

A large field trial to assess the short-term and long-term effects of 5 insecticides used to control the pollen beetle on parasitic hymenoptera in oilseed rape

Jean-Pierre Jansen¹, Gilles San Martin Y Gomez¹

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), Boravi WG (Phosmet) and Pyrinex (Chlorpyrifos-ethyl) used to control the pollen beetle *Meligethes aeneus* (F.) (Col.; Nitidulidae) on pest and beneficial arthropods' populations in winter oilseed rape. The insecticides were applied at their commercial rate soon 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 weekly sampled with the help of beating methods and sweep net from one day 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 of parasitic hymenoptera related to 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, Boravi WG, Pyrinex and Mavrik 2F were effective to control the adult pollen beetle population and had also an 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 to control 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 adult parasitic hymenoptera population compared to the control. Plenum had limited effects while the other insecticides reduced of 59% to 72% the captures in the sweep net samplings. The main effects were observed on adult of the Tersilochinae family that are mainly specialised in the parasitism of pollen beetle larvae. Biscaya had also a significant impact on Pteromalidae captures, 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, that dropped to less than 15% compared to 43.2% in the control. Pyrinex also decreased the parasitism rate with only 23% of larval parasitism, but the differences were not significant. Biscaya and, to a lesser extent Pyrinex, reduced the balance of parasitic hymenoptera/pollen beetle while Plenum, Boravi WG and Mavrik 2F led to the end of the growing season to the same ratio than 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.

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.*, 2006, 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 chlorpyrifos-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). 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 0.5kg/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 samplings 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 testes insecticides were Plenum (Pymetrozine, 500 g/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, Boravi WG (Phosmet, 750 g/kg, WG) applied at 1.5 kg/ha and Pyrinex (Chlorpyrifos-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 followed and the climatic conditions: beatings (plastic trays and funnels) and sweep net methods. The details of the sampling methods used at each dates are given in Table 1.

Before the first occurrence of pollen beetle larvae, beating methods in plastic vessels were used to assess the adult pollen beetle population. The terminal part (stem and flower buds) of 20 plants randomly selected into each plots were shaken just above a plastic tray (40 x 30 x 8 cm). The insects that fell on the trays (mainly pollen beetle) were directly identified, counted and released. Several weevils were also collected and counted.

When the first larvae of pollen beetle were detected, this method was replaced by “funnel beatings”, that allowed to harvest pollen beetle larvae. This method was also used to assess parasitic hymenoptera populations when the sweep net samplings were not possible, due to very bad weather conditions (vegetation continuously wetted). 20 terminal part of plants (stem, flowers and flower bud) randomly selected into each plots were shaken just above a plastic funnel (Ø 30 cm) placed under a plastic bottle (250 ml). The insects that fell on the funnel were rinsed with water (+ commercial soap) to collect them on the plastic bottle that were brought back to the laboratory for counting, identification and determination of the parasitism rates for the pollen beetle larvae.

For parasitic hymenoptera sampling, reinforced nets (Ø 35 cm) were used, with 10 go-back moves (sampling of around 3-4 m²). The sweep nets were emptied under a funnel, identical to those used for the funnel samplings. Adult 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).

The samplings started 5 days before the application of products and were performed 1 day after the product applications (DAT1) and then at 7 (\pm 1 day) interval till 50 DAT, corresponding to the end of flowering.

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.

Presentation of the results

The short term effects, that concerned the field where the products were applied and the longer-term effects that concern the pest/beneficial balance for the following years were both taken into consideration.

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

The long-term effects were assessed on pollen beetle larvae populations and pollen beetle larvae parasitism. These two results were used to estimate the balance between adult parasitoid and adult pollen beetle that will be produced by the treated area for the next growing season. These balances were calculated as follow for each plot:

$$R = (L_p/L_{np}) * 100$$

R = balance; L_p = pollen beetle larvae parasitized sampled in the corresponding plot; L_{np} = pollen beetle larvae unparasitized sampled in the corresponding plot

A R value = 100 is indicating that the corresponding plot will produce the next year the same number of adult parasitoid than adult pollen beetle, taking as working hypothesis that all larvae gave an adult for the next season. This hypothesis is probably false but the factors that affect the success of the larval development of both groups into the soil after the season are mainly depending on the tillage methods and the winter conditions and not of the insecticides applied as spray. Thus, the estimation of these ratios could be a basis for a comparison of long-term adverse effects of products and untreated control.

