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A large field trial to assess the short-term and long-term effects of 4 insecticides used to control the pollen beetle on parasitic hymenoptera in oilseed rape

Jean-Pierre Jansen and Gilles San Martin Y Gomez
Plant Protection and Ecotoxicology unit, Life Sciences Department, Walloon Agricultural Research Centre, Gembloux, Belgium

Abstract: A large-scale field trial was performed in spring 2013 to assess the effects of Plenum (pymetrozine), Mavrik 2F (tau-fluvalinate), Biscaya (thiacloprid) and Pyrinex (Chlorpyriphos-ethyl) used to control the pollen beetle *Meligethes aeneus* (F.) (Col.; Nitidulidae) on the populations of pests and beneficial arthropods in winter oilseed rape. The insecticides were applied at their commercial rate just before flowering on large strips of oilseed rape (30 m x 200 m), divided into four plots of 50 m x 30 m. A strip was left untreated as control. Insects were sampled by plant beating methods and sweep netting the day after treatment and thereafter weekly up to 50 days after product application. The direct effects of the products were assessed on adult pollen beetle (target pest), adult cabbage seed weevil *Ceutorrhynchus obstrictus* (Marsham) (Col.; Curculionidae) (secondary pest) and adult parasitic hymenoptera associated with these insects (Tersilochinae and Pteromalidae). In the context of IPM, long term effects were assessed on pollen beetle larvae to determine their number, the parasitism rate and to estimate the balance of parasitic hymenoptera/pollen beetle that could be produced by the field for the next season.

Biscaya, Pyrinex and Mavrik 2F were effective in controlling the adult pollen beetle population and also had activity on cabbage seed weevil, despite the fact that this pest only occurred 2 or 3 weeks after the application of product. Plenum was only effective in controlling the pollen beetle population 1 day after treatment and had no significant impact on cabbage seed weevil.

All the insecticides tested had a significant impact on the population of adult parasitic hymenoptera compared to the control. Plenum had limited effects while the other insecticides reduced by 59-72% the numbers captured in the sweep net samples. The main effects were observed on adults of the Tersilochinae family that are mainly specialised in the parasitism of pollen beetle larvae. Biscaya also had a significant impact on numbers of Pteromalidae caught, a family containing species specialised in the parasitism of weevils, despite the 4-week delay between the day of the treatments and the first arrival of these hymenoptera in the crop.

Biscaya significantly reduced the parasitism rate of the pollen beetle larvae, which was reduced to less than 15% compared to 43.2% in the control. Pyrinex also decreased the parasitism rate with only 23% larval parasitism, but the difference was not significant. Biscaya and, to a lesser extend Pyrinex, reduced the balance of parasitic hymenoptera: pollen beetle, while Plenum and Mavrik 2F led to the same ratio as the untreated control. These results suggest that the regular use of Biscaya and/or Pyrinex on a large scale before flowering is favourable to the long term development of pollen beetle populations by negatively impacting the populations of their parasitoids, despite their good short-term efficacy to control this pest.

Key words: insecticide, side effects, pollen beetle, parasitic hymenoptera, parasitism

Introduction

The pollen beetle has become one of the most important pest problems in oilseed rape in Western Europe during these last years, mainly by the apparition of population resistant to insecticides (Thieme *et al.*, 2010). Now, in the context of the development of IPM, a specific attention is given to the possible natural control of this pest by its natural enemies. Specific hymenoptera belonging to the Tersilochinae subfamily (Hym.; Ichneumonidae) appeared to be as the key pollen beetle natural enemy (Ulber *et al.*, 2010a), with larval parasitism rates across Europe in the range of 25-50% in unsprayed crops, or even higher in some cases. However, these beneficial insects are exposed to several insecticides applied in spring in oilseed rape, when they are foraging for host location or in the crop where the adults emerged after the winter spent in the soil, mainly wheat drilled after the oilseed rape.

Several insecticides still in use in oilseed rape have already been assessed on pollen beetle parasites. Most of the products still in use were highly toxic for the adults, with reduction of the adult populations regularly higher than 50% compare to the untreated control. These products had also a negative impact on pollen beetle parasitism (Halden, 2004, Ulber *et al.*, 2010b). Compared to other pyrethroids insecticides, tau-fluvalinate (Mavrik 2F) appeared as the less toxic product, but the effects cannot be considered as negligible.

