 'Favorite' and 'Titan'. To further analyse the influence of different plant growth stages on population growth, pollen beetles were released into caged plots at two different growth stages of oilseed rape in semi-field experiments.

Materials and methods

Field experiment

Experimental design: Three field experiments were conducted at Goettingen (N 51°33'456.38, E 9°56'56.43), Germany, in 2007-2010. In each year, four cultivars of winter oilseed rape were grown in four replicated plots in a randomized block design (plot size 75m²). Two cultivars were categorized as early flowering and two cultivars were categorized as late flowering, each represented by a hybrid ('Elektra' vs. 'Titan') and an open-pollinated

cultivar ('NK Passion' vs. 'Favorite') were selected for the experiments. To reduce edge effects the field experiment was set up within a field grown with a late flowering cultivar of oilseed rape.

Pollen beetle abundance: The overwintered pollen beetles were counted every third day from the start of immigration during bud stage until full flowering. In each plot the number of beetles on 25 randomly selected plants was counted by beating the main raceme onto a plastic funnel (diameter 25cm). Beetles were released after counting to avoid effects on beetle density and oviposition. To calculate beetle abundance per square metre the number of beetles per plant was related to the plant density of each cultivar which was assessed at the end of March. On each occasion, the BBCH growth stage of the cultivars was recorded (Lancashire et al., 1991).

Eggs and first instar larvae (L1): The number of pollen beetle eggs in the buds was assessed on 24 April 2008, 8 April and 17 April 2009 and 26 April 2010, respectively. In 2008, the main raceme, first and second side raceme (total of 3 racemes/ plant presented in table 2) and in 2009 and 2010 the main raceme, first, third and fifth side raceme (total of 4 racemes/ plant presented in table 2), respectively, were collected from the top of 10 plants per plot and stored in plastic bags at 2°C. To compare the egg numbers and the feeding injuries of pollen beetles on buds of the four cultivars, all buds were examined under a binocular microscope (magnification 25-40x). Feeding injuries were recognized by uneven holes in the bud, while oviposition holes were characterized as distinct narrow holes at the base of the buds. The bud size (<2mm; 2-3mm; >3mm) was measured and the buds with oviposition holes were dissected to count the number of eggs and L1 larvae. The total supply of buds was analysed by relating buds with feeding and oviposition injury per main raceme and each of 3 side racemes on one sampling occasion in 2009 and 2010, respectively.

Second instar larvae (L2): Before mature L2 larvae started to migrate to the ground for pupation, 15 water traps (17cm x 12.5cm, with detergent) were distributed randomly on the ground of each plot. The traps were emptied every fourth day until the end of flowering and the larvae caught were stored in 70% Ethanol. First and second instar larvae were separated by measuring their head capsule widths (Nilsson, 1988b). To determine the level of larval parasitism, 100 L2 larvae per plot were dissected under a binocular microscope (magnification 25x). The cuticle of each larva was cut off longitudinal under water and the body contents were squeezed out by using a fine needle. Immature stages of the parasitoids *Phradis* spp. and *Tersilochus heterocerus* were identified morphologically (Osborne, 1960). Additionally, subsamples of larvae were transferred to vials containing loamy soil in the laboratory for pupation (Nitzsche, 1998). Adult parasitoids emerging from parasitized hosts were identified to species level (Horstmann, 1971, 1981).

New generation pollen beetle abundance: The emergence of new generation pollen beetles was assessed by using two randomly distributed ground photoeclectors (0,25m², ecoTech GmbH) per plot. Numbers of beetles caught were checked every third day during the pod stage in June and July.

Semi-field experiment

Experimental design: In 2009 and 2010, the open pollinated oilseed rape cultivars 'NK Passion' and 'Favorite' were grown on unsprayed fields at Goettingen (N 51°33'456.38, E 9°56'56.43), Germany. Ten plots of each cultivar were enclosed by gauze cages (2m wide x 4m long x 2m high; mesh size 0.3mm) to protect the plants from natural beetle infestation. To establish two different dates of plant colonization during the bud stage, five beetles/plant (150 beetles/m²) were released into the cages at an interval of 7 days in 2009. The "early infestation" was on 3 April ('NK Passion' BBCH 51/ 'Favorite' BBCH 50-51) while the "late infestation" was on 10 April ('NK Passion' BBCH (52) 53-55/ 'Favorite' BBCH 52-53). The BBCH growth stages of both cultivars were recorded from April until June. In 2010, oviposition of beetles released into the cages was on a very low level and the data could not be analysed.

Adult beetles were collected from hibernation sites in a deciduous forest in Mecklenburg-Western Pomerania in Germany, three days before release into the cages. They were maintained at 14°C and a photoperiod of 16L:8D in gauze cages on potted plants of oilseed rape (cv. 'Miniraps'). The beetles were starved for 24h before releasing them into field cages. Larvae dropping to the ground for pupation were caught in 6 water traps (17cm x 12.5cm; with detergent) per plot. The traps were emptied during the flowering stage every four to six days and the L1 and L2 larvae were counted. The emergence of new generation beetles was recorded by using one ground photoelector (0,25m², ecoTech GmbH) per plot during the pod stage. Emerging beetles were counted every third day.

Data analyses

Infestation: To compare numbers of overwintered adult pollen beetles on the four cultivars across the bud and flowering period, cumulative beetle-days were calculated by using the formula by Ruppel (1983): Cumulative beetle-days = $\sum (X_{i+1} - X_i) [(Y_i + Y_{i+1})/ 2]$, where X_i and X_{i+1} are consecutive counting occasions, Y_i is the density of individuals on day i and Y_{i+1} is the density of individuals on day $i+1$.

Emergence rate: The emergence rate of new generation pollen beetles was calculated by relating the accumulated number of emerging beetles in June and July to the accumulated number of L2 larvae dropping to the ground for pupation. Because parasitism of larvae causes mortality of prepupae in soil, the expected emergence rate of beetles was corrected by excluding the number of parasitised L2 larvae from the calculation (Schierbaum-Schickler, 2005).

Population growth rate: To estimate the growth of the pollen beetle population within one year the abundance of new-generation beetles emerging in summer was related to peak abundance of overwintered beetles on the plants in spring.

Statistical analyses: The software STATISTICA, version 9.1 (StatSoft, Inc., 2010) was used for all statistical analyses. Data of each year were treated separately. The influence of cultivar on each explanatory variable (cumulative beetle-days, pollen beetle abundance, numbers of eggs, larval abundance, new generation beetle abundance, emergence rate, corrected emergence rate, parasitism rate, growth rate, numbers of buds and proportion of buds used for feeding and oviposition) was analysed by Kruskal-Wallis test. The

nonparametric test was used because the data were not normally distributed and not determined by homogeneity of variance.

Simple linear regression models were used to examine the relationship between the length of time from the start of pollen beetle immigration to full flowering stage and the emergence rate. Correlations between the numbers of first instar larvae dropping to the ground and the numbers of second instar larval of all three years were analysed together. In the semi-field experiment, the effect of colonization date on numbers of L2 larvae and new-generation beetles on each cultivar was tested using Mann-Whitney-U test. Treatment means were compared at $p \leq 0.05$.

Results

Field experiment

Generally, the infestation level of the four cultivars by pollen beetles increased considerably over the three years of study. Mean peak abundance of overwintered pollen beetles increased from 63.04 ± 8.02 to 290.76 ± 31.42 and 526.9 ± 33.64 (mean \pm SEM) beetles per m^2 in 2008, 2009 and 2010, respectively. In 2008, when the maximum daily temperature increased to $>20^\circ\text{C}$ not before mid April, the first beetles colonised the plants 16 days later than in 2009 and 2010 (Figure 1). In all three years the infestation level showed one or two peaks and thereafter declined until the beginning of petal fall.

In 2008, the overwintered beetles clearly preferred the early flowering cultivars 'NK Passion' and 'Elektra' (Figure 1A). The inflorescences of these cultivars developed faster than the inflorescences of the late flowering cultivars 'Favorite' and 'Titan' (Figure 1A). Beetle abundance on plants peaked on 27 April, when the main racemes of the early flowering cultivars had up to 40% open flowers and the late flowering cultivars were in the yellow bud stage. The higher attractiveness of cv. 'Elektra' and particularly of cv. 'NK Passion' in 2008 was also demonstrated by the cumulative beetle-days (Table 1).

In 2009, forced by rapidly increasing temperatures in March/April, there was little difference between the growth stages of the four cultivars across the observation period (Figure 1B). At peak abundance of pollen beetles on 14 April, all cultivars were in the green to yellow bud stage. Although all cultivars showed the same growth stage pollen beetles were more abundant on the hybrids 'Elektra' and 'Titan' than on the open pollinated cvs. 'NK Passion' and 'Favorite' (Figure 1B). However, the cumulative beetle-days were not significantly different between the four cultivars (Table 1).

In 2010, the growth of early flowering cultivars was slightly advanced and the cultivars 'Elektra' and 'NK Passion' were 5 days earlier in flower than the cultivars 'Favorite' and 'Titan' (Figure 1C). At this time pollen beetles were already present on plants since 23 days. Plant infestation by pollen beetles increased from 16 April to 19 April and decreased at low maximum temperature on 22 April. The ranking of cumulative beetle-days showed the order 'Elektra' $>$ 'Titan' $>$ 'NK Passion' $>$ 'Favorite', thereby confirming the higher attractiveness of hybrids for pollen beetles compared with open pollinated cultivars (Table 1).

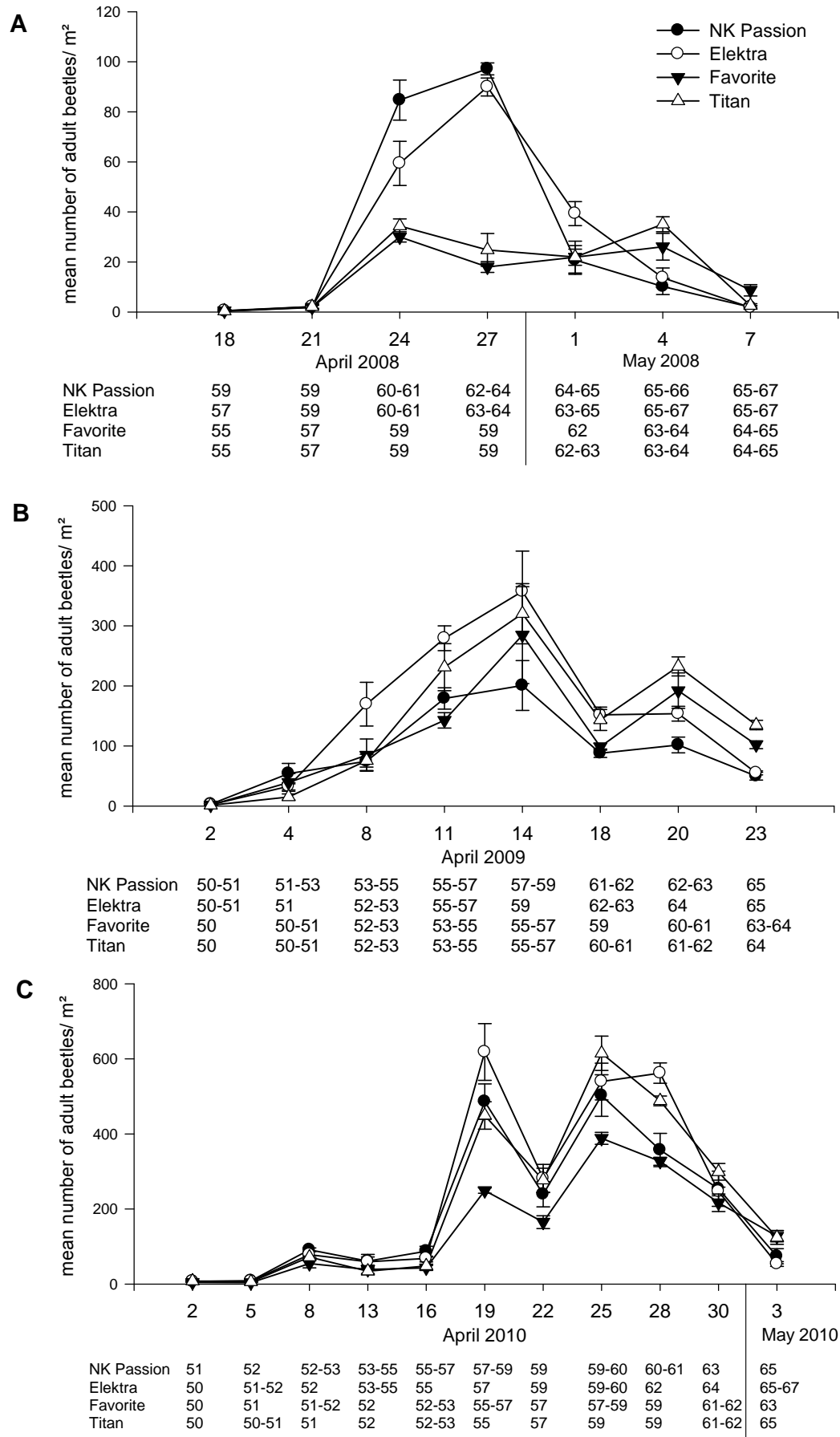


Figure 1: Abundance of overwintered pollen beetles (mean \pm SEM) and BBCH growth stages of four winter oilseed rape cultivars in 2008 (A), 2009 (B) and 2010 (C).

Table 1: Cumulative beetle-days (mean \pm SEM) of overwintered pollen beetles on four oilseed rape cultivars in 2008-2010 (upper case letters indicate significant differences between cultivars in one year; $p \leq 0.05$; Kruskal-Wallis test).

Cultivar	2008		2009		2010	
	mean	(\pm SEM)	mean	(\pm SEM)	mean	(\pm SEM)
NK Passion	706.9	(\pm 36.2) A	2259.5	(\pm 153.8) A	6233.5	(\pm 637.8) AB
Elektra	682.5	(\pm 17.7) AB	3706.4	(\pm 400.5) A	7211.9	(\pm 436.6) A
Favorite	328.0	(\pm 29.8) B	2771.0	(\pm 453.1) A	4480.8	(\pm 104.9) B
Titan	383.3	(\pm 31.9) AB	3339.8	(\pm 360.4) A	6775.4	(\pm 196.1) AB

Table 2: Mean abundance of pollen beetle adults, eggs and larvae on four oilseed rape cultivars in field experiments 2008-2010 (PB = pollen beetles; sampling date 1 = 24 April 2008 and 08 April 2009; sampling date 2 = 17 April 2009 and 26 April 2010) (upper case letters indicate significant differences between cultivars in one year; $p \leq 0.05$; Kruskal-Wallis test).

Year	Cultivar	Overwintered PB/ m ²		Eggs/ m ²		L1 larvae/ m ²		Mature L2 larvae/ m ²		New-generation PB/ m ²			
		(\pm SEM)		(\pm SEM)		(\pm SEM)		(\pm SEM)		(\pm SEM)			
2008	NK Passion	97 (\pm 2.4)	A	23 (\pm 9.3)	A	-	-	653 (\pm 96.2)	A	214 (\pm 13.7)	A		
	Elektra	90 (\pm 3.6)	AB	61 (\pm 28.6)	A	-	-	733 (\pm 68.7)	A	285 (\pm 16.4)	A		
	Favorite	30 (\pm 2.1)	B	25 (\pm 7.4)	A	-	-	605 (\pm 51.6)	A	315 (\pm 37.0)	A		
	Titan	35 (\pm 3.0)	AB	44 (\pm 10.7)	A	-	-	569 (\pm 87.7)	A	301 (\pm 42.9)	A		
2009	NK Passion	201 (\pm 41.6)	A	41 (\pm 20.9)	A	991 (\pm 318.6)	A	477 (\pm 83.7)	AB	2262 (\pm 393.3)	AB	407 (\pm 85.1)	A
	Elektra	357 (\pm 67.1)	A	177 (\pm 38.2)	A	1958 (\pm 632.2)	A	672 (\pm 104.8)	AB	4130 (\pm 375.5)	A	650 (\pm 117.3)	A
	Favorite	285 (\pm 80.7)	A	45 (\pm 21.8)	A	659 (\pm 245.1)	A	172 (\pm 56.2)	A	1058 (\pm 154.2)	B	272 (\pm 54.1)	A
	Titan	320 (\pm 50.2)	A	115 (\pm 41.3)	A	1711 (\pm 395.2)	A	692 (\pm 126.3)	B	3236 (\pm 216.6)	AB	554 (\pm 72.8)	A
2010	NK Passion	486 (\pm 47.2)	A	-	-	1361 (\pm 261.7)	A	122 (\pm 65.4)	A	3376 (\pm 640.5)	A	338 (\pm 80.9)	A
	Elektra	618 (\pm 75.6)	A	-	-	2734 (\pm 508.6)	A	129 (\pm 27.4)	A	4824 (\pm 391.8)	A	298 (\pm 19.2)	A
	Favorite	388 (\pm 15.9)	A	-	-	1288 (\pm 153.4)	A	37 (\pm 11.1)	A	2638 (\pm 220.1)	A	289 (\pm 21.6)	A
	Titan	615 (\pm 45.8)	A	-	-	1829 (\pm 359.4)	A	195 (\pm 142.7)	A	4748 (\pm 455.1)	A	403 (\pm 55.5)	A

In 2008, peak abundance of overwintered pollen beetles in spring was significantly lower on cv. 'Favorite' than on cv. 'NK Passion' (Table 2). The number of overwintered beetles was not correlated with the number of eggs, L2 larvae and new-generation beetles emerging in summer (Table 2). Larval mortality caused by premature larval dropping and by parasitism did not differ significantly between cultivars (Table 3). Only the specialist parasitoids *T. heterocerus* and *P. interstitialis* were identified from larvae. The emergence rate of new-

generation beetles tended to be higher in late flowering cultivars than in early flowering cultivars. The corrected emergence rate which was taking only non-parasitised larvae into the calculation ranged from 58.4% to 90.3%. The growth rate of the pollen beetle population was significantly higher on cv. 'Favorite' and 'Titan' than on the early flowering cv. 'NK Passion' (Table 3).

Table 3: Population development and mortality factors of pollen beetle on four oilseed rape cultivars in field experiments 2008-2010 (Growth rate = ratio new-generation beetles:overwintered beetles) (upper case letters indicate significant differences between cultivars in one year; $p \leq 0.05$; Kruskal-Wallis test).

Year	Cultivar	Premature larval dropping		Parasitism rate		Emergence rate		Corrected emergence rate		Growth rate	
		L1/ m ² (± SEM)	A	[%] (± SEM)	A	[%] (± SEM)	A	[%] (± SEM)	A	(± SEM)	A
2008	NK Passion	24 (± 10.4)	A	41.4 (± 1.1)	A	34.3 (± 4.4)	A	58.4 (± 6.9)	A	2.2 (± 0.1)	A
	Elektra	58 (± 18.4)	A	42.3 (± 2.9)	A	39.4 (± 2.6)	A	68.7 (± 4.9)	A	3.2 (± 0.3)	AB
	Favorite	68 (± 15.4)	A	39.1 (± 1.2)	A	53.4 (± 8.6)	A	87.2 (± 12.4)	A	10.9 (± 2.0)	B
	Titan	47 (± 12.4)	A	41.0 (± 1.6)	A	53.2 (± 2.8)	A	90.3 (± 4.5)	A	8.9 (± 1.8)	B
2009	NK Passion	476 (± 66.4)	A	46.3 (± 5.5)	A	17.7 (± 2.0)	A	33.8 (± 4.2)	A	2.1 (± 0.4)	A
	Elektra	453 (± 44.0)	A	44.2 (± 3.2)	A	15.5 (± 1.9)	A	27.7 (± 2.5)	A	2.0 (± 0.4)	A
	Favorite	349 (± 63.0)	A	42.2 (± 4.2)	A	26.2 (± 4.1)	A	46.3 (± 7.7)	A	1.2 (± 0.4)	A
	Titan	572 (± 74.3)	A	45.4 (± 6.2)	A	17.2 (± 2.4)	A	32.3 (± 4.4)	A	1.9 (± 0.5)	A
2010	NK Passion	1461 (± 175.3)	AB	72.6 (± 2.5)	A	9.8 (± 0.8)	AB	36.5 (± 3.6)	A	0.7 (± 0.1)	A
	Elektra	1661 (± 82.7)	A	77.3 (± 3.9)	A	6.3 (± 0.7)	A	30.2 (± 6.1)	A	0.5 (± 0.1)	A
	Favorite	960 (± 124.5)	B	70.8 (± 2.1)	A	11.0 (± 0.5)	B	38.6 (± 4.4)	A	0.7 (± 0.1)	A
	Titan	1478 (± 134.3)	AB	71.0 (± 2.4)	A	8.4 (± 0.5)	AB	29.1 (± 1.0)	A	0.7 (± 0.1)	A

In 2009, when higher numbers of eggs were laid and higher numbers of L1 and L2 larvae developed in hybrid cultivars than in open pollinated cultivars (Table 2), the emergence rate and growth rate of pollen beetle populations was considerably smaller than in 2008 and did not differ between cultivars (Table 3). In buds of cv. 'Titan' a significantly higher number of L1 larvae was recorded compared to cv. 'Favorite'. The number of mature L2 larvae was significantly higher on cv. 'Elektra' than on cv. 'Favorite'.

In 2010, higher numbers of eggs, L1 and L2 larvae were found in hybrids than in open-pollinated cultivars, however, the number of individuals was not significantly different between cultivars (Table 2). The level of the larval parasitism increased from 42.2-46.3% in

2009 to 70.8-77.3% in 2010. The emergence rate of new generation beetles significantly differed between cv. `Elektra` and cv. `Favorite` (Table 3).

Premature larval dropping significantly increased with increasing abundance of mature L2 larvae ($r^2 = 0.7057$, $p = 0.000$, $y = -87.0985 + 0.3001 \cdot x$, $n = 48$) over the three years. In each year, the emergence rate of new-generation beetles was significantly influenced by the length of time from the start of beetle immigration to full flowering of the tested cultivars (2008: $r^2 = 0.4453$, $p = 0.0047$; 2009: $r^2 = 0.4175$, $p = 0.0068$; 2010: $r^2 = 0.6352$, $p = 0.0002$) (Figure 2). The precipitation during the emergence period differed between the three years. A high amount (87mm) and moderate amount (53mm) of rainfall occurred in a period of 22 days and 28 days in 2008 and 2009, respectively. In 2010, the precipitation decreased drastically to only 19mm in 25 days.

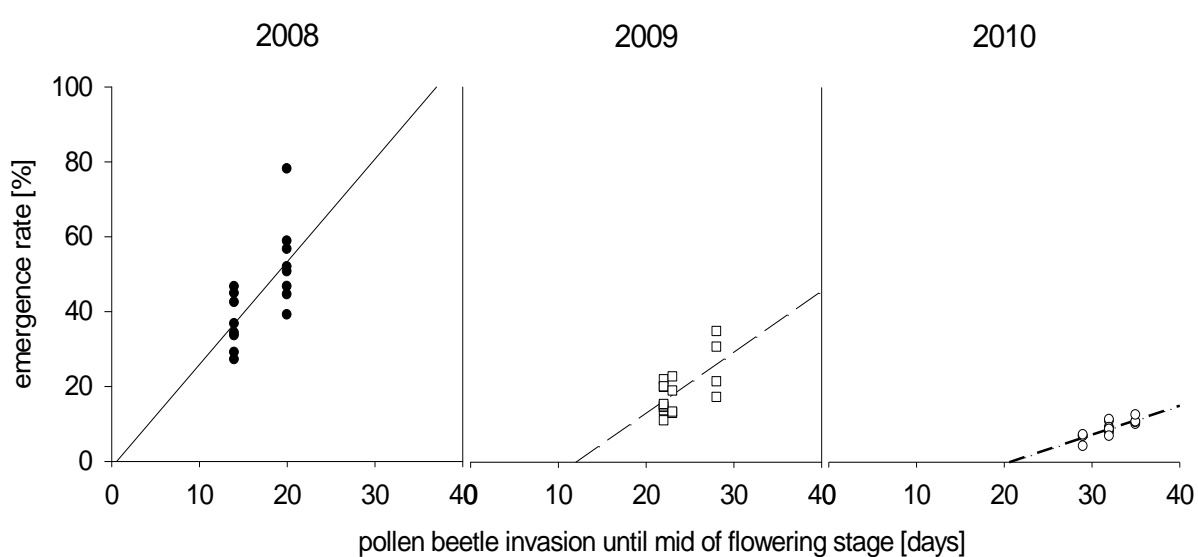


Figure 2: Effect of the length of time from the beginning of pollen beetle immigration until full flowering stage on emergence of adults in three years (2008: $-1.5308 + 2.7424 \cdot x$, 2009: $y = -19.5782 + 1.6315 \cdot x$, 2010: $y = -15.9682 + 0.7764 \cdot x$).

The dissection of buds sampled from all cultivars showed that females deposited eggs only into buds of the size 2-3mm and >3mm. No eggs were found in buds <2mm. In samples collected on 26 April 2010, the maximum proportion of buds used for feeding and oviposition was $66.1 \pm 3.5\%$ (mean \pm SEM) (first side raceme of `Elektra`, bud size > 3mm) and $47.1 \pm 4.9\%$ (mean \pm SEM) (third side raceme of `Elektra`, bud size 2-3mm) (Table 4). Significant differences were recorded between cultivars; cv. `Elektra` was most attractive for oviposition and feeding. On 17 April 2009, the rank order of cultivars used for feeding and oviposition was `Titan` > `Elektra` > `NK Passion` > `Favorite` (bud size 2-3mm). On cv. `Titan` the highest percentage of the buds >3mm was used for oviposition and feeding (Table 4).

Table 4: Proportion (mean \pm SEM [%]) of buds showing feeding and oviposition holes in relation to the total number of available buds (size 2-3mm and > 3mm) and mean number (\pm SEM) of eggs per bud on four oilseed rape cultivars in 2009 and 2010 ($p \leq 0.05$ = significant difference between cultivars within line; Kruskal-Wallis test).

