Nachrichtenbl. Deut. Pflanzenschutzd., **58** (1), S. 1–5, 2006, ISSN 0027-7479.

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First steps to analyse pyrethroid resistance of different oil seed rape pests in Germany

Erste Untersuchungen auf Pyrethroid-Resistenz verschiedener Rapsschädlinge in Deutschland

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Abstract

Laboratory experiments were conducted testing the effects of active substances of pyrethroids on different pest insects of rape collected in different areas of Germany. As test method an adult-vial-test was used. The pyrethroids (λ-cyhalothrin and cypermethrin) were applied in glasstubes of 30 ml content and the insects were exposed to the dried residues of insecticides for up to 24 hours. Tests have been carried out on *Meligethes aeneus, Phyllotreta* spp., *Ceutorhynchus assimilis*, *C. pallidactylus, C. napi,* and *Dasineura brassicae*. Besides some *M. aeneus* samples showing drastic reduction in sensitivity to pyrethroids, out of 25 samples of other oil seed rape pest species only two samples, one each of *C. napi* and *C. pallidactylus,* showed a lower level of sensitivity to pyrethroids. The laboratory methods used showed good reproducibility and the field collection method of the different pest species resulted in sufficient number of test insects in many cases. Data obtained from the laboratory can not directly be used to predict possible resistance in the field but have to be validated with results from the field.

Key words: Pyrethroids, oil seed rape, resistance, laboratory test, *Phyllotreta* spp., *Ceutorhynchus assimilis, C. pallidactylus, C. napi, Dasineura brassicae, Meligethes aeneus*

Zusammenfassung

Im Rahmen eines Monitorings zur Resistenz von Rapsschädlingen (*Meligethes aeneus, Phyllotreta* spp., *Ceutorhynchus assimilis*, *C. pallidactylus, C. napi* und *Dasineura brassicae)* wurden aus verschiedenen Regionen Deutschlands Tierproben mit Hilfe eines Adult-Vial-Tests hinsichtlich ihrer Reaktion auf Pyrethroide getestet. Dazu wurden 30-ml-Schnappdeckelgläser mit den Wirkstoffen λ-Cyhalothrin oder Cypermethrin beschichtet und die zu testenden Tiere in diesen Gläsern 24 Stunden exponiert. Einige Proben des Rapsglanzkäfers zeigten eine deutlich verminderte Wirkung der Pyrethroide. Von den weiteren 25 getesteten Populationen der übrigen Rapsschädlinge zeigten zwei Proben, eine von *C. napi* und eine weitere von *C. pallidactylus,* eine verringerte Sensitivität. Insgesamt lieferte das benutzte Testsystem gute, reproduzierbare Ergebnisse und erwies sich auch für bisher noch nicht getestete Schädlingsarten als praktikabel. Die Sammelmethoden bei den verschiedenen Schädlingen lieferten in den meisten Fällen eine für die Tests ausreichende Anzahl an Organismen. Laborergebnisse können nicht direkt für eine Beurteilung der Resistenzsituation im Feld genutzt werden, dazu ist eine Validierung der Ergebnisse mit Hilfe von Felduntersuchungen erforderlich.

Stichwörter: Pyrethroide, Resistenz, Rapsschädlinge, Labor-Tests**,** *Phyllotreta* spp., *Ceutorhynchus assimilis, C. pallidactylus, C. napi, Dasineura brassicae, Meligethes aeneus*

Introduction

Due to EU regulations of plant protection products and increasing demands for human and environmental safety issues the number of active substances which can be used to control pest insects were reduced in the last years in the EU. In Germany only pyrethroids are available for the control of most pest insects in oil seed rape at the moment. Therefore resistance development of pest insects to pyrethroids is very relevant for IPM. In the past years *Meligethes aeneus* has developed resistance to pyrethroids in different European regions (Ballanger et al., 2003; Derron et al., 2004; Hansen, 2003; Wegorek, 2005) and resistant *M. aeneus* populations seem to spread also in Germany (Burghause and Jörg, 2005; Heimbach, 2005; Nauen, 2005 and unpublished data of Nauen, 2005; Sattler and Slater, 2005; Thieme, 2005). No information on possible development of resistance to the other pest insects of rape is available, though they often are exposed to more than one pyrethroid application per season similar to *M. aeneus*. According to EU pesticide regulation, resistance issues need to be addressed during registration process of pesticides. In an EPPO paper guidance is given for this purpose and also sensitivity data for pest organisms at resistance risk are demanded, preferably from laboratory tests (EPPO, 2003). To foster sensitivity testing and method development as well as to get more knowledge on the resistance status of oil seed rape pest insects, a resistance monitoring for most relevant pest insects in oil seed rape was started in Germany.