Due to resistance problems, several other insecticides were recently registered in oilseed rape, as chlorpyriphos-ethyl and thiacloprid. These product have not yet been assessed on Tersilochinae species, but at each time they were tested on a parasitic hymenoptera species, they were highly toxic (Van de Veire & Tirry, 2003; Medina *et al*., 2008; Jansen, 2010). Another insecticide with a totally different mode of action, pymetrozine, known to be effective mainly on aphids, has also been recently authorized in oilseed rape. This product was considered as selective for a large set of beneficial arthropods (Jansen *et al.*, 2011), including the parasitic wasp *Aphidius rhopalosiphi* (DeStefani-Perez) (Hym.; Aphidiidae), often used as an indicative species for the parasitic hymenoptera group. The aim of this work was to assess in the field the effects of these new products and of Mavrik 2F on parasitic hymenoptera, with specific assessments on pollen beetle parasitism.

Material and methods

Test products

The test was implemented in a commercial oilseed rape field cropped under the current agricultural practices for the area. The field was located at Onhaye (Ferme de Lenne, 50°13'24.82"N, 4°52'36.13"E) and 3.00 ha of the total field site (17.0 ha) was dedicated to the trial. No insecticides were applied before and during the tests, except the test products, while the rest of the field was treated on the 24th April with Mavrik 2F. The entire field was treated with a fungicide during flowering (Cantus 0.5 kg/ha, Boscalid 50% WG). The field site was divided into 5 strips of 30 m x 200 m that received one of the 4 insecticide treatment or was left untreated (control). Each strip was divided into four plots of 30 m x 50 m, each plot being considered as a replicate. All samples and visual counts were done in the 20 m x 40 m central part of each plot. The field was nearly completely surrounded by woods.

The test insecticides were: Plenum (Pymetrozine, 500g/l, WG), applied at 150 g/ha, Biscaya (Thiacloprid, 240 g/l, OD) applied at 300 ml/ha, Mavrik 2F (tau-fluvalinate, 240 g/l, EW) applied at 200 ml/ha, and Pyrinex (Chlorpyriphos-ethyl, 240 g/l CS) applied at 750 ml/ha. The doses correspond to the commercial recommended rates. The insecticides were applied on the 26th of April at the end of the bud stage (GS 3.6-3.7), on basis of a volume of 200 l spray mixture/ha.

Sampling techniques and organization

Different sampling techniques were used during the experiments, according to the main target insects and the climatic conditions: plant beatings (into plastic trays and funnels) and sweep netting methods. The details of the sampling methods used on each assessment dates are given in Table 1.

Table 1. Schedule of the sampling methods used. DAT= days after treatment, DBT= days before treatment.

Sampling method	DBT5	DAT1	DAT8	DAT13	DAT22	DAT29	DAT36	DAT43	DAT50
Tray beating	X	X	X	X					
Funnel beating					X	X			
Sweep net		(X)[1]	X	X			X	X	X

[1] only in the control to detect first adults of parasitic hymenoptera. Not found at this date and not performed in the other plots.

Before the first occurrence of pollen beetle larvae, plant beating was used to assess the adult pollen beetle population. The terminal part (stem and flower buds) of 20 plants randomly selected in each plot were shaken just above a plastic tray (40 x 30 x 8 cm). The insects that fell onto the trays (mainly pollen beetle) were immediately identified, counted then released. Several weevils were also counted and recorded.

When the first larvae of pollen beetle were detected, the “funnel beatings” method was used for sampling, to allow the collection of pollen beetle larvae. This method was also used to assess populations of parasitic hymenoptera when sweep net sampling was not possible, due to wet weather conditions. During ‘funnel beating’, 20 terminal racemes were randomly selected from each plot and shaken just above a plastic funnel (Ø 30 cm) placed into a plastic bottle (250 ml). The insects that fell on the funnel were rinsed with water (+ commercial soap) to collect them into the plastic bottle and they were returned to the laboratory for counting, identification and determination of the parasitism rates for the pollen beetle larvae.

For sampling of parasitic hymenoptera, reinforced nets (Ø 35 cm) were used, with 10 side-to-side sweeping moves (sampling of around 3-4 m^2). The sweep nets were emptied over a funnel, identical to those used for the funnel samplings. Adults and larvae of pollen beetle and adult weevils were also collected with this method, with results comparable to the funnel samplings (based on a comparison made in the control).

Sampling started 5 days before the application of products and were performed 1 day after the product applications (DAT1) and then at 7 (± 1 day) intervals until 50 DAT, corresponding to the end of flowering.

Analysis

The short term effects, that concerned the field where the products were applied and the longer-term effects that concern the pest: beneficial ratio for the following years were both considered.