Statistical analysis

The results were analysed with the help of R software. One-way Anova tests were performed at 0.05 level. Pair-wise comparisons were performed using Tukey (multiple comparisons) and Dunet-test (comparison to the control for efficacy results) at 0.05 level. Several data were log transformed 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 91% to 96%. 8 days after treatments, Mavrik 2F, Boravi WG, Biscaya and Pyninex reduced significantly the populations, with a range of 82% to 96% efficacy. Plenum was not longer effective with only 20% of reduction. At DAT13, only Pyninex and Boravi WG reduced significantly the pollen beetle populations compared to the control. It must be noted that the populations have dropped down in the control at this date and that the populations were low in all objects with proportionally higher variability of the results. The residual population of pollen beetles sampled after these dates were not considered, as the crop was no longer sensitive to pollen beetle, due to the general flowering of the plants.

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 in a same time as the pollen beetle were high and allowed to assess the impact of the insecticides on this insect. The results are listed in Table 3. The data have been pooled in two groups to facilitate the statistical analysis: the early populations (DAT1-13), from bud stage to beginning of flowering with a first peak of population at DAT13 (9.0 beetles in the control) and the late populations, when the plants were flowering (DAT22-50), with a peak population at DAT36 (16.25 beetles 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. These results are indicating that these insecticides, except Plenum, applied before flowering to control pollen beetle populations, can also have a non negligible impact on seed weevil populations.

Table 2: Mean number of adult pollen beetle collected \pm sd (mean of 4 replicates) at different dates after treatments (DAT) and efficacy compared to control.

	DAT1	DAT8	DAT13	Total
Control	16.25 \pm 4.2 a	11.25 \pm 4.6 a	6.50 \pm 2.1 a	34.00 \pm 2.2 a
Plenum (pymetrozine)	1.50 \pm 1.9 b	9.00 \pm 3.9 a	9.00 \pm 6.7 a	19.50 \pm 12.1 ab
Mavrik 2F (tau-fluvalinate)	1.00 \pm 0.8 b	1.75 \pm 2.1 b	3.00 \pm 1.8 ab	5.75 \pm 3.3 c
Biscaya (thiacloprid)	1.25 \pm 0.5 b	2.00 \pm 1.4 b	3.25 \pm 1.9 ab	6.50 \pm 2.6 bc
Boravi WG (Phosmet)	1.00 \pm 1.4 b	0.50 \pm 0.6 b	0.75 \pm 1.5 c	2.25 \pm 2.1 c
Pyrinex (chlorpyriphos-ethyl)	0.50 \pm 1.0 b	0.50 \pm 1.0 b	2.00 \pm 1.2 bc	3.00 \pm 1.6 c
<i>Control</i>	- a	- a	- a	- a
<i>Plenum</i>	91% b	20% a	-38% a	43% a
<i>Mavrik 2F</i>	94% b	84% b	54% a	83% b
<i>Biscaya</i>	92% b	82% b	50% a	81% b
<i>Boravi WG</i>	94% b	96% b	88% b	93% b
<i>Pyrinex</i>	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 \pm sd (mean of 4 replicates) at different dates after treatments (DAT) and efficacy compared to control.

	DAT1-13	DAT22-50	Total
Control	13.00 \pm 0.8 a	29.00 \pm 13.3 a	42.00 \pm 13.5 a
Plenum (pymetrozine)	9.25 \pm 2.6 ab	22.50 \pm 9.4 ab	31.75 \pm 9.7 b
Mavrik 2F (tau-fluvalinate)	3.50 \pm 0.6 c	17.00 \pm 13.4 bc	20.5 \pm 13.6 c
Biscaya (thiacloprid)	3.25 \pm 1.7 c	8.00 \pm 5.2 c	11.25 \pm 4.0 c
Boravi WG (Phosmet)	5.50 \pm 3.0 bc	9.00 \pm 4.2 c	14.50 \pm 5.8 c
Pyrinex (chlorpyriphos-ethyl)	7.25 \pm 2.1 bc	11.75 \pm 4.4 c	19.0 \pm 6.1 c
<i>Control</i>	- a	- a	- a
<i>Plenum</i>	29% a	22% a	24% b
<i>Mavrik 2F</i>	73% b	41% b	51% b
<i>Biscaya</i>	73% b	75% b	72% b
<i>Boravi WG</i>	58% b	69% b	65% b
<i>Pyrinex</i>	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 adults

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 hymenoptera family 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). A few adults of the Braconidae *Diospilus capito* (0 to 0.5/sample in total) were also harvested. As this species was also related to the 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 sampled but not analysed separately, as the populations were very low (0.0 to 0.5 specimen by object in total).

