Chapter 12
Chemical Control of Insect Pests and Insecticide Resistance in Oilseed Rape

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Abstract Public concerns about environmental hazards and widespread resistance in pollen beetle populations on oilseed rape in Europe are threatening the availability of a variety of insecticidal modes of action for pest control on the crop. For a sustainable use of insecticides any overuse has to be avoided to minimize risk of resistance development. Pollen beetles are present in the crop for a long period during the season and are therefore exposed to most insecticide applications carried out, irrespective of any need for control above threshold values. To avoid resistance development active substances with several modes of action are needed to ensure sustainable control of pollen beetle. Insecticide resistance is an example of an evolutionary process in which insecticide resistant genotypes are selected in treated populations. The available insecticides, their modes of action, methods for identifying resistance and both, factors affecting the dynamics of insecticide resistance in the field as well as management strategies to prevent or delay the evolution of resistance, are reported.

12.1 Introduction

Pest insects in oilseed rape, *Brassica napus* L., among them the pollen beetle, *Meligethes aeneus* (Fabricius), are one of the main target groups for development of new insecticides by the ever-shrinking number of pesticide manufacturers.

Some 40 years ago, the insecticides dominating the pollen beetle control market were mostly cyclodiene organochlorines, DDT, carbamates and organophosphates (OPs); pyrethroids were introduced into this market from about 1980. Insecticides in the former groups were usually not systemic, were relatively persistent, most were highly toxic to users, and not just to the target pests but also to many beneficial insects; many have since been withdrawn under pressure from environmental and human health aspects. The EU regulation of pesticides has accepted only 43 out

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of 218 insecticidal active substances that had been used in the EU countries before 1993. Since 1993, only 11 new insecticidal active substances have been accepted (January 2009). Pyrethroid insecticides have replaced more and more the other groups, but showing relatively persistent activity they also lack systemic activity. Similar to OPs they have also broad-spectrum effects on many non-target insects but, as a very positive aspect for pollen beetle control, pyrethroids are relatively bee safe and less toxic for users and mammals compared to OPs.

Increasing public concerns about environmental hazards and widespread resistance in pest populations are threatening the availability of a variety of insecticidal modes of action. For the effectiveness of insecticides to continue, there is a need to avoid any overuse to minimize risk of resistance development. In oilseed rape, several pest insects need to be controlled within a few weeks resulting in a high selection pressure for resistance. Pollen beetles in particular are present in the crop for a long period from early spring to summer and are therefore exposed to most insecticide applications carried out in spring including those on other target pests such as the weevils (*Ceutorhynchus napi, C. pallidactylus* and *C. obstrictus* syn. *assimilis*) and the brassica pod midge (*Dasineura brassicae*), irrespective of any need for control because of appearance in numbers above threshold values. In the last 10 years, the demand for safer insecticides for human health and the environment has stimulated the development of some novel groups. Some have properties that are ideal for pollen beetle control, including the neonicotinoids, thiacloprid and acetamiprid, both of which are bee safe and quite safe for several other environmental aspects.

To avoid resistance development several active substances with different modes of action are needed to ensure sustainable control of the pollen beetle and other pest insects in oilseed rape. Pyrethroids are an important tool for pest management because they show high efficacy for all pests in oilseed rape even at lower temperature, they have a moderate price and are quite user-friendly. However any overuse leads to selection for resistance.

Insecticide resistance is an example of an evolutionary process in which mutations conferring protection against insecticides are selected in treated populations. During the last 25 years, advances have been made in the characterization and understanding of such adaptations. These have delivered valuable information about the origin and nature of selection and evolution in farm land.

The evolution of insecticide resistance has contributed to overall increases in the application of chemicals to crops. Besides seed treatment with insecticides, oilseed rape in Europe is sprayed with insecticides on average about twice but, in some cases, up to five times, with large differences between regions (Richardson 2008). Despite this, resistant insects, such as the pollen beetle, continue to affect agricultural productivity. It is proving impossible to fight back resistance by being focused only on application of insecticides. The development of a new insecticide takes eight to 10 years at a cost of € 15–35 million, and the rate of discovery of new molecules, unaffected by cross resistance, appears to be declining. Only by monitoring, characterizing and predicting the appearance and spread of resistant pollen beetles can we hope to use chemical tools in a sustainable manner.

12.2 Chemical Control of Insect Pests

The pollen beetle is a serious pest of oilseed rape throughout Europe and a major target pest for control with insecticides (Williams Chapter 1 this volume). It is polyphagous and damages many agricultural and horticultural crops; the adults are generalist feeders on pollen of oilseed rape and many other plants, including cauliflower, rose, blackberry, chamomile and lime trees; the presence of beetles has an impact on the market value of horticultural crops. On oilseed rape, the pollen beetle causes primary damage by adult feeding on pollen within the flower buds, thereby destroying them. Damage occurs also by larval feeding in the buds and flowers. However, where populations are potentially exposed to intensive applications of insecticides with the same mode of action, there is a high level of concern regarding the increased potential for selection pressure and resistance development.

12.2.1 Choice of Insecticide

In cases where a pest reaches the damage threshold farmers need to choose an effective insecticide. Table 12.1 lists the range of insecticides approved for use against the pollen beetle in oilseed rape in Germany in 2009 (https://portal.bvl.bund.de/psm/jsp/ListeMain.jsp?page=1&ts=1237033472771). This list differs from that for other countries, but it does illustrate the choice of available insecticides these days.

Pyrethroids have been the tower of strength for pollen beetle control as well as for the control of other oilseed rape pest insects for nearly the last 20 years in Europe. However, neonicotinoids are now also approved for pollen beetle control

Table 12.1 List of insecticides approved to control the pollen beetle on oilseed rape in Germany as notified in the German Pesticide Guide (BVL, 2009)

			Control activity on populations	
Insecticide	Class	Properties	Sensitive to pyrethroids	Resistant to pyrethroids
Chlorpyrifos-ethyl*	OP	Cont	***	***
Chlorpyrifos-methyl*	OP	Cont	***	***
Bifenthrin	Pyrethroid	Cont	****	**
Alpha-cypermethrin	Pyrethroid	Cont	****	*
Beta-cyfluthrin	Pyrethroid	Cont	****	*
Lambda-cyhalothrin	Pyrethroid	Cont	****	*
Zeta-cypermethrin	Pyrethroid	Cont	***	*
Deltamethrin	Pyrethroid	Cont	****	*
Esfenvalerate	Pyrethroid	Cont	***	*
Etofenprox	Pyrethroid	Cont	***	**
Tau-fluvalinate	Pyrethroid	Cont	**	**
Thiacloprid	Neonicotinoid	Cont, Syst	***	***

*Approved only for emergency actions with high populations densities of pollen beetles for 120 days in 2009 for a limited area; cont, contact; syst, systemic.

in many countries, but the use of many active substances with different modes of action showing some control action of pollen beetle (e.g., pymetrozine, spinosad, indoxacarb) is still restricted to the control of other pests on other crops.