Sampling date	Bud size	Position of raceme	cv. NK Passion		cv. Elektra		cv. Favorite		cv. Titan		H _{3,0,0.95}	p
			mean	\pm SEM	mean	\pm SEM	mean	\pm SEM	mean	\pm SEM		
17 April 2009	2-3mm	main raceme	11.8	4.4	24.5	2.2	11.6	1.9	32.8	10.0	6.949	0.074
		side raceme 1	10.5	3.9	28.7	8.1	3.7	1.8	37.8	2.9	10.277	0.016
		side raceme 3	6.7	3.2	24.2	5.8	3.3	2.1	28.4	1.2	10.863	0.013
		side raceme 5	7.2	1.3	17.6	5.4	3.3	3.3	19.5	7.1	6.750	0.080
	>3mm	main raceme	20.9	4.0	28.6	1.8	35.4	4.2	40.8	4.2	8.559	0.036
		side raceme 1	40.0	3.9	39.1	5.0	33.2	5.3	56.1	6.2	6.904	0.075
		side raceme 3	33.9	3.6	47.1	4.9	27.5	6.0	56.9	4.7	9.593	0.022
		side raceme 5	26.1	4.8	42.5	6.1	15.7	5.0	50.0	6.1	9.420	0.024
		eggs/bud			1.7		1.9		3.2		3.3	
	26 April 2010	2-3mm	main raceme	18.2	2.8	28.6	5.9	35.4	3.4	30.3	4.1	4.610
side raceme 1			14.8	5.1	39.1	2.9	33.2	4.7	24.0	2.5	3.199	0.362
side raceme 3			11.5	2.8	47.1	2.0	27.5	3.8	24.8	4.8	6.772	0.080
side raceme 5			6.7	0.5	42.5	3.0	15.7	1.6	19.7	3.1	8.912	0.031
>3mm		main raceme	44.0	6.4	61.9	4.9	47.1	4.8	48.0	3.1	5.537	6.137
		side raceme 1	40.2	3.9	66.1	3.5	44.1	3.9	51.3	2.7	10.743	0.013
		side raceme 3	26.3	1.1	55.9	1.3	43.0	5.1	50.9	2.5	11.404	0.010
		side raceme 5	22.7	3.2	43.3	2.4	34.4	10.0	41.8	6.0	5.272	0.153
		eggs/bud			1.6		2.0		1.7		1.7	

A high proportion of infested buds contained one egg ($44.4 \pm 4.3\%$) (mean \pm SEM), while two eggs and three eggs per bud were found in $32.1 \pm 3.54\%$ (mean \pm SEM) and $7.9 \pm 1.32\%$ (mean \pm SEM) of buds, respectively. A maximum clutch size of 11 eggs was found in $0.1 \pm 0.05\%$ (mean \pm SEM) of buds. In 2009, females in average deposited more eggs into the buds of hybrid cultivars. The number of buds per plant did not differ between cultivars in 2009 (Table 5) while in plants sampled in 2010, the number of buds per plant significantly differed between cultivars.

Table 5: Mean number (\pm SEM) of buds per plant on four oilseed rape cultivars in 2009 and 2010.

Sampling date	Bud size	cv. NK Passion		cv. Elektra		cv. Favorite		cv. Titan		H _{3,0,0.95}	p
		mean	\pm SEM	mean	\pm SEM	mean	\pm SEM	mean	\pm SEM		
17 April 2009	2-3mm	28.5	5.7	39.1	6.9	38.2	4.5	45.6	10.3	2.206	0.531
	>3mm	77.7	5.8	67.7	8.1	49.4	13.1	64.4	3.7	3.507	0.320
26 April 2010	2-3mm	34.1	6.1	34.5	1.9	47.3	4.5	33.3	3.3	8.824	0.032
	>3mm	76.7	10.0	92.2	12.9	51.2	13.6	78.1	12.9	10.478	0.015

Semi-field experiment

In 2009, the accumulated number of second instar larvae and of emerging new-generation pollen beetles was significantly higher at early infestation than at late infestation of cv. `NK Passion´ by overwintered pollen beetles (second instar larvae: $p = 0.022$, new-generation beetles: $p = 0.02$) (Figure 3). In cv. `Favorite´ the same trend was observed, however, the numbers in early and late infestations were not significantly different.

Migration of L2 larvae to the ground started on 21 April and increased in the early infestation treatment of both cultivars until 12 May 2009. The new generation beetles emerged within a period of four weeks. In the early and late infestation treatments of cv. `NK Passion´ maximum numbers of emerging beetles occurred on 14 June. On cv. `Favorite´ maximum numbers occurred in early and late infestation treatments three and six days later, respectively, than on cv. `NK Passion´. On both cultivars, maximum numbers of emerging beetles were higher in early infestation treatments (`NK Passion´ mean $268.0 \pm \text{SEM } 59.3$ beetles/m², `Favorite´ mean $215.2 \pm \text{SEM } 31.8$ beetles/m²) than in late infestation treatments (`NK Passion´ mean $120.0 \pm \text{SEM } 37.9$ beetles/m², `Favorite´ mean $198.4 \pm \text{SEM } 52.5$ beetles/m²).

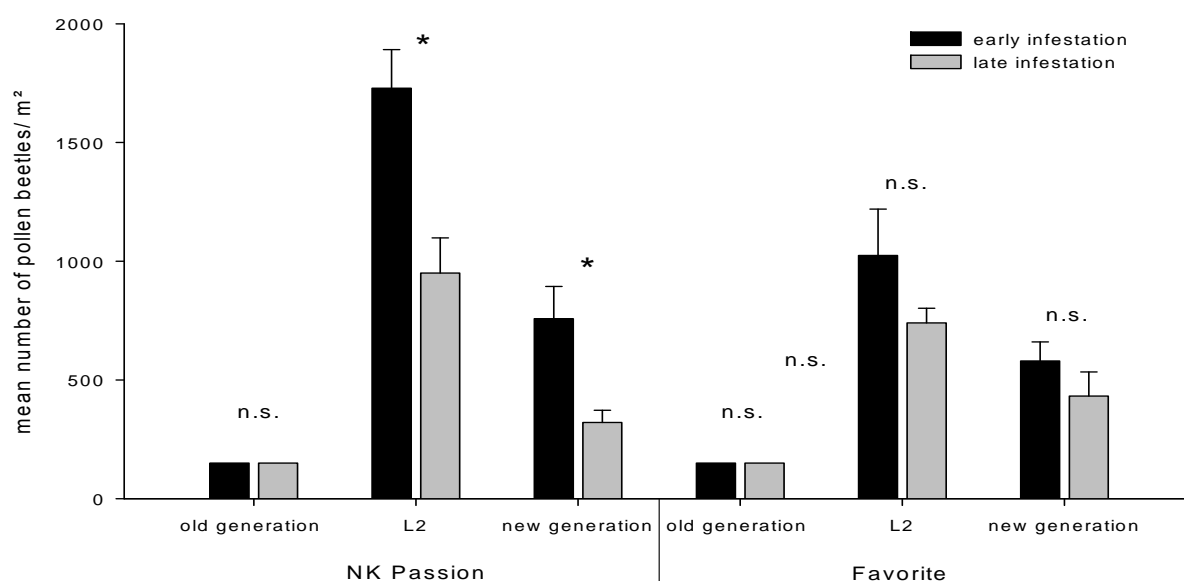


Figure 3: Cumulative number of second instar larvae and new-generation beetles in a semi-field experiment in 2009 (* indicate significant differences with $p \leq 0.05$ between treatments; Mann-Whitney-U test).

Discussion

Adult pollen beetles locate their host plants by odour cues using upwind anemotaxis (Evans & Allen-Williams, 1994; Cook et al., 2002; Williams et al., 2007). Host plant volatiles, particularly isothiocyanates, are carried downwind from the crop. The yellow and green colours of the plants are important cues for orientation. In spring 2008, our field experiment showed that winter oilseed rape cultivars in the early flowering stage were preferred by over-

wintered pollen beetles at the beginning of crop colonization. It has been previously reported that the plant growth stage has a significant effect on the colonization behaviour of pollen beetles (Free & Williams, 1978). Beetles were more abundant on plants in flower or the yellow bud stage than in the green bud stage (Free & Williams, 1978). The host plant preference was tested in polytunnel experiments, offering a turnip rape cultivar, a conventional cultivar of oilseed rape and a cultivar with low isothiocyanate emissions in the flower and bud stage. Irrespective of plant species or cultivar, the numbers of pollen beetles on the plants in flowering stage were significantly higher (Cook et al., 2006b). As all four cultivars developed to the green and yellow bud growth stage simultaneously in our field experiments in 2009 and 2010, we expected that all cultivars might be colonized by similar numbers of adult pollen beetles. However, the cumulative beetle-days showed that the hybrids 'Elektra' and 'Titan' were more attractive than the open pollinating cultivars when all were in a similar growth stage. In earlier studies, oilseed rape cultivars with higher amounts of glucosinolates were more attractive to adult pollen beetles compared to cultivars with low content of glucosinolates (Giamoustaris & Mithen, 1996, Cook et al., 2006b). Giamoustaris & Mithen (1996) concluded that after biting of adult beetles through the sepals the release of isothiocyanates may be repellent or deterrent. Further investigations are necessary to find out whether the glucosinolate content of the inflorescences could be deterrent or repellent and a factor for low numbers of adult pollen beetles in winter oilseed rape cultivars tested in this study.

Other behavioural responses to chemical cues may have affected orientation and distribution and influenced pollen beetle abundance on cultivars in our experiments. Aggregations of pollen beetles were observed on individual plants of spring rape (Free & Williams, 1978; Nielsen & Axelsen, 1988b). Cook et al. (2006a) found no evidence for pollen beetle male-produced aggregation pheromones in laboratory experiments. They suggested that visual cues may play a more important role for aggregation. Furthermore they identified an epideictic pheromone released by females. Pollen beetles significantly moved away from a group of 200 females on one plant (Cook et al., 2006a).

Pollen beetle oviposition in spring depends on the buds available on oilseed rape plants (Ekbom & Borg, 1996). Recent studies as well as our experiments have shown that females deposit their eggs into *B. napus* buds with the size of 2-3mm and >3mm (Nilsson, 1988c; Ekbom & Borg, 1996). The mean number of 2-3 eggs per bud was similar to the number reported by Nilsson (1988c) from winter rape, but in contrast to 3-4 eggs/bud found in spring rape (Hokkanen, 2000). The bud stage is one of the most relevant phenological stages of rape plants determining population development of pollen beetles (Nilsson, 1988c; Ekbom & Borg, 1996; Hokkanen, 2000). Ekbom and Borg (1996) found the greatest number of eggs in plants when more than one third of all buds was medium-sized (2-3mm). Females prefer this bud stage because the eggs and first instar larvae are protected within closed buds (Nilsson, 1988c). The availability of medium-sized buds had no apparent influence on oviposition into the four cultivars in our studies. On 17 April 2009, the number of eggs deposited on cv. 'Favorite' amounted to only one third of the number of eggs on cv. 'Elektra', the cultivar with the greatest number of eggs. However, the supply of buds with the size of 2-3mm was on the same level on both cultivars. In 2010, although the number of buds of 2-3mm size was higher

on cv. 'Favorite' than on cv. 'Elektra', nearly half of the egg numbers deposited into buds of cv. 'Elektra' were found in buds of cv. 'Favorite'. There was no evidence that the supply of buds influenced the oviposition preference for the hybrids 'Elekta' and 'Titan' in 2009 and 2010. Obviously, there was no shortage of resources for oviposition: in both years of our field experiments, the proportion of buds with oviposition and feeding injuries did not exceed 66%. However, this data represents only one single sampling occasion and multiple samples are required for precise estimates of the whole number of eggs and feeding injuries during the bud stage. In 2009, the four cultivars developed nearly simultaneously and the bud stage continued 16 and 18 days after pollen beetle immigration into the early flowering and late flowering cultivars, respectively. In 2010, the possible main egg laying period was extended to 23 and 28 days of the early flowering and late flowering cultivars, respectively. Increasing numbers of eggs, L1 larvae and L2 larvae might result from an extended duration of available buds and favourable annual weather conditions (Nilsson, 1988d).

In our field experiments the larger number of eggs and larvae in hybrids did not result in a higher abundance of emerged pollen beetles compared to open pollinated cultivars. It can be assumed that intraspecific competition between larvae was a reasonable factor. Second instar larvae are very mobile and move from flower to flower to feed on pollen (Williams & Free, 1978); they may force the smaller first instar larvae out of the flower (Hokkanen, 2000). Mortality due to dropping of pre-mature larvae was also described by Nilsson (1988d). In the three years, significant correlations between the numbers of first and second instar larvae indicate that higher numbers of second instar larvae cause a higher rate of first instar larvae dropping to the ground. Intraspecific competition is a density depending effect that may cause high larval mortality.

Hymenopterous parasitoids and carabid beetles are important mortality factors of pollen beetle larval stages and can reduce the emergence rate of new generation beetles (Nilsson, 1988d; Büchi, 2002). In previous studies parasitism rates exceeding 50% were found (Nitzsche, 1998; Billquist & Ekbohm, 2001; Büchi, 2002). In our field experiments the parasitism ranged between 39.1% and 77.3%. The parasitism rate depends on synchronisation between host and parasitoid phenology (Nitzsche, 1998). The abundance and phenology of parasitoids is influenced by a large range of factors like landscape structure (Thies & Tschardtke, 1999), weather conditions and crop management (Nilsson, 2003). The field experiments at Goettingen were conducted in a complex landscape structure with hedges that may have favoured the occurrence of the parasitoids within the three years. In addition, entomopathogenic fungi and nematodes were reported as effective antagonists that kill the larvae in soil (Nielsen & Philipsen, 2005; Hokkanen, 2008). However, these antagonists in agricultural soils apparently have no relevant effect on pollen beetle mortality in Europe (Hokkanen, 2008); this aspect needs to be studied further.

In 2008, the corrected emergence rate of new-generation beetles with 58.4 to 90.3% was very high. In the following two years it decreased significantly. In addition to antagonists, mortality during pupation in soil might have been increased by unfavourable weather conditions (Ellis et al., 2004; Schierbaum-Schickler, 2005). From 18 June to 12 July 2010, the amount of precipitation with 19mm was on a low level. Ellis et al. (2004) assumed that larvae of the Nitidulide *Aethina tumida* died in dry soils because of predrying or suffocation.

In our study, the emergence rate was significantly correlated with the time period available for egg-laying and larval development of pollen beetle during the bud and flowering stage. In 2008 in particular, but also in 2009 and 2010, a short time period caused a low emergence rate. The period required for development of eggs, first and second instar larvae depends on temperature and relative humidity (Fritzsche, 1957; Nielsen & Axelsen, 1988a). Developmental of eggs and larvae takes 20-25 days in winter rape (Nilsson, 1988d). In this period their numbers are limited by the supply of buds and flowers as a source of food. If the larvae are not mature for pupation at the end of flower and petal fall, a high mortality might be caused by premature pupation of larvae (Nielsen & Axelsen, 1988a). Cook et al. (2004) confirmed this statement by laboratory experiments, demonstrating the influence of food quantity on larval development and pupation: less second instar larvae, prepupae, pupae and adult pollen beetles developed after a diet without pollen. Despite the higher adult pollen beetle abundance on the early flowering cultivars 'NK Passion' and 'Elektra', the emergence rate and population growth rate were on a low level compared to the late flowering cultivars in 2008. Thus, a shorter time for development in flower may have increased the risk of larvae to suffer from food shortage, preventing successful pupation. The results of the semi-field experiment in 2009 confirmed the impact of food quantity on larval development. The delay of beetle colonization by 7 days caused significantly lower numbers of second instar larvae and newly emerged pollen beetles in the cv. 'NK Passion'.

The growth rate of the pollen beetle population differed widely between years. The ratio between the abundance of overwintered beetles and new-generation beetles varied from 1:0.5 and 1:11. Similar results between 1:1 and 1:10 were reported by Nilsson (1988d). This author mentioned that minor changes, for example in crop management, can influence the survival during development of pollen beetles. Plant growth characteristics of cultivars, like the number of buds and duration of flowering period, are important for the survival of immature stages. In 2008, the cvs. 'NK Passion' and 'Elektra' began to flower 6 days after beetle immigration and maintained flowering for 13 days. This short time period resulted in a limited population growth which was reflected by a lower ratio between the abundance of overwintered beetles and their progeny compared to cv. 'Titan' and 'Favorite'.

In conclusion, the population growth of pollen beetle can be negatively influenced by early flowering winter oilseed rape cultivars. The temperature, the date of colonization and the plant growth stage were major restricting factors for larval development and the abundance of new-generation beetles. Early flowering cultivars have potential to be used in integrated pest management systems for limiting the population outcome in summer and in spring of the following year.

Acknowledgements

This research was supported by the Federal Ministry of Food, Agriculture and Consumer Protection. We wish to thank BioTest Labor (BTL), Sagerheide, Germany for providing pollen beetles. The technical assistance of Dorothea Mennerich, Hubertus Reintke and Rainer

Wedemeyer is highly appreciated. We thank Welf Gehrke, Ulrike Jeché, Ruth Lefering, Joshua Sikhu Okonya, Sonja Schneider, Kim Stey, Florian Rekate and Ines Vollhard for field and laboratory work and Heike Beckmann for helpful comments to an earlier draft of the manuscript.

References

- Alford, D. V. (2003). Insect pest of oilseed rape crops. In: Alford D.V. (ed.) *Biocontrol of oilseed rape pests*. Oxford, UK: Blackwell Publishing, 9-41.
- Billquist, A. & Ekblom, B. (2001). The influence of host plant species on parasitism of pollen beetles (*Meligethes* spp.) by *Phradis morionellus*. *Entomologia Experimentalis et Applicata* 98, 41-47.
- Börjesdotter, D. (2000). *Barbarea verna* and *Barbarea vulgaris* as host plants for the pollen beetle (*Meligethes aeneus*). *Journal of Agricultural Science* 134, 213-220.
- Büchi, R. (2002). Mortality of pollen beetle (*Meligethes* spp.) larvae due to predators and parasitoids in rape fields and the effect of conservation strips. *Agriculture, Ecosystems and Environment* 90, 255-263.
- Cook, S. M., Bartlett, E., Murray, D. A. & Williams, I. H. (2002). The role of pollen odour in the attraction of pollen beetles to oilseed rape flowers. *Entomologia Experimentalis et Applicata* 104, 43-50.
- Cook, S. M., Murray, D. A., Watts, N. P. & Williams, I. H. (2006a). Responses of pollen beetle (*Meligethes aeneus*) for conspecific odours. *Integrated Control in Oilseed Crops, IOBC/wprs Bulletin* 29, 143-150.
- Cook, S. M., Murray, D. A. & Williams, I. H. (2004). Do pollen beetles need pollen? The effect of pollen on oviposition, survival, and development of a flower-feeding herbivore. *Ecological Entomology* 29, 164-173.
- Cook, S. M., Smart, L. E., Martin, J. L., Murray, D. A., Watts, N. P. & Williams, I. H. (2006b). Exploitation of host plant preferences in pest management strategies for oilseed rape (*Brassica napus*). *Entomologia Experimentalis et Applicata* 119, 221-229.
- Ekblom, B. & Borg, A. (1996). Pollen beetle (*Meligethes aeneus*) oviposition and feeding preferences on different host plant species. *Entomologia Experimentalis et Applicata* 78, 291-299.
- Ellis, J., Hepburn, R. & Elzen, P. (2004). Effects of soil type, moisture, and density on pupation success of *Aethina tumida* (Coleoptera : Nitidulidae). *Environmental Entomology* 33, 794-798.
- Evans, K. A. & Allen-Williams, L. J. (1994). Laboratory and field response of the pollen beetle, *Meligethes aeneus*, to the odour of oilseed rape. *Physiological Entomology* 19, 285-290.
- Free, J. B. & Williams, I. H. (1978). The responses of the pollen beetle, *Meligethes aeneus*, and the seed weevil, *Ceuthorrhynchus assimilis*, to oil-seed rape, *Brassica napus*, and other plants. *Journal of Applied Ecology* 15, 761-774.
- Fritzsche, R. (1957). Zur Biologie und Ökologie der Rapsschädlinge aus der Gattung *Meligethes*. *Sonderdruck aus Zeitschrift für angewandte Entomologie* 40, 222-280.
- Giamoustaris, A. & Mithen, R. (1996). The effect of flower colour and glucosinolates on the interaction between oilseed rape and pollen beetles. *Entomologia Experimentalis Et Applicata* 80, 206-208.
- Hokkanen, H. M. T. (2008). Biological control methods of pest insects in oilseed rape. *IOBC/wprs Bulletin* 38, 104-109.
- Hokkanen, H. M. T. (2000). The making of a pest: recruitment of *Meligethes aeneus* onto oilseed Brassicas. *Entomologia Experimentalis et Applicata* 95, 141-149.
- Horstmann (1971). Revision der Europäischen Tersilochinen I (Hymenoptera: Ichneumonidae). *Veröffentlichung der zoologischen Staatssammlung München* 15, 47-138.
- Horstmann, K. (1981). Revision der Europäischen Tersilochinen II (Hymenoptera: Ichneumonidae). *Spixiana, Suppl.* 4, 1-76.
- Lancashire, P. D., Bleiholder, H., Vandenboom, T. & Langeluddeke, P. (1991). A uniform decimal code for growth stages of crops and weeds. *Annals of Applied Biology* 119, 561-601.
- Mithen, R. (1992). Leaf glucosinolate profiles and their relationship to pest and disease resistance in oilseed Rape. *Euphytica* 63, 71-83.
- Nielsen, O. & Philipsen, H. (2005). Susceptibility of *Meligethes* spp. and *Dasyneura brassicae* to entomopathogenic nematodes during pupation in soil. *BioControl* 50, 623-634.

- Nielsen, P. S. & Axelsen, J. (1988a). Developmental time and mortality of the immature stages of the pollen beetle (*Meligethes aeneus* F) under natural conditions. *Journal of Applied Entomology* 105, 198-204.
- Nielsen, P. S. & Axelsen, J. (1988b). Spatial-distribution of the pollen beetle (*Meligethes aeneus* F) in spring sown rape (*Brassica napus* L) in Denmark, and its importance for sampling. *Journal of Applied Entomology* 105, 35-40.
- Nilsson, C. (1988a). Pollen beetles (*Meligethes aeneus* F.) and flowering in rape. *Swedish J. agric. Res.* 18, 113-118.
- Nilsson, C. (1988b). The number of larval instars of *Meligethes aeneus* (F.) in southern Sweden. *Växtskyddsnotiser* 52, 151-152.
- Nilsson, C. (1988c). The pollen beetle (*Meligethes aeneus* F.) in winter and spring rape at Alnarp 1976-1978. II. Oviposition. *Växtskyddsnotiser* 52, 139-144.
- Nilsson, C. (1988d). The pollen beetle (*Meligethes aeneus* F.) in winter and spring rape at Alnarp 1976-1978. III. Mortality factors. *Växtskyddsnotiser* 52, 145-150.
- Nilsson, C. (1994). Pollen beetles (*Meligethes* spp.) in oil seed rape crops (*Brassica napus* L.): Biological interactions and crop losses. Dissertation, Swedish University of Agricultural Sciences, Department of Plant Protection Sciences, Faculty of Agriculture.
- Nilsson, C. (2003). Parasitoids of pollen beetles. In: Alford D. V. (ed.) *Biocontrol of oilseed rape crops*. Oxford, UK: Blackwell Publishing, 73-85.
- Nitzsche, O. (1998). Auftreten und Effizienz von Parasitoiden als natürliche Gegenspieler von Schadinsekten in Winterraps unter besonderer Berücksichtigung unterschiedlicher Bodenbearbeitungsmaßnahmen nach Winterraps. Diss. Georg-August-Universität.
- Osborne, P. (1960). Observations on the natural enemies of *Meligethes aeneus* (F.) and *M. viridescens* (F.) [Coleoptera: Nitidulidae]. *Parasitology* 50, 91-110.
- Ruppel, R. F. (1983). Cumulative insect-days as an index of crop protection. *Journal of Economic Entomology* 76, 375-377.
- Schierbaum-Schickler, C. (2005). Einfluß einer mehrjährig differenzierten Bodenbearbeitung auf die Befallsdichte und Populationsentwicklung von Schadinsekten in Winterrapskulturen. Diss. Göttingen: Georg-August-Universität Göttingen.
- Singh, D. & Singh, A. (2005). *Disease and insect resistance in plants*. Enfield, NH, USA: Science Publishers.
- Thies, C. & Tschardtke, T. (1999). Landscape structure and biological control in agroecosystems. *Science* 285, 893-895.
- Williams, I. H., Frearson, D., Barari, H. & McCartney, A. (2007). Migration to and dispersal from oilseed rape by the pollen beetle, *Meligethes aeneus*, in relation to wind direction. *Agricultural and Forest Entomology* 9, 279-286.
- Williams, I. H. & Free, J. B. (1978). The feeding and mating behaviour of pollen beetles (*Meligethes aeneus* Fab.) and seed weevils (*Ceutorhynchus assimilis* Payk.) on oil-seed rape (*Brassica napus* L.). *Journal of Agricultural Science, Cambridge* 91, 453-459.
- Williams, I. H. & Cook, S. M (2010) Crop location by oilseed rape pests and host location by their parasitoids. In: Williams, I. H. (ed.) *Biocontrol-based integrated management of oilseed rape pests*. Netherlands: Science+Business Media B. V. Springer, 215-243.
- Winfield, A. L. (1961). Field observations on the control of blossom beetles (*Meligethes aeneus* F.) and cabbage-seed weevils (*Ceuthorhynchus assimilis* Payk.) on mustard-seed crops in East Anglia. *Annals of Applied Biology* 49, 539-555.