The adults of Tersilochinae were affected by all the treatments. Plenum was the less toxic product for this family, with a reduction of 32.6% of the captures. The other products have a similar pattern with 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 parasitic hymenoptera adult population reductions followed the same trends than the Tersilochinae results, with a limited effect of Plenum and a more important effect of all other treatments. This could easily be explained by the weight of the Tersilochinae records, that were the most abundant group in the samples.

Table 4: Mean number of adult parasitic wasps collected \pm sd (mean of 4 replicates) between DAT1 and DAT50 and reduction of the populations compared to the control. Tersilochinae (pollen beetle parasites), Pteromalidae (seed weevil parasite) and total, including the other families.

	Tersilochinae	Pteromalidae	Total
Control	36.00 \pm 9.8 a	7.75 \pm 2.2 a	43.75 \pm 10.6 a
Plenum (pymetrozine)	24.25 \pm 6.2 b	6.00 \pm 3.2 a	30.75 \pm 5.6 b
Mavrik 2F (tau-fluvalinate)	10.50 \pm 6.6 c	5.25 \pm 2.4 a	16.25 \pm 6.8 c
Biscaya (thiacloprid)	10.00 \pm 7.0 c	3.25 \pm 2.1 b	13.50 \pm 8.7 c
Boravi WG (Phosmet)	7.25 \pm 3.8 c	5.75 \pm 1.9 a	13.25 \pm 5.6 c
Pyrinex (chlorpyrifos-ethyl)	6.50 \pm 7.0 c	5.25 \pm 1.5 a	12.25 \pm 8.5 c
<i>control</i>	- a	- a	- a
<i>Plenum</i>	32.6% b	23% a	30% b
<i>Mavrik 2F</i>	70.8% b	32% a	63% b
<i>Biscaya</i>	72.2% b	58% b	69% b
<i>Boravi WG</i>	79.9% b	26% a	70% b
<i>Pyrinex</i>	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).

Long term effects – Pollen beetle larvae and parasitic hymenoptera larvae

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

All products except Plenum significantly reduced the number of pollen beetle larvae produced by the treated plots. Pynrex was the most toxic. The analysis of the parasitism rate of these larvae indicated that all products except Biscaya (significant differences) have no impact on the parasitism rate of the pollen beetle larvae. Pynrex has also a higher effect on the parasitism rates but the differences to the control were only marginally significant ($0.05 < p < 0.10$). The ratio adult parasite that will be produced by the treated plots by 100 pollen beetles confirm these observations with a significant impact of Biscaya and Pynrex, compared to the control and no differences between the control, Mavrik 2F, Boravi WG and Plenum.

Table 5: Mean number of pollen beetle larvae collected \pm sd (mean of 4 replicates) between DAT1 and DAT50, parasitism rate and balance adult parasite/pollen beetle.

	Total larvae	% parasitism	Adult parasite/100 pollen beetle
Control	122.3 \pm 60.8 a	43.2% \pm 8.2% a	79.1 \pm 27.9 a
Plenum (pymetrozine)	92.5 \pm 50.5 a	34.5% \pm 5.2% a	53.4 \pm 11.4 ab
Mavrik 2F (tau-fluvalinate)	30.3 \pm 14.2 b	32.3% \pm 6.6% ab	48.7 \pm 15.0 ab
Biscaya (thiacloprid)	19.8 \pm 18.1 bc	14.5% \pm 4.4% b	17.2 \pm 6.1 c
Boravi WG (Phosmet)	14.5 \pm 9.5 bc	36.6% \pm 27.2% a	80.0 \pm 72.6 a
Pynrex (chlorpyrifos-ethyl)	8.5 \pm 1.30 c	23.3% \pm 8.0% ab	31.4 \pm 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).

Discussion

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

Most of the insecticides applied before flowering had also an impact on adults of the cabbage seed weevil, even if the first significant population of this insect was detected at DAT13 in the control and the timing of application of the insecticide therefore not ideal. Plenum was ineffective against the seed weevil while Biscaya, Boravi WG, Mavrik 2F and Pynrex reduced the population in the range 49-70% compared to the control. These values could perhaps not be sufficient for an effective chemical control of this pest, but the residual activity of treatments made 2