Pyrethroid resistance in pest insects of oil seed rape in Germany

Udo Heimbach, Andreas Müller, Thomas Thieme*

Federal Biological Research Centre for Agriculture and Forestry, Institute for Plant Protection in Arable Crops and Grassland 38104 Braunschweig, Germany;
**BTL Bio-Test Lab Sagerheide, 18184 Sagerheide, Germany Email: u.heimbach@BBA.DE*

Abstract

Pyrethroid resistant pollen beetles (*Meligethes aeneus*) are widely distributed in Germany after first resistant beetles were detected only about 6 years before. The reason of the fast development and spread of resistance is that at present only pyrethroids are registered for spraying in oil seed rape to control pest insects. Thus less sensitive beetles were selected by every insecticide application in rape in the last years. Results of a monitoring program on pollen beetles show that pyrethroid resistant beetles were found in about 75 % of the oil seed rape area in Germany resulting 2006 in big yield losses because of limited control of pollen beetles.

Additionally a monitoring on other oils seed rape pests was carried out in order to find out if also other species might have developed resistance already. No clear resistance was detected until now in several of these other pest species including *Ceutorhynchus* spp., *Dasyneura* spp. and *Phyllotreta* spp., but distinct differences in sensitivity were detected for some populations of *Ceutorhynchus* spp.. The laboratory method used to determine the sensitivity of pollen beetles is capable of producing reproducible and valid results which can be used for monitoring of resistance and the control of strategies to reduce it.

Key words: Oil seed rape, *Brassica napus*, *Meligethes aeneus*, pyrethroid resistance, adult vial-test

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 until 2006. 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* seem to spread also in Germany (Burghause & Jörg, 2005, Heimbach et al., 2006, Nauen, 2005). No information on possible development of resistance to 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 carried out in Germany.

Material and Methods

Sampling of 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 direct hand collecting, using sweep nets, or yellow water traps. When yellow water traps were used, they had to be emptied several times a day to avoid harming the beetles caught. 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.

Insects not collected by the Institute for Plant Protection in Arable Crops and Grassland were sent by collectors to Braunschweig for the tests. Most samples arrived in good conditions.

Insecticide tests in the laboratory, adult-vial-test

Similar to the method presented now as "IRAC 11" glass vials of 30 ml (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 a pyrethroid dissolved in acetone in different concentrations. 1.3 ml of the solution was given into each vial. The vials were kept open on a "Stuart Roller Mixer" for about 60 minutes until the acetone was evaporated, resulting in a film of the active substance on the walls and bottom of the glass

vials. Thereafter the vials were closed with a lid and stored at 8 °C in the dark for up to 14 days before the insects were transferred into the vials.

As test substance lambda-cyhalothrin was used. As far as possible, besides a control, several doses of the pyrethroid were tested, depending on the number of insects available. Rates used for l-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 doses (up to 0.75 µg/cm²) were tested, depending on the number of insects available. The dose of 0.015 µg/cm² was chosen to distinguish differences in population sensitivity, because at this dose all samples of *M. aeneus* with known field resistance showed in the tests less than 100% mortality 5 hours after exposure.

Insects were kept for at least one day in the laboratory, and only healthy alive beetles, were used for the test. About 10 individuals were exposed per vial, which was closed with a lid. Up to 4 replicates were carried out per dosage tested. The vials with the exposed insects were kept at a temperature of about 20 °C and at constant light. Assessments were made after one, five, and 24 hours. Assessments after five hours were chosen to be reported, because control mortality often increased at 24 hours already, whereas a significant increase of effects was detected between one and five hours and only a small increase between 5 and 24 hours, which is the expectation for the fast acting l-cyhalothrin. All results presented were not corrected for control mortality.

Results

Samples of *M. aeneus* were more sensitive in 2005 with about 20 % of 9 samples showing 100 % mortality at the dose of 0.015 µg/cm² compared to about 10 % only in 2006 (Fig 1). Additionally minimum mortality in 2005 were about 30 % whereas in 2006 they were about 10 % only.