The short-term effects were assessed on adults of pollen beetle (target pest), adults of seed weevils (secondary pest) and adults of the parasitic hymenoptera, identified at the family level for Tersilochinae (mainly pollen beetle larval parasitoids) and Pteromalidae (mainly seed weevil larval parasitoids).

The long-term effects were assessed on pollen beetle larval populations and pollen beetle larval parasitism. These two results were used to estimate the balance between the numbers of adult parasitoids and adult pollen beetles produced by the treated area for the next growing season. These were calculated as follows for each plot:

$$R = (Lp/Lnp) * 100$$

R = balance; Lp = pollen beetle larvae parasitized sampled in the corresponding plot; Lnp = pollen beetle larvae unparasitized sampled in the corresponding plot

An R balance value of 100 indicates that the plot will produce the next year the same number of adult parasitoids as adult pollen beetles, taking as working hypothesis that all larvae are viable, and would produce an adult for the next season. This hypothesis is probably false as several factors can affect the success of the larval development, especially for the parasitoids which overwinter in the soil and are susceptible to tillage effects, but this method is simpler than the direct assessment of the emergence of adult beetles and parasitoids (Nilsson, 2010) However, as the factors affecting the larval development of both pollen beetle and parasitoid are not dependent on the experimental treatments, this method provides a useful rough assessment to compare the effects of different insecticides regimes.

Statistical analysis
The data were analysed for differences using one-way Anova tests (R software). Pair-wise comparisons were performed using Tukey (multiple comparisons) and Dunet-test (comparison to the control for efficacy results) at the 0.05 level. Data were log transformed if necessary to normalise the distribution before analysis.

Results

Adults of pollen beetle
The number of adult pollen beetles sampled at the different dates is given in Table 2. All treatments were effective one day after product application, with a reduction of pollen beetle population ranging from 82-100%. Eight days after treatments, Mavrik 2F, Biscaya and Pyrinex reduced significantly the populations, with a range of 76-100% efficacy. Plenum was no longer effective with only 20% reduction. At DAT13, only Pyrinex reduced significantly the pollen beetle populations compared to the control. It must be noted that the populations were reduced in the control on this date and that the populations were low in all treatments with proportionally higher variability. Some adult pollen beetles were also sampled from DAT22 to DAT50 but these results were not taken into consideration as numbers were very low (mean of 1.1 pollen beetle/assessment in the control) and the crop was no longer sensitive to pollen beetle, due to having progressed well into the flowering growth stages (during which time the crop is no longer susceptible to pollen beetle damage).

When the assessments carried out at the different dates were pooled together, the results indicated that all the treatments significantly reduced pollen beetle population when directly compared to the control, but Plenum was less effective than the 3 other insecticides.

Cabbage seed weevil
The cabbage seed weevil was not the target of the trial for the selection of products and the timing of the application, but the populations sampled at the same time as the pollen beetle were high and allowed us to assess the impact of the insecticides on this species. The results

are listed in Table 3. The data have been pooled in two groups to facilitate statistical analysis: the early populations (DAT1-13), from bud stage to the beginning of flowering with a first population peak at DAT13 (9.0 weevils in the control) and the later population, when the plants were flowering (DAT22-50), with a peak at DAT36 (16.25 weevils in the control). The results showed that all products except Plenum had an impact on the cabbage weevil population; even if the effects were lower than for the Pollen beetle.

Table 2. Mean number of adult pollen beetles collected ± sd at different dates after treatments (DAT) and efficacy compared to control.

	DAT1	DAT8	DAT13	Total
Control	16.25 ± 4.2 a	11.25 ± 4.6 a	6.50 ± 2.1 ab	34.00 ± 2.2 a
Plenum (pymetrozine)	1.50 ± 1.9 bc	9.00 ± 3.9 ab	9.00 ± 6.7 a	19.50 ± 12.1 ab
Mavrik 2F (tau-fluvalinate	1.00 ± 0.8 bc	1.75 ± 2.1 cd	3.00 ± 1.8 ab	5.75 ± 3.3 cd
Biscaya (thiacloprid)	1.25 ± 0.5 bc	2.00 ± 1.4 cd	3.25 ± 1.9 ab	6.50 ± 2.6 c
Pyrinex (chlorpyriphos-ethyl)	0.50 ± 1.0 bc	0.50 ± 1.0 cd	2.00 ± 1.2 bc	3.00 ± 1.6 cd
Control	*- a*	*- a*	*- a*	*- a*
Plenum	*91% b*	*20%a*	*-38% a*	*43% b*
Mavrik 2F	*94% b*	*84% b*	*54% a*	*83% b*
Biscaya	*92% b*	*82% b*	*50% a*	*81% b*
Pyrinex	*96% b*	*96% b*	*69% b*	*91% b*

GLM, One-way Anova (p = 0.05 level) followed by pair-wise Tukey test for population (results followed by the same letter are not statistically different) and Dunet test for efficacy (a = not different to control, b = different to control).