Neonicotinoids were first discovered in the early 1970s, but they were not developed for use in agriculture until 1991, when imidacloprid (Elbert et al. 1990, Altmann and Elbert 1992) was introduced to the market. Imidacloprid had the required photostability, insecticidal activity, and residual persistence to be marketed for a wide range of uses. It is a systemic broad-spectrum insecticide, with translaminar activity and with contact and stomach action. Other insecticides developed within this group include thiacloprid (Elbert et al. 2000, Jeschke et al. 2001), and acetamiprid (Takahashi et al. 1992). All have pollen beetle control properties, but some are more active than others. Among the neonicotinoids, only thiacloprid and acetamiprid have low bee toxicity, though their effectivity on pollen beetles and some other pests in oilseed rape is limited.

12.2.2 Modes of Action

To understand the differences in properties of the novel insecticides now being used for pollen beetle control, their modes of action are recapitulated here.

12.2.2.1 Carbamates and Organophosphates

Carbamates and organophosphates are systemic insecticides with contact and stomach action. Both are acetylcholinesterase (AchE) inhibitors. They interrupt the transmission of nerve impulses across the synaptic gap between two nerve cells by preventing the breakdown of the neurotransmitter, acetylcholine (Tomizawa and Casida 2003). This results in paralysis that destroys the ability of animals to respond to external stimuli. Both groups of insecticides tend to have high toxicity to honey bees, fish and mammals (Plapp 1991).

12.2.2.2 Pyrethroids

Pyrethroids prevent the voltage-gated sodium channel from functioning, so that no nerve impulses travel along the nerve axon. Pyrethroids have high toxicity to fish but low mammalian toxicity. To have maximum effect they need to be applied just at the point when beetle numbers exceed threshold values. But it is very difficult to establish threshold values and they differ quite markedly between European countries and regions (see Williams Chapter 1 this volume). Additionally, it is quite difficult to monitor the number of pollen beetles with the required precision.

12.2.2.3 Neonicotinoids

Neonicotinoids act as antagonists by binding to post-synaptic nicotinic acetylcholine-receptors (nAChR), affecting the synapses in the insect central nervous system (Ishaaya and Horowitz 1998, Nauen et al. 2001, 2003, Tomizawa and Casida 2003). These substances are xylem-mobile and therefore suitable for seed treatment and soil application. They have low mammalian toxicity due to differences between the nAChRs of insects and those of mammals (Tomizawa and Casida 2003), but some have high toxicity to birds (Anonymous 1993) and honey bees.

Imidacloprid and acetamiprid, applied topically to the upper surface of leaves, showed translaminar (movement across leaf layers) and acropetal (movement towards leaf margins) activity against pests (Bucholz and Nauen 2002). They also suppress feeding of pollen beetle when treated with sublethal concentrations, even though the beetles are not killed (Nauen *pers comm*).

The long persistence of imidacloprid may be explained by the slow degradation on or in plants and the toxicity of its metabolites (Nauen et al. 1998). Most of the imidacloprid was metabolized within 97 days (Westwood et al. 1998). This neonicotinoid is metabolized by loss of the nitro group, hydroxylation, hydrolysis and formation of conjugates.

Thiacloprid is an acute contact stomach poison with systemic properties. It has a short half-life with a good safety for bees (Elbert et al. 2002), making it ideal for spray application. Its mode of action is similar to that of the other neonicotinoids.

12.2.2.4 Adjuvants for Use Against Resistant Populations

The efficacy of some insecticides can be improved by the use of adjuvants in the mix. For example, piperonyl butoxide (PBO) improves the efficacy of lambda-cyhalothrin and other pyrethroids against the pollen beetle, especially if they have pyrethroid resistance. This is attributed to a degradation of monooxygenase in the targeted beetles. But PBO also causes some mortality of pyrethroid resistant beetles in the laboratory if applied without any insecticide though interestingly PBO is always reported as a non-toxic adjuvant.

12.2.3 Application

All insecticides for control of the pollen beetle are applied as sprays, often in response to the economic threshold. However, the only forecasting scheme to guide such usage in Germany is presented by proPlant (http://www.proplant.de/german/pp_frameset/frs_home/pp_home_data.htm) (see also Johnen et al. Chapter 15 this volume). It is difficult to estimate how many farmers make use of threshold values, as usage of insecticides has tended to remain constant or even increase from year to year, irrespective of the pest pressure. The use of pyrethroids has increased since fungicide use during flowering of oilseed rape was introduced and made the use of tank mixtures with insecticides attractive. The reduction of cost for the application of tank mixtures made insecticide use more economic for farmers and also increased the prophylactic use of insecticides which was additionally supported by the low price of pyrethroids. A major problem for good insecticide coverage of plants seems to be induced by the tendency to reduce the amount of water for application. When farmers observe that many pollen beetles are still present after spraying of an insecticide they usually argue that the product is failing even if good agricultural practice was not followed. This is the case if the amount of water sprayed was to low for proper coverage of plants or spraying took place at an inappropriate time.

12.2.4 Thresholds for Control

Thresholds for control of the pollen beetle are useful to encourage rational use of pesticides. However, the applied thresholds were calculated many years ago and differ quite drastically between countries and regions, the crop health conditions and crop growth stage (Richardson 2008, Williams Chapter 1 this volume). Their use is therefore hampered by the lack of information about their relevance to changed modern cropping systems, e.g., reduction in the number of plants per m^2 and use of cultivars which start flowering much earlier and have a much stronger ability to compensate loss of flower buds than the older cultivars.

12.3 Insecticide Resistance in the Pollen Beetle

The economic importance of the pollen beetle as a pest has resulted in intensive selection by insecticides and led to the evolution of resistance mechanisms. Here, we focus on their characterization by bioassay, the molecular basis of resistance, their impact on insecticide efficacy and, where known, the dynamics of resistance in pollen beetle populations and the factors driving them.