Material and methods

Sampling of the different oil seed rape pests

Species collected were *Ceutorhynchus assimilis* (CEUTAS), *C. napi* (CEUTNA), *C. pallidactylus* (CEUTQU), *Dasineura brassicae* (DASYBR), *Meligethes aeneus* (MELIAE), and *Phyllotreta* spp. (PHYLSP).

All species except *D. brassicae* were collected in oil seed rape fields in Germany by either direct hand collecting, using sweep nets or yellow water traps. When yellow water traps filled with

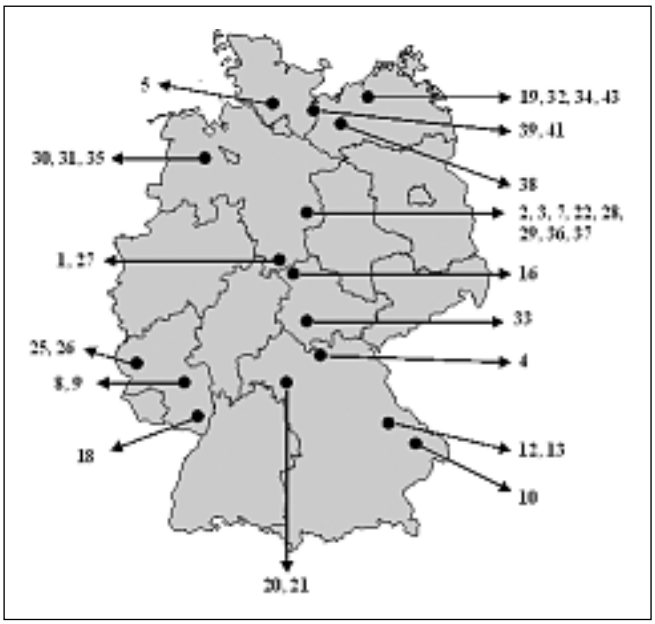

Figure 1. Areas of collection of oil seed rape pest insects in Germany.

water were used, they had to be emptied several times a day to avoid harming the collected beetles. Collected animals were transported and stored in boxes with some oil seed rape leaves as food. The boxes had an inlet of paper to avoid the building of condense water. Storing of the insects took place at low temperatures of about 10 °C.

For the collection of *D. brassicae* infested pods of oil seed rape were collected and kept in hatching cages in the laboratory. Larvae leaving the pods pupated in a small amount of soil supplied below the pods. Midges hatching were kept only for up to 3 days at low temperature and high humidity before they were used in the tests. No food was supplied.

All insects which were not collected by the BBA were mailed to Braunschweig for the tests. Most samples arrived in good conditions in Braunschweig. Only 4 out of 38 samples could not be used for tests anymore, because the insects did not survive the transport. In 6 out of a total of 12 samples of *D. brassicae* no or not enough midges hatched.

Sampling of test insects

The samples collected and tested are presented in Table 1. Figure 1 illustrates the locations of the sampling in Germany. In some cases *M. aeneus* samples were collected in areas with known resistance shown by reduced field effects after application (samples No. 9, 19, 25, 34, 43).

Insecticide tests in the laboratory, adult-vial-test

Similar to an unpublished method used by THIEME in his laboratory, glass vials of 30 ml content (6.5 cm long and 2.4 cm diameter) were used for the test. Prior to testing the vials were coated with the active substance of pyrethroids dissolved in acetone in different concentrations. 1.3 ml of the solution was given into each vial. The vials were kept open on a rolling bank for about 90 minutes until the acetone was evaporated, resulting in an even film of the active substance on the walls and bottom of the glass vials. In trials with the formulated product Karate Zeon® water was used as solvent. Drying of the vials on the rolling bank with