Effects of insecticides on abundance of pollen beetles (*Meligethes aeneus* Fabricius) and yields in oilseed rape

Abstract

Pollen beetle, *Meligethes aeneus* (Fabricius) (Col.: Nitidulidae), is a major pest of oilseed rape throughout Europe. Chemical control is impeded by widespread resistance to pyrethroid insecticides and new insecticidal agents are needed for regulating resistant populations and control of pest outbreaks. In three-year field experiments, this study investigates the effect of synthetic insecticides on the abundance of various life stages of pollen beetle (overwintered adults, eggs, first and second instar larvae, new generation beetles) and subsequent bud and yield losses in oilseed rape. Two active ingredients, the organophosphate chlorpyrifos-methyl and the neonicotinoide thiacloprid were applied in a sequence during the bud and flowering stage of winter oilseed rape. The abundance of overwintered pollen beetles was significantly reduced by insecticide treatments in two years. The efficacy of insecticides on adult beetles varied widely between 2.3% and 77.4%, depending on the time of application. Likewise, numbers of second instar larvae and new generation beetles were significantly reduced by insecticides in all three years. Compared to untreated plots, percentage reduction of the abundance of second instar larvae and of emerging new-generation beetles ranged from 83.2% to 90.6% and from 82.5% to 95.5%, respectively. Although bud losses caused by pollen beetles was significantly higher in untreated plots, there were no significant differences between seed yields of oilseed rape in untreated and treated plots. In conclusion, our results indicate that adult populations may remain on high levels after repeated insecticide applications in spring, whereas the abundance of new generation beetles can be strongly inhibited.

Introduction

Pollen beetle, *Meligethes aeneus* (Fabricius) (Col.: Nitidulidae), is a common pest of oilseed rape, *Brassica napus* L. (Brassicaceae) in Europe (Alford et al., 2003). In March/April, during the green and yellow bud stage of the crop, adult beetles bite into buds to feed on pollen, thereby causing bud abortion and reduced development of pods (Winfield, 1961; Nilsson, 1988). Yield losses eventually can exceed 80% (Ahuja et al., 2010). Oviposition starts a few days after crop colonization and continues as long as buds are available. Females deposit one to six eggs into buds of various brassicaceous plants (Ekbohm & Borg, 1996). The univoltine pollen beetle has two larval instars which develop in 3–4 weeks within the buds and later on in open flowers (Williams & Free, 1978; Nilsson, 1994). In May/June the full-grown second instar larvae drop to the ground for pupation in upper soil layers. New generation beetles emerge in June/July to feed on pollen of *Brassica* plants and many other plant species before overwintering (Nilsson, 1994).

With increasing areas grown with oilseed rape, the population density and damage potential of *M. aeneus* has increased as well (Hokkanen, 2000). Effective control measures are required when beetle numbers exceed the application threshold of 3–8 beetles per plant in the green bud stage (Alford et al, 2003; Williams, 2010). In 2010 and 2011, oilseed rape was sprayed with insecticides in Germany three times in average per growing period, of which the highest intensity was applied on pollen beetle (Freier et al., 2012). Due to the limited choice of registered insecticides and the extensive use of pyrethroid insecticides, pollen beetle populations have built up widespread resistance to these compounds in recent years across Europe (Slater et al., 2011). High levels of resistance to pyrethroids and corresponding high levels of plant infestation by pollen beetles have resulted in estimated yield losses of 22-25 million EUR in 2006 (Zlof, 2008). Following a severe reduction in sensitivity to pyrethroids in Germany (Heimbach et al., 2006), alternative insecticides with different modes of action, such as organophosphorous and neonicotinoid compounds, were recommended for control of pyrethroid-resistant pollen beetles (Slater et al., 2011, Heimbach & Müller, 2013). The organophosphate chlorpyrifos-methyl and the neonicotinoid thiacloprid are effective by systemic and contact activity, acting as acetylcholinesterase inhibitor and agonist on the nicotinic acetylcholin receptor in the central nervous system of insects, respectively (Elbert et al., 2008). In addition to direct mortality, sublethal effects of insecticides on beetle physiology and behaviour may affect longevity, fecundity and orientation of adults as well as larval development (Desneux et al. 2007; Stark and Banks, 2003). Effects of insecticides on the abundance of immature stages and new-generation pollen beetles, however, have not been studied.

The objective of the present study was to determine the effects of successive applications of thiacloprid and chlorpyrifos-methyl on the abundance of overwintered adults, eggs, first and second instar larvae, and new-generation adults of pollen beetles in winter oilseed rape. We hypothesized that (i) insecticide applications against adult pollen beetles would reduce their reproduction, resulting in lower abundance of new generation beetles emerging in summer, and that (ii) repeated insecticide sprays would reduce the damage caused by beetles and consequently increase yields of winter oilseed rape.

Materials and methods

The study was conducted in the region of Goettingen (N 51°33'456.38, E 9°56'56.43), Northern Germany. The effect of insecticide application on pollen beetle abundance and plant damage was tested under field conditions from 2008-2010. Each year, four winter oilseed rape cultivars ('NK Passion', 'Elektra', 'Favorite', 'Titan') were grown in a randomized block design with four replicated plots. The BBCH code of Lancashire et al. (1991) was used for characterizing the growth stages of winter oilseed rape. The data obtained from all cultivars have been pooled to examine the main factor insecticide treatment. Plot size was 150m² and half of the plots (75m²) was sprayed two to three times with insecticides during the bud and early flowering stage (Table 1). The commercial insecticides Biscaya (a.i. thiacloprid) and Reldan 22 (a.i. chlorpyrifos-methyl) were applied at

recommended dose rates of 0.3L/ha and 1.5L/ha, respectively, in 300L of water per ha. The abundance of adult pollen beetles on plants was assessed at seven, eight and eleven sampling dates in 2008, 2009 and 2010, respectively, before and following the insecticide applications. On each sampling date, the number of pollen beetles was recorded on the main raceme of 50 randomly selected plants per plot by beating each raceme onto a white plastic funnel (diameter 25cm). Plant density was assessed to calculate the number of beetles per square metre. Bud samples of main racemes and of lateral racemes (overall 3 racemes/plant in 2008; 4 racemes/plant in 2009 and 2010) of 20 randomly selected plants per plot were collected on 24 April 2008, on 8 and 17 April 2009 and on 26 April 2010. Samples were stored in plastic bags at 2°C and later dissected under a binocular microscope (magnification 25-40x) to count the number of eggs and first instar larvae (L1). To collect second instar larvae (L2) dropping from plants for pupation in soil, 30 trays (17cm x 12.5cm) containing water + detergent were randomly distributed on the ground of each plot. The traps were emptied at four-day intervals until the end of flowering (BBCH 69). The larvae were counted and their number computed to obtain the total larval abundance per m². The abundance of new generation beetles was assessed by four ground photoelectors (0.25m², ecoTech GmbH) per plot during the emergence period of three weeks in June (BBCH 71-85). During the pod stage (BBCH 79-83), the number of pods and blind stalks on the main raceme and one lateral raceme (2008: second lateral raceme from top; 2009 and 2010: third lateral raceme from top) was counted on 20 randomly selected plants per plot. At maturity (BBCH 97), yields were estimated from a subplot of 50m² per plot. In 2008, a hailstorm caused complete loss of harvest. In 2009 and 2010, thousand-seed-weights were additionally determined. Seed yield was recorded at 9% water content of seeds.

Table 1: Dates of insecticide applications to winter oilseed rape in field experiments 2008-2010.

Year	Date of application	Growth stage (BBCH)	Trade name	Active ingredient	Application rate [l/ha]
2008	24 April	59-61	Biscaya	240 g/l thiacloprid	0.3
	6 May	64-67	Biscaya	240 g/l thiacloprid	0.3
2009	6 April	51-53	Reldan 22	225 g /l chlorpyrifos-methyl	1.5
	14 April	55-59	Biscaya	240 g/l thiacloprid	0.3
	21 April	60-64	Biscaya	240 g/l thiacloprid	0.3
2010	8 April	51-53	Reldan 22	225 g /l chlorpyrifos-methyl	1.5
	20 April	55-59	Biscaya	240 g/l thiacloprid	0.3
	26 April	57-60	Biscaya	240 g/l thiacloprid	0.3

Based on the number of adult pollen beetles per m², cumulative beetle-days were calculated across the period of infestation (Ruppel, 1983) to compare the level of infestation in sprayed and unsprayed plots. Differences in beetle-days between treated and untreated plots were analysed using the nonparametric Mann-Whitney-U test. The efficacy of insecticide treatments was calculated according to the formula of Henderson & Tilton (1955).

Percentage bud loss resulting from adult beetle attack was calculated as the proportion of blind stalks in relation to the total number of mature pods and blind stalks. Differences in bud loss, yield, thousand-seed-weight and numbers of immature stages of pollen beetle between treated and untreated plots were analysed by the parametric two-sample t-test. All statistical tests were performed using the software STATISTICA, version 9.1 (StatSoft, Inc., 2010).

Results

In 2008 and 2009, the cumulative beetle-days of adult pollen beetles were significantly lower in all plots treated with insecticides (Figure 1) than in untreated plots. The efficacy of insecticide applications which was estimated three to five days following insecticide applications varied substantially in all years (Table 2). For example, in 2009, treatment with thiacloprid showed a very low efficacy at the third application but a high efficacy at the second application.

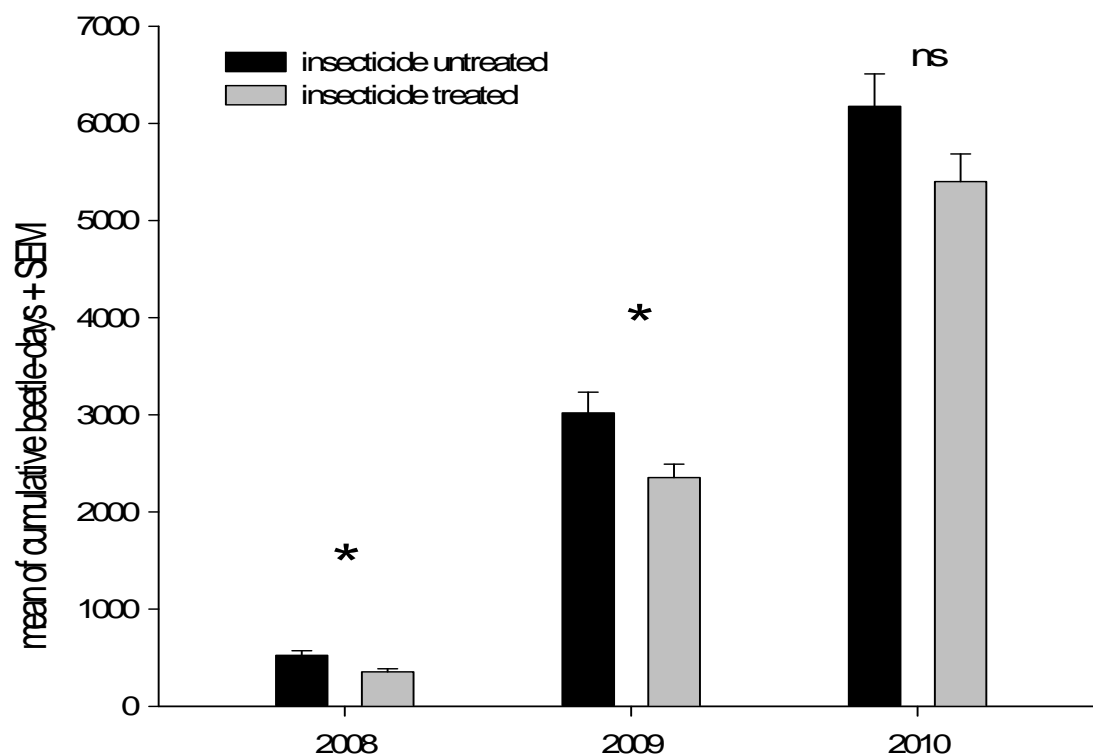


Figure 1: Cumulative beetle-days (mean \pm SEM) of pollen beetles in insecticide treated and untreated plots of winter oilseed rape cultivars in 2008-2010 (* significant differences between treated and untreated plots within four cultivars, Mann-Whitney-U test, $p \leq 0.05$).

Table 2: Mean efficacy (mean \pm SEM) of 2 - 3 insecticide applications per year on adult pollen beetles (Henderson & Tilton, 1955) of winter oilseed rape in 2008-2010.

Year	Date of application	Active ingredient	Days after treatment	Mean (\pm SEM) insecticide efficacy [%]
2008	24 April	thiacloprid	3	51.2 (\pm 8.2)
	6 May	thiacloprid	3	56.8 (\pm 10.3)
2009	6 April	chlorpyrifos-methyl	3	68.1 (\pm 5.1)
	14 April	thiacloprid	4	77.4 (\pm 5.1)
	21 April	thiacloprid	3	2.3 (\pm 2.3)
2010	8 April	chlorpyrifos-methyl	5	68.9 (\pm 7.9)
	20 April	thiacloprid	3	42.2 (\pm 4.2)
	26 April	thiacloprid	3	27.3 (\pm 6.2)

Table 3: Abundance of eggs and L1 larvae within buds (3 racemes in 2008, 4 racemes in 2009 and 2010), abundance of L2 larvae and abundance of new generation beetles (NGB) in insecticide-treated and untreated plots during bud stage of winter oilseed rape (data of four cultivars pooled, $p \leq 0.05$ = significant differences within a row between treatments, two-sample t-test).

Year	Sampling date	Instar	Mean no. (\pm SEM) of ind./m ² in untreated plots	Mean no. (\pm SEM) of ind./m ² in treated plots	t	df	p
2008	24 April	Eggs	38 (\pm 8.3)	15 (\pm 2.4)	-3.20	28	0.003
	9 – 23 May	L2	640 (\pm 38.3)	61 (\pm 18.3)	-13.48	17.9	0.000
	13 June – 4 July	NGB	279 (\pm 17.3)	19 (\pm 5.6)	-14.59	17.9	0.000
2009	8 April	Eggs	95 (\pm 20.4)	40 (\pm 11.2)	-1.36	27.8	0.186
	17 April	Eggs	1330 (\pm 233.0)	707 (\pm 101.1)	-1.88	26.3	0.071
		L1	503 (\pm 69.1)	265 (\pm 52.2)	-2.70	28.8	0.011
	25 April – 13 May	L2	2672 (\pm 324.5)	461 (\pm 104.3)	-7.32	26.1	0.000
5 June – 2 July	NGB	470 (\pm 53.3)	19 (\pm 4.0)	-14.78	25.7	0.000	
2010	26 April	Eggs	1803 (\pm 214.8)	497 (\pm 61.3)	-7.66	29.9	0.000
		L1	121 (\pm 36.6)	272 (\pm 34.6)	4.33	23.2	0.000
	6 May – 1 June	L2	3896 (\pm 313.5)	400 (\pm 141.3)	-11.65	19.5	0.000
	18 June – 12 July	NGB	332 (\pm 25.7)	59 (\pm 14.1)	-10.24	20.1	0.000

In 2008 and 2010, the number of eggs/m² differed significantly between plants collected from treated and untreated plots. In 2009, the number of L1 larvae in the buds was significantly lower three days after insecticide application. In contrast, in 2010 the number of L1 larvae was significantly higher in treated plots than in untreated plots (Table 3). The third insecticide treatment was carried out after sampling of buds for dissection (Table 1). In each year, the abundance of second instar larvae and new generation beetles was significantly reduced in all treated plots (Table 3). The L2 larvae in treated plots accounted only for $10.4 \pm 3.5\%$, 16.8

$\pm 3.3\%$ and $9.4 \pm 2.9\%$ (mean \pm SEM) in 2008, 2009 and 2010, respectively, compared to the number of L2 larvae in untreated plots. The proportion of emerging beetles in treated plots was similarly reduced to $7.1 \pm 1.9\%$, $4.5 \pm 1.0\%$ and $17.5 \pm 3.2\%$ (mean \pm SEM) in 2008, 2009 and 2010, respectively. The last insecticide application in flowering stage was conducted three, four and ten days before the first assessment of L2 abundance in 2008, 2009 and 2010, respectively. (Table 1, Table 3).

In 2009 and 2010, bud losses on the main and lateral raceme were significantly decreased by insecticide applications (Table 4). In 2008, the mature pods were completely destroyed by a hailstorm few days before harvest. In 2009 and 2010, seed yield was higher in insecticide treated plots but there was no significant difference to untreated plots (Table 4). Similarly, the thousand-seed-weight did not differ significantly between insecticide treated and untreated plots.

Table 4: Bud loss as percentages of total number of buds set, yields and thousand-seed-weights (TSW) (mean \pm SEM) measured on insecticide treated and untreated plots in three consecutive years (MR = main raceme, SR = side raceme; $p \leq 0.05$ = significant difference between treatments within years, two-sample t-test).

Year	Characteristic	Raceme	Insecticide untreated mean (\pm SEM)	Insecticide treated mean (\pm SEM)	t	df	p
2008	Bud loss [%]	MR	9.7 (\pm 1.0)	7.9 (\pm 0.6)	-1.38	25.6	0.181
		SR	16.7 (\pm 1.5)	16.5 (\pm 1.4)	-0.07	29.8	0.848
2009	Bud loss [%]	MR	26.4 (\pm 2.5)	18.1 (\pm 2.3)	-2.64	30.0	0.013
		SR	20.9 (\pm 2.3)	14.7 (\pm 2.1)	-2.21	30.0	0.035
	Yield [dt/ha]		32.0 (\pm 1.1)	33.2 (\pm 1.3)	0.72	28.9	0.479
	TSW [g]		5.2 (\pm 0.1)	5.2 (\pm 0.1)	0.08	30.0	0.940
2010	Bud loss [%]	MR	25.3 (\pm 2.4)	14.1 (\pm 1.3)	-4.26	25.7	0.000
		SR	15.0 (\pm 1.1)	6.0 (\pm 0.6)	-7.15	28.7	0.000
	Yield [dt/ha]		40.9 (\pm 1.2)	42.9 (\pm 0.8)	1.33	24.4	0.195
	TSW [g]		4.6 (\pm 0.1)	4.7 (\pm 0.1)	0.21	28.9	0.837

Discussion

Despite huge variation in the efficacy of insecticides on adult pollen beetle abundance in spring, we recorded strong effects on the immature stages and new-generation beetles of this pest. According to our expectation, insecticide application reduced oviposition and resulted in lowered abundances of larvae and new generation beetles in summer which account for the level of infestation in the following spring. Although bud losses caused by adult beetles in insecticide treated plots in two years were significantly reduced, seed yields were not increased over yields of untreated plots.

The insecticide treatments significantly decreased the cumulative beetle-days in 2009 and 2008. Applications of thiacloprid and chlorpyrifos-methyl had no significant effect on cumulative beetle-days at very high levels of infestation in 2010. When leading the focus

from the cumulative-beetle days to the efficacy of insecticides, a more accurate picture may be obtained.

In 2009 and 2010, Reldan 22 achieved a moderate efficacy of 68-69% three and five days after insecticide application, respectively, despite the high infestation of adult beetles. This efficacy is in agreement with the manufacturer who claimed a long effectiveness of chlorpyrifos-methyl of approximately one week for control of pollen beetle (DowAgro, 2010). Following application, the ambient temperatures in both years were below the minimum temperature of 15 to 25°C required for optimum efficacy, which may explain the moderate effectiveness of chlorpyrifos-methyl. In 2009, the efficacy of the first application of thiacloprid was relatively high (77.4%), but dropped considerably at the second application date. In 2010, exceptionally high numbers of pollen beetles may have resulted in low efficacy of insecticides. Furthermore, the effect on adult beetles is influenced by their migratory activity and the time they spent on the host plant. Pollen beetles take up the active ingredient thiacloprid not only by feeding (Elbert et al., 2008) but also by contact exposure (Thieme et al., 2010). Due to the small size of insecticide-treated plots the efficacy may have been limited by extensive migratory flights at the time of insecticide application. In field experiments, Schroeder et al. (2009) documented a higher effectiveness of thiacloprid in large plots of 10ha as compared to small plots of 60m². Migration of adult pollen beetles is influenced by temperature. Sedivy (1993) observed a high flight activity at maximum air temperatures above 20°C. During the infestation period maximum daily temperatures exceeded 20°C on four, seven and four days in 2008, 2009 and 2010, respectively. Therefore, we assume that the effectiveness of both insecticides might have been reduced by high migratory activity of adult beetles in combination with high population densities of pollen beetle.

Despite a moderate effectiveness of the applied insecticides in our study, the numbers of eggs, first instar larvae, second instar larvae and new-generation pollen beetles were drastically reduced in insecticide treated plots in all three years. Lower numbers of pollen beetles following insecticide treatment may have reduced oviposition into buds, resulting in lower numbers of eggs and larvae in treated plots. Besides the direct effect on adult mortality, additional toxic effects to the eggs and larvae might have occurred because the immature stages were present when the insecticides were applied during the oviposition and flowering period. Further, L2 larvae were topically exposed in the open flowers to the final treatment of thiacloprid in each year. An ovicidal and larvicidal effect of organophosphorous and neonicotinoid insecticides has also been observed in other studies. For example, thiacloprid and chlorpyrifos-methyl showed larvicidal and ovicidal effectiveness to the small fruit tortrix *Grapholita lobarzewskii* on apple (Charmillot et al., 2007). In another study, the emergence rate of *Conotrachelus nenuphar* larvae from plums was reduced and only a few larvae survived 30 days after treatment with thiacloprid (Hoffmann et al., 2009). In addition to lethal effects, sub-lethal effects of insecticides on fertility and fecundity as well as on foraging and reproductive behaviour have been reported from various arthropod species (Stark & Banks, 2003). Field dose rates of different neonicotinoid insecticides like thiacloprid reduced oviposition of spider mites resulting in decreased population growth (Ako et al., 2004).

Damage caused by adult pollen beetles to the buds is evident from blind-stalks (Winfield, 1961). Despite high levels of pollen beetle infestation in 2009 and 2010, the insecticide applications led to a considerable reduction of bud losses. In addition to the infestation level, yield losses by pollen beetles are mainly determined by the amount of precipitation and the temperature (Hansen, 2004). Climatic conditions are particularly important during the bud and flowering stage, because the assimilate availability during this period regulates pod density and seed set (Habekotte, 1993). Nilsson (1994) reported that a high compensatory reaction of oilseed rape may increase the number of side racemes and the thousand corn-weight following pollen beetle infestation. It can be assumed that conditions for compensation were favourable during the study period in all three years. Apparently the time of crop colonization and the duration of flowering are major determining factors for pod formation (Vietinghoff, 1985). If beetles feed on small buds, damage can be high due to bud abortion. The period from crop colonization to full flowering took 20, 21 and 31 days in 2008, 2009 and 2010, respectively. Particularly in 2008, the beetles colonized the oilseed rape fields late in the green bud and the yellow bud stage, and there was only short time for feeding on buds. In 2010, the infestation period from colonization to full flowering stage was extended and favourable for compensation.

Our results indicate that different life stages of pollen beetle show different susceptibilities to the insecticides thiacloprid and chlorpyrifos-methyl. The effect on overwintered adults during the bud and flowering stage is highly related to their mobility, which in turn depends on abiotic factors, mainly on temperature. In addition to lethal effects on adult pollen beetles and immature stages, sublethal effects of the neonicotinoid thiacloprid may have affected oviposition and larval fitness. The lower number of immature stages results in a lower population outcome of new-generation beetles. More research is needed to determine the susceptibility of immature stages to various insecticides and application dates rather than focussing only on the target life stage of a pest.

Acknowledgements

This research was supported by the Federal Ministry of Food, Agriculture and Consumer Protection. The technical assistance of Dorothea Mennerich and Hubertus Reintke is highly appreciated. We thank Ulrike Jeche, Ruth Lefering, Joshua Sikhu Okonya, Sonja Schneider and Kim Stey for field work. We also thank Heike Beckmann and Katharina Lohaus for helpful comments to an earlier draft of the manuscript.