With the expansion of oilseed rape in Europe more farmers became aware of pest problems in their crop (Kirk-Spriggs 1996, Hansen 2003) which resulted in an increasing number of insecticide applications. In the late 1980s, resistance to insecticides was identified in populations of pollen beetles in Poland (Lakocy 1977). Since the late 1990s, further insecticide resistance has been found in different countries and is still increasing (Hansen 2003, Ballanger et al. 2003, 2007, Derron et al. 2004, Heimbach 2005, Nauen 2005, Heimbach and Müller 2006, Heimbach et al. 2006, Thieme et al. 2006, Wegorek and Zamojska 2006). In 2007, an EPPO workshop on pollen beetle resistance took place in which the relevance for all European countries was discussed (EPPO Bulletin 38, 2008).

More than 40 years ago, DDT resistant pollen beetles were documented in Poland (Table 12.2). By switching to insecticides with other mode of action this resistance was broken.

The first documented case of reduced pyrethroid susceptibility in the pollen beetle was reported in 1999 in the Champagne region in North-Eastern France (Table 12.2). Anecdotal reports on pyrethroid resistance development date back to 1997, although it should be noted that a number of these reports have not been validated. A similar phenomenon was observed in the Scandinavian countries, such as Denmark and Sweden. First rumours of reduced pyrethroid efficacy in Germany came in 2001 in the federal state of Rhineland-Palatinate. These rumours were confirmed in 2002, and since then pyrethroid resistance has spread all over Germany; since 2006, it has affected more than 50% of the winter oilseed rape acreage (Nauen

Table 12.2 Insecticide resistant pollen beetles reported from oilseed rape in Europe (after IRAC 2006)

Compounds resisted		Resistance first recorded
Insecticide	MOA	Year and country
Carbaryl	Carbamates	1974 PL[a]
Dioxacarb	Carbamates	1974 CZ[b], 1985 PL[a]
Propoxur	Carbamates	1974 PL[a]
Aldrin	Cyclodiene organochlorines	1974 PL[a]
Dieldrin	Cyclodiene organochlorines	1974 PL[a]
Endosulfan	Cyclodiene organochlorines	1974 PL[a], 1984 CZ[c]
Kelevan	Cyclodiene organochlorines	1974 CZ[b], 1985 PL[a]
Toxaphene	Cyclodiene organochlorines	1974 PL[a]
DDT	DDT	1965 PL[d], 1974 CZ[b]
Methoxychlor	DDT	1974 PL[a]
HCH-gamma	Cyclodiene organochlorines	1974 PL[a], 1974 CZ[b]
Cartap	Cartap	1974 CZ[b]
Bromophos	Organophosphates	1974 PL[a]
Chlorfenvinphos	Organophosphates	1974 PL[a]
Diazinon	Organophosphates	1974 PL[a]
Dimethoate	Organophosphates	2003 DK[e]
Methamidophos	Organophosphates	1974 PL[a]
Methidathion	Organophosphates	1974 PL[a]
Parathion-methyl	Organophosphates	1974 PL[a]
Phoxim	Organophosphates	1974 PL[a]
Pirimiphos-methyl	Organophosphates	1974 PL[a]
Lambda-cyhalothrin	Pyrethroids	2003 DK[e], 2005 D[f]
Alpha-cypermethrin	Pyrethroids	2004 PL[g]
Esfenvalerate	Pyrethroids	2003 DK[e]
Tau-fluvalinate	Pyrethroids	2003 DK[e]

[a] Lakocy (1974).
[b] Anonymous (1974).
[c] No entry FAO.
[d] Wegorek (1965).
[e] Hansen (2003).
[f] Heimbach et al. (2006).
[g] Wegorek (2005).

2007). In 2008, more than 80% of German populations showed high or very high resistance.

Periodically, pyrethroid resistance can escalate, as in 2007, when samples taken from a range of crops contained high proportions of resistant beetles. This apparent instability most probably reflected selection gradually acting through the response of growers to up-to-date advice on resistance management. In more recent years, high pyrethroid resistance has become more common in EU field samples, and has now also been found in the UK. Variation in susceptibility to pyrethroids has been documented previously among geographically-distinct pollen beetle populations and annual evaluations of susceptibility to pyrethroids have been conducted for insects collected from oilseed rape for many years.

12.3.1 Identification of Resistance by Bioassay

Although many laboratory bioassay methods have been developed to detect resistance, most of them are limited to defining phenotypes and provide little or no information on the underlying mechanisms. Nonetheless, bioassays remain the indispensable tower of strength of most large-scale resistance monitoring programs and are essential prior to the development of alternative diagnostic techniques based on the knowledge of the mechanism of resistance.

The phenotypic expression of resistance is assessed in small-scale laboratory bioassays by exposing pollen beetles to different applications of insecticides. The response of potentially insecticide-resistant populations is compared with known insecticide-susceptible standards. The only way of clearly identifying resistant populations needs baseline studies to be carried out prior to the use of the active substance. Such baseline studies are nowadays required by the pesticide regulation procedure (EPPO PP 213(2)) and hopefully will be available for new compounds. With pyrethroids and pollen beetles no such studies were carried out before about 20 years of use. Really sensitive pollen beetle populations may be extremely rare and therefore, in the majority of instances, susceptibility is a relative rather than an absolute concept.

The increase in resistant populations of pollen beetle is demonstrated by the response to lambda-cyhalothrin (applied at the recommended field rate in a adult vial test) for pollen beetles collected each year from 2002 to 2007 from a locality in Germany subjected to extensive use of insecticides (Fig. 12.1) and by results of a monitoring of pollen beetle resistance in Germany organised by the Julius Kühn-Institute (Table 12.3).