Table 3. Mean number of adult seed weevils collected ± sd at different dates after treatments (DAT) and efficacy compared to the control.

	DAT1-13	DAT22-50	Total
Control	13.00 ± 0.8 a	29.00 ± 13.3 a	42.00 ± 13.5 a
Plenum (pymetrozine)	9.25 ± 2.6 b	22.50 ± 9.4 a	31.75 ± 9.7 ab
Mavrik 2F (tau-fluvalinate	3.50 ± 0.6 bcd	17.00 ± 13.4 bc	20.5 ± 13.6 bc
Biscaya (thiacloprid)	3.25 ± 1.7 cd	8.00 ± 5.2 c	11.25 ± 4.0 c
Pyrinex (chlorpyriphos-ethyl)	7.25 ± 2.1 bc	11.75 ± 4.4 bc	19.0 ± 6.1 c
Control	*- a*	*- a*	*- a*
Plenum	*29% a*	*22% a*	*24% a*
Mavrik 2F	*73% b*	*41% b*	*51% b*
Biscaya	*73% b*	*75% b*	*72% b*
Pyrinex	*55% b*	*44% b*	*59% b*

GLM, One-way Anova (p = 0.05 level) followed by pair-wise Tukey test for population (results followed by the same letter are not statistically different) and Dunet test for efficacy (a = not different to control, b= different to control).

Parasitic hymenoptera

The numbers of adult parasitoids sampled during the experiments are listed in Table 4. Results of the different assessments have been pooled for statistical analysis. The two main families of hymenoptera found were Tersilochinae (mainly endoparasitoids of pollen beetle larvae) and Pteromalidae (mainly exoparasitoids of seed weevil larvae). The Tersilochinae were mostly sampled between DAT1 and 13, with more than 60% of the adults sampled at this last date in the control. The Pteromalidae were collected later with a first record at DAT29. These dates were linked to the presence of the hosts (pollen beetle larvae and seed weevil larvae). The identification of these two families to the species level is planned. A few adults of the Braconidae *Diospilus capito* (0 to 0.5/sample in total) were also identified from the samples. As this species is also associated with pollen beetle larvae, these records were added to the results of the Tersilochinae. A few adult parasites of other families, probably parasitoids of brassica pod midge, were also identified but not analysed separately, as the populations were very low (0.0 to 0.5 specimens by object in total).

Table 4. Mean number of adult parasitic wasps collected ± sd between DAT1 and DAT50 and reduction of the populations compared to the control. Tersilochinae (pollen beetle parasitoids), Pteromalidae (seed weevil parasitoid) and total, including the other families.

	Tersilochinae	Pteromalidae	Total
Control	36.00 ± 9.8 a	7.75 ± 2.2 a	43.75 ± 10.6 a
Plenum (pymetrozine)	24.25 ± 6.2 b	6.00 ± 3.2 a	30.75 ± 5.6 b
Mavrik 2F (tau-fluvalinate	10.50 ± 6.6 c	5.25 ± 2.4 a	16.25 ± 6.8 c
Biscaya (thiacloprid)	10.00 ± 7.0 c	3.25 ± 2.1 b	13.50 ± 8.7 c
Pyrinex (chlorpyriphos-ethyl)	6.50 ± 7.0 c	5.25 ± 1.5 a	12.25 ± 8.5 c
control	*- a*	*- a*	*- a*
Plenum	*32.6% b*	*23% a*	*30% b*
Mavrik 2F	*70.8% b*	*32% a*	*63% b*
Biscaya	*72.2% b*	*58% b*	*69% b*
Pyrinex	*81.9% b*	*32% a*	*72% b*

GLM, One-way Anova (p = 0.05 level) followed by pair-wise Tukey test for population (results followed by the same letter are not statistically different) and Dunet test for efficacy (a = not different to control, b = different to control).

The adults of Tersilochinae were affected by all the treatments. Plenum was the least toxic product for this family, with a reduction of 32.6% compared with the control. The other products have a similar pattern with a reduction of adult populations of 70-80% compared to the control.

Only one insecticide, Biscaya, significantly affected the Pteromalidae adult population when directly compared to the control.