References

Ahuja, I., Rohloff, J. & Bones, A. M. (2010). Defence mechanisms of *Brassicaceae*: implications for plant-insect interactions and potential for integrated pest management. A review. *Agronomy for Sustainable Development* 33, 311-348

- Ako, M., Borgemeister, C., Poehling, H. M. & Nauen, R. (2004). Effects of neonicotinoid insecticides on the bionomics of twospotted spider mite (Acari : Tetranychidae). *Journal of Economic Entomology* 97, 1587-1594.
- Alford, D. V., Nilsson, C. & Ulber, B. (2003). Insect pests of oilseed rape crops. In: Alford D.V. (ed.) *Biocontrol of oilseed rape pests*. Oxford, UK: Blackwell Publishing, 9-41
- Charmillot, P., Pasquier, D., Salamin, C. & Ter-Hovannesyanyan, A. (2007). Ovicidal and larval effectiveness of insecticides applied by dipping apples on the small fruit tortrix *Grapholita lobarzewskii*. *Pest Management Science* 63, 677-681.
- DowAgro (2010). www.dowagro.com/de/produkte/PRODUKTSEITEN/reldan22.htm.
- Ekbom, B. & Borg, A. (1996). Pollen beetle (*Meligethes aeneus*) oviposition and feeding preferences on different host plant species. *Entomologia Experimentalis et Applicata* 78, 291-299.
- Elbert, A., Haas, M., Springer, B., Thielert, W. & Nauen, R. (2008). Applied aspects of neonicotinoid uses in crop protection. *Pest Management Science* 64, 1099-1105.
- Freier, B., Sellmann, J., Strassemeyer, J., Schwarz, J., Klocke, B., Moll, E., Gutsche, V. & Hornbach, W. (2012). Netz Vergleichsbetriebe Pflanzenschutz Jahresbericht 2011 - Analyse der Ergebnisse der Jahre 2007 bis 2011. *Berichte aus dem Julius Kühn-Institut* 166.
- Habekotte, B. (1993). Quantitative-analyses of pod formation, seed set and seed filling in winter oilseed rape (*Brassica napus* L.) under field conditions. *Field Crops Research* 35, 21-33.
- Hansen, L. (2004). Economic damage threshold model for pollen beetles (*Meligethes aeneus* F.) in spring oilseed rape (*Brassica napus* L.) crops. *Crop Protection* 23, 43-46.
- Heimbach, U., Müller, A. & Thieme, T. (2006). First steps to analyse pyrethroid resistance of different oil seed rape pests in Germany. *Nachrichtenblatt Deutscher Pflanzenschutzdienst* 58, 1-5.
- Heimbach, U. & Müller, A. (2013): Incidence of pyrethroid-resistant oilseed rape pests in Germany. *Pest Management Science* 69, 209-216.
- Henderson, C. & Tilton, E. (1955). Test with acaricides against the brown wheat mite. *Journal of Economic Entomology* 48, 157-161.
- Hoffmann, E. J., Vandervoort, C. & Wise, H. C. (2009). Curative activity of insecticides against plum curculio (Coleoptera: Curculionidae) in tart cherries. *Journal of Economic Entomology* 102, 1864-1873.
- Hokkanen, H. M. T. (2000). The making of a pest: recruitment of *Meligethes anaenus* onto oilseed Brassicas. *Entomologia Experimentalis et Applicata* 95, 141-149.
- Lancashire, P.D., Bleiholder, H., Boom, T. van den, Langelueddeke, P., Strauss, R., Weber, E. & Witzemberger, A. (1991) A uniform decimal code for growth stages of crops and weeds. *Annals of Applied Biology* 119, 561-601.
- Nilsson, C. (1988). Pollen beetles (*Meligethes aeneus* F.) and flowering in rape. *Swedish J. agric. Res.* 18, 113-118.
- Nilsson, C. (1994). Pollen beetles (*Meligethes* spp.) in oil seed rape crops (*Brassica napus* L.): Biological interactions and crop losses. PhD thesis Swedish University of Agricultural Sciences.
- Ruppel, R. F. (1983). Cumulative insect-days as an index of crop protection. *Journal of Economic Entomology* 76, 375-377.
- Schroeder, G., Politz, B., Wolff, C. & Krueger, B. (2009). Alternatives for an effective control of pyrethroid-resistant pollen beetle populations - results of proficiency test of several states. *Gesunde Pflanzen* 61, 19-30.
- Sedivy, J. (1993). Variation in the population density of pollen beetles (*Meligethes aeneus* F.) in winter rape. *Ochrana Rostlin* 29, 9-15.
- Slater, R., Ellis, S., Genay, J., Heimbach, H., Huart, G., Sarazin, M., Longhurst, C., Müller, A., Nauen, R., Rison, J. L. & Robin, F. (2011). Pyrethroid resistance monitoring in European populations of pollen beetle (*Meligethes* spp.): a coordinated approach through the Insecticide Resistance Action Committee (IRAC). *Pest Management Science* 67, 633-638.
- Stark, J. D. & Banks, J. E. (2003). Population-level effects of pesticides and other toxicants on arthropods. *Annual Review of Entomology* 48, 505-519.
- Thieme, T., Gloyna, K. & Zellner, M. (2010). How to determine the susceptibility of *Meligethes aeneus* to neonicotinoids? *Journal of Applied Entomology* 134, 55-60.
- Vietinghoff, J. (1985). Untersuchungen zur Schadwirkung und Befallsprognose des Rapsglanzkäfers (*Meligetes aeneus* F.). Diss. Rostock: Wilhelm-Pieck-Universität Rostock.
- Williams, I. H. & Free, J. B. (1978). The feeding and mating behaviour of pollen beetles (*Meligethes aeneus* Fab.) and seed weevils (*Ceutorhynchus assimilis* Payk.) on oil-seed rape (*Brassica napus* L.). *Journal fo Agricultural Science, Cambridge* 91, 453-459.
- Williams, I. H. (2010). The major insect pests of oilseed rape in Europe and their management: An overview. In: Williams, I. H. (ed.) *Biocontrol-based integrated management of oilseed rape pests*. Netherlands: Science+Business Media B. V. Springer, 1-44

- Winfield, A. L. (1961). Field observations on the control of blossom beetles (*Meligethes aeneus* F.) and cabbage-seed weevils (*Ceuthorrhynchus assimilis* Payk.) on mustard-seed crops in East Anglia. *Annals of Applied Biology* 49, 539-555.
- Zlof, V. (2008). Recommendations and conclusions of the Ad hoc EPPO-Workshop on insecticide resistance of *Meligethes* spp. (pollen beetle) on oilseed rape. *IOBC/wprs Bulletin* 38, 65-67.

Effects of site-specific factors and insecticide application on parasitisation of *Meligethes aeneus* (Fabricius)

Abstract

Pollen beetle, *Meligethes aeneus* (Fabricius) (Col.: Nitidulidae) is a major pest of oilseed rape, *Brassica napus* (L), across Europe. Hymenopteran parasitoids can exert substantial natural control on pollen beetle populations. We examined the impact of site-specific factors, such as field size, plant density, proportion of crop area grown with oilseed rape, and insecticide application during bud and flowering stage on parasitism by the larval parasitoids *Tersilochus heterocerus* and *Phradis* spp. (Hym.: Ichneumonidae) across Germany. In 2008 and 2009, the level of parasitism of *M. aeneus* was determined from 36 and 42 commercial crops of winter oilseed rape, respectively, which were distributed from the north to the south of Germany. The total parasitism rate ranged from 1.6% to 55.9% in 2008 and from 1.0% to 81.3 % in 2009. The parasitoids *T. heterocerus* and *Phradis* spp. were the most common species. In 2008, both were equally abundant while in 2009 *T. heterocerus* was the dominating species. Univariate statistical analyses using a tree model were applied for identification of the most influencing factors. The level of parasitism was strongly influenced by the geographic location within Germany, the crop area grown with oilseed rape and the field size, indicating an impact of climate and site-specific factors. Insecticides applied during the main period of parasitoid activity in the bud and flowering stage of oilseed rape reduced the parasitism rates. The results show that there is potential to enhance the effectiveness of major parasitoids by temporal targeting of insecticides according to pest damage threshold.

Introduction

The pollen beetle, *Meligethes aeneus* (Fabricius) (Col.: Nitidulidae), is one of the most destructive pests of brassicaceous crops in Europe (Alford, 2003). Adult beetles cause extensive damage by feeding on the buds (Nilsson, 1988). Parasitoids of pollen beetle are considered as key natural control agents in integrated pest management systems on oilseed rape crops (Williams, 2006; Ulber et al., 2010). Depending on the year, country and meteorological factors, total larval parasitism rates can vary from 0% to more than 90% in Europe (Nilsson & Andreasson, 1987; Nielsen & Axelsen, 1988; Hokkanen, 2000; Büchi, 2002; Williams, 2006; Thies et al., 2008, Ulber et al., 2010). Landscape structure has been shown to directly affect parasitism of pollen beetles, and a complex habitat structure including conservation strips and fallows enhance the parasitisation (Büchi, 2002; Thies et al., 2008; Zaller et al., 2009). Studies on the migration and temporal occurrence of parasitoids of pollen beetle indicate that insecticide application during late bud and flowering stage of oilseed rape crops can be detrimental to these natural enemies (Jönsson et al., 2005; Jönsson & Anderson, 2008, Neumann, 2010). However, so far no research on the

effects of insecticides and site-specific factors in fields under common farming practice has been conducted.

The two larval instars of *M. aeneus* develop in the closed buds and later in open flowers (Fritzsche, 1957). They are hosts to several endoparasitoids (Jourdhueil, 1960). In Europe, in winter oilseed rape the most abundant parasitoids of pollen beetle are the univoltine species *Phradis interstitialis* (Thomson), *P. morionellus* (Holmgren) and *Tersilochus heterocerus* (Holmgren) (Hym.: Ichneumonidae-Tersilochinae) (Nilsson, 2003, Ulber et al., 2010). In addition, the multivoltine parasitoid species *Diospilus capito* (Haliday) (Hym.: Braconidae) has been recorded to parasitise *M. aeneus* in northern and central Europe. The tersilochine parasitoids of pollen beetle are koinobionts, i. e. parasitised hosts can complete their larval development and are killed after migration to soil for pupation. Adult parasitoids diapause in a cocoon within their pupal chamber until the following spring (Jourdhueil, 1960). During the diapause, their number can be strongly reduced by inversion tillage techniques. Several studies have shown negative effects of ploughing and grubbing after harvest on the abundance of emerging parasitoids (Klingenberg & Ulber, 1994; Nitzsche, 1998; Williams, 2006). In spring, parasitoids locate their host plants by using upwind-anemotaxis (Williams et al., 2007). Olfactory and colour cues of oilseed rape flowers can enhance attraction of the parasitoid *T. heterocerus* (Jönsson et al., 2005). Insecticides applied during the bud and flowering stage of oilseed rape can be harmful to all three species (Cook and Denholm, 2008) and *T. heterocerus* is particularly affected during the flowering stage.

In the present study, we investigated the effects of geographic location, proportion of oilseed rape crop area, field size, plant density and insecticide treatments in the bud and flowering stage on the level of parasitism of *M. aeneus* in the years 2008 and 2009 at 36 and 42 sites, respectively, across Germany.

Materials and methods

In 2008 and 2009, samples of pollen beetle larvae were collected from 36 and 42 crops, respectively, located in major oilseed rape-growing regions of Germany. The sampling sites were randomly distributed from the north (N 54°34'08 in 2008, N 54°38'33 in 2009) to the south (N 48°21'56 in 2008, N 48°35'00 in 2009) across Germany. They were not selected with regard to distinct crop management and landscape criteria. All winter oilseed rape crops were grown in accordance with current farming practices. Larval samples were collected at the end of flowering during April and May by farmers and agricultural advisers using a standardised sampling protocol. A standardised questionnaire was conceptualized to obtain information on site and crop-specific characteristics which might impact the parasitisation of *M. aeneus*. Informations on insecticide application during bud or flowering stage, average insecticide usage in previous 5 years, level of pyrethroid resistance of pollen beetle, geographical latitude, field size, plant density of the sampled crops and proportion of oilseed rape crop area were specified by participating farmers and agricultural advisers. The level of pollen beetle resistance to the pyrethroid lambda-cyhalothrin was determined according to the standardised pyrethroid sensitivity monitoring method (Müller et al., 2008). The sensitivity

of pollen beetle to lambda-cyhalothrin was classified in 5 groups: very sensitive - class 1, sensitive - class 2, moderate resistant - class 3, resistant - class 4 and high resistant - class 5.

To collect the larvae of pollen beetle, at least 50 randomly selected main inflorescences were beaten over a white plastic tray. The larvae were stored in 70% Ethanol. A total number of 100 (minimum 70) second instar larvae per sample was dissected under a binocular microscope (magnification 25-40x). The abdomen and thorax of each larva were cut off longitudinally and the body contents were squeezed out by a needle in water. The larvae of the parasitoids *Phradis* spp. as well as *D. capito* and the eggs of *T. heterocerus* were identified by morphological characters (Osborne, 1960). Larvae of the braconid *D. capito* are characterized by a black, heavily sclerotised head-capsule, a colourless semi-transparent body and a characteristically shaped final abdominal segment. In contrast, larvae of *Phradis* spp. have a slightly sclerotised and unpigmented head-capsule and a creamy-white body. As morphological separation between the larvae of *P. interstitialis* and *P. morionellus* is not possible (Rusch et al., 2011), these were assigned to *Phradis* spp.. The eggs of *T. heterocerus* can be easily identified by their dark brown to black chorion.

The total parasitism rate, the parasitism by individual parasitoid species, superparasitism and multiparasitism were calculated from larval samples collected from individual crops in each year. The arcsin root transformed data were analysed by univariate regression tree analyses (Breiman et al., 1984). To identify the explanatory variables with the highest impact on parasitism and to test for correlations between these variables, the response variables total parasitism rate and parasitism rate by individual parasitoids were analysed by using the tree-package (Ripley, 2010). In the figures transformed data are shown, except for the total parasitism rates in 2009 (Figure 2) when analyses using transformed data gave the same results as analyses using untransformed data. Correlations between parasitism by *T. heterocerus* and *Phradis* spp., between parasitism by *T. heterocerus* and superparasitism, and between parasitism by *Phradis* spp. and superparasitism were evaluated using simple linear regression models. Statistical analyses were carried out using the software R 2.10.0 (R Development Core Team, 2009).

Results

In May 2008, total parasitism rates of pollen beetle larvae sampled from 36 sites in Germany varied between 1.6% and 55.9%. The parasitoid *Phradis* spp. was present at all sampling sites while *T. heterocerus* was not determined from 5 sampled oilseed rape crops. The braconid *D. capito* was only recorded with very low parasitism rates from 19.4% of the sampling sites. In 2008, the mean parasitism rate by *T. heterocerus* and *Phradis* spp. were assessed at the same level (Table 1).

In 2009, total parasitism rates of larvae sampled from 42 sites across Germany varied between 1.0% and 81.3%. Overall, the mean parasitism rate was at 25.1% higher than in 2008 (22.6%). Mean parasitism and superparasitism by *T. heterocerus* were higher than the mean parasitism and superparasitism by *Phradis* spp. The average mean multiparasitism by

T. heterocerus and *Phradis* spp. was nearly the same in both years with 1.4% and 1.5% (Table 1). Parasitism of larvae by *D. capito* was found only at few locations, with low parasitism rates in both years. Therefore this species was not included in the statistical analyses.

Table 1: Frequency and levels of parasitism of *M. aeneus* larvae collected from 38 and 42 crops of oilseed rape across Germany in 2008 and 2009, respectively.

Mortality factor	Year	Sites with parasitism [%]	mean \pm SEM parasitism [%]	Min-max. parasitism [%]	Mean \pm SEM multi-parasitism [%]	Mean \pm SEM super-parasitism [%]
Total parasitism	2008	100.0	22.6 \pm 2.7	1.6-55.9	1.4 \pm 0.4	
	2009	100.0	25.1 \pm 3.2	1.0-81.3	1.5 \pm 0.5	
<i>T. heterocerus</i>	2008	86.1	11.1 \pm 2.2	0.0-50.0		1.4 \pm 1.5
	2009	92.9	17.2 \pm 2.9	0.0-79.7		4.6 \pm 1.5
<i>Phradis</i> spp.	2008	100.0	11.1 \pm 1.6	1.0-47.5		0.1 \pm 0.0
	2009	64.3	7.6 \pm 1.5	0.0-41.0		0.0 \pm 0.0
<i>D. capito</i>	2008	19.4	0.4 \pm 0.2	0.0-6.8		0.0 \pm 0.0
	2009	7.1	0.3 \pm 0.2	0.0-5.0		0.0 \pm 0.0

In both years, the mean number of insecticide applications during previous years had no effect on the total parasitism rates in Germany. In 2008, the proportion of crop area grown with oilseed rape within the region of the sampled crop had a positive effect on the parasitism rate (Figure 1). With a threshold exceeding 20% of oilseed rape this was the most influential factor. In addition, if the area grown with oilseed rape exceeded 20%, the parasitism rate was positively affected by field sizes larger than 18.3ha. If the plant density exceeded 40 plants/m², parasitism rate was increased compared to lower plant densities (Figure 1).

In 2009, the most important explanatory variable for the total parasitism rate was the number of insecticide applications during flowering. Insecticide application reduced the level of parasitisation. On untreated crops, fields smaller than 23.5ha had a negative effect on total parasitism, and pyrethroid resistance of pollen beetle populations (>class 4) increased parasitism as compared to moderate levels of resistance (Figure 2).



Figure 1: Tree-model analyses of factors influencing the total parasitism rate [%] in the year 2008 (transformed data; threshold values next to explanatory variable; right hand branch of the tree indicates the next explanatory variable exceeding the threshold; left hand branch of the tree indicates the next explanatory variable smaller than the threshold, mean values of parasitism rates are given at the bottom end of branches).

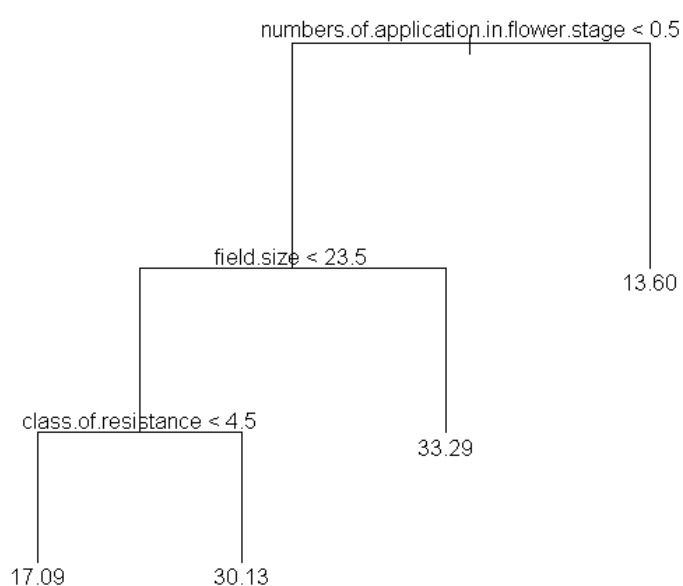


Figure 2: Tree-model analyses of factors influencing the total parasitism rate [%] in the year 2009 (untransformed data; for explanation see fig. 1).

In 2008, parasitism by *T. heterocerus* was higher in regions north of the geographic latitude of 52.5° in Germany and were influenced negatively by field sizes exceeding 19.5ha. In the southern regions of Germany (latitude <52.5°), the explanatory variable 'pyrethroid resistance' was negative associated with parasitism rates by *T. heterocerus* (Figure 3).

In 2009, the field size affected the parasitism rate by *T. heterocerus* at two levels. Fields with a size larger than 14.7ha and 39.4ha had a positive impact on parasitism rates by *T.*

heterocerus. Insecticide applications in the bud stage of winter oilseed rape had negative effects on the parasitism by *T. heterocerus* (Figure 4).

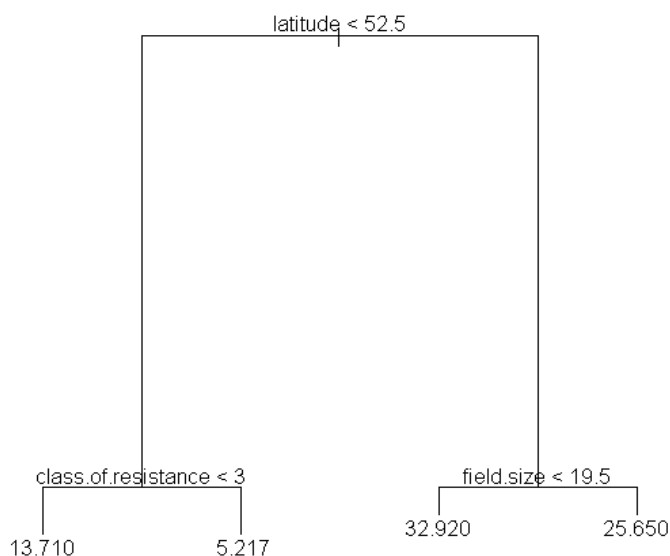


Figure 3: Tree-model analyses of factors influencing the parasitism rate by *T. heterocerus* [%] in the year 2008 (transformed data; for explanation see fig. 1).

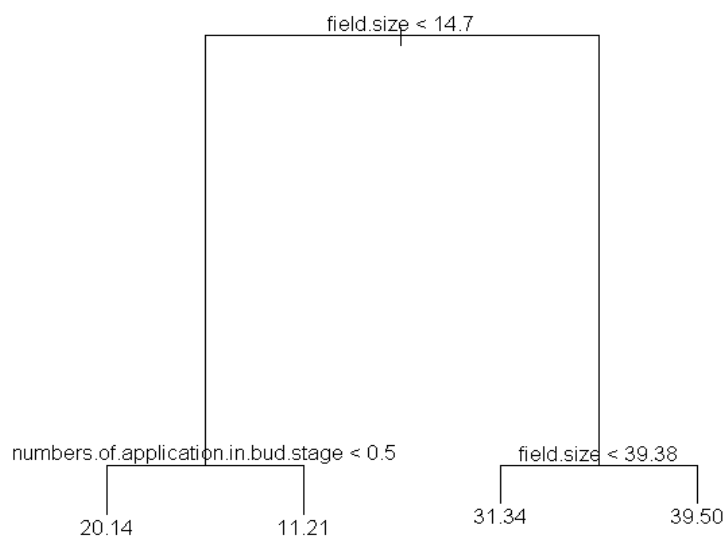


Figure 4: Tree-model analyses of factors influencing the parasitism rate by *T. heterocerus* [%] in the year 2009 (transformed data; for explanation see fig. 1).

In 2008, the parasitism by *Phradis* spp. was influenced by increasing field size both in the north and in the south of Germany. In the north the parasitism rate was increased at field sizes exceeding 18.3ha and in the south at field sizes exceeding 4.1ha (Figure 5).

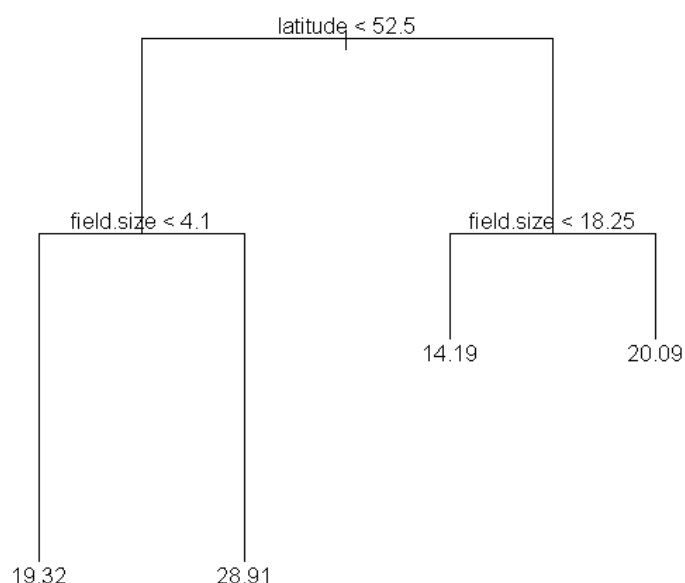


Figure 5: Tree-model analyses of factors influencing the parasitism rate by *Phradis* spp. [%] in the year 2008 (transformed data; for explanation see fig. 1).

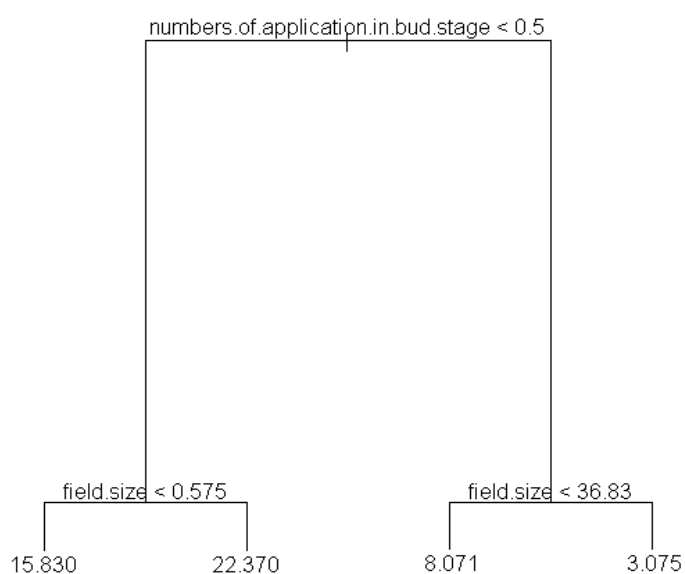


Figure 6: Tree-model analyses of factors influencing the parasitism rate by *Phradis* spp. [%] in the year 2009 (transformed data; for explanation see fig. 1).

In 2009, the most important explanatory variable was the application of insecticides during bud stage. Insecticides reduced the parasitism rate by *Phradis* spp.. Particularly on fields exceeding a size of 36.9ha, insecticide application during bud stage caused lower parasitism by *Phradis* spp.. Insecticide treatments during bud stage also reduced parasitism by *Phradis* spp. on fields smaller than 0.6ha (Figure 6).

Generally, parasitism by *T. heteroceris* and *Phradis* spp. was not significantly correlated (Table 2). In both years, a significantly positive correlation between the total parasitism and the multiparasitism was recorded. Particularly the superparasitism by *T. heteroceris*

increased with increasing total parasitism. Superparasitism by *Phradis* spp. increased significantly with increasing total parasitism only in 2009 (Table 2).

Table 2: Results of linear regression analyses of parasitism rates, superparasitism and multiparasitism by *T. heterocerus* and *Phradis* spp. in 2008 and 2009.

Regression	Year	Beta	R ²	F	p
Parasitism by <i>T. heterocerus</i> vs. <i>Phradis</i> spp.	2008	0.192	0.014	0.487	0.490
	2009	0.068	0.014	0.574	0.453
Total parasitism vs. multiparasitism	2008	0.365	0.612	54.51	<0.0001
	2009	0.301	0.509	41.48	<0.0001
Total parasitism vs. superparasitism by <i>T. heterocerus</i>	2008	0.302	0.400	22.71	<0.0001
	2009	0.607	0.675	83.14	<0.0001
Total parasitism vs. superparasitism by <i>Phradis</i> spp.	2008	0.053	0.180	7.454	0.010
	2009	0.009	0.022	0.886	0.352

Discussion

The large variability between the parasitism rates of pollen beetle at various sites in Germany (1.6% to 55.9% in 2008, 1.0% to 81.3% in 2009) indicates a high susceptibility of parasitoids to environmental factors. Average levels of parasitism within the range of 25-50% have been reported from several countries in Europe, for example from Austria, Finland, Germany, Sweden, Switzerland and the United Kingdom (Ulber et al., 2010).

The abundance and occurrence of parasitoids can be affected by various factors, such as local climate and weather conditions, area of oilseed rape crops grown during previous years and cultivation techniques (Nilsson, 2003). In 2008, site-specific factors, such as geographical region, proportion of oilseed rape crop area, field size and plant density were most influential for levels of parasitism, while the insecticide application in addition to site-specific factors had a strong impact in 2009. In 2008, a high proportion of oilseed rape on the total crop area had a positive effect on the total parasitism rate, if field size exceeded 18.3ha. In Austria, the combination of high pollen beetle abundance and high proportions of rape crop area and non-crop habitats, like roadside strips and hedges, increased the parasitism of pollen beetle (Zaller et al., 2009). Non-crop areas may have a strong effect on parasitisation because flowering plants in hedges and roadside strips provide pollen and nectar sources to adult parasitoids (Nilsson, 2003). In their investigations on the effect of interannual changes of the size of rape crop area on pollen beetle parasitism, Thies et al. (2008) found different responses of the parasitoid species. *Phradis* spp. was more sensitive to interannual changes of the rape crop area than *T. heterocerus*. The total parasitism rate decreased when the size of the rape crop area increased between consecutive years. In structurally complex landscapes with abundant field margin strips and old fallow habitats the larval parasitism was higher, indicating that survival and activity of the parasitoids is affected by the type of landscape (Thies & Tschardtke, 1999). In our study, other site-specific factors like field size

and geographical region may have influenced the parasitism via their relation to local size of rape crop area.