Susceptibility of these beetles to lambda-cyhalothrin decreased from 2005 to 2007, hence they have to be classified as being resistant in the last 3 years, according to the definition of EPPO (Heimbach et al. 2006). These analyses also showed that the resistance to lambda-cyhalothrin of pollen beetles from other locations increased

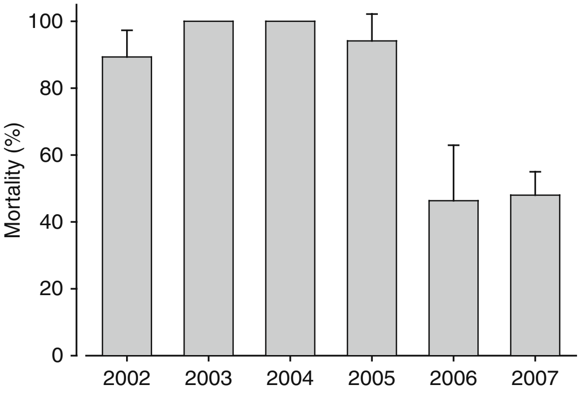


Fig. 12.1 Susceptibility to lambda-cyhalothrin of pollen beetles from a location with extensive use of insecticides in Germany

Table 12.3 Frequency of resistance classes of pollen beetle populations collected in Germany from 2006 to 2008 (from class 1 = very sensitive to class 5 = very resistant)

Resistance class	2006 (*n*=103)	2007 (*n*=281)	2008 (*n*=297)
1	7.0	3.2	0.3
2	8.0	5.3	2.4
3	18.0	19.9	15.8
4	41.0	38.8	40.4
5	26.0	32.7	41.1

up to 2007, even in large fields where there had been no previous application of insecticides (Thieme et al. 2008). This indicates that pollen beetles are very good at dispersing and makes it difficult to use field experiments for characterizing resistance. It is hardly possible to identify the recolonization of a field by insects that are such active flyers as pollen beetles.

The classification of beetles for resistance can be dependent on sampling time. Their great mobility allows fast mixing with beetles from other locations. Therefore, it is difficult to implicate a relationship between the application of an insecticide and resistance. This was demonstrated in an experiment on a 10 ha field sprayed with a pyrethroid. The application took place in the evening, the number of pollen beetles on plants and their susceptibility to lambda-cyhalothrin were assessed just before spraying, the next morning and until up to 10 days after application (Fig. 12.2). Because of immigration of beetles from other areas, the resistance identified directly after spraying was dramatically different from that on the second day after application. Therefore, it is essential to judge the susceptibility/resistance of a population of mobile insects by combining field results with laboratory analyses.

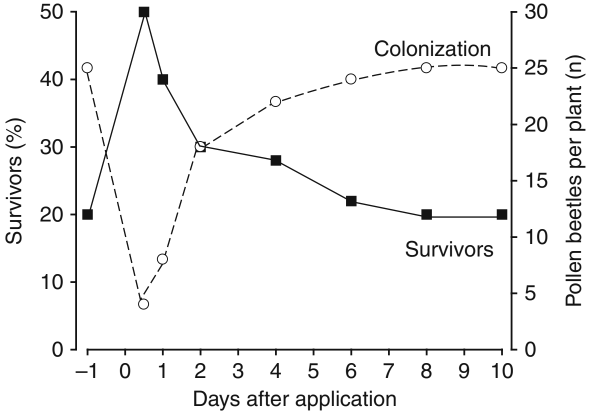


Fig. 12.2 Colonization of oilseed rape by pollen beetles and their survival after 5 h exposure to 0.075 μg lambda-cyhalothrin/cm^2 applied to glass tubes (Assessments of 20 plants per time and sampling were done in April 2007 on a 10 ha field in North-East Germany)

12.3.1.1 Problems with Bioassays

The response of pollen beetles to the active ingredient may differ from their response to the formulated product (Thieme et al. 2008). This is especially important when determining the sensitivity for products that seem to fail in the field.

Often, it is easier to run bioassays with pure technical substances. This has the advantage that they are not influenced by any formulation change which often occurs with products. Whereas technical substances can be dissolved in acetone, formulated products usually need at least some water which increases the time of drying test tubes. It is often argued that all insecticides with technical substances belonging to the same mode of action group should be cross-resistant. Thus, one might expect that, when Karate Zeon® is applied in the field, because of the many years of selection for resistance to lambda-cyhalothrin in a pollen beetle population, these beetles must be cross-resistant to all other pyrethroids. This is supported by bioassays using pure active substances, but not when formulated products are applied. Whereas formulated lambda-cyhalothrin shows resistance, formulated beta-cyfluthrin shows less resistance (Fig. 12.3a). For one sample of pollen beetles, a dosage of 1.41 g/ha lambda-cyhalothrin induced the same mortality (50%) as 1.24 g a.i./ha of the formulated product. However, for the same sample, a dosage of 1.74 g/ha beta-cyfluthrin induced the same mortality (50%) as 0.62 g a.i./ha of the formulated product. Similar results were obtained for etofenprox and its formulation (Fig. 12.3b). Therefore, results obtained using a formulated product and its active substance should not be generalized to other pyrethroids.

In bioassays suitable for testing insecticides that have best efficacy if consumed by feeding (e.g., Biscaya®), complete inflorescences or excised flower buds of oilseed rape that have been dipped into the insecticide can be used. Although more labour intensive and time consuming (in terms of preparation), bioassays using this

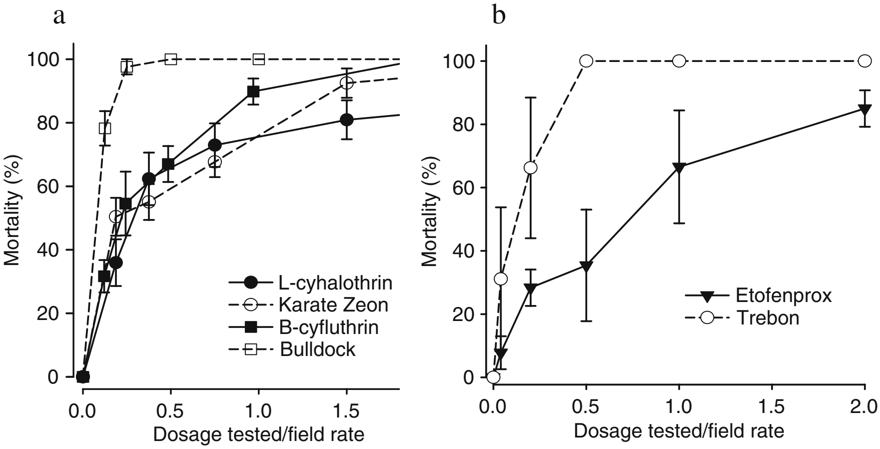
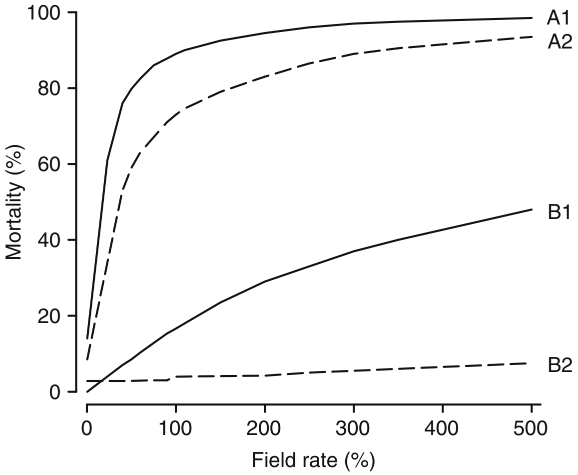