Table 1. Test number, date of collection, collector, and number of insects tested at a rate of 0.015 µg/cm² λ-cyhalothrin

Sample Number	Date of collection	Institution	Collector	Species (EPPO Code)	No. tested at 0.015 µg/cm² λ-cyhalothrin
1	20. 03. 05	Univ. of Göttingen	Ulber/Wedemeyer	CEUTNA	50 in 5 repl.
2	24. 03. 05	BBA Braunschweig	Müller	CEUTNA	18 in 2 repl.
3	24. 03. 05	BBA Braunschweig	Müller	CEUTQU	15 in 3 repl.
4	24. 03. 05	LWA Coburg	Hemmer	CEUTQU	30 in 5 repl.
5	24. 03. 05	Spiess-Urania	Goebel	CEUTQU	20 in 4 repl.
7	04. 04. 05	BBA Braunschweig	Müller	CEUTQU	8 in 1 repl.
8	04. 04. 05	DLR Rheinhessen	Burghause	PHYLSP	38 in 4 repl.
9	04. 04. 05	DLR Rheinhessen	Burghause	MELIAE	30 in 3 repl.
10	04. 04. 05	LWA Deggendorf	Thalhammer	CEUTQU	40 in 4 repl.
12	04. 04. 05	LWA Regensburg	Rupprecht	CEUTQU	30 in 3 repl
13	04.04.05	LWA Regensburg	Rupprecht	CEUTNA	10 in 1 repl.
16	08. 04. 05	LWA Leinefelde	Eiselt	CEUTQU	20 in 2 repl.
18	13. 04. 05	BASF Limburgerhof	Landvogt	CEUTQU	10 in 1 repl.
19	06. 04. 05	BTL Bio-Test Labor	Thieme	MELIAE	10 in 1 repl.
20	14. 04. 05	LWA Würzburg	Rüdinger	MELIAE	10 in 1 repl.
21	17. 04. 05	LWA Würzburg	Seifert	MELIAE	20 in 2 repl.
22	04. 05. 05	BBA Braunschweig	Müller	PHYLSP	23 in 2 repl.
25	10. 05. 05	DLR Westeifel	Schackmann	MELIAE	40 in 4 repl.
26	10. 05. 05	DLR Westeifel	Schackmann	PHYLSP	51 in 4 repl.
27	11. 05. 05	Univ. of Göttingen	Ulber	CEUTAS	17 in 2 repl.
28	23. 05. 05	BBA Braunschweig	Müller	PHYLSP	21 in 2 repl.
29	24. 05. 05	BBA Braunschweig	Müller	MELIAE	42 in 4 repl.
30	25. 05. 05	PSA Oldenburg	Schröder	MELIAE	37 in 4 repl.
31	25. 05. 05	PSA Oldenburg	Schröder	CEUTAS	32 in 3 repl.
32	02. 05. 05	BTL Bio-Test Labor	Bergmann	CEUTAS	22 in 2 repl.
33	24. 05. 05	LWA Hildburghausen	Hartmann	CEUTAS	9 in 1 repl.
34	25. 04. 05	BTL Bio-Test Labor	Thieme	MELIAE	42 in 4 repl.
35	01. 06. 05	PSA Oldenburg	Schröder	DASYBR	48 in 4 repl.
36	02. 06. 05	BBA Braunschweig	Müller	DASYBR	21 in 2 repl.
37	02. 06. 05	BBA Braunschweig	Müller	DASYBR	9 in 1 repl.
38	06. 06. 05	PSA Schwerin	Rehm	DASYBR	24 in 2 repl.
39	06. 06. 05	ALR Lübeck	Landschreiber	DASYBR	51 in 4 repl.
41	05. 06. 05	ALR Lübeck	Kaak	DASYBR	23 in 2 repl.
43	15. 06. 05	BTL Bio-Test Labor	Thieme	MELIAE	36 in 4 repl.

water as solvent lasted up to 24 hours. The vials were closed then with a lid and stored at 8 °C in the dark for up to 14 days before the insects were exposesed in the vials.