In our study, in 2008 the parasitism by *Phradis* spp. increased with increasing field size, while the mortality of pollen beetle larvae resulting from parasitism by *T. heterocerus* decreased when the field size increased. In contrast, in 2009 larger fields favoured parasitism by *T. heterocerus* as well. Moreover, parasitism by *Phradis* spp. on large and small fields was influenced by insecticide treatment in 2009. These results show that parasitoids may respond very sensitive to environmental influences. Site-specific factors like field size and geographical latitude also may determine the level of parasitisation via their effects on mobility and migration of parasitoids. After emerging from old rape fields in spring, parasitoids migrate to the new rape fields (Jourdeuil, 1960). This migration is affected by wind and by the distance between old and new oilseed rape fields. Due to the fact that the parasitoids locate the habitat of their hosts by using upwind anemotaxis migration flights depend on wind direction (Williams et al., 2007). The spatial distribution within fields was found to differ between parasitoid species: *Phradis interstitialis* similar to pollen beetle larvae was patchily distributed, while *T. heterocerus* was spread evenly across the field (Ferguson et al., 2003). As parasitoids of pollen beetle use upwind anemotaxis for locating the rape crop (Williams & Cook, 2010) it can be assumed, that in larger fields of oilseed rape the hosts and its parasitoids occurred at higher densities at the downwind side of the field and that the upwind side is not as representative for taking samples of larvae as the downwind side.

Another site-specific factor which could not be included in our analyses is soil tillage. In regions where ploughing is a common tillage practice, the parasitisation of pollen beetle larvae may be on a lower level because the abundance of parasitoids emerging from soil in spring is strongly reduced by ploughing as compared to no-tillage or conservation tillage practices (Nilsson, 1985; Klingenberg & Ulber, 1994).

In 2009, insecticide application during flowering of oilseed rape reduced the total parasitism rates, and application during the bud stage reduced the parasitism rate by both individual parasitoid species. In earlier studies at Goettingen, *P. interstitialis* was found to emerge earlier from hibernation fields in spring than *T. heterocerus* (Ulber & Nitzsche, 2006). Depending on temperature in spring, immigration of parasitoids occur in the late bud stage or in the flowering stage of the oilseed rape crop (Nilsson, 1994, 2003; Ulber & Nitzsche, 2006). Consequently, insecticide application during this time period might cause high mortality of parasitoids. It has been shown that adult parasitoids are killed immediately after direct exposure to dry residues of insecticides, i. e. gamma-cyhalothrin, bifentrin, lambda-cyhalothrin and indoxacarb (Haseeb et al., 2004; Carmo et al., 2010). Further, sublethal effects of insecticides may have impact on life span, fertility, fecundity and behaviour, like feeding, searching and oviposition (Stark & Banks, 2003). These effects might reduce the parasitisation in addition to lethal effects. For example, females of *Aphidius ervi* (Hym.: Aphidiinae) showed a reduced oviposition activity when they were exposed to the LD₂₀ of lambda-cyhalothrin (Desneux et al., 2003). Insecticide residues can change the attractiveness of host plants to parasitoids, leading to a reduced time span for host searching and avoidance of sprayed plants by adult parasitoids (Hardin et al., 1995; Neumann, 2010).

Repellency of deltamethrin to parasitoids for up to two days after application has been reported (Longley & Jepson, 1996). These direct and indirect effects of insecticides in combination with large field sizes may have reduced total parasitism rates and parasitism rates by *T. heterocerus* and *Phradis* spp. in our investigation in 2009.

At host location, the parasitoids of pollen beetle respond to visual cues, i. e. the yellow flower colour and olfactory cues released by the oilseed rape crop (Williams, 2010). In addition to oviposition sites, flowering rape provide food resources to adults. In larger fields these cues might have stronger effects on immigration and spatial distribution of parasitoids within the field.

The impact of the pyrethroid resistance status of the sampled populations of *M. aeneus* on the level of larval parasitism was contradictory: With increasing level of resistance the parasitisation decreased in 2008 and increased in 2009. There is no information in the literature indicating that insecticide-resistant larvae might be more susceptible to parasitism than insecticide-sensitive larvae. As the mortality and reproduction of adult pollen beetles may be affected by the resistance status to widely applied insecticides (Müller et al., 2008), the abundance of the larval hosts might be higher at increasing resistance status of the host population. Host density was found to directly affect the level of parasitism (Nilsson & Andreasson, 1987; Billquist & Ekbohm, 2001; Zaller et al., 2009), however, host density on sampled crops could not be assessed in this study.

The main parasitoids *Phradis* spp. and *T. heterocerus* occurred at the same level in 2008, whereas *T. heterocerus* was predominant in 2009. In both years, *D. capito* was rarely observed on the winter oilseed rape fields selected across Germany. In studies from France and Switzerland, *T. heterocerus* was also the most frequent species (Büchi, 2002; Rusch et al. 2011). Generally, the parasitoids *P. interstitialis* and *T. heterocerus* were found to be the most abundant species in central Europe, while in northern Europe *P. morionellus* and *D. capito* are more abundant, particularly on spring oilseed rape (Nilsson & Andreasson, 1987; Ulber, 2010). In both years of study, superparasitism by *T. heterocerus* and multiparasitism significantly increased with increasing levels of total parasitism. These results provide a confirmation of previous studies (Nilsson, 1994; Ulber & Nitzsche, 2006). The parasitism by *T. heterocerus* and *Phradis* spp. was not correlated in our studies. As *P. interstitialis* is occurring within green bud stage, *P. morionellus* within green bud to flowering stage and *T. heterocerus* within the flowering stage, these parasitoid species minimise interspecific competition by niche segregation (Williams, 2010)

Finally, our results support the hypothesis that the main parasitoid species of the pollen beetle are influenced by insecticide and site-specific factors. Therefore, it is essential to consider multiple factors, such as in-field habitat management and site-specific factors to enhance the potential of conserving the biological control agents of *M. aeneus* in integrated pest management systems on crops of oilseed rape.

Acknowledgements

This research was supported by the Federal Ministry of Food, Agriculture and Consumer Protection. We thank Dorothea Mennerich, Katja Fischer and Jaqueline Platzek for technical

assistance and Gerrit Kruger for correcting the English. We would like to thank all colleagues who supported our investigation by collecting larval samples of pollen beetle populations in Germany.

References

- Alford, D. V. (2003). Insect pest of oilseed rape crops. In: Alford D.V. (ed.) *Biocontrol of oilseed rape pests*. Oxford, UK: Blackwell Publishing, 9-41.
- Billquist, A. & Ekblom, B. (2001). The influence of host plant species on parasitism of pollen beetles (*Meligethes* spp.) by *Phradis morionellus*. *Entomologia Experimentalis et Applicata* 98, 41-47.
- Breiman, L., Friedman, J. H., Olshen, R. A. & Stone, C. J. (1984). *Classification and regression trees*. Belmont, California: Wadsworth International Group.
- Büchi, R. (2002). Mortality of pollen beetle (*Meligethes* spp.) larvae due to predators and parasitoids in rape fields and the effect of conservation strips. *Agriculture, Ecosystems and Environment* 90, 255-263.
- Carmo, E., Bueno, A. & Bueno, R. (2010). Pesticide selectivity for the insect egg parasitoid *Telenomus remus*. *BioControl* 55, 455-464.
- Cook, S. M. & Denholm, I. (2008). Ecological approaches to the control of pollen beetles in oilseed rape. *Bulletin OEPP/EPPO, Bulletin* 38, 110–113.
- Desneux, N., Pham-Delegue, N. & Kaiser, L. (2003). Effects of sub-lethal and lethal doses of lambda-cyhalothrin on oviposition experience and host-searching behaviour of a parasitic wasp, *Aphidius ervi*. *Pest Management Science* 60, 381-389.
- Ferguson, A. W., Campbell, J. M., Warner, D. J., Watts, N. P., Schmitdt, J. E. U. & Williams, I. H. (2003). Spatio-temporal distributions of *Meligethes aeneus* and its parasitoids in an oilseed rape crop and their significance for crop protection. In: *Proceedings of Proc. 11th Int. Rapeseed Congress, Copenhagen, 6-10 July 2003*, 1057-1059.
- Fritzsche, R. (1957). Zur Biologie und Ökologie der Rapsschädlinge aus der Gattung *Meligethes*. *Sonderdruck aus Zeitschrift für angewandte Entomologie* 40, 222-280.
- Hardin, M., Benrey, B., Coll, M., Lamp, W., Roderick, G. & Barbosa, P. (1995). Arthropod pest resurgence - an overview of potential mechanisms. *Crop Protection* 14, 3-18.
- Haseeb, M., Liu, L. & Jones, W. (2004). Effects of selected insecticides on *Cotesia plutellae*, endoparasitoid of *Plutella xylostella*. *BioControl* 49, 33-46.
- Hokkanen, H. M. T. (2000). The making of a pest: recruitment of *Meligethes anaenus* onto oilseed Brassicas. *Entomologia Experimentalis et Applicata* 95, 141-149.
- Jönsson, M., Lindkvist, A. & Anderson, P. (2005). Behavioural responses in three ichneumonid pollen beetle parasitoids to volatiles emitted from different phenological stages of oilseed rape. *Entomologia Experimentalis Et Applicata* 115, 363-369.
- Jönsson, M. & Anderson, P. (2008). Emission of oilseed rape volatiles after pollen beetle infestation; behavioural and electrophysiological responses in the parasitoid *Phradis morionellus*. *Chemoecology* 17, 201-207.
- Jourdheuil, P. (1960). Influence de quelques facteurs ecologiques sur les fluctuations de population d'une biocénose parasitaire: Étude relative à quelques Hyménoptères (Ophioninae, Diopsilinae, Euphorinae) parasites de divers Coléoptères inféodés aux Crucifères. *Annales des Epiphyties* 11, 445-658.
- Klingenberg, A. & Ulber, B. (1994). Investigations on the occurrence of tersilochinae (Hym, Ichneumonidae) as parasitoids of oilseed rape pests in the Göttingen region in 1990 and 1991, and on their emergence following various tillage techniques. *Journal of Applied Entomology* 117, 287-299.
- Longley, M. & Jepson, P. (1996). Effects of honeydew and insecticide residues on the distribution of foraging aphid parasitoids under glasshouse and field conditions. *Entomologia Experimentalis Et Applicata* 81, 189-198.
- Müller, A., Heimbach, U. & Thieme, T. (2008). Pyrethroid sensitivity monitoring in Germany of oilseed rape pest insects other than pollen beetle. *IOBC/wprs Bulletin* 38, 85-90.
- Neumann, N. (2010). Lethal and sublethal effects of insecticides on mortality, migration and host searching behaviour of tersilochine parasitoids on winter oilseed rape. Diss. Göttingen: Georg-August Universität.

- Nielsen, P. S. & Axelsen, J. (1988). Developmental time and mortality of the immature stages of the pollen beetle (*Meligethes aeneus* F.) under natural conditions. *Journal of Applied Entomology* 105, 198-204.
- Nilsson, C. (1985). Impact of ploughing on emergence of pollen beetle parasitoids after hibernation. *Zeitschrift für angewandte Entomologie* 100, 302-308.
- Nilsson, C. (1988). Pollen beetles (*Meligethes aeneus* F.) and flowering in rape. *Swedish J. agric. Res.* 18, 113-118.
- Nilsson, C. (1994). Pollen beetles (*Meligethes* spp.) in oil seed rape crops (*Brassica napus* L.): Biological interactions and crop losses. Diss. Swedish University of Agricultural Sciences.
- Nilsson, C. & Andreasson, B. (1987). Parasitoids and predators attacking pollen beetles (*Meligethes aeneus* F.) in spring and winter rape in Southern Sweden. *IOBC/wprs Bulletin* 10, 64-73.
- Nilsson, C. (2003). Parasitoids of pollen beetles. In: Alford D. V. (ed.) *Biocontrol of oilseed rape crops*. Oxford, UK: Blackwell Publishing 73-85.
- Osborne, P. (1960). Observations on the natural enemies of *Meligethes aeneus* (F.) and *M. viridescens* (F.) [Coleoptera: Nitidulidae]. *Parasitology* 50, 91-110.
- R Development Core Team (2009). *R: A language and environment for statistical computing*. Vienna, Austria: Foundation for Statistical Computing. <http://www.R-project.org>.
- Ripley, B. (2010). tree: Classification and regression trees. <http://cran.rproject.org/web/packages/tree/index.html>.
- Rusch, A., Valantin-Morison, M., Sarthou, J.-P. & Roger-Estrade, J. (2011). Multi-scale effects of landscape complexity and crop management on pollen beetle parasitism rate. *Landscape Ecology* 26, 473–486.
- Stark, J. D. & Banks, J. E. (2003). Population-level effects of pesticides and other toxicants on arthropods. *Annual Review of Entomology* 48, 505-519.
- Thies, C., Steffan-Dewenter, I. & Tscharntke, T. (2008). Interannual landscape changes influence plant-herbivore-parasitoid interactions. *Agriculture, Ecosystems and Environment* 125, 266-268.
- Thies, C. & Tscharntke, T. (1999). Landscape structure and biological control in agroecosystems. *Science* 285, 893-895.
- Ulber, B. & Nitzsche, O. (2006). Phenology of parasitoids (Hym, Ichneumonidae-Tersilochinael of oilseed rape pests in northern Germany from 1995-1997. *Integrated Control in Oilseed Crops*, IOBC/wprs Bulletin 29, 175-181.
- Ulber, B., Williams, I., Klukowski, Z., Luik, A. & Nilsson, C. (2010). Parasitoids of oilseed rape pests in Europe: key species for conservation biocontrol. In: Williams I. H. (ed.) *Biocontrol-based integrated management of oilseed rape pests*. Netherlands: Springer Science+Business Media B. V., 45-76
- Williams, I. H. (2006). Integrating parasitoids into management of pollen beetle on oilseed rape. *Agronomy Research* 4(special issue), 465-470.
- Williams, I. H., Frearson, D., Barari, H. & McCartney, A. (2007). Migration to and dispersal from oilseed rape by the pollen beetle, *Meligethes aeneus*, in relation to wind direction. *Agricultural and Forest Entomology* 9, 279-286.
- Williams, I. & Cook, S. M. (2010). Crop location by oilseed rape pests and host location by their parasitoids. In: Williams I. H. (ed.) *Biocontrol-based integrated management of oilseed rape pests*. Netherlands: Springer Science+Business Media B. V., 215-244.
- Williams, I. H., Frearson, D. J., Barari, H. & McCartney, A. (2007). First field evidence that parasitoids use upwind anemotaxis for host-habitat location. *Entomologia Experimentalis et Applicata* 123, 299-307.
- Zaller, J. G., Moser, D., Drapela, T., Schmoeger, C. & Frank, T. (2009). Parasitism of stem weevils and pollen beetles in winter oilseed rape is differentially affected by crop management and landscape characteristics. *Biocontrol* 54, 505-514.
- Zlof, V. (2008). Recommendations and conclusions of the Ad hoc EPPO-Workshop on insecticide resistance of *Meligethes* spp. (pollen beetle) on oilseed rape. *IOBC/wprs Bulletin* 38, 65-67.

Effect of trap cropping and spatial targeting of insecticide application on the abundance and spatio-temporal distribution of pollen beetle (*Meligethes aeneus* Fabricius) on oilseed rape

Abstract

The pollen beetle *Meligethes aeneus* (Fabricius) (Col.: Nitidulidae) is one of the most damaging pests of oilseed rape throughout Europe. Alternative control strategies are needed in order to reduce the current extensive use of insecticides. We investigated the potential of turnip rape, *Brassica rapa*, grown as a trap crop on border strips for protecting the main crop of winter oilseed rape, *Brassica napus*, from infestation by pollen beetles. In addition to trap cropping, the effect of insecticide sprays targeted to the border strip on pollen beetle colonization of the crop was tested. In three-year field experiments, we studied the influence of turnip rape trap crop borders in combination with insecticide treatment on the spatio-temporal distribution and damage of pollen beetles in the main crop. As long as the main crop of winter oilseed rape was in bud stage, the turnip rape trap crop was significantly more attractive to pollen beetle than oilseed rape. However, the preference of beetles for turnip rape in the border strips did not result in lower numbers of pollen beetles on the adjacent main crop. In our study the plant development of turnip rape was only 3-4 days in advance of the oilseed rape. Insecticide applications to the border strips had no significant effect on pollen beetle abundance in the adjacent untreated oilseed rape crop. Pollen beetle numbers were only significantly reduced in plots sprayed overall with insecticides. Damage of pollen beetles to the buds of oilseed rape was not significantly different between all treatments. The results show the importance of the relative growth stage and attractiveness of the turnip rape plants to be effective as a trap crop.

Introduction

Trap cropping is applied to concentrate or intercept the pest population on the trap crop in order to protect the target crop plants from infestation, with the additional option to control the pest on the trap crop (Hokkanen, 1991; Shelton & Badenes-Perez, 2006). The principle of insect control by trap cropping has been considered since 1860 (Hokkanen, 1991). A trap crop in combination with targeted insecticide treatment has proven to provide effective control against several pests, for example pepper maggot *Zonosemata electa* (Dip.: Tephritidae) (Boucher et al., 2003) and cabbage seedpod weevil *Ceuthorrhynchus obstrictus* (Col.: Curculionidae) (Carcamo et al., 2007). In Finland the pollen beetle colonization of cauliflower has been successfully reduced by trap plants treated with insecticides in several experiments (Hokkanen, 1991). Recently, the use of trap cropping in oilseed rape became of interest to reduce potential negative effects of insecticide application on pest resistance and natural pest control (Shelton & Badenes-Perez, 2006). Insecticide resistance of pollen beetle to pyrethroids was recorded for the first time and afterwards widely distributed in Europe

since 1997 (Hansen, 2003; Heimbach et al., 2006; Kazachkova et al., 2007; Délos, 2008; Tiilikainen & Hokkanen, 2008; Wegorek & Zamoyska, 2008). Due to the increase of the pest population and the difficulties involved with chemical control, alternative control strategies for pollen beetle are needed (Hokkanen, 2000; Cook et al., 2007).

The pollen beetle, *Meligethes aeneus* (Col.: Nitidulidae), is a major pest of oilseed rape throughout Europe (Alford, 2003). Through damaging the oilseed rape crop in the bud stage, the beetle causes substantial yield losses (Winfield, 1961). Pollen beetles are *Brassica* specialists and show preferences for some *Brassica* species over others (Ekbohm & Borg, 1996; Cook et al., 2006). The adult beetles locate their host plants by using a combination of visual cues, i. e. the yellow colour of the flowers, and host plant odours, e.g. the isothiocyanates, breakdown products of the glucosinolates (Mithen, 1992; Giamoustaris & Mithen, 1996; Cook et al., 2002). Further, the inflorescence growth stage of the plants is important for host selection of pollen beetles (Frearson et al., 2005).

Brassica rapa (turnip rape) is preferred by *M. aeneus* to *B. napus* and shows good potential as trap crop (Büchi, 1995; Cook et al., 2006, 2007). The spatial distribution of the adult beetles within the field can be manipulated by surrounding the main crop with a border strip of turnip rape (Cook et al., 2004). In field experiments using spring oilseed rape, beetles were retained in the turnip rape border; the number of beetles in the centre was reduced compared to the control plot entirely grown with spring oilseed rape (Cook et al., 2004). Pollen beetles are attracted by the advanced growth stage of turnip rape (Cook et al., 2004) and by volatile cues, including phenylacetaldehyde and indole of turnip rape (Cook et al., 2007). The economic benefits including reduction of insecticide use may motivate farmers to use trap crops (Hokkanen, 1991). So far, trap cropping as control strategy for pollen beetle was only investigated in spring oilseed rape in small plot experiments.

The aim of this study was to investigate the effect of trap cropping on pollen beetle infestation of oilseed rape. Field experiments were designed in a block with borders of different treatments along large commercial fields of winter oilseed rape. The impact of a turnip rape trap crop on the spatio-temporal distribution of pollen beetles on the main crop was compared to the distribution and infestation of oilseed rape plots without trap crop. In addition, the effect of insecticide application targeted to the borders on spatial distribution of the beetles was investigated.

Materials and methods

Field experiments were conducted in crops of oilseed rape (*B. napus*) in the region of Goettingen, Germany. In three consecutive years the replicated treatments were distributed across multiple locations for reasons of space: four replicates over three, six replicates over three and four replicates over two sites in 2008, 2009 and 2010, respectively (Table 1). Individual plots were bordered at one side with either a trap crop strip of turnip rape *Brassica rapa* (cv. 'Perko') or by an equally-sized extension of the main crop *Brassica napus* (L.) as control. In 2008 and 2009, the border strips were 6.5m wide, and in 2010 17.5m wide. They were 50-100m long according to the plot size of the main crop (Table 1, Figure 1).

Depending on the field size, the area of the strips in relation to the total plot area varied from 8 to 20% (Table 1).

Different insecticide compounds (Table 1) were applied for targeted control of adult pollen beetles either in the plots grown with oilseed rape overall, in border strips of oilseed rape or in border strips of turnip rape. The effect of the insecticide treatment on spatial distribution of pollen beetle on the main crop was compared with the distribution of pollen beetle on untreated oilseed rape plots and on untreated oilseed rape crop bordered with an untreated strip with turnip rape (Figure 1).

Table 1: Field characteristics and insecticide application in field experiments 2008-2010 (OSR = oilseed rape).

Year	Site no.	OSR Cultivar	Plot size [m]	Relative area covered by border crop [%]	Field size [ha]	Date of insecticide application	Insecticide	dose rate [(g) a.i./300 l water]
2008	1	NK Fair	50 x 57	11.6	5.5	24 April	thiacloprid	240
	2	NK Fair	50 x 57	11.6	5.5	26 April	thiacloprid	240
	3	Taurus	50 x 57	11.6	4.0	24 April	thiacloprid	240
2009	1	Visby	70 x 77	8.5	13.5	09 April	bifenthrin	80
						16 April	thiacloprid	240
	2	Visby	50 x 57	11.6	7.0	07 April	chlorpyrifos-methyl	225
						15 April	thiacloprid	240
3	Hybride PR031	70 x 77	8.5	11.5	13 April	bifenthrin	80	
2010	1	Dimension	70 x 88	20	13.7	13 April	bifenthrin	80
						21 April	thiacloprid	240
	2	Visby	100 x 118	14.9	9.0	13 April	bifenthrin	80
						21 April	thiacloprid	240

Insecticides were applied when the economic threshold for pollen beetle was exceeded. In Germany, the economic threshold of pollen beetle is 3-4 beetles/plant, 7-8 beetles/plant and >8 beetles/plant at BBCH growth stage 50-51, 52-53 and 55-59, respectively (Williams, 2010). Cultivation and fertilization was applied in accordance with common farming practice. The abundance and spatial distribution of pollen beetles on the plants was assessed six times at intervals of 3 to 4 days from the bud to the full flowering stage. On each occasion, the number of adult pollen beetles was recorded on the main raceme of 50 plants selected at random in a central line in the strip and at a distance of 10m, 20m and 40m from the strip in each plot (50 plants x 4 distances). Adult pollen beetles were collected by beating each raceme onto a white plastic funnel (diameter: 25cm). On each occasion, the BBCH growth stage of winter oilseed rape and turnip rape plants was recorded (Lancashire et al., 1991). The numbers of pods and blind stalks of the main raceme was counted on 128 plants (32 plants x 4 distances) in each plot during the pod stage.

	<	50-100 m	>>	50-100 m	>>	50-100 m	>>	50-100 m	>>	50-100 m	>	
Border 7-18 m		OSR untreated		TR strip untreated		OSR strip treated		TR strip treated		OSR treated		
50 m		OSR untreated		OSR untreated		OSR untreated		OSR untreated		OSR treated		
50 m		OSR untreated		OSR treated		OSR untreated		OSR untreated		OSR untreated		
Border 7-18 m		TR strip untreated		OSR treated		TR strip treated		OSR untreated		OSR strip treated		
		<	50-100 m	>>	50-100 m	>>	50-100 m	>>	50-100 m	>>	50-100 m	>

Figure 1: Design of field experiments comprising 5 treatments randomly distributed on each side of a field grown with winter oilseed rape (TR = turnip rape, OSR = oilseed rape; background dark-gray = insecticide treated).

Data of each year and sampling occasion were analysed separately. The average number of pollen beetles per plant in the five different treatments was compared at any distance. To estimate the bud loss caused by adult feeding the proportion of blind stalks in relation to total number of ripen pods and blind stalks was computed. The data of bud loss were compared between the five different treatments within each distance. The nonparametric Kruskal-Wallis test was used because the data were not normally distributed and not determined by homogeneity of variance. Treatment averages were compared at $p \leq 0.05$. The software STATISTICA, version 9.1 (StatSoft, Inc., 2010) was used to perform all analyses.

Results

In 2008 and 2009, the abundance of pollen beetles increased during the first week following migration into the crop (Figure 2-3). In 2008, the colonization by pollen beetle started when the oilseed rape was in the green bud stage and in the yellow bud stage, while turnip rape was in the early flowering stage (Figure 2). On turnip rape the number of beetles remained on a higher level than on oilseed rape until the oilseed rape came into flower. However, these numbers were not significantly different. Insecticide application following the third sampling reduced the number of pollen beetles in the treated strips and in the oilseed rape plot totally treated. No significant effect of turnip rape strips or insecticide treated strips on spatial distribution of pollen beetles within the main crop, at 10, 20 and 40m distance from strips, was observed (Figure 2).