Fig. 12.3 **a**, **b** Mortality of pollen beetles induced by different dosages of pyrethroids (active substances compared with formulated products)

Fig. 12.4 Mortality of pollen beetles induced by 24 h exposure to Biscaya® (A) and formulated lambda-cyhalothrin (B) applied either to excised flower buds (1) or to a complete inflorescence (2) of oilseed rape. The beetles were collected in May 2007 near Dommritz/Saxony (Germany)



method allow oral application of formulated products. However, results can differ with the oilseed rape material to which the insecticide is applied as demonstrated for a pollen beetle population collected from rape in 2007 in Saxony. Although highly resistant to Karate Zeon®, these beetles were susceptible to Biscaya® (Fig. 12.4). But, for both products, exposure to excised and dipped buds resulted in significantly higher mortality than exposure to dipped complete inflorescences. For Biscaya® the hypothesis for equality ($\chi^2 = 31.76$; d. f. = 2; P = 0.000) and also the hypothesis of parallelism ($\chi^2 = 9.06$; d. f. = 2; P = 0.002) were rejected. Also for Karate Zeon® the hypothesis for equality was rejected ($\chi^2 = 48.49$; d. f. = 2; P = 0.000) as well as the hypothesis of parallelism ($\chi^2 = 7.96$; d. f. = 2; P = 0.005).

These differences may be caused by the larger size of container needed for the complete inflorescence, allowing the beetles to disperse more in space (which may result in a reduced consumption of treated plant material). The differences may also be caused by a stronger concentration of volatiles in the smaller glass tubes containing the excised buds. These volatiles are attractive to the pollen beetle and are not sex related (Cook et al. 2002). Further experiments will show if beside attractive floral volatiles, damage volatiles too are olfactory cues inducing increased consumption of treated plant material.

12.3.1.2 Do We Have to Know the Species?

Pest monitoring surveys have shown that *Meligethes* spp. differ in their susceptibility to insecticides (Derron et al. 2004). Currently insecticide resistance is determined under the assumption of testing only the true and most-commonly found species of pollen beetle, namely *M. aeneus*. But it is strikingly evident that samples of the genus *Meligethes* collected in the field can include several different species. Ignoring this may result in apparent susceptibility. The occurrence of different species in both winter and spring oilseed rape is not new (Reitter 1871, Friederichs

1921, Kauffmann 1925, Bollow 1950, Nolte and Fritzsche 1952, Scherney 1953, Fritzsche 1955, Kirk-Spriggs 1996, Marczali and Keszthelyi 2003). The problem is that a time-consuming dissection of male and female genitalia is necessary to distinguish between most species and, in monitoring surveys often, if at all, only those species are recorded that can be distinguished by colour or by morphometric characters that are easy to recognize (as in *M. viridescens*). Analyses have shown that in Germany and the UK, species of *Meligethes* other than *M. aeneus* occur in samples collected from oilseed rape fields (Thieme et al. 2006; Figs. 12.5 and 12.6).

As already reported by Derron et al. (2004), *M. viridescens* is not resistant and is present in variable proportions in relation to collection time and origin. Insecticide

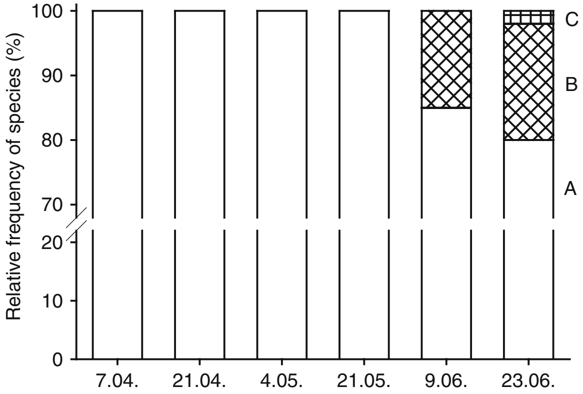


Fig. 12.5 Frequency of *Meligethes* species in samples collected in 2004 near Schwerin/Germany (A – *M. aeneus*; B – *M. viridescens*; C – *M. nigrescens, M. coeruleovirus and M. coracinus*)

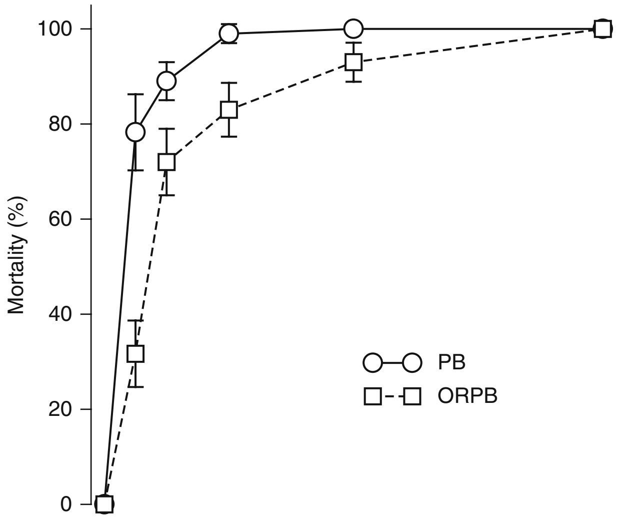


Fig. 12.6 Mortality induced by lambda-cyhalothrin to a mixture of different pollen beetle species (PB) and to *M. aeneus* (ORPB) collected in July 2007 near Rothamsted, UK

resistance should be determined only for the true oilseed rape pollen beetle *M. aeneus*. Ignoring this distorts bioassay results.

Additionally, other aspects can influence the outcome of bioassays such as time of beetle collection (see below), the location within a field, any pyrethroid application prior to the collection, the fitness of the beetles, as well as their weight (Tiilikainen and Hokkanen 2008).

12.3.1.3 Relevance of Bioassays to Field Control

In addition to quantifying resistance in bioassays, it is important to establish whether resistance quantified in this manner is of practical importance in the field. One way to do this is to apply insecticides in a more realistic manner under field conditions. These approaches ensure that bioassays are correlated with potential field control problems.