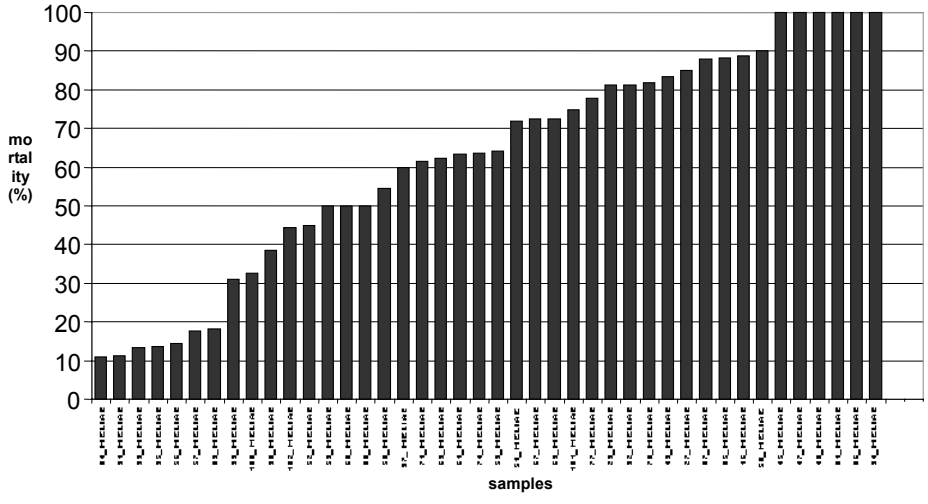

Fig 1 Mean mortality of adult *M. aeneus* collected in 2006, five hours after exposure to one dose of l-cyhalothrin (0.015 µg/cm²)

Increasing resistance is also detectable for a population collected in 2005 and 2006 from the same location near Braunschweig (Fig. 2). In 2005 a dose of 0.015 µg/cm² induced 100 % mortality but in the following year about 5 % of the population survived even at a dose of 0.75 µg/ha.

In contrast to the population in Fig. 2 most beetles from other locations tested showed a clear dose response with increasing mortality at higher rates.

Other rape pest insects showed usually 100 % mortality at the rate of 0.015 l-cyhalothrin µg/cm² in 2005 and 2006 (Fig. 3). In 2005 only one *C. napi* and one *C. pallidactylus* sample showed some survivors at this dose out of 32 samples of different pest insects species tested (except *M. aeneus*). Many samples were also very sensitive at a four times lower dose. But in 2006 already seven samples tested were not fully sensitive any more. But all samples tested at higher doses (0.0375 µg/cm² or more, although in the case of the least sensitive population only 0.075 µg/cm² was tested as the next higher rate) showed 100 % mortality.

Discussion

The results indicate for Germany a fast development of resistance to l-cyhalothrin of pollen beetles within few years, which is caused by the registration situation in Germany where only pyrethroids are registered for control of rape pest insects in spring. Sensitive individuals within pollen beetle populations are affected by the regular spring of pyrethroids and only resistant ones survive and are able to reproduce. Monitoring in Germany (unpublished data) showed that about 75 % of the rape growing area is affected by resistance which is similar to the 90 % of populations being resistant in the populations shown in Fig. 1. The populations tested indicate that a pyrethroid application into a population, as shown in Fig. 2 with 5 % highly resistant individuals, will results in about 50 % resistant individuals in the next spring providing that 95 % control is achieved on the sensitive individuals, there is no mixture of different populations, and that there is no difference in mortality during

winter time between susceptible and resistant beetles. So in the next year instead of about 95 % control only about 70 % control has to be expected using a pyrethroid again, which would explain the fast development of resistance in Germany. The nature of resistance was shown to be metabolic due to increased monooxygenase activities (Nauen, pers. comm.). Although not identified for *M. aeneus* until now, "knock down" resistance may develop in this pest as described for other arthropods. But this needs to be analysed in future studies.

The lesson to be learned from the resistance development of pollen beetle is that other pest insects with similar exposure should be kept under observation for resistance development. The data of other pest insects showed reduced sensitivity to pyrethroids for *Ceutorhynchus* spp. in 2006 compared to 2005. It is not clear yet whether this is natural variation in sensitivity or already the beginning of resistance. Further monitoring and research is needed to clarify this situation. Field data are needed to support the laboratory data if populations are showing less sensitivity in the laboratory. Sensitivity data of standardised laboratory trials on species in risk produced before the introduction of a pesticide into the market (as asked for in the resistance risk analysis paper of EPPO 2003) would enable a fast decision if relevant changes of sensitivity occurred or not.

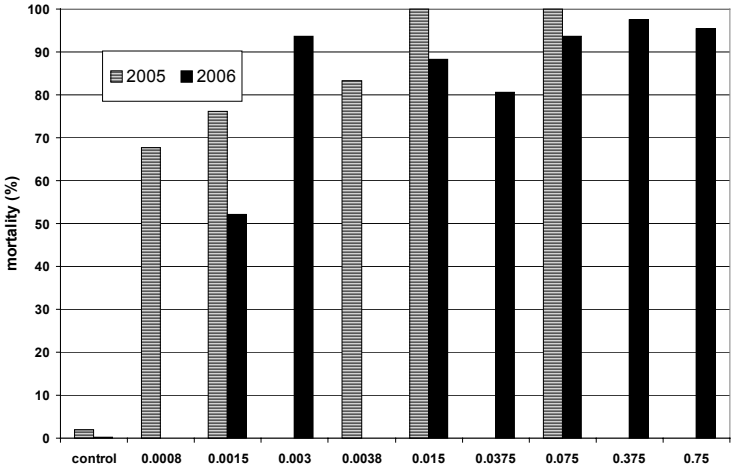

Fig. 2. Mean mortality of adult *M. aeneus* from one field near Braunschweig sampled in 2005 and 2006, five hours after exposure to different doses of l-cyhalothrin.