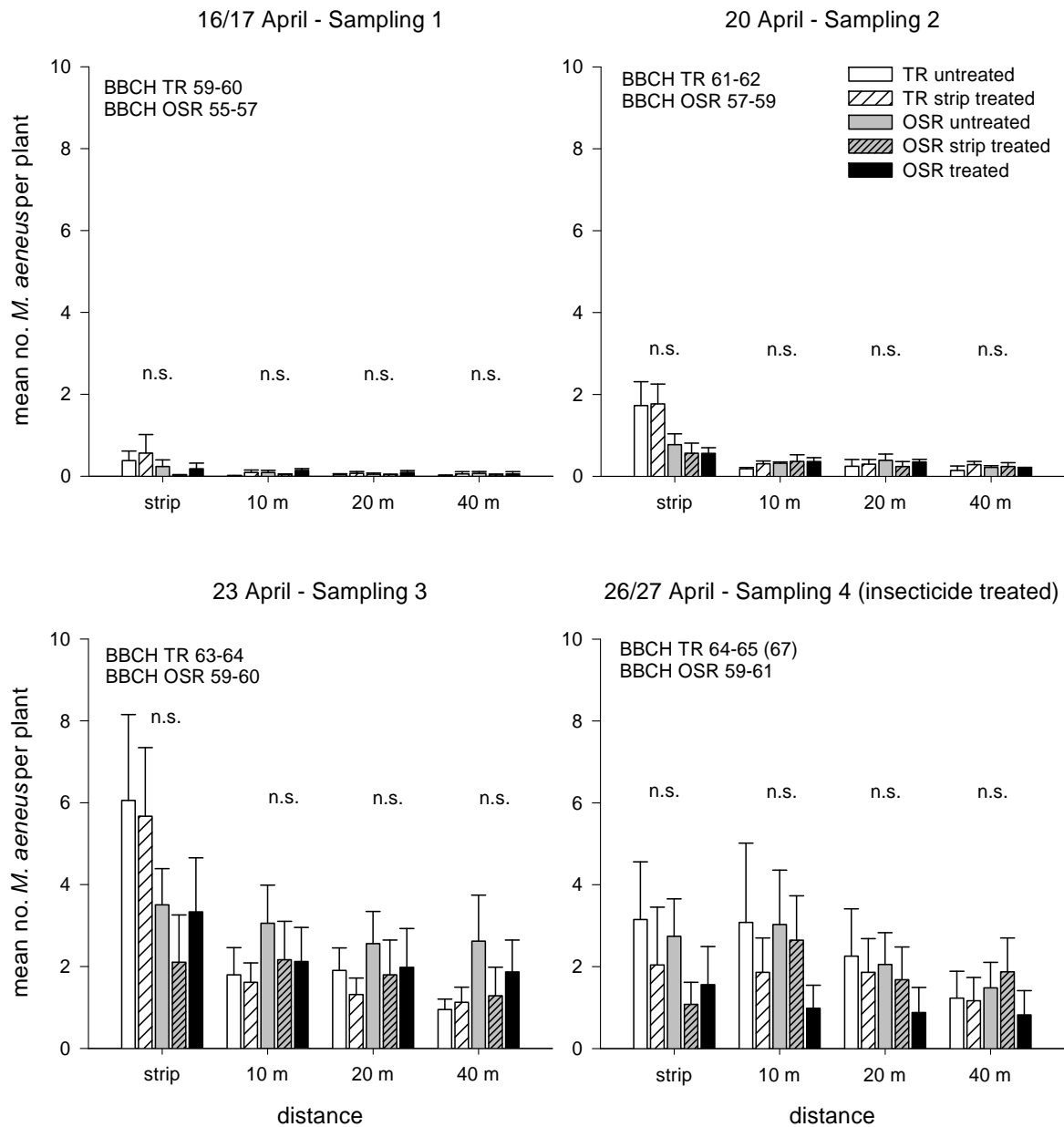


Figure 2: Number of pollen beetles per plant (mean + SEM) in plots spatially distributed (strip, 10m, 20m, 40m in field) of turnip rape (TR) and oilseed rape (OSR) untreated, strip treated or entire plot treated with insecticide treatment after sampling 3 in 2008 (n.s. = not significant differences with $p \leq 0.05$ between treatments; Kruskal-Wallis test).

In 2009, beetle immigration started in the bud stage of turnip rape and oilseed rape (Figure 3). The turnip rape strip was more attractive to pollen beetles particularly on the second and third samplings. After insecticide treatment the turnip rape strip showed significantly higher numbers of adult beetles than the strip of the plot oilseed rape treated overall on sampling 3 ($p = 0.008$). Following peak abundance in turnip rape the beetles spread evenly over the whole plot in oilseed rape. Insecticide application after sampling 4 significantly decreased the numbers of pollen beetles in the strip of the treated oilseed rape compared to untreated turnip rape strip (strip of oilseed rape treated in strip: $p = 0.01$, strip of oilseed rape totally treated: $p = 0.001$). In the main crop the direct treatment of oilseed rape showed the lowest

number of pollen beetles. Pollen beetle numbers on the inner parts of the field (10, 20 and 40m distance) were not influenced by turnip rape border from bud to flowering stage (Figure 3).

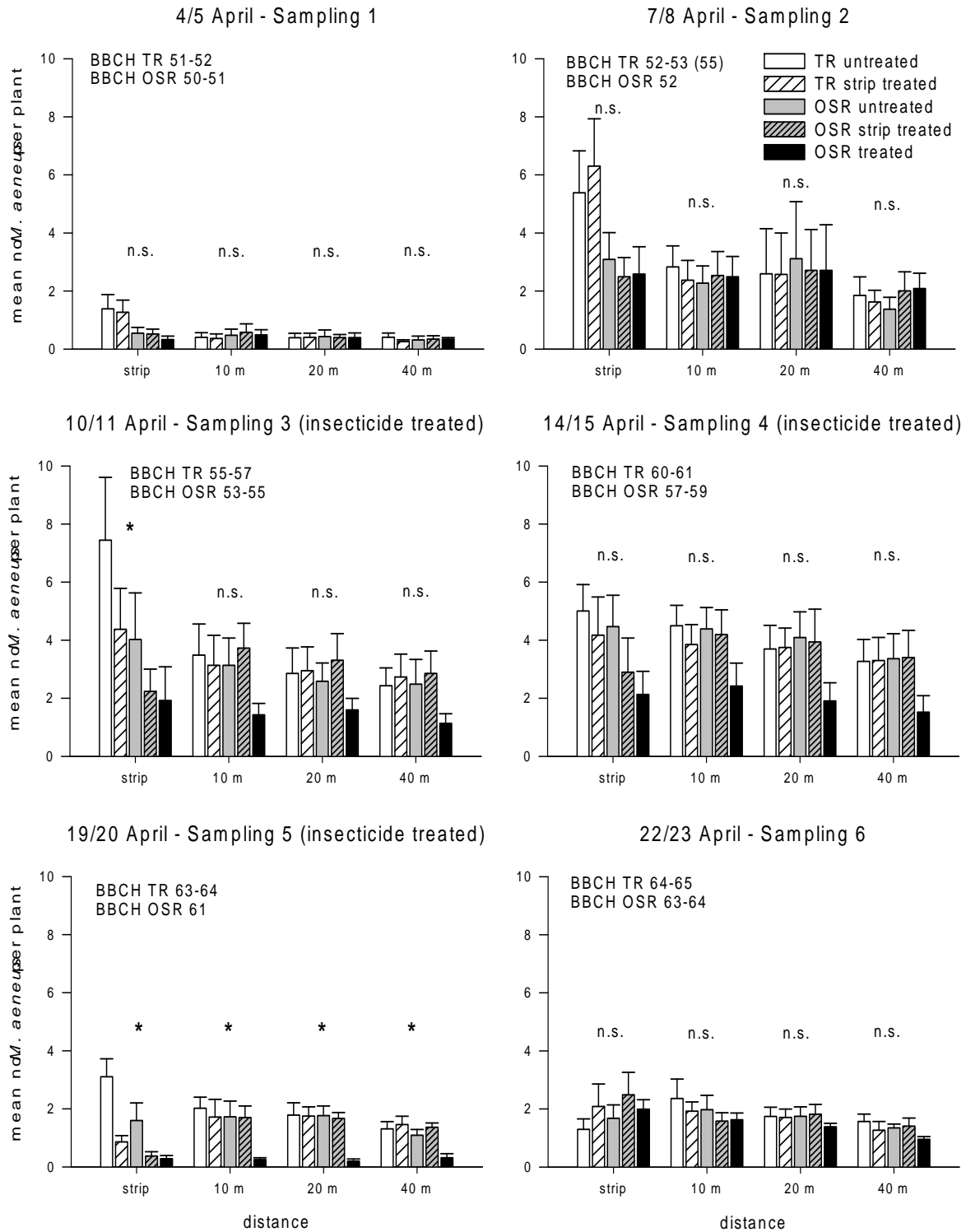


Figure 3: Number of pollen beetles per plant (mean + SEM) in plots spatially distributed (strip, 10m, 20m, 40m in field) of turnip rape (TR) and oilseed rape (OSR) untreated, strip treated or entire plot treated with insecticide treatment after sampling 2 (site no. 1 and 2), 3 (site no. 3) and 4 (site no. 1 and 2) in 2009 (n.s. = not significant differences or *significant differences with $p \leq 0.05$ between treatments; Kruskal-Wallis test).

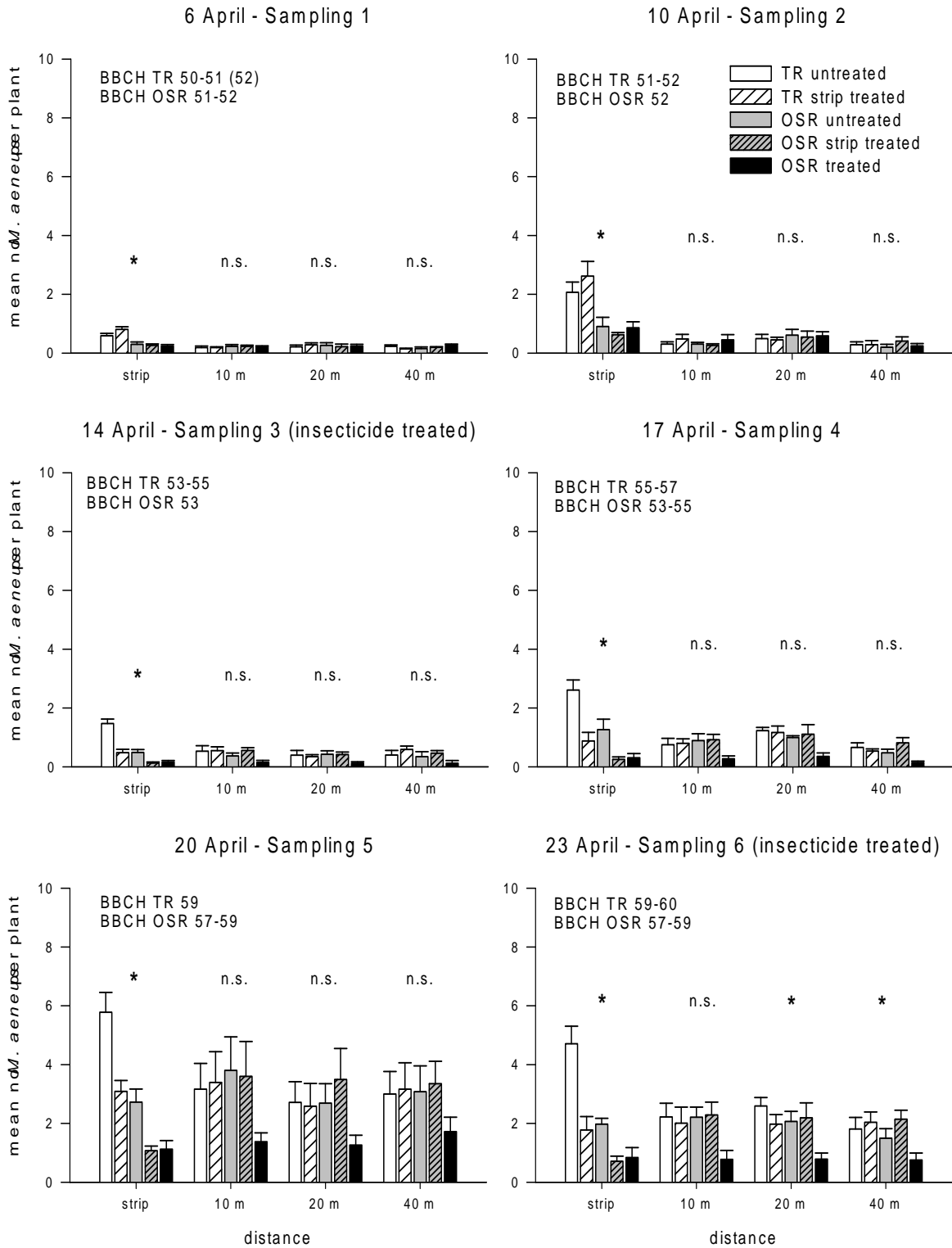


Figure 4: Number of pollen beetles per plant (mean + SEM) in plots spatially distributed (strip, 10m, 20m, 40m in field) of turnip rape (TR) and oilseed rape (OSR) untreated, strip treated or entire plot treated with insecticide spraying after sampling 2, 3 and 5 in 2010 (n.s. = not significant differences or *significant differences with $p \leq 0.05$ between treatments; Kruskal-Wallis test).

In 2010, pollen beetles colonized the turnip rape and oilseed rape plots in the early bud stage and peak abundance occurred at the fifth assessment (Figure 4). Even though the growth

stage of turnip rape strips was similar to the growth stage of oilseed rape, pollen beetles were more strongly attracted to the strip of turnip rape during the early bud to early flowering stage in April. On the first and second sampling date, pollen beetle abundance was significantly different between insecticide untreated turnip rape strips and untreated oilseed rape strips ($p = 0.04$; $p = 0.04$). The number of pollen beetles differed significantly between the strips on sampling dates 3, 4, 5 and 6. Insecticide application to these strips (sampling 3: $p = 0.005$; sampling 4: $p = 0.02$; sampling 5: $p = 0.006$; sampling 6: $p = 0.01$) and to the entire oilseed rape plot (sampling 3: $p = 0.01$; sampling 4: $p = 0.02$; sampling 5: $p = 0.01$; sampling 6: $p = 0.02$) significantly reduced the number of beetles in oilseed rape strip compared to the number of beetles in untreated turnip rape strip. Reduced numbers of adult beetles on the main crop (10, 20 and 40 m distance) were only recorded following insecticide application on the entire oilseed rape plot (Figure 4).

No significant difference of bud loss per main raceme was recorded between the treatments in 2008 and 2009 (Figure 5). In 2008, the insecticide-treated turnip rape strip showed the lowest bud loss, followed by the untreated turnip rape strip. Bud loss of treated oilseed rape was decreased at distances of 20m and 40m compared to the other four treatments. In 2009, oilseed rape plots treated overall with insecticide showed the lowest bud loss. The unsprayed strips of turnip rape and oilseed were more heavily damaged by pollen beetle than the sprayed strips (Figure 5).

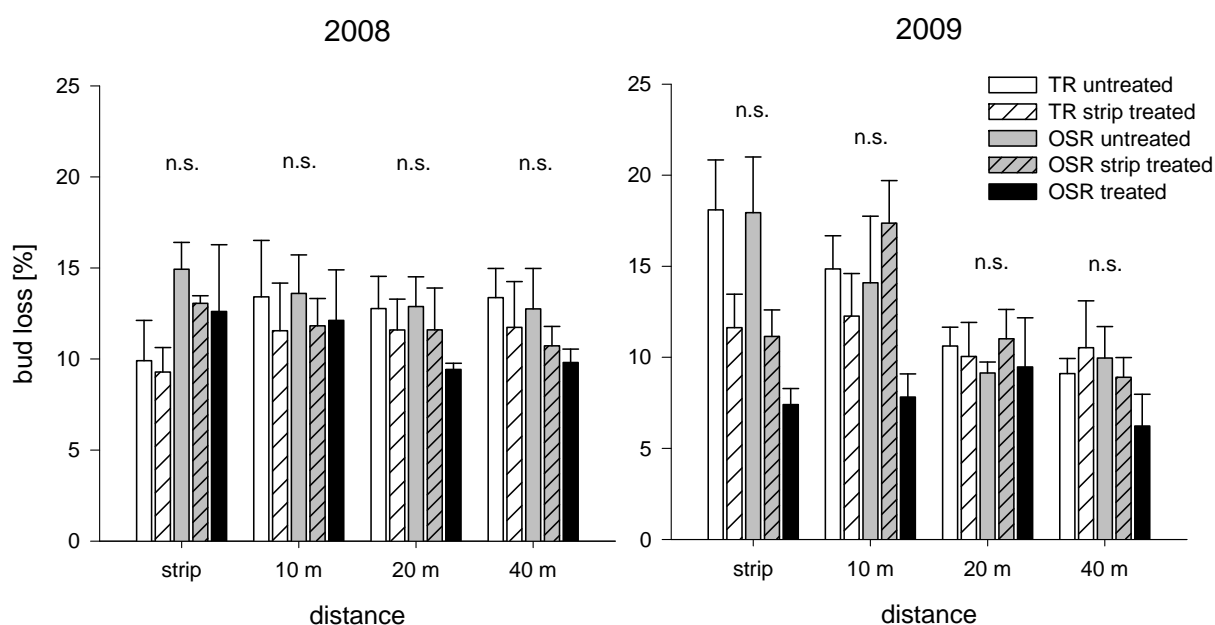


Figure 5: Bud loss per main raceme due to pollen beetle feeding (mean + SEM) in bordering and adjacent plots in different distances (strip, 10m, 20m, 40m in field) of turnip rape (TR) and oilseed rape (OSR) untreated, strip treated or entire plot treated with insecticide in 2008 and 2009 (n.s. = not significant differences with $p \leq 0.05$ between treatments; Kruskal-Wallis test).

Discussion

The success of *B. rapa* as trap crop in spring oilseed rape crops was attributed to more advanced plant development and associated volatile cues, including phenylacetaldehyde and indole (Cook et al., 2006, 2007). In our field experiments, pollen beetles were more abundant in the turnip rape border compared to oilseed rape from the beginning of colonization until the main crop came into flower. Results of several studies indicate that turnip rape has potential as a trap crop for pollen beetles (Büchi, 1989, 1995; Hokkanen, 1991). Pollen beetles were significantly more abundant on turnip rape than on oilseed rape when both species were in the bud stage, but no significant preference of adult beetles was observed in a polytunnel bioassay when both species were in the flowering stage (Cook et al., 2007). Irrespective of *Brassica* species, the species in flower attracted more pollen beetles than the species in bud stage (Cook et al., 2006). Similar to our field experiments, the host selection of pollen beetles in many field studies was found to be mainly regulated by the plant growth stage, particularly by the high attractiveness of the flowering stage (Büchi, 1995; Cook et al., 2006; Valentin-Morison et al., 2007). However, the winter turnip rape strips did not serve their expected purpose as trap crop in our experiments. The reasons for this might be as follows: synchronisation between flowering stage of turnip rape and bud stage of winter oilseed rape, immigration and dispersal behaviour of pollen beetles into the crop after hibernation and weather conditions.

The distribution of pollen beetles in the fields is complex and reflects the interplay of location and selection of host plants with various environmental factors, such as the distance and direction of overwintering sites and the wind direction (Ferguson et al., 2003). Despite the preference for turnip rape, the spatial distribution and dispersal of the beetles on the main crop was not delayed in our study. The main crop of oilseed rape was also attractive for colonization by pollen beetles. Gravid females are attracted by plants of *B. napus* when the bud size suitable for oviposition is available (Ekbohm & Borg, 1996). The availability of buds is an important parameter for the residence time of pollen beetle on the plant (Frearson et al., 2005). Triggered by their host plant preference and dispersal behaviour, adult pollen beetles rapidly spread over the whole plot in our field experiment. Even when the width of the trap crop strips was extended to 18m in 2010, compared to 7m in 2008 and 2009, no effect on the spatial distribution of *M. aneneus* on the main crop was found.

Free and Williams (1978) reported that pollen beetles initially colonize brassicaceous and non-brassicaceous plants in flower growing on verges of oilseed rape fields. However, when the crop started to flower they became more abundant at field edges of oilseed rape crops. In our studies, during the initial period of immigration, pollen beetle numbers were not increased on the oilseed rape margins. Independent of plant species grown in the border strips, similar numbers of adult beetles colonized the main crop at distances of 10, 20 and 40m from the border. Flight activity of pollen beetles in spring starts at temperatures exceeding 12°C (Fritzsche, 1957). Temperatures exceeding 15°C cause mass colonization of oilseed rape crops. During the observation period, maximum ambient temperature exceeded 12°C on 14 of 18 days, 19 of 20 days and 13 of 18 days in 2008, 2009 and 2010, respectively. This

supports the observation that temperatures in all years favoured massive flight activity of pollen beetle, resulting in abundant immigration of beetles all over the plots.

The synchronisation of trap crop flowering and initial colonization by pollen beetle depends on annual weather conditions like temperature and precipitation (Hokkanen, 1991). Using trap crops is only appropriate for crop protection management when the pest is retained on the trap crop during the entire sensitive growth stage of the main crop; according to Holden et al. (2012), the trap crop retention of the pest is the most important factor in trap cropping. In our study the plant development of turnip rape was only 3 to 4 days ahead of the main crop. Particularly in 2009 and 2010, the colonization of pollen beetles began in the early bud stage of both plant species. Büchi (1995) concluded that it is difficult to find the time frame of insecticide spraying of the trap crop border with turnip rape. However, in our study the trap crop did not affect the number and spatial distribution of pollen beetles in the centre of the plot. In our field experiments, the number of pollen beetles was only reduced by direct application of insecticides in the border strips and in the main crop.

The proportion of blind stalks in relation to the total number of ripen pods and blind stalks ('bud loss') of oilseed rape did not differ between the trap crop treatments. This may have resulted from the unsuccessful suppression of pollen beetle abundance on the main crop during the bud and flowering stage. Bud loss was slightly, not significantly reduced in the insecticide-treated strips and in oilseed rape plots treated overall, compared to the untreated parts of the plot. In 2009, the direct application of insecticides decreased the pollen beetle abundance below the economic threshold (Williams, 2010) while in 2010 the economic threshold was only exceeded in untreated turnip rape. Despite lower numbers of adult beetles in 2010, bud loss was higher than in 2009. In 2010, the weather conditions may have inhibited plant compensation through production of new buds, leading to high damage caused by pollen beetle. When the conditions for assimilate production due to climate conditions and nutrition are favourable during the bud and flower stage, pod density will increase (Habekotte, 1993).

The trap crop strategy is only effective when the development stage of the trap crop is well advanced to the main crop. Then the main oilseed rape crop might be protected from pollen beetle damage during its susceptible bud stage through the flowering trap crop (Frearson et al., 2005). Plant growth stage is affected by weather conditions and crop management techniques, such as the sowing time (Hokkanen, 1991). In our study, the use of turnip rape as a trap crop for reducing beetle infestation and damage was less effective than spraying insecticides all over the plot. Turnip rape might be more effective as a trap crop in spring oilseed rape because the inflorescence stages of spring turnip rape usually are more advanced than spring oilseed rape (Cook et al., 2004). For trap cropping on the abundance of pollen beetle an early flowering winter turnip rape cultivar might be more suitable in winter oilseed rape crops.

Acknowledgements

This research was supported by the Federal Ministry of Food, Agriculture and Consumer Protection. We wish to thank the participating farmers. We thank Ulrike Jeche, Nadine Neumann, Joshua Sikhu Okonya, Kim Stey, Florian Rekate and Ines Vollhard for field work and Gerrit Kruger for comments on the manuscript.

References

- Alford, D. (2003). Insect pest of oilseed rape crops. In: Alford D.V. (ed.) *Biocontrol of oilseed rape pests*. Oxford, UK: Blackwell Publishing, 9-41.
- Boucher, T., Ashley, R., Durgy, R., Sciabarrasi, M. & Calderwood, W. (2003). Managing the pepper maggot (Diptera : Tephritidae) using perimeter trap cropping. *Journal of Economic Entomology* 95, 420-432.
- Büchi, R. (1989). Modelle für die Verteilung des Rapsglanzkäfers, *Meligethes aeneus* F., auf Raps und Rübsenpflanzen. *Journal of Applied Entomology* 108, 363-371.
- Büchi, R. (1995). Combination of trap plants (*Brassica rapa* var. *Silvestris*) and insecticide use to control rape pests. *IOBC/wprs Bulletin* 18, 102-121.
- Carcamo, H., Dunn, R., Dossall, L. & Olfert, O. (2007). Managing cabbage seedpod weevil in canola using a trap crop - A commercial field scale study in western Canada. *Crop Protection* 26, 1325-1334.
- Cook, S. M., Bartlet, E., Murray, D. A. & Williams, I. H. (2002). The role of pollen odour in the attraction of pollen beetles to oilseed rape flowers. *Entomologia Experimentalis et Applicata* 104, 43-50.
- Cook, S. M., Rasmussen, H. B., Birkett, M. A., Murray, D. A., Pye, B. J., Watts, N. P. & Williams, I. H. (2007). Behavioural and chemical ecology underlying the success of turnip rape (*Brassica rapa*) trap crops in protecting oilseed rape (*Brassica napus*) from the pollen beetle (*Meligethes aeneus*). *Arthropod-Plant Interactions* 1, 57-67.
- Cook, S. M., Smart, L. E., Martin, J. L., Murray, D. A., Watts, N. P. & Williams, I. H. (2006). Exploitation of host plant preferences in pest management strategies for oilseed rape (*Brassica napus*). *Entomologia Experimentalis et Applicata* 119, 221-229.
- Cook, S. M., Watts, N. P., Hunter, F., Smart, L. E. & Williams, I. H. (2004). Effects of a turnip rape trap crop on the spatial distribution of *Meligethes aeneus* and *Ceutorhynchus assimilis* in oilseed rape. *Bulletin IOBC/wprs: Integrated Control in oilseed crops* 27, 199-206.
- Délos, M. (2008). *Meligethes* du colza: Evolution et gestion de resistances aux pyrethrinoides en France et recommandations pour gerer le risque. *IOBC/wprs Bulletin* 38, 75-78.
- Ekbom, B. & Borg, A. (1996). Pollen beetle (*Meligethes aeneus*) oviposition and feeding preferences on different host plant species. *Entomologia Experimentalis et Applicata* 78, 291-299.
- Ferguson, A. W., Klukowski, Z., Walczak, B., Clark, S. J., Mugglestone, M. A., Perry, J. N. & Williams, I. H. (2003). Spatial distribution of pest insects in oilseed rape: implications for integrated pest management. *Agriculture Ecosystems and Environment* 95, 509-521.
- Frearson, D. J. T., Ferguson, A. W., Campbell, J. M. & Williams, I. H. (2005). The spatial dynamics of pollen beetles in relation to inflorescence growth stage of oilseed rape: implications for trap crop strategies. *Entomologia Experimentalis et Applicata* 116, 21-29.
- Free, J. B. & Williams, I. H. (1978). The responses of the pollen beetle, *Meligethes aeneus*, and the seed weevil, *Ceuthorhynchus assimilis*, to oil-seed rape, *Brassica napus*, and other plants. *Journal of Applied Ecology* 15, 761-774.
- Fritzsche, R. (1957). Zur Biologie und Ökologie der Rapsschädlinge aus der Gattung *Meligethes*. *Sonderdruck aus Zeitschrift für angewandte Entomologie* 40, 222-280.
- Giamoustaris, A. & Mithen, R. (1996). The effect of flower colour and glucosinolates on the interaction between oilseed rape and pollen beetles. *Entomologia Experimentalis Et Applicata* 80, 206-208.
- Habekotte, B. (1993). Quantitative-analyses of pod formation, seed set and seed filling in winter oilseed rape (*Brassica napus* L) under field conditions. *Field Crops Research* 35, 21-33.
- Hansen, L. M. (2003). Insecticide-resistant pollen beetles (*Meligethes aeneus* F) found in Danish oilseed rape (*Brassica napus* L) fields. *Pest Management Science* 59, 1057-1059.