Field experiments have been used to study the performance of various established insecticides applied at recommended field rates against the pollen beetle carrying resistance to insecticides. To decide if the population is resistant or not they need to be combined with bioassays with adequate sampling of beetles shortly before and/or after the spraying. Bioassay results need then to be compared to known resistant or susceptible strains. Unfortunately, it is not yet possible to culture the pollen beetle for several generations in the laboratory. Therefore, it is impossible to compare bioassay results of well-defined strains that may support the development of biochemical and molecular characterization.

12.3.2 Biochemistry and the Molecular Basis of Resistance

The information provided by the use of bioassay techniques is essential. However, attention is being focused increasingly on the development of more sophisticated diagnostics that not only offer greater precision and throughput, but also identify the specific mechanism(s) present and even the genotype of the resistant insect. This is exemplified by research on aphids where significant advances have led to rapid and precise methods for the detection of different resistance mechanisms in individual insects.

But one of the disadvantages of the pollen beetle is that it is not yet possible to culture characterized strains in the laboratory.

In Europe, the pollen beetle possesses at least two resistance mechanisms: (i) metabolic resistance, caused by overproduced monooxygenase conferring resistance to pyrethroids (Nauen 2007), and (ii) target site resistance (kdr) to pyrethroids (Kristensen pers comm, Nauen pers comm, http://www.jki.bund.de/fileadmin/dam_uploads/_A/resistenz/insektizide_akarizide/5te_Kurzprotokoll zur 5. Sitzung des Fachausschuss Pflan.pdf). The latter mechanism confers strong resistance to virtually all available pyrethroids. It is now possible to diagnose both mechanisms in beetles using an immunoassay for the overproduced monooxygenase, and a molecular diagnostic for the *kdr* allele.

12.3.2.1 Metabolic Resistance Mechanism

In recent years, the majority of insecticides used to control the pollen beetle on oilseed rape have belonged to the pyrethroid class, which is an ester. Their bonds are particularly prone to cleavage and resistance to these active substances is commonly mediated by the enhanced hydrolysis of ester bonds. An association between resistance in bioassays and enhanced esterase activity was established in aphids (*M. persicae*) over 30 years ago (Needham and Sawicki 1970). This discovery was central to the understanding of this type of resistance in aphid species.

Biochemical studies were performed by Nauen (2007) with pyrethroid-susceptible and pyrethroid-resistant pollen beetle populations. No differences were found in the overall esterase activity and glutathione S-transferase activity but monooxygenase levels differed significantly. This suggested a metabolic mechanism of resistance contributing to the differences in pyrethroid susceptibility. The overproduced monooxygenase sequester or degrade insecticide esters before they reach their target sites in the nervous system and confer strong resistance to pyrethroids.

12.3.2.2 Target Site Resistance Mechanism

A target site mechanism, termed knockdown resistance (kdr), has been identified as the main threat of resistance to pyrethroid insecticides (Martinez-Torres et al. 1999). Target site resistance is conferred by mutations in the voltage-gated sodium channel gene (Soderlund and Knipple 2003). Initial recognition of the kdr mechanism was dependent on a molecular biological approach in which a highly conserved point mutation in the sodium channel gene was identified based on studies in kdr houseflies, aphids and other pest species with the same mechanism (Martinez-Torres et al. 1997, 1999, Devonshire et al. 1998). A replacement of leucine by phenylalanine in domain II of transmembrane segment II-S6 is the most common amino acid substitution in the *kdr* allele. This homologous mutation has been documented in many insects (Dong 1997, Park et al. 1997, Jamroz et al. 1998, Martinez-Torres et al. 1998) and was initially particularly well-studied in the house fly (Williamson et al. 1993, 1996a, b). Molecular diagnostic methods continue to be essential in understanding the role of this mechanism in resistant aphids (Guillemaud et al. 2003*)*.The kdr mechanism was found recently in pollen beetle populations in Denmark (see above).

The biochemical and molecular genetic diagnostics described will enable a greater understanding of the incidence and effect of the different resistance mechanisms in the pollen beetle.

12.3.3 Factors Affecting the Dynamics of Insecticide Resistance in the Field

12.3.3.1 Selection Pressures

Insecticide Application Factors

The use of reduced rates, poor application techniques, or too early applications before most of the beetles arrive, will lead to substantial survival and population increase. It causes selection of resistant beetles when the insecticide is present at concentrations lower than those expected at the time of correct treatments. Such conditions also arise from the natural decay of the insecticide with time, the application at unfavorable conditions (e.g., pyrethroids show reduced efficacy applied at high temperatures) or the application of reduced rates, either to cut cost or to target other pests. This demonstrates that variation in susceptibility is the first evolutionary step towards resistance. In Germany at least, studies were recently launched to analyze the fitness costs or advantages of maintaining a fluctuating polymorphism of susceptible and resistant pollen beetle populations despite prolonged and often intense selection by insecticides.

Ecological Factors

The diversity of resistance mechanisms known to date leads to the assumption that no insecticide is immune to the appearance of genes conferring resistance. The probability of these achieving detectable frequencies depends instead on a suite of ecological and genetic factors and how these interact with insecticide usage patterns. As a consequence, the same product can face very different resistance risks with different pest species and even within the same species in different cropping systems (Foster et al. 2007). So selection pressure and resistance risk seem to be much higher in spring oilseed rape crops than in winter oilseed rape crops (Hansen 2008).

One of the basic principles of resistance management is that resistant forms are less fit than the susceptible genotype in the absence of insecticides; otherwise, the former would probably be present at higher frequencies prior to selection by insecticides. Some of the best evidence of pleiotropic effects of resistance genes comes from recent work on aphids where field and laboratory studies suggest the existence of adverse selection in the form of poor winter survival, maladaptive behavior, and reduced reproductive fitness imposed primarily during times of stress. Significant selection pressure from synthetic insecticides dates back only 40 years, but the intensity of usage already has imposed extremely strong selection.

Nevertheless, susceptible beetles persist and could increase in proportion within a population when insecticide selection pressure is reduced, for example, over winter months when pollen beetles have settled within hibernation places and are untreated. The fitness of resistant forms in the absence of insecticides has not been studied. Monitoring of pollen beetle populations in Germany did not show a fall in the frequencies of resistant beetles during the winter months in bioassays (Thieme et al. 2008, Fig. 12.7); the same level of resistance was found in pollen beetles caught during infestation of oilseed rape crops in spring/summer as in those caught in the previous autumn.