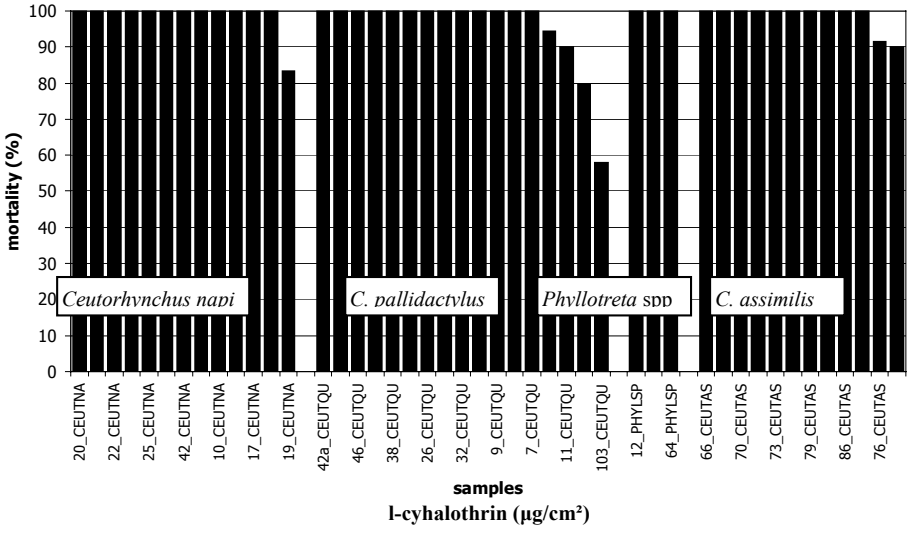

Fig. 3. Mean mortality of different oilseed rape pests collected in 2006, five hours after exposure to one dose of l-cyhalothrin (0.015 µg/cm²)

Conclusions

EPPO (2003) 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.

Because field tests are time consuming and expensive to screen for resistance, 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 an efficacy problem. Sensitivity data as demonstrated in this paper can show if there is a shift or change to resistance already in an early stage. Distinct differences in the results of laboratory tests between different populations from different regions or years indicate possible resistance.

The method as presented here is able to analyse rising and already existing problems with pollen beetles resistant to pyrethroids. But the method and interpretation of results can not be used for all other types of products (e.g. not for those that have systemic action, are acting slowly or have high volatility). It also needs to be validated by field experiences, if other pest insects (e.g. aphids probably not) can be tested in this way.

Acknowledgements

We thank all collectors of field samples (Schackmann, Burghause, Mayer, Raiser, Ostermeier, Faber, Schemmel, Nagel, Erichsen, Eichstaedt, Münkel, Ulber, Ernst, Ziegler, Rupprecht, Rippel, Klingenhagen, Weiske, Mühlberg, Krull, Eiselt, Ohnmacht, Vonnahme, Renzel, Havers, Kayser, Bayer, Schaper-Viedt, Graser, Kettel, Köhler, Dömpke, Landschreiber, Baumgartner, Kahl, Parker, Maker, Steingroever, Kühne, Schulz, Hanschke, Lehradt, Roßbach, Hoppe, Schröder, Weiser, Scheid) and the UFOP for a financial contribution.

References

Ballanger, Y., Detourne, D., Delorme, R., Pinochet, X. (2003). Difficulties to control pollen beetle (*Meligethes aeneus* F.) in France revealed by unusual high level infestations in winter rape fields - GCIRC, 11th International Rapeseed Congress, Copenhagen, 6-10 July 2003, **3**: 1048-1050.

Burghause, F., Jörg, E. (2005). Bald keine Wirkung mehr? DLG Mitteilungen Heft **4**, 40-44.

EPPO (2003). EPPO/OEPP-Standard PP1/213 (2), Resistance Risk Analysis. EPPO Bulletin **33**, 37 – 63.

Derron, J.O., Le Clech, E., Bezençon, N., Goy, G. (2004). Résistance des méligèthes du colza aux pyréthrinoïdes dans les bassin lémanique. Revue Suisse Agriculture **36**, 237-242.

Hansen, L.M. (2003). Insecticide-resistant pollen beetles (*Meligethes aeneus* F) found in Danish oilseed rape (Brassica napus L) fields. Pesticide 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 des Deutschen Pflanzenschutzdienstes **58**, 1-5.

Nauen, R. (2005). Insecticide resistance in European agriculture: Research instead of rumours. Proceedings BCPC – Crop Science & Technology 3A-1, 123-130.

Wegorek, P. (2005). Preliminary data on resistance appearance of Pollen Beetle PB (*Meligethes aeneus* F.) to selected pyrethroids, organophosphorous and chloronicotynyls insecticide, in 2004 year in Poland. Resistant Pest Management Newsletter **14**, No. 2: 19-21.