As insecticides the active substances of lambda-cyhalothrin and cypermethrin were used and in some of the trials additionally a formulated product with the active substance λ-cyhalothrin (Karate Zeon®) was used. As far as possible, besides a control, several rates of the pyrethroids were tested, depending on the number of insects available. Rates used for λ-cyhalothrin were: 0.075 µg/cm² of glass surface which is representing the registered field rate in Germany of 7.5 g a.s./ha. Additionally lower (down to 0.00075 µg/cm²) and higher rates (up to 0.75 µg/cm²) were tested, depending on the number of insects available. The rate of 0.015 µg/cm² was chosen to distinguish differences in population sensitivity, because at this rate all samples of *M. aeneus* with known field resistance showed less than 100 % mortality 5 hours after exposure. Cypermethrin was used in rates between 0.003 and 3.0 µg/cm² (0.3 µg/cm² is the equivalent to the field rate of 30 g a.s./ha that has been used in Germany in the past).

Insects were kept for at least 1 day in the laboratory before only those, appearing to be unaffected and active, were exposed to the residues. About 10 individuals were exposed per vial which was closed with a lid, having a small prick for ventilation. If possible up to 5 replications were carried out per test rate. The vials with the exposed insects were kept at a constant temperature of 16 °C and at constant light. Assessments were carried out after 1, 5, and 24 hours. Assessments after 5 hours were chosen to be reported, because control mortality often was increased at 24 hours already, whereas a significant increase of effects was detected between 1 and 5 hours and only a small increase any more between 5 and 24 hours, which is the expectation for the fast acting pyrethroids. All results presented were not corrected for control mortality values.

Results and discussion

Two samples of *M. aeneus* were taken, to test whether the use of the active substance and the formulated product on the glass surface of the vials may result in different effects using the adult-

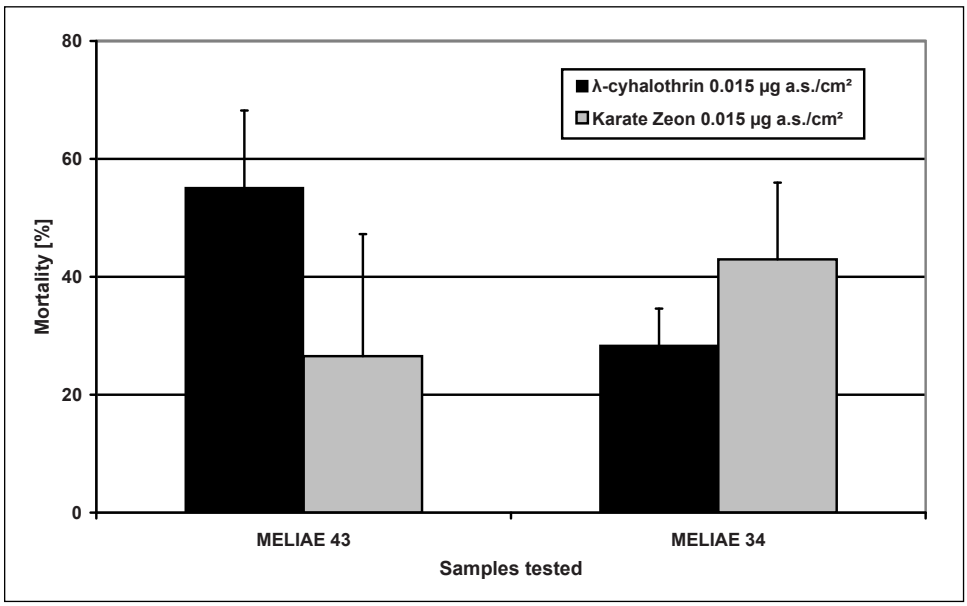

Figure 2. Mean mortality (± SD) of two *M. aeneus* samples with the formulated product Karate Zeon and the active substance λ-cyhalothrin using the same rate of a.s. of 0.015 µg/cm². (For the number of insects used per test see Tab. 1).