- Heimbach, U., Müller, A. & Thieme, T. (2006). First steps to analyse pyrethroid resistance of different oil seed rape pests in Germany. *Nachrichtenblatt Deutscher Pflanzenschutzdienst* 58, 1-5.
- Hokkanen, H. M. T. (1991). Trap cropping in pest management. *Annual Review of Entomology* 36, 119-138.
- Hokkanen, H. M. T. (2000). The making of a pest: recruitment of *Meligethes anaenus* onto oilseed Brassicas. *Entomologia Experimentalis et Applicata* 95, 141-149.
- Holden, M. H., Ellner, S. P., Lee, D.-H., Nyrop, J. P. & Sanderson J. P. (2012). Designing an effective trap cropping strategy: the effects of attraction, retention and plant spatial distribution. *Journal of Applied Entomology* 49, 715-722.
- Kazachkova, N., Meijer, J. & Ekbohm, B. (2007). Genetic diversity in pollen beetles (*Meligethes aeneus*) in Sweden: role of spatial, temporal and insecticide resistance factors. *Agricultural and Forest Entomology* 9, 259-269.
- Lancashire, P. D., Bleiholder, H., Vandenboom, T. & Langeluddeke, P. (1991). A uniform decimal code for growth stages of crops and weeds. *Annals of Applied Biology* 119, 561-601.
- Mithen, R. (1992). Leaf glucosinolate profiles and their relationship to pest and disease resistance in oilseed rape. *Euphytica* 63, 71-83.
- Shelton, A. M. & Badenes-Perez, E. (2006). Concepts and applications of trap cropping in pest management. *Annual Review of Entomology* 51, 285-308.
- Taimr, L., Sedivy, J., Bermannova, E. & Hanker, I. (1967). Further experience obtained in studies on dispersal flights of *Meligethes aeneus* F., marked with P³² (Coleoptera). *Acta ent. bohemoslov.* 64, 325-332.
- Tiilikainen, T. M. & Hokkanen, H. M. T. (2008). Pyrethroid resistance in Finnish pollen beetle (*Meligethes aeneus*) populations - is it around the corner? *IOBC/wprs Bulletin* 38, 99-103.
- Valentin-Morison, M., Meynard, J. & Doré, T. (2007). Effects of crop management and surrounding field environment on insect incidence in organic winter oilseed rape (*Brassica napus* L.). *Crop Protection* 26, 1108-1120.
- Wegorek, P. & Zamoyska, J. (2008). Current status of resistance in pollen beetle (*Meligethes aeneus* F.) to selected active substances of insecticides in Poland. *IOBC/wprs Bulletin* 38, 91-94.
- Williams, I. (2010). The major insect pests of oilseed rape in Europe and their management: An overview. In: Williams, I. H. (ed.) *Biocontrol-based integrated management of oilseed rape pests*. Netherlands: Springer, 1-44.
- Winfield, A. L. (1961). Field observations on the control of blossom beetles (*Meligethes aeneus* F.) and cabbage-seed weevils (*Ceuthorrhynchus assimilis* Payk.) on mustard-seed crops in East Anglia. *Annals of Applied Biology* 49, 539-555.

General discussion

The pollen beetle *Meligethes aeneus* (Fabricius) (Col.: Nitidulidae) is one of the most important pests during the bud stage of oilseed rape (Alford, 2003). Feeding of adult pollen beetles on rape buds causes podless stalks (Winfield, 1961) and yield reductions up to 80% (Ahuja et al., 2009). The frequent use of insecticides and the limited supply of registered insecticide products for control the pollen beetle populations have resulted in widespread resistance to pyrethroids in Europe in recent years (Hansen, 2003; Heimbach et al., 2006; Kazachkova et al., 2007; Délos, 2008; Tiilikainen & Hokkanen, 2008; Wegorek & Zamoyska, 2008). To minimise insecticide exposure of beetles and larvae new ecological approaches are required (Cook & Denholm, 2008). To integrate different control strategies in integrated pest management systems, a better understanding of the factors governing the relationships between the pest and its host plant is fundamental (Schoonhoven et al., 2005).

In contrast to its wide host range for feeding, *M. aeneus* on oviposition is specialized on various *Brassica* species (Ekbon & Borg, 1996; Börjesdotter, 2000). There is a strong correlation between the oviposition preference and the size of buds of oilseed rape (Ekbon & Borg, 1996). Several life stages of pollen beetle are related to the bud and flowering stage of *Brassica* spp. (Fritzsche, 1957). So far, only a few studies have been conducted to investigate the impact of host plant phenology on pollen beetle population dynamics (Nielsen & Axelsen, 1988; Cook et al., 2004a). The results of our study regarding plant phenology will be integrated in the following discussion. The analyses focused on factors regulating the reproduction, population growth, population dynamics and damage of pollen beetle. This knowledge might be included in integrated pest management strategies in the future.

Factors regulating the population dynamics and population growth

There are several factors which potentially have an influence on the population dynamics and population growth of *M. aeneus*. Among these, weather conditions, host location, quality and quantity of host plants and natural enemies have major impact.

The main stimulus for immigration of adult pollen beetles into crops of oilseed rape is determined by temperature above 15°C (Fritzsche, 1957). Furthermore, weather conditions including air temperature and sunshine duration are influential for the pollen beetle migration (Fritzsche, 1957; Sedivy & Kocourek, 1994). In our study, the maximum air temperatures increased over 15°C on 21, 1 and 6 April in 2008, 2009 and 2010, respectively.

Adult beetles locate their host plants by odour and colour cues (Evans & Allen-Williams, 1994; Cook et al., 2002). Host plant volatiles, particularly isothiocyanates which are carried downwind from the crop, and the yellow and green colour of the plant are important cues for orientation. In our field experiments, first beetles arrived on the crop on 18 April in 2008 and on 2 April in 2009 and 2010, respectively. In 2008, the overwintered pollen beetles preferred the winter oilseed rape cultivars in the flowering stage for the first 6 days following immigration, compared to the cultivars in the bud stage (Chapter II). This is in agreement with other field studies which have shown that flowers provide more important cues than

buds for host selection of pollen beetle during flowering (Free & Williams, 1978; Cook et al., 2002, 2006; Frearson et al., 2005).

When beetles arrive before crop flowering, they locate their host plants by olfactory stimuli of rape leaves, stems and buds in addition to visual stimuli (Ruther & Thiemann, 1997; Williams & Cook, 2010). A cultivar with low content of alkenyl glucosinolates in the leaves was less attractive for pollen beetles than a conventional cultivar (Cook et al., 2006). In 2009 and 2010, *M. aeneus* colonized the cultivars in the early bud stage. Under these conditions, beetles were more abundant on the hybrids, particularly on cv. 'Elektra' (Chapter II), which may have resulted from a higher glucosinolate content of this oilseed rape cultivar compared to the other cultivars. Even though we did not analyse the glucosinolate content of the cultivars, the higher number of eggs and first instar larvae in the buds of the hybrids 'Elektra' and 'Titan' in comparison with the open pollinated cultivars 'NK Passion' and 'Favorite' might reflect the preference of adults for cultivars with a higher alkenyl glucosinolate content (Chapter II). In addition to odour cues, colour cues might have an influence on the preference of adult beetles for the winter oilseed rape cultivars in the bud stage as well. Pollen beetles have been shown to aggregate on oilseed rape flowers with simulated pollen beetles drawn on them (Free & Williams, 1978).

Before females start ovipositing into the buds of *Brassica* plants, they feed on plants of different families or on oilseed rape crops in spring. Ovary maturation of females depends on weather conditions, such as temperature and humidity, and on the feeding period (Fritzsche, 1957; Ekbohm & Ferdinand, 2003). Field-collected beetles showed variable egg loads after a change from cold and rainy to warm and sunny weather conditions. The proportion of gravid females was higher at the beginning of the oviposition period than at the end (Ekbohm & Ferdinand, 2003). Free and Williams (1979) reported that egg-laying of pollen beetle females began 3 weeks after their arrival on winter oilseed rape. In comparison, females immigrating into spring oilseed rape had fully developed eggs. In our field experiments, we found eggs in the buds 6 days after immigration in 2008 and 2009. In 2009 the number of eggs increased 24 days after immigration. In addition, first larvae were found in the buds at that time (Chapter II). This suggests that gravid females are already present at the beginning of crop infestation and oviposition increases with increasing abundance of adult pollen beetles in our experiments.

The host plant quality influences not only the herbivore abundance (Bach, 1990) but also their reproduction (Preszler & Price, 1988). Hopkins and Ekbohm (1999) examined the oviposition rate of pollen beetle females on different host plants. Beetles that moved from *B. napus*, a "high-accepted" plant, to *Sinapis alba*, a "low-accepted" plant, reduced their oviposition rate whereas in the opposite case, beetles migrating from *S. alba* to *B. napus*, the rate of oviposition increased (Hopkins & Ekbohm, 1999). This either suggests that beetles adapt to host plant quality to save resources for future egg production or that the oogenesis is arrested or incomplete on a "low-quality" host plant. An incomplete oogenesis is indicated by reduced egg production rates when pollen beetle females are exposed to "low-quality" host plants (Ekbohm & Popov, 2004). In 2009 and 2010, lower numbers of eggs and second instar larvae on the open pollinated cultivars compared to hybrids were recorded from plants in the same growth stage (Chapter II). This indicates that there may be other factors

influencing the host-quality of winter oilseed rape cultivars for oviposition by pollen beetle females.

Females prefer buds with a length of 2-3mm for egg deposition (Nilsson, 1988a; Ekbohm & Borg, 1996). In our field experiments, buds >3mm were also used for oviposition and feeding (Chapter II). In 2009, all four cultivars showed a similar development, and first flowering started 16 and 18 days after pollen beetle invasion in the early flowering and late flowering cultivars, respectively. In 2010, the main egg laying period was extended because flowering started after 23 days and after 28 days of pollen beetle invasion in the early flowering and late flowering cultivars respectively. Due to the extended oviposition period and increased number of eggs, the L1 and L2 stages might have been affected positively by the longevity of available buds and annual weather conditions (Nilsson, 1988b).

During the egg and first instar larval stages, several factors may impact the mortality rate. Damage to pistils and ovaries within buds lead to bud abortion (Williams & Free, 1978). A higher mortality rate of eggs due to bud abortion can occur when high population densities are present in the early developmental stage of oilseed rape (Nilsson, 1988b). Nilsson (1988b) made this observation primarily in spring oilseed rape where a large proportion of buds used for oviposition is smaller than 2mm. In our study in winter oilseed rape no eggs were found in buds smaller than 2mm. Not more than 66% of buds >2mm were used for oviposition and feeding (Chapter II). The probability of a high egg mortality rate due to an insufficient number of sizeable buds apparently was low because high numbers of buds larger than 2mm were available on the winter rape plants in our study.

Premature larval dropping due to competition between larvae, completion of the flowering period or parasitoid attack has been observed (Hokkanen, 2000; Nilsson, 1988b). Increased larval dropping with increasing numbers of pollen beetle larvae suggest density dependency (Chapter II). Competition between larvae in different larval stages was recorded by Hokkanen (2000). Second instar larvae are very mobile and can migrate from flower to flower (Williams & Free, 1978). During migration, larvae are easily exposed to parasitoids, particularly to *Tersilochus heterocerus* that oviposits into second instar larvae in open flowers (Osborne, 1960). Furthermore, larval mortality rates may be enhanced during the last days of flowering when larvae are feeding in open flowers. This increases the risk of premature larval dropping together with the petals falling to the ground at the end flowering.

The phenology of host plant and pest needs to coincide for optimal population development of the pest (Singh & Singh, 2005). Changes of abiotic factors such as temperature can cause a disturbance of this coincidence (Schoonhoven et al., 2005). Earlier flowering of oilseed rape resulted in insufficient synchronisation of seed weevil *Ceutorhynchus obstrictus* and its host plant (Haye et al., 2010). In our experiment in 2008, high temperatures in the flowering stage increased the number of pollen beetle on the early flowering cultivars 'NK Passion' and 'Elektra' (Chapter II). Despite of this, a reduction of overall population growth was observed. Even though there was a higher abundance of adult pollen beetles on the early flowering cultivars 'NK Passion' and 'Elektra', the emergence rate of new-generation beetles and the population growth rate were lower than on late flowering cultivars. The number of eggs is strongly correlated with the availability of buds and the growth stage of the host plant (Nilsson, 1994; Ekbohm & Borg, 1996). In 2008, reproduction of pollen beetles was negatively

affected due to an advanced flowering period and a delayed colonization of adult beetles. This means that the larvae originating from eggs deposited in the late flowering period had not gained maturity at the end of flowering of the early flowering cvs. 'NK Passion' and 'Elektra' (Chapter II). Similar results were also obtained in our semi-field experiment: In the 'late infestation' treatment, lower numbers of second instar larvae and new-generation pollen beetles were recorded than in the 'early infestation' treatment. Poor pollen supply restricts the larval fitness and results in incomplete development of the larvae, thus in reduced fitness of pollen beetles in the following life stages (Nielsen & Axelsen, 1988; Cook et al., 2004a). Nielsen and Axelsen (1988) suggested that delayed oviposition may result in incomplete larval development due to advanced flowering. Hence we can assume that early flowering winter oilseed rape cultivars have potential to reduce the population growth when the colonization of pollen beetles occurs in the late bud stage.

In 2009 and 2010, the emergence rates of new-generation pollen beetles were on a low level (Chapter II). Survival of *M. aeneus* seemed to be negatively affected by dry soil conditions. Similar effects on population dynamics of the Nitidulid *Aethina tumida* have been reported: More beetles died in dry soils because of predrying or suffocation (Ellis et al., 2004). Reduced growth rates of pollen beetle populations may also result from attack by natural enemies (Bellows et al., 1992). Parasitoids have major impact on the mortality rate of several rape pests, particularly of pollen beetle (Ulber et al., 2010b). In our studies, the average parasitism rate increased over the years 2008, 2009 and 2010 from 41.0% over 44.5% to 72.9%, respectively (Chapter II). Similarly, the mortality rate of cabbage seedpod weevil *Ceutorhynchus obstrictus* between larval dropping and emergence of adults varied between 60-90% (Haye et al., 2010). The author suggested entomopathogenic fungi and polyphagous predators such as ground beetles (Coleoptera: Carabidae) as important mortality factors. Nuss (1999) and Büchi (2002) found 4% and 16-27% mortality, respectively, of pollen beetle caused by predators such as ground beetles, spiders and staphylinid beetles on oilseed rape crops in Germany and Switzerland.

The relationship between the abundance of overwintered beetles and new-generation pollen beetles can be drastically influenced by weather conditions, host-quality and natural antagonists. The population growth rate ranged from 1:0.5 to 1:11 in our field experiments (Chapter II). Nilsson (1988b) found similar results between 1:1 and 1:10. This author mentioned that small changes, for example growth characteristics of the cultivars, can influence the impact of several mortality factors within the development period of pollen beetles.

Population dynamics of *M. aeneus* may also be influenced by mortality during hibernation. Overwintering pollen beetles are adapted to loamy soil in shadowed and moderately humid microhabitats of deciduous forests (Müller, 1941). In Finland, a high mortality of pollen beetle during hibernation of 85-98% was due to high levels of infection by entomopathogenic fungi (Hokkanen, 1993). In addition to the impact of entomopathogenic fungi, the fitness and cold tolerance of overwintering beetles is influenced by environmental factors, thereby affecting the level of mortality during overwintering (Somme, 1999).

Effects of insecticide application on population growth and damage

In two years of our field experiments, the effectiveness of the insecticide chlorpyrifos-methyl was on a similar level (68-69%). In contrast, the effectiveness of thiacloprid ranged widely between 2.3% and 77.4% (Chapter III). Chlorpyrifos-methyl is active by contact and by dietary uptake (Anonymus, 2005). Neonicotinoide insecticides are distributed acropetally and have a systemic and translaminar activity in plants. They are widely used for control of sucking pests (Elbert et al., 2008). Pollen beetles ingest the active ingredient by feeding. In addition, residuals of thiacloprid are toxic through contact activity (Thieme et al., 2010). The sensitivity of adult beetles can be influenced by their mobility and the time they spent on the host plant. Pollen beetles are very mobile on small plots (Winfield, 1961). A low effectiveness of insecticides might result from small plot size (75 m²) and a high dispersal ability of adult pollen beetles.

Insecticide application during the inflorescence stage reduces the abundance of adult pollen beetles on oilseed rape which in turn decreases oviposition into buds, resulting in lower numbers of larvae (Winfield, 1961; Schroeder et al., 2009, Chapter III). In all three years of our field tests, percentage reduction of second instar larvae and of emerging beetles ranged from 83.2% to 90.6% and from 82.5% to 95.5%, respectively (Chapter III). Lethal effects of topical application of thiacloprid to L2 larvae in open flowers might have caused a high larval mortality rate (Williams & Free, 1978). Further, ovicidal and larvicidal effects might be responsible for increased egg and larval mortality in our studies. Thiacloprid has shown a high larvicidal and low ovicidal effectiveness to the small fruit tortrix *Grapholita lobarzewskii* in apples (Charmillot et al., 2007). In another study, emergence of *Conotrachelus nenuphar* larvae from fruits was reduced and only a few larvae survived 30 days after treatment with thiacloprid (Hoffmann et al., 2009). Furthermore, sublethal effects of insecticides may have an impact on the longevity, fertility, fecundity and behaviour, such as feeding, searching and oviposition of arthropods (Stark & Banks, 2003).

Following insecticide application during the bud stage and early flowering stage of oilseed rape in our experiments, the development of pollen beetle was interrupted (Chapter III). After decline of the insecticidal activity, adult beetles are able to re-colonize the treated plots for oviposition and feeding. The oviposition period may extend up to 2 months as long as mature females are present on the plants (Ekbom & Ferdinand, 2003). However, the plant development will proceed following insecticide application, leading to a reduced availability of buds suitable for oviposition of re-colonizing females. Oviposition into buds of the size >3mm may have resulted in an increased number of larvae which could not complete their development until the end of the flowering stage (Nielsen & Axelsen, 1988).

In 2009 and 2010, the insecticide treatments caused a significant decrease of bud losses compared to untreated plots (Chapter III). The insecticides had significant effects on adult beetle abundance resulting, in reduced feeding and bud abortion. However, the seed yield did not significantly differ in both years. With respect to the pollen beetle, the level of damage may originate from several factors: the number of adult beetles, the temporal occurrence of the adult beetles on the crop, the level of plant nutrition, the development stage of the host plant, and the weather conditions (Fritzsche, 1957). Oilseed rape shows a high

compensatory ability (Nilsson, 1994). After feeding of pollen beetles the number of racemes and the thousand seed-weight increased. This is particularly the case on the racemes of second order (Vietinghoff, 1985). The compensatory responses are enhanced by favourable nitrogen supply and weather conditions (Habekotte, 1993). In an expanded flowering period the plants increase the assimilate availability to enhance their pod density and seed set. High plant compensation might have been responsible for a high yield in the insecticide treated and untreated plots in our investigation (Chapter III).

Effects of site-specific factors and insecticide application on parasitisation

Average levels of parasitism by specialised parasitoids of pollen beetle within the range of 25-50% have been reported from several countries in Europe (Ulber et al., 2010b). In our study, the average level of parasitism was 22.6% and 25.1% in 2008 and 2009, respectively (Chapter IV). Widely varying levels from 1.6% to 55.9% in 2008 and 1% to 81.3% in 2009 at individual locations indicate a high impact of biotic and abiotic factors. The abundance and phenology of parasitoids depend, among other factors, on local climate and weather conditions, the area of oilseed rape crops grown in previous years and the soil cultivation techniques (Nilsson, 2003). In our study, site-specific factors such as the field size, the proportion of rape crop area within the region and the geographical location influenced the levels of parasitism. This indicates that parasitoids response sensitive to environmental factors (Chapter IV). To evaluate the effectiveness of parasitoids as natural control agents these factors have to be taken into consideration in future studies.

In structurally complex landscapes with field margin strips and old fallow habitats the parasitism is higher compared to structurally simple landscapes (Thies & Tschardtke, 1999). Thies & Tschardtke (1999) suggested that 6 year old and undisturbed field margins near hibernation sites may enhance the parasitoid population. Zaller et al. (2009) found similar results: The combination of high pollen beetle abundance and high levels of rape crop areas and non-crop habitats such as roadside strips and hedges increased the parasitisation of pollen beetle.

In our study, clear effects of insecticide application during the bud and flowering stage on parasitism were observed (Chapter IV). In 2009, Insecticide treatment reduced the level of total parasitism and of parasitism by *T. heterocerus* and *Phradis* spp.. The side-effects of insecticides on parasitoids depend on several factors such as mode of action, dosage, persistency and temporal and spatial application (Ulber et al., 2010a). Mortality of parasitoids can occur through direct contact with spray droplets or through chemical residues on the plant surface. Parasitoids of pollen beetle occur during the late bud and flowering stage of oilseed rape crops when they search for host larvae (Jönsson et al., 2005; Jönsson et al., 2007). In an olfactometer experiment, *P. morionellus* was more attracted by odours emitted from oilseed rape plants infested with host larvae than by odours from uninfested plants (Jönsson et al., 2007). In two-choice tests, the three tersilochine parasitoids preferred the odour of oilseed rape buds. In addition, *T. heterocerus* preferred the odour of flowers (Jönsson & Anderson, 2008). When the odour stimuli were combined with visual stimuli, the

three parasitoid species responded differently. When odours of flowering rape were combined with the colour yellow, and odours of the bud stage were combined with the colour green, *P. interstitialis* was equally attracted to both combinations, and *T. heterocerus* showed an increased preference for flower odours, while *P. morionellus* was not affected by the visual stimuli (Jönsson & Anderson, 2008). As a result, *T. heterocerus* is particularly exposed to insecticides during host searching in the flowering stage of oilseed rape. Considering parasitoids' behaviour during the flowering stage, the insecticide application against pollen beetles in this stage should be avoided to enhance the parasitisation.

Effect of trap crops to control pollen beetle

Turnip rape (*Brassica rapa*) has shown potential as trap crop to decrease the numbers of adult pollen beetles (Büchi, 1995; Cook et al., 2004b, 2006). However, a combination of turnip rape with spatial targeted insecticide application to the trap crop was not effective (Büchi, 1995, Chapter V). A higher number of pollen beetles in the border strips of winter turnip rape did not change the spatial distribution in the main crop. Consequently, the insecticide spray in the border strip had no effect on the number of pollen beetles in the main crop (Chapter V). Even though the adult beetles were attracted by turnip rape until the main crop of oilseed rape came into flower. Pollen beetles show a preference for yellow petals of flowering oilseed rape (Giamoustaris & Mithen, 1996; Frearson et al., 2005) and respond to rape odours (Cook et al., 2002). This visual cue is important for orientation (Williams, 2010). Trap cropping might be more successful when the turnip rape plants remain in flower during the infestation period of pollen beetles and the main crop is in the susceptible bud stage at the same time (Winfield, 1961; Cook et al., 2006). Divergent to a successful control of pollen beetles in Finland where the trap crop flowers two weeks earlier than the main crop (Hokkanen et al., 1986), our study was influenced by an earlier plant development of turnip rape by 3 to 4 days compared to winter oilseed rape. Because of the rapid increase of temperatures and the early immigration of pollen beetles in the early bud stage of both plant species, the protection of winter oilseed rape from pollen beetle infestation by winter turnip rape was insufficient in April in 2009 and 2010 (Chapter V). An early flowering winter turnip rape cultivar might be more suitable than winter oilseed rape crops for trap cropping.

In addition to the phenology of a trap crop cultivar, the design of the trap crop is important for an effective control. Accounts need to be taken of the fact that there are different colonization types of herbivores into a field (Potting et al., 2005). For a successful control of pollen beetle numbers, it is advised to have a trap crop at the surrounding border of a field to intercepts the population and reduces the movement to the main crop (Frearson et al., 2005; Potting et al., 2005). In our study a trap crop border was grown on two sides of the field in a block-design. Immigrating beetles might have colonized the main crop from the sides without turnip rape borders (Chapter V), but this has not been studied in detail. The colonization of oilseed rape depends essentially on two factors in spring: Overwintering location and wind direction. After overwintering, the beetles fly upwind towards the fields (Williams et al., 2007).

Bordering the whole field with the trap crop may avoid the colonization of adult beetles directly in the main crop.