In Germany, a large field experiment was undertaken to show the consequences of different treatment regimes for the build-up of metabolic resistance. To check if any reduction of selection pressure has effects on the sensitivity of the populations present, a region in North-East Germany (diameter about 6–8 km) with strong resistance problems in 2006 was selected in which no pyrethroids were used on oilseed rape crops after 2007 during periods when pollen beetles were present. No clear change in sensitivity (Fig. 12.8) was detected; this may have been caused either by too high mobility of the beetles, too short a period for the analysis, or a learning effect of near-by non-regulated farmers also avoiding the use of pyrethroids. The

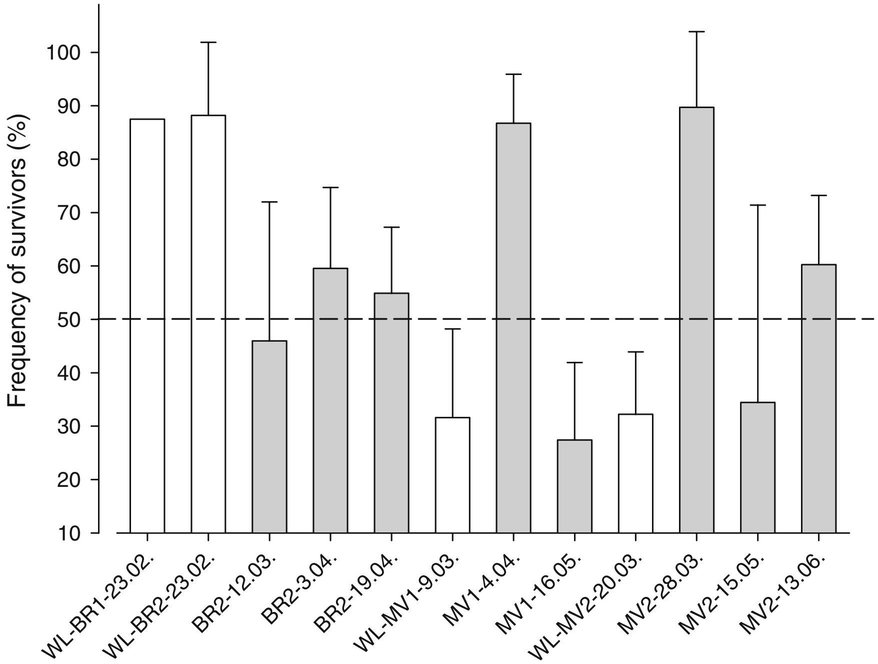


Fig. 12.7 Mean percentage survivors (± SD) of pollen beetles collected at the same location during different periods in Brandenburg (BR) and Mecklenburg-West Pomerania (MV) in hibernation places (WL) and in the field after 5 h exposure to 0.075 μg lambda-cyhalothrin/cm^2 in an adult vial test (*dashed line*: threshold for resistance class ‘highly resistant’ according Heimbach et al. 2006)

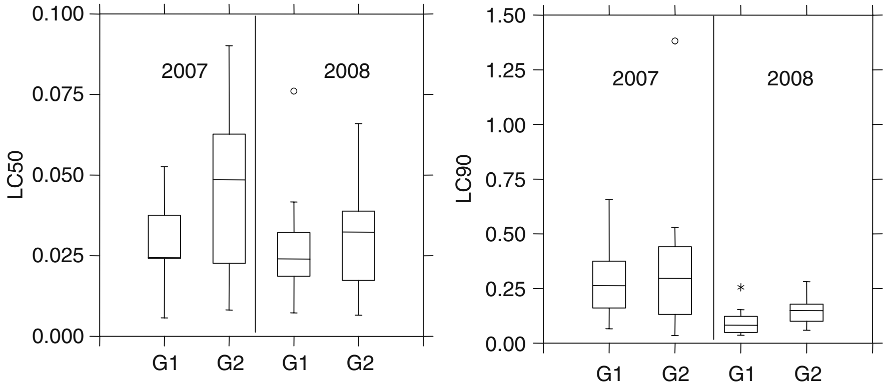


Fig. 12.8 LC50 and LC90 (μg a.i./cm^2) values for pollen beetles exposed to lambda-cyhalothrin in an adult vial test. The beetles belonging to populations sampled at different times in 2007 and 2008 in a German region without pyrethroid use (G1) and a non-regulated region nearby (G2)

latter may be relevant because, in 2008, an improvement in the resistant status of pollen beetles was detected in North East Germany in contrast to the situation in Germany as a whole where resistance is still increasing. This might support the idea that a reduction of the selection pressure will result in less resistant populations and that replacement of pyrethroids seems to be an efficient resistance management strategy.

12.3.4 Resistance Management Strategies

The possibility of resistance highlights the need to develop and implement resistance management strategies to prevent or delay the evolution of resistance to new insecticides. These strategies are dependent on the development of effective resistance monitoring programs capable of early detection of resistance that will allow implementation of appropriate management decisions in a timely manner. The initial steps in implementing such programs include development of appropriate bioassay techniques and establishment of baseline susceptibility data among populations across the geographic range of the target species. With this information, potential population susceptibility changes in response to selection with a distinct insecticide can be identified.

Variation in susceptibility to pyrethroids has been documented previously among geographically-distinct *M. aeneus* populations and annual evaluations of susceptibility to pyrethroids are conducted for insects collected from oilseed rape. The objective of different studies was to establish a baseline of susceptibility to pyrethroids from geographically-distinct populations of *M. aeneus* collected from oilseed rape, emphasizing areas where there is likely to be exposure to insecticides.

The variation in baseline susceptibility to insecticides may reflect differences in vigour among parental populations, attributes that are the product of genotype, the maternally-determined nutritional status of the egg, age, size and health status. Variation in susceptibility to insecticidal toxins has been reported from repeated bioassays against larvae from single strains of Colorado potato beetle and diamondback moth (Robertson et al. 1995) and European corn borer (Marcon et al. 1999). Intrapopulation variation in response to insecticides is a common phenomenon when any bioassay is repeated. The extents of both inter- and intra-population variation in susceptibility to a given insecticide should be investigated before biologically-important changes can be identified with any certainty. This should be done before the product is used commercially rather than after resistance is already widespread.