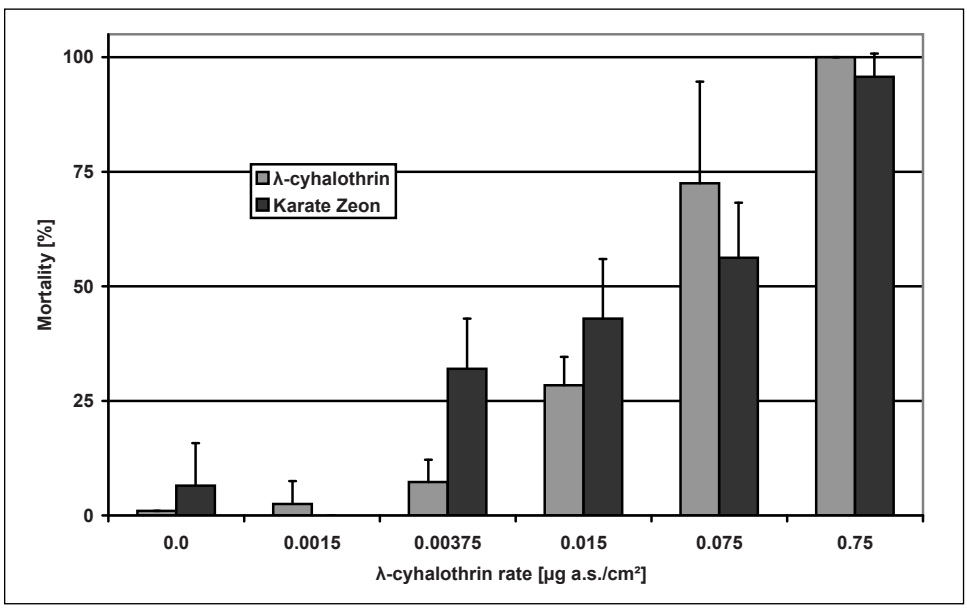

Figure 3. Mean mortality (± SD) of a *M. aeneus* sample (no. 34) in adult-vial-tests after an exposure of 5 hours with the formulated product Karate Zeon and the active substance λ-cyhalothrin using different rates. (For the number of insects used per test see Tab. 1).

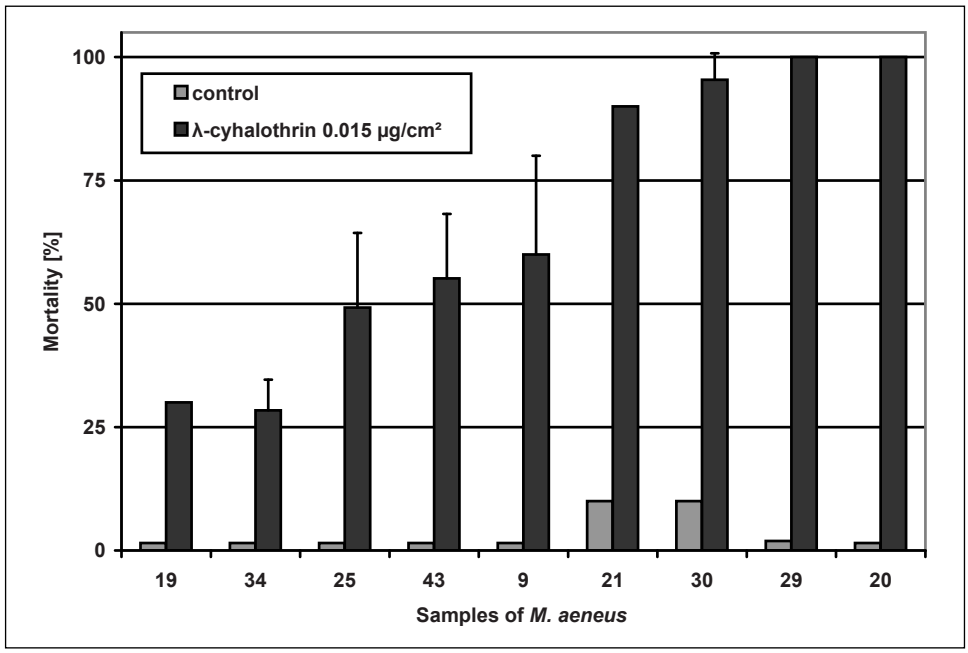

Figure 4. Mean mortality (± SD) of several *M. aeneus* samples in adult-vial-tests after an exposure of 5 hours with λ-cyhalothrin using the same rate of a.s. of 0.015 µg/cm². (For the number of insects used per test see Tab. 1).

vial-test design. The results were quite similar in two experiments thus showing that no major difference was detected between the use of the active substance λ-cyhalothrin and the formulated product Karate Zeon in the test system (Fig. 2). This is supported by comparing the effects of the active substance and the product for several rates of λ-cyhalothrin and Karate Zeon on a sample of *M. aeneus* with known resistance in the field. For each test rates similar mortality values were obtained between effects of the active substance and the product (Fig. 3). Even at the highest test rate (equivalent to 10 times the field rate) no full control of *M. aeneu*s was achieved which was also the case for the samples 19, 25, 43.