The total adult parasitic hymenoptera population reductions followed the same trends as the Tersilochinae results, with a limited effect of Plenum and a more important effect of all other treatments. This could be explained by the Tersilochinae records, which were the most abundant group in the samples.

Long term effects – Pollen beetle larvae and parasitic hymenoptera larvae

The number of pollen beetle larvae and their parasitism rates are listed in Table 5. The long term effects of the product, assessed by estimating the number of parasitoids produced for 100 pollen beetles is also included in this table.

Table 5. Mean number of pollen beetle larvae collected ± sd between DAT1 and DAT50, parasitism rate and balance adult parasite/pollen beetle.

	Total larvae	% parasitism	Adult parasitoid/100 pollen beetle
Control	122.3 ± 60.8 a	43.2% ± 8.2% a	79.1 ± 27.9 a
Plenum (pymetrozine)	92.5 ± 50.5 a	34.5% ± 5.2% a	53.4 ± 11.4 a
Mavrik 2F (tau-fluvalinate	30.3 ± 14.2 b	32.3% ± 6.6% ab	48.7 ± 15.0 ab
Biscaya (thiacloprid)	19.8 ± 18.1 bc	14.5% ± 4.4% b	17.2 ± 6.1 c
Pyrinex (chlorpyriphos-ethyl)	8.5 ± 1.30 c	23.3% ± 8.0% ab	31.4 ± 13.0 bc

GLM, One-way Anova (p = 0.05 level) followed by pair-wise Tukey test (results followed by the same letter are not statistically different).

All products except Plenum significantly reduced the number of pollen beetle larvae found in the treated plots. Pyrinex was the most toxic product. The analysis of the parasitism rate of these larvae indicated that all products except Biscaya had no significant impact on the parasitism rate of the pollen beetle larvae compared with control plots. Pyrinex also had a large effect on the parasitism rates but the differences to the control were only marginally significant ($0.05 < p < 0.10$). The ratios of adult parasitoids that will be produced by the treated plots per 100 pollen beetles confirm these observations, with a significant negative impact observed for Biscaya and Pyrinex, compared to the control and no differences between the control, Mavrik 2F and Plenum.

Discussion

The products assessed during this study have shown different effects on pest and beneficial insect populations. In terms of adult pollen beetle control, all treatments have good efficacy directly after application. Plenum had however limited effect one week after application while the other products were still effective. In the test conditions, with the treatment applied just before flowering, all products, except perhaps Plenum, can be used to control adult pollen beetle.

Most of the insecticides applied to control pollen beetle before flowering had also an impact on adults of the cabbage seed weevil, even if the first significant population of this insect was detected until DAT13 in the control. Plenum was ineffective against the seed weevil while Biscaya, Mavrik 2F and Pyrinex reduced the population in the range of 49-70% compared to the control. These values may not perhaps be sufficient for effective chemical control of this pest, but timing of treatment was not ideal. Our data indicate the residual activity of treatments made 2 weeks before arrival of the pest are not negligible and perhaps sufficient for control in crops with a low seed weevil pressure.

The effects of the products on adult parasitic wasps were high for most of the products, with a reduction of 60-70% of the populations. Plenum was the only product with limited effects (30%) when compared to the control. When the hymenoptera families were identified and related to their expected hosts, the main effects were observed on adults of Tersilochinae, mostly associated to pollen beetle. The effects on Pteromalidae, which are mainly associated to the cabbage seed weevil, were limited and non-significant for all of the products, except Biscaya. As these hymenoptera arrived later in the season (first observation 29 days after the application of the insecticides), these results indicated that Plenum, Mavrik 2F and Pyrinex, applied soon before flowering to control pollen beetle, had no significant impact on the main cabbage seed weevil parasitoid family. The only exception was Biscaya, with a significant impact on these insects despite the 4-week delay between the application and the colonization of the crop by these beneficials.

The effects of the insecticides on pollen beetle larvae indicated that most of the products reduced the total number of pollen beetle larvae found on the plants, and the parasitism rates by Tersilochinae were not affected compared to the control with Mavrik 2F and Plenum. However, Biscaya and, to a lesser extent, Pyrinex, had a different effect profile and reduced the parasitism rate of the pollen beetle larvae and the ratio of parasitoids: pest, with significant differences compared to the control. This could indicate that the repeated use of these insecticides could affect the population of beneficial parasitic hymenoptera and should not be recommended in the context of IPM.

These conclusions were, however, only based on one year’s trial data and need to be carefully replicated in future experiments.

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