Resistance strategies should be implemented before a resistance problem becomes acute. This was apparently not the case for pyrethroid-resistant pollen beetles. Now, in several countries, resistance groups have been established. For example, in Germany, resistance strategies for insecticidal use in oilseed rape have been discussed and published since 2006, to try to ensure effective control of the pollen beetle as well as at the same time to slow down the development of resistance (http://www.jki.bund.de/no_cache/de/startseite/fachinformationen/pflanzenschutz/

pflanzenschutzresistenz/insektizide-akarizide.html). Such strategies need support by all relevant parties including pesticide regulators, industry, pesticide merchants, advisors and last, but not least, farmers. The German strategy still relies on the use of pyrethroids because of the limited availability of other control options. OPs are implemented but show only low control options on stem weevils and can only be used clearly before flowering because of bee safety issues. Bee-safe neonicotinoids are less effective on relevant oilseed rape pests than pyrethroids and not sufficient under high pollen beetle pressure. The use of some pyrethroid active substances, such as tau-fluvalinate, etofenprox and bifenthrin, which sometimes still seem to be less affected by resistance (e.g., Hansen 2008), is the only chance, though the risk of further resistance selection is very high and, in Denmark, kdr resistance has already been detected (see above) after the use of such active substances for some years. The same is true for additives such as PBO, which have not been accepted for use in Germany. There is a strong need for new modes of action with good efficacy on several pest insects of oilseed rape, no cross resistance, bee safety and a competitive price.

Resistance management strategies should also more and more implement new growing schemes for oilseed rape such as border rows with early flowering varieties or other attractive crops (Cook and Denholm 2008) as well as biological control options (Hokkanen 2008). But there is only limited experience with such growing forms and often they are associated with more economic risk for farmers. So, at the moment, only chemical control is accepted by farmers.

In 2007, an ‘Insecticide Resistance Action Committee’ (IRAC) Pollen Beetle Working Group was established to bring together expertise from agrochemical companies and independent researchers in order to monitor the development of insecticide resistance in oilseed rape pests and to provide guidance and advice on the best practices to monitor and to prevent further insecticide resistance development. The monitoring showed the wide distribution of resistant populations in Europe (http://www.irac-online.org/documents/pdf_pb_monitoring_%202008_v2%202.pdf).

All resistance groups and participants of the EPPO workshop in 2006 (Zlof 2008) have concluded that an efficient strategy to reduce the resistance level or to slow down resistance development requires at least 2 additional modes of action with no cross resistance to pyrethroids, the correct use of the full dose with the correct equipment, and insecticide application only in those situations in which relevant pest damage is expected. Such strategies need also to take account of other insect pests of oilseed rape. In Germany, many populations of other pests (*Ceutorhynchus* spp. weevils), have been monitored with no clear incidence of any resistance yet (Müller et al. 2008), although there seems to be an increase in their survival rate from 2005 to 2008 (Fig. 12.9).

Development of baseline susceptibility data represents the first step toward the development of a monitoring program designed to detect changes in susceptibility that may result from repeated and prolonged exposure to a given insecticide. These data also may provide information that will allow development of diagnostic bioassays that would be more efficient in detection of resistant populations. However, it

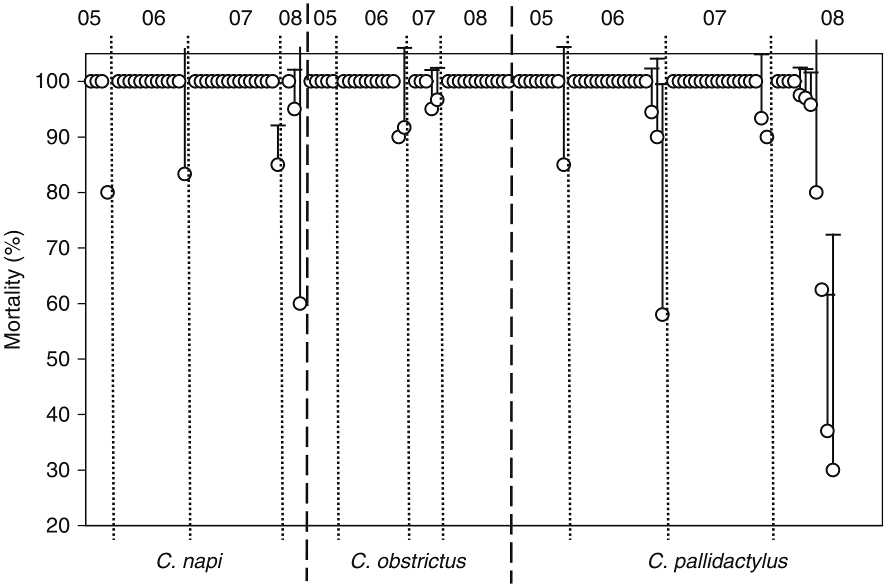


Fig. 12.9 Mortality of populations of different *Ceutorhynchus* spp. 5 h after exposure to 0.015 μg/cm^2 lambda-cyhalothrin in adult vial tests. The beetles were collected in Germany between 2005 and 2008. Each *dot* represents a different population

should be noted that pollen beetle populations are exposed to multiple applications of pyrethroids for a long time. As a result, designation of a shift in susceptibility to pyrethroids may be difficult to achieve because of the lack of field populations not being exposed to these insecticides.

12.4 Conclusions

The battle to control the pollen beetle and other pest insects of oilseed rape will continue as long as these pests compete with us for food resources. The new insecticides such as neonicotinoids with novel modes of action, will provide more effective control in the future. Their use will achieve less exposure of potentially risky chemicals to non-target organisms and farmers compared to older insecticides from the last century. In future, conventional breeding programs for resistance or genetic modified plants may provide more options for control. But there is also a need to develop oilseed rape growing systems which reduce pollen beetle pressure and reduce the need to control. Integrated pest management protocols for oilseed rape pollen beetle problems are needed. Sustainable control of pest insects on oilseed rape needs to alternate the use of different active substances having no cross resistance and needs a selection of optimal active substances with good efficacy for the different pest species and climatic condition including bee safety aspects. Such an optimal choice is not possible at the moment in Europe with only one or two types of active substances being available in many countries.

As a consequence, insecticidal control of oilseed rape pests continues to select for resistance and is clearly a case of co-evolution demonstrating how such processes generate biological diversity. Over the past 15 years, scientists have made great progress in monitoring and characterizing insecticide resistance and in understanding some of the factors that affect the speed of its development. Regardless of the progress that has been made, the competition between insect evolution and human inventiveness will continue to present major challenges. In order to meet these, it will be necessary to increase understanding of the processes that mediate the development of resistance in pollen beetles. There is a strong need for empirical research on the mechanisms conferring resistance to new chemical groups, and an understanding of the width of resistance that these mechanisms confer. There is also a lack of information on the ecological factors that mediate resistance development – fitness costs for resistant beetles, and the effects of dispersal and migration.

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