The effects of λ-cyhalothrin at a rate of 0.015 µg/cm² clearly show differences between the nine samples of *M. aeneus* tested. The samples originating from regions or fields with known pyrethroid resistance react differently from areas where no resistance is known yet. This differences at a rate of 0.015 µg/cm² seem to allow to separate between populations being still sensitive compared to those with detectable reduction of efficacy in the field (Fig. 4). Control mortality in most cases was equal or below 10 % in tests, which was also the case for the other beetles species (average control mortality of 1.86 % after 5 hours in 28 tests) but higher for *D. brassicae* (average of 11.95 % after 5 hours in 6 tests). After 24 hours the mean control mortality was 11.98 % for beetles and 30.67 % for midges.

Five samples of different pest species were tested comparing the active substances of cypermethrin and λ-cyhalothrin at rates of 0.06 µg/cm² of cypermethrin and 0.015 µg/cm² of λ-cyhalothrin. Results were similar in all tests (Fig. 5). This is indicating that the two *M. aeneus* samples show cross resistance to this pyrethroids.

Comparing the results of all samples tested (Fig. 6) it is obvious that a main resistance problem seems to exist with *M. aeneus* only. But it has to be kept in mind, that sampling was not randomly, but more concentrated in areas with known resistance for *M. aeneus,* whereas for the other species tested, sampling was carried out mainly in regions where no resistance is known yet. Especially from northern Germany, the region with most

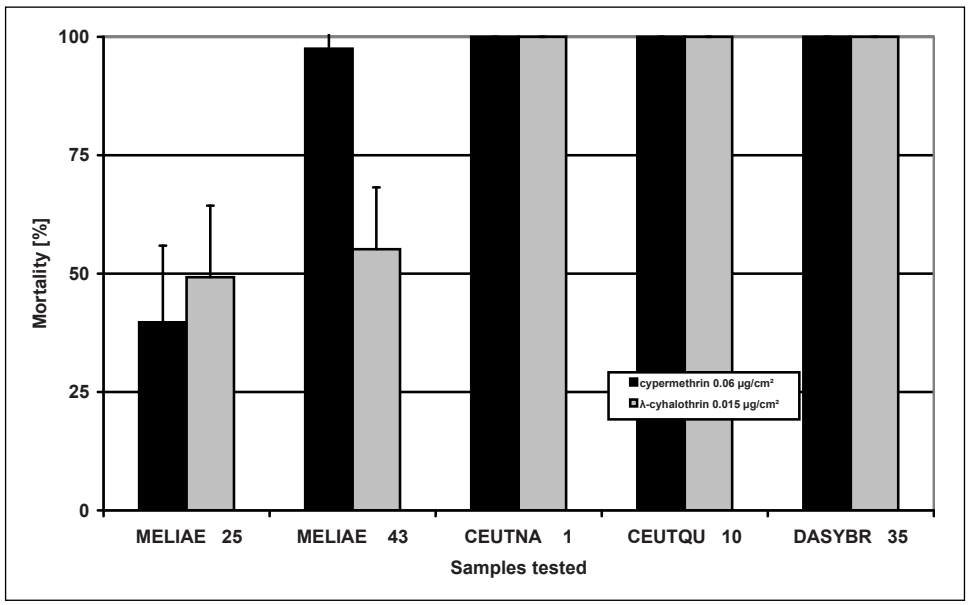

Figure 5. Mean mortality (± SD) of different oil seed rape pest insects in adult-vial-tests after an exposure of 5 hours with the 2 active substances cypermethrin (0.06 µg/cm²) and λ-cyhalothrin (0.015 µg/cm²). (For the number of insects used per test see Tab. 1).