Approaches of integrating pest management strategies in oilseed rape

The aim of integrated pest management is to avoid insecticide applications by reducing the incidence of damage by herbivores (Börner, 1997). In order to reach this goal, methods such as conservation of natural enemies, trap cropping, breeding of resistant varieties and cultivation techniques in oilseed rape crop management are used (Evans & Scarisbrick, 1994). When using insecticides, economic thresholds should be considered. The economic threshold is based on the biology of the pest, the potential damage to the crop, the effects on yield and its response to insecticides (Evans & Scarisbrick, 1994). It is also affected by the plant growth stage. In Germany for example, the application threshold for pollen beetles in winter oilseed rape is 3-4 beetles/plant, 7-8 beetles/plant and >8 beetles/plant at growth stage 50-51, 52-53 and 55-59, respectively (Williams, 2010).

Farmers are advised to use active monitoring methods, such as visiting the crop and counting pollen beetle numbers per plant, in order to determine the accurate timing of insecticide application (Williams, 2010). Knowledge about the spatio-temporal distribution of a pest may allow temporal and spatial targeting of insecticides to reduce insecticide application and conserve beneficial insects (Warner et al., 2000). The spatial distribution of *M. aeneus* within a field is complex and can be manipulated by host-plant volatiles, trap crops or pheromones (Ferguson et al., 2003). In field studies, a turnip rape trap crop in small plots of spring oilseed rape was effective to control pests of oilseed rape, including the pollen beetle (Ferguson et al., 2003; Cook et al., 2006). However, the turnip rape trap crop strategy is only effective when the growth stage of the trap crop is more advanced. The flowering stage of the trap crop is only able to protect the main crop in the bud stage against pest infestation (Frearson et al., 2005). However, plant development depends on weather conditions and cultivation techniques such as the sowing time (Hokkanen, 1991). In our study, the trap cropping system was not effective for the control of adult pollen beetles because the growth development of turnip rape was only 3-4 days faster compared to the main crop winter oilseed rape (Chapter V). Further studies are needed to develop a suitable model.

In contrast, the study including early flowering cultivars of oilseed rape showed potential of these cultivars for integrated pest management (Chapter II). The population growth of pollen beetles can be negatively influenced by early flowering winter oilseed rape cultivars. Depending on the temperature, the date of crop colonization and the plant growth stage were restricting factors for larval development and the number of new-generation pollen beetles. Early flowering cultivars can be used in integrated pest management to achieve a smaller population outcome in the summer and for the following year after hibernation. However, this effect is largely dependent on the annual weather conditions.

Parasitoids have no effect on damage by adult beetles at the susceptible plant stage because they target only the larval stages. The specialised parasitoids *Tersilochus*

heterocerus and *Phradis* spp. are univoltine and koinobiont endoparasitoids. They parasitise the larvae, thereby causing a significant reduction of newly emerging pollen beetles (Jourdeuil, 1960). Farmers might not appreciate the conservation of biological agents in integrated pest management strategies because these agents do not have a direct effect on the feeding damage of beetles in winter oilseed rape.

In their native locations, a threshold parasitism rate by parasitoids of 32% was found to be sufficient for successful biological control of several species (Hawkins & Cornell, 1994). Parasitism rates of pollen beetles exceeding 50% have been reported from several European countries (Ulber et al., 2010b). Parasitism rates differ widely between various sites in Germany. In our study, the parasitism even exceeded 81.3% (Chapter IV). This indicates that there might be a high potential to act as natural control agents in an integrated pest management system in oilseed rape. The avoidance of unnecessary insecticide treatments would help to conserve parasitoids (Cook & Denholm, 2008) and increase the parasitism rate. If possible, insecticides should only be applied before the main periods of parasitoid activity on the oilseed rape crops.

References

- Ahuja, I., Rohloff, J. & Bones, A. (2010). Defence mechanisms of Brassicaceae: implications for plant-insect interactions and potential for integrated pest management. A review. *Agronomy for Sustainable Development* 30, 311-348.
- Alford, D. (2003). Insect pest of oilseed rape crops. In: Alford D.V. (ed.) *Biocontrol of oilseed rape pests*. Oxford, UK: Blackwell Publishing, 9-41.
- Bach, C. (1990). Plant successional stage and insect herbivory: Flea beetles on sand-dune willow. *Ecology* 71, 598-609.
- Bellows, T. S., Van Driesche, R. G. & Elkinton, J. S. (1992). Life-table construction and analyses in the evaluation of natural enemies. *Annual Review of Entomology* 37, 587-614.
- Börjesdotter, D. (2000). *Barbarea verna* and *Barbarea vulgaris* as host plants for the pollen beetle (*Meligethes aeneus*). *Journal of Agricultural Science* 134, 213-220.
- Börner, H. (1997). *Pflanzenkrankheiten und Pflanzenschutz*. 7. Aufl. Stuttgart: Verlag Eugen Ulmer.
- Büchi, R. (2002). Mortality of pollen beetle (*Meligethes* spp.) larvae due to predators and parasitoids in rape fields and the effect of conservation strips. *Agriculture, Ecosystems and Environment* 90, 255-263.
- Büchi, R. (1995). Combination of trap plants (*Brassica rapa* var. *Silvestris*) and insecticide use to control rape pests. *IOBC/wprs Bulletin* 18, 102-121.
- Charmillot, P., Pasquier, D., Salamin, C. & Ter-Hovannesyanyan, A. (2007). Ovicidal and larval effectiveness of insecticides applied by dipping apples on the small fruit tortrix *Grapholita lobarzewskii*. *Pest Management Science* 63, 677-681.
- Cook, S. M., Bartlett, E., Murray, D. A. & Williams, I. H. (2002). The role of pollen odour in the attraction of pollen beetles to oilseed rape flowers. *Entomologia Experimentalis et Applicata* 104, 43-50.
- Cook, S. M. & Denholm, I. (2008). Ecological approaches to the control of pollen beetles in oilseed rape. *IOBC/wprs Bulletin* 38, 110-113.
- Cook, S. M., Murray, D. A. & Williams, I. H. (2004a). Do pollen beetles need pollen? The effect of pollen on oviposition, survival, and development of a flower-feeding herbivore. *Ecological Entomology* 29, 164-173.
- Cook, S. M., Smart, L. E., Martin, J. L., Murray, D. A., Watts, N. P. & Williams, I. H. (2006). Exploitation of host plant preferences in pest management strategies for oilseed rape (*Brassica napus*). *Entomologia Experimentalis et Applicata* 119, 221-229.
- Cook, S. M., Watts, N. P., Hunter, F., Smart, L. E. & Williams, I. H. (2004b). Effects of a turnip rape trap crop on the spatial distribution of *Meligethes aeneus* and *Ceutorhynchus assimilis* in oilseed rape. *Bulletin IOBC/wprs: Integrated Control in oilseed crops* 27, 199-206.
- Délos, M. (2008). *Meligethes* du colza: Evolution et gestion de resistances aux pyrethrinoides en

- France et recommandations pour gerer le risque. *IOBC/wprs Bulletin* 38, 75-78.
- Ekbom, B. & Borg, A. (1996). Pollen beetle (*Meligethes aeneus*) oviposition and feeding preferences on different host plant species. *Entomologia Experimentalis et Applicata* 78, 291-299.
- Ekbom, B. & Ferdinand, V. (2003). Field oviposition rates and egg load dynamics of pollen beetles (*Meligethes aeneus* Fab.) (Coleoptera: Nitidulidae). *Agricultural and Forest Entomology* 5, 247-252.
- Ekbom, B. & Popov, S. A. (2004). Host plant affects pollen beetle (*Meligethes aeneus*) egg size. *Physiological entomology* 29, 118-122.
- Elbert, A., Haas, M., Springer, B., Thielert, W. & Nauen, R. (2008). Applied aspects of neonicotinoid uses in crop protection. *Pest Management Science* 64, 1099-1105.
- Ellis, J., Hepburn, R. & Elzen, P. (2004). Effects of soil type, moisture, and density on pupation success of *Aethina tumida* (Coleoptera : Nitidulidae). *Environmental Entomology* 33, 794-798.
- Evans, K. A. & Allen-Williams, L. J. (1994). Laboratory and field response of the pollen beetle, *Meligethes aeneus*, to the odour of oilseed rape. *Physiological Entomology* 19, 285-290.
- Evans, K. A. & Scarisbrick, D. (1994). Integrated insect pest-management in oilseed rape crops in Europe. *Crop Protection* 13, 403-412.
- Ferguson, A. W., Klukowski, Z., Walczak, B., Clark, S. J., Mugglestone, M. A., Perry, J. N. & Williams, I. H. (2003). Spatial distribution of pest insects in oilseed rape: implications for integrated pest management. *Agriculture Ecosystems and Environment* 95, 509-521.
- Frearson, D. J. T., Ferguson, A. W., Campbell, J. M. & Williams, I. H. (2005). The spatial dynamics of pollen beetles in relation to inflorescence growth stage of oilseed rape: implications for trap crop strategies. *Entomologia Experimentalis et Applicata* 116, 21-29.
- Free, J. B. & Williams, I. H. (1978). The responses of the pollen beetle, *Meligethes aeneus*, and the seed weevil, *Ceuthorrhynchus assimilis*, to oil-seed rape, *Brassica napus*, and other plants. *Journal of Applied Ecology* 15, 761-774.
- Free, J. B. & Williams, I. H. (1979). The infestation of crops of soil-seed rape (*Brassica napus* L.) by insect pests. *Journal for Agricultural Science, Cambridge* 92, 203-218.
- Fritzsche, R. (1957). Zur Biologie und Ökologie der Rapsschädlinge aus der Gattung *Meligethes*. *Sonderdruck aus Zeitschrift für angewandte Entomologie* 40, 222-280.
- Giamoustaris, A. & Mithen, R. (1996). The effect of flower colour and glucosinolates on the interaction between oilseed rape and pollen beetles. *Entomologia Experimentalis Et Applicata* 80, 206-208.
- Habekotte, B. (1993). Quantitative-analyses of pod formation, seed set and seed filling in winter oilseed rape (*Brassica napus* L) under field conditions. *Field Crops Research* 35, 21-33.
- Hansen, L. M. (2003). Insecticide-resistant pollen beetles (*Meligethes aeneus* F) found in Danish oilseed rape (*Brassica napus* L) fields. *Pest Management Science* 59, 1057-1059.
- Hawkins, B. & Cornell, P. M. (1994). Maximum parasitism rates and successful biological control. *Science* 266, 1886.
- Haye, T., Mason, P. G., Dossall, L. M. & Kuhlmann, U. (2010). Mortality factors affecting the cabbage seedpod weevil, *Ceutorrhynchus obstrictus* (Marsham), in tis origin: A life table analyses. *Biological Control* 54, 331-341.
- Heimbach, U., Müller, A. & Thieme, T. (2006). First steps to analyse pyrethroid resistance of different oil seed rape pests in Germany. *Nachrichtenblatt Deutscher Pflanzenschutzdienst* 58, 1-5.
- Hoffmann, E. J., Vandervoort, C. & Wise, H. C. (2009). Curative activity of insecticides against plum curculio (Coleoptera: Curculionidae) in tart cherries. *Journal of Economic Entomology* 102, 1864-1873.
- Hokkanen, H., Granlund, H., Husberg, G. & Markkula, M. (1986). Trap crops used successfully to control *Meligethes aeneus* (Col., Nitidulidae), the rape blossom beetle. *Annales Entomologici Fennici* 52, 115-120.
- Hokkanen, H. M. T. (1991). Trap cropping in pest management. *Annual Review of Entomology* 36, 119-138
- Hokkanen, H. M. T. (1993). Overwintering survival and spring emergence in *Meligethes aeneus* - effects of body-weight, crowding, and soil treatment with *Beauveria bassiana*. *Entomologia Experimentalis et Applicata* 67, 241-246.
- Hokkanen, H. M. T. (2000). The making of a pest: recruitment of *Meligethes anaenus* onto oilseed Brassicas. *Entomologia Experimentalis et Applicata* 95, 141-149.
- Hopkins, R. J. & Ekbom, B. (1999). The pollen beetle, *Meligethes aeneus*, changes egg production rate to match host quality. *Oecologia* 120, 274-278.
- Jönsson, M. & Anderson, P. (2008). Emission of oilseed rape volatiles after pollen beetle infestation; behavioural and electrophysiological responses in the parasitoid *Phradis morionellus*. *Chemoecology* 17, 201-207.

- Jönsson, M., Lindkvist, A. & Anderson, P. (2005). Behavioural responses in three ichneumonid pollen beetle parasitoids to volatiles emitted from different phenological stages of oilseed rape. *Entomologia Experimentalis Et Applicata* 115, 363-369.
- Jönsson, M., Rosdahl, K. & Anderson, P. (2007). Responses to olfactory and visual cues by overwintered and summer generations of the pollen beetle, *Meligethes aeneus*. *Physiological ecology* 32, 188-193.
- Jourdheuil, P. (1960). Influence de quelques facteurs ecologiques sur les fluctuations de population d'une biocénose parasitaire : Étude relative à quelques Hyménoptères (Ophioninae, Diopsilinae, Euphorinae) parasites de divers Coléoptères inféodés aux Crucifères. *Annales des Epiphyties* 11, 445-658.
- Kazachkova, N., Meijer, J. & Ekbohm, B. (2007). Genetic diversity in pollen beetles (*Meligethes aeneus*) in Sweden: role of spatial, temporal and insecticide resistance factors. *Agricultural and Forest Entomology* 9, 259-269.
- Müller, H. J. (1941). Weitere Beiträge zur Biologie des Rapsglanzkäfers, *Meligethes aeneus* F. (Über das Winterlager und die Massenbewegung im Frühjahr.). *Zeitschrift für Pflanzenkrankheiten (Pflanzenpathologie) und Pflanzenschutz* 51, 529-595.
- Nielsen, P. S. & Axelsen, J. (1988). Developmental time and mortality of the immature stages of the pollen beetle (*Meligethes aeneus* F) under natural conditions. *Journal of Applied Entomology* 105, 198-204.
- Nilsson, C. (1988a). The pollen beetle (*Meligethes aeneus* F.) in winter and spring rape at Alnarp 1976-1978. II. Oviposition. *Växtskyddsnotiser* 52, 139-144.
- Nilsson, C. (1988b). The pollen beetle (*Meligethes aeneus* F.) in winter and spring rape at Alnarp 1976-1978. III. Mortality factors. *Växtskyddsnotiser* 52, 145-150.
- Nilsson, C. (1994). Pollen beetles (*Meligethes* spp.) in oil seed rape crops (*Brassica napus* L.): Biological interactions and crop losses. Diss. Swedish University of Agricultural Sciences.
- Nilsson, C. (2003). Parasitoids of pollen beetles. In: Alford D. V. (ed.) *Biocontrol of oilseed rape crops*. Oxford, UK: Blackwell Publishing 73-85.
- Nuss, H. (1999). Zur Bedeutung der Laufkäfer (Coleoptera, Carabidae) als natürliche Gegenspieler von Rapsschädlingen. Diplomarbeit: Technische Universität Braunschweig.
- Osborne, P. (1960). Observations on the natural enemies of *Meligethes aeneus* (F.) and *M. viridescens* (F.) [Coleoptera: Nitidulidae]. *Parasitology* 50, 91-110.
- Potting, R., Perry, J. N. & Powell, W. (2005). Insect behavioural ecology and other factors affecting the control efficacy of agro-ecosystem diversification strategies. *Ecological Modelling* 182, 199-216.
- Preszler, R. & Price, P. (1988). Host quality and sawfly populations: A new approach to life table analyses. *Ecology* 69, 2012-2020.
- Ruther, J. & Thiemann, K. (1997). Response of the pollen beetle *Meligethes aeneus* to volatiles emitted by intact plants and conspecifics. *Entomologia Experimentalis et Applicata* 84, 183-188.
- Schoonhoven, L., van Loon, J. & Dicke, M. (2005). *Insect-plant biology*. Second edition. Oxford, UK: University Press.
- Schroeder, G., Politz, B., Wolff, C. & Krueger, B. (2009). Alternatives for an effective control of pyrethroid-resistant pollen beetle populations - results of proficiency test of several states. *Gesunde Pflanzen* 61, 19-30.
- Sedivy, J. & Kocourek, F. (1994). Flight activity of winter rape pests. *Journal of Applied Entomology* 117, 400-407.
- Singh, D. & Singh, A. (2005). *Disease and insect resistance in plants*. Enfield, NH, USA: Science Publishers.
- Somme, L. (1999). The physiology of cold hardiness in terrestrial arthropods. *European Journal of Entomology* 96, 1-10.
- Stark, J. D. & Banks, J. E. (2003). Population-level effects of pesticides and other toxicants on arthropods. *Annual Review of Entomology* 48, 505-519.
- Thies, C. & Tschardtke, T. (1999). Landscape structure and biological control in agroecosystems. *Science* 285, 893-895.
- Tiilikainen, T. M. & Hokkanen, H. M. T. (2008). Pyrethroid resistance in Finnish pollen beetle (*Meligethes aeneus*) populations - is it around the corner? *IOBC/wprs Bulletin* 38, 99-103.
- Ulber, B., Klukowski, Z. & Williams, I. (2010a). Impact of insecticides on parasitoids of oilseed rape pests. IN: Williams I. H. (ed.) *Biocontrol-based integrated management of oilseed rape pests*. Netherlands: Springer Science+Business Media B. V., 337-335

- Ulber, B., Williams, I., Klukowski, Z., Luik, A. & Nilsson, C. (2010b). Parasitoids of oilseed rape pests in Europe: key species for conservation biocontrol. In: Williams I. H. (ed.) *Biocontrol-based integrated management of oilseed rape pests*. Netherlands: Springer Science+Business Media B. V., 45-76
- Warner, D., Allen-Williams, L. J., Ferguson, A. & Williams, I. (2000). Pest-predator spatial relationships in winter rape: implications for integrated crop management. *Pest Management Science* 56, 977-982.
- Wegorek, P. & Zamoyska, J. (2008). Current status of resistance in pollen beetle (*Meligethes aeneus* F.) to selected active substances of insecticides in Poland. *IOBC/wprs Bulletin* 38, 91-94.
- Williams, I. H. (2010). The major insect pests of oilseed rape in Europe and their management: An overview. In: Williams I. H. (ed.) *Biocontrol-based integrated management of oilseed rape pests*. Netherlands: Springer Science+Business Media B. V., 1-44
- Williams, I. & Cook, S. M. (2010). Crop location by oilseed rape pests and host location by their parasitoids. In: Williams I. H. (ed.) *Biocontrol-based integrated management of oilseed rape pests*. Netherlands: Springer Science+Business Media B. V., 215-244
- Williams, I. H., Frearson, D., Barari, H. & McCartney, A. (2007). Migration to and dispersal from oilseed rape by the pollen beetle, *Meligethes aeneus*, in relation to wind direction. *Agricultural and Forest Entomology* 9, 279-286.
- Williams, I. H. & Free, J. B. (1978). The feeding and mating behaviour of pollen beetles (*Meligethes aeneus* Fab.) and seed weevils (*Ceutorhynchus assimilis* Payk.) on oil-seed rape (*Brassica napus* L.). *Journal of Agricultural Science, Cambridge* 91, 453-459.
- Winfield, A. L. (1961). Field observations on the control of blossom beetles (*Meligethes aeneus* F.) and cabbage-seed weevils (*Ceuthorhynchus assimilis* Payk.) on mustard-seed crops in East Anglia. *Annals of Applied Biology* 49, 539-555.
- Zaller, J. G., Moser, D., Drapela, T., Schmoger, C. & Frank, T. (2009). Parasitism of stem weevils and pollen beetles in winter oilseed rape is differentially affected by crop management and landscape characteristics. *Biocontrol* 54, 505-514.

Summary

Factors regulating the population dynamics and damage potential of pollen beetle (*Meligethes aeneus* F.) on crops of oilseed rape

The pollen beetle *Meligethes aeneus* (Fabricius) (Col.: Nitidulidae) is one of the most important pests of oilseed brassicas in Europe. Due to extensive use of pyrethroids and the limited supply of registered insecticide products pollen beetle populations have built up widespread resistance to pyrethroids in recent years. Alternative integrated pest management strategies are needed to regulate the population growth and damage caused by pollen beetle below threshold levels. In this study, we investigated various factors which might have an impact on population development, behaviour, damage potential and natural control of this pest. The effect of different cultivars of winter oilseed rape, *Brassica napus*, on the reproduction and population growth of pollen beetle was studied in field experiments over three years. Further, the effectiveness of the alternative insecticides thiacloprid and chlorpyrifos-methyl, applied for control of adult beetles in the bud and early flowering stage of oilseed rape, on population growth and plant damage was tested in these field experiments. As hymenopterous parasitoids can exert substantial natural control of pollen beetle populations, we analysed the impact of site-specific factors and insecticide application on the level of larval parasitism of *M. aeneus* in a monitoring study, collecting samples from various locations across Germany. In addition, the effect of trap cropping in combination with targeted insecticide treatment on the spatio-temporal distribution of adults within the field was studied as an integrating pest management strategy for pollen beetle.

Four *Brassica napus* (L.) cultivars with different flowering behaviour were grown in field experiments in 2008-2010. The population growth of pollen beetle on these cultivars was determined with respect to the effects of annual weather conditions on the phenology of pollen beetle infestation and plant growth stage. In 2008, the early flowering cultivars `NK Passion` (open pollinated) and `Elektra` (hybrid) developed more rapidly than the late flowering cultivars `Favorite` (open pollinated) and `Titan` (hybrid). This delay of flowering by 6 days significantly reduced the pollen beetle abundance and significantly increased the population growth of pollen beetle on cv `Favorite` compared to cv. `NK Passion`. In 2009 and 2010, when the phenological growth of the cultivars was nearly synchronously, no cultivar effects on population growth could be measured, although the adult beetles significantly preferred the buds of the hybrid cultivars for oviposition. The emergence rate of the new pollen beetle generation in relation to the abundance of second instar larvae migrating to soil for pupation was significantly correlated with the period available for egg laying and larval development in the bud and flowering stage in each year.

The application of the organophosphate insecticide chlorpyrifos-methyl and the neonicotinoide insecticide thiacloprid significantly reduced the abundance of adult pollen beetles in two of three years of study. However, although the abundance of adult beetles was only little affected by insecticide treatment in the third year, the number of second instar larvae and of newly emerged beetles was significantly reduced. In 2008, 2009 and 2010,

percentage reduction of second instar larvae and emerging beetles by insecticides ranged between 83.2% to 90.6% and between 82.5% to 95.5%, respectively. Although the pollen beetles caused high bud losses in untreated plots, no significant effect of insecticide treatment on yield of oilseed rape was recorded.

The parasitism rates of pollen beetle larvae by the parasitoids *Tersilochus heterocerus* and *Phradis* spp. (Hym.: Ichneumonidae) varied widely between locations and years in Germany. On the 36 and 42 crops of winter oilseed rape sampled in 2008 and 2009, the total levels of parasitism varied from 1.6% to 55.9% and from 1.0% to 81.3%, respectively. The parasitism was significantly influenced by site-specific factors like geographical region/latitude, proportion of oilseed rape crop and field size. Insecticides applied during the main period of parasitoid activity during the bud and flowering stage of oilseed rape significantly reduced the parasitism rates.

Despite the preference of immigrating adult pollen beetles for turnip rape (*Brassica rapa*), a trap crop border strip of turnip rape had no significant effect on the spatio-temporal within-field distribution of pollen beetles on the main crop of winter oilseed rape, as compared to plots with a border strip of oilseed rape. Higher numbers of beetles remained on turnip rape until the oilseed rape began to flower. Further, insecticide application targeted to the border strip did not significantly reduce the abundance of adult beetles in the centre of the field. Plant damage was not significantly different between the treatments. These insignificant differences may have occurred because the plant growth stage of the turnip rape trap crop was only 3-4 days in advance of the oilseed rape main crop in all three years of the field experiments.

In summary, these results present new approaches for alternative control strategies of pollen beetle which might be included in an integrated pest management system. The population growth of pollen beetles can be reduced by growing early flowering cultivars of winter oilseed rape and by targeted insecticide treatments within the bud and early flower stage. However, insecticide applications should be minimised because they can cause high mortality of parasitoids which are substantial biological agents of pollen beetle. The use of turnip rape as trap crop depends strongly on weather conditions.

Acknowledgements

I would like to thank my super adviser Prof. Dr. Stefan Vidal for the reviewing of this thesis and the many helpful comments. Many thanks to Prof. Dr. von Tiedeman for the co-reviewing. Special thanks to Dr. Bernd Ulber for making me interested in the topic, supporting me in my experiments, for the discussions and positive critics during my work. A lot of thanks to Dorothea Mennerich, who did mostly of the larvae dissection, and for the technical assistance. Hubertus Reintke I thank for helping me with the field work. I would like to thank all colleagues who supported our investigation by collecting larvae samples of pollen beetle populations in Germany. Also many thanks to Dr. Thomas Thieme, Dr. Michael Zellner, Dr. Udo Heimbach, Kai Gloya, Iris Dotterweich and Andreas Müller for the nice meetings and discussions.

I am also grateful to: Nadine Neumann, Rainer Wedemeyer, Welf Gehrke, Ulrike Jeche, Ruth Lefering, Joshua Sikhu Okonya, Sonja Schneider, Kim Stey, Florian Rekate, Katja Fischer, Jaqueline Platzek, Eugen Hodyl and Ines Vollhard for helping me in the field, in the greenhouse and in the laboratory and for the nice collaboration. I am very thankful to Heike Beckmann, Gerrit Kruger and Mario Schumann for correcting the English. I like to thank all colleagues in the Entomology group for such great atmosphere. Finally a lot of hugs to my family and Christian.

This research was supported by the Federal Ministry of Food, Agriculture and Consumer Protection.

Curriculum vitae

- 12th December 1979** born in Gifhorn, Germany
- 1999** Matriculation at Grammar School "Wilhelm-Gymnasium",
Brunswick, Germany
- 1999-2001** Apprenticeship as Gardener in a tree nursery at the
Department of Gardens and Landscaping of the City of
Brunswick
- 2001-2006** Study of horticultural sciences at Leibniz University
Hanover, Horticulture Diploma thesis at the Federal
Biological Research Centre for Agriculture and Forestry
in Brunswick: "Studies for the oospore production of
Phytophthora ramorum in Rhododendron"
- 2007-2011** PhD-study at Georg-August-University Goettingen,
Department of Crop Sciences, Agricultural Entomology

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

Göttingen, den 9. Mai 2014