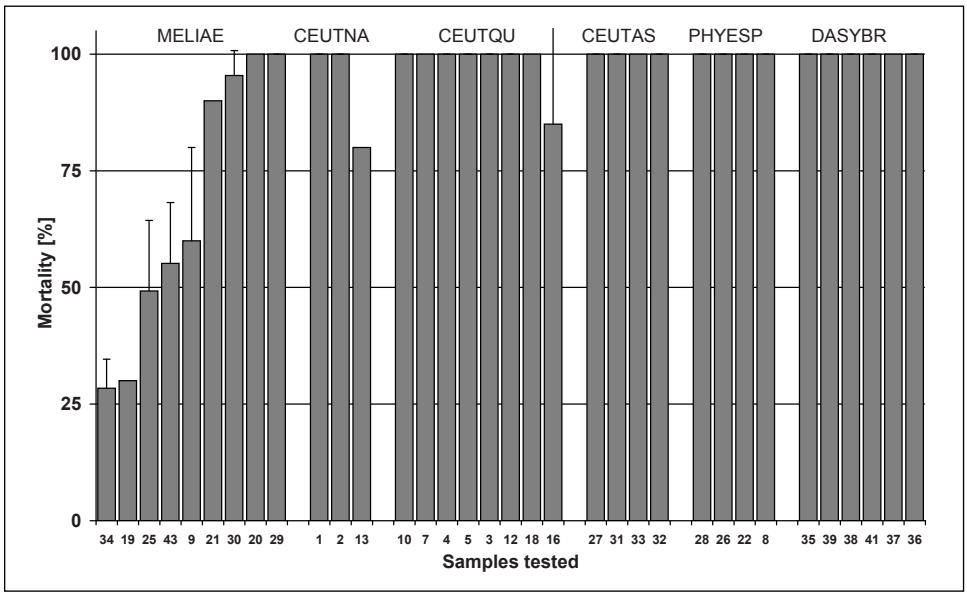

Figure 6. Mean mortality (± SD) of monitored oil seed rape pest insect samples in adult-vial-tests after an exposure of 5 hours and 0.015 µg/cm² λ-cyhalothrin. Species tested were: *Meligethes aeneus* (MELIAE), *C. napi* (CEUTNA), *C. pallidactylus* (CEUTQU), *Ceutorhynchus assimilis* (CEUTAS), *Phyllotreta* spp. (PHYLSP), *Dasineura brassicae* (DASYBR). (For the number of insects used per test see Tab. 1).

pyrethroid resistance problems, only few pest insects samples except *M. aeneus* could be tested, because of low pest density in 2005 in this region.

Results of two samples of stem weevils (No. 13 and 16) indicate that there might be a rising problem with this species in some areas. But to be sure if there is resistance developing or not, generally laboratory data need to be validated with real field data. More analyses must be carried out for the other oil seed rape pests to find out if relevant sensitivity changes are developing which might finally end in field resistance.

It also has to be kept in mind, that all tests were carried out on small numbers of populations of different insect species and therefore it is not possible to show first signs of resistance unless very high numbers are tested. The results only can give a rough indication of the percentage of individuals with resistance.

Conclusions

EPPO defines resistance as a clear reduction of effectiveness in the field at the registered rate of a plant protection product compared to field effectiveness reached in the past. Field failure alone can not answer the question if there is resistance in a population of insects or not, because several factors can result in failure of an application of insecticides such as reinvasion of pest insects, unfavourable conditions during or after application etc.

Field tests are also too time consuming and expensive to screen for resistance. Therefore adequate and standardised laboratory tests should be developed which are validated by field experiences and then easily can be used in cases of field failure of a product to decide if resistance or something else caused the problem. Sensitivity data as demonstrated in this paper can support the decision if there is resistance in a population of insects or not. Distinct differences in the results of laboratory tests between different populations from different regions or years indicate a possible resistance.

The method presented here seems to be able to analyse rising and already existing resistance problems with pyrethroids in oil seed rape. But the method can not be used for all other types of products (e.g. not for those that have systemic action, that are acting slowly or that have high volatility). It also needs to be checked carefully, if other pest insects (e.g. aphids probably not) can be tested in this way.

Acknowledgements

We thank all collectors of samples (see Table 1) for supporting the monitoring, the UFOP for a financial contribution, and the companies BASF and Syngenta for supply of the active substances.

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Accepted: October 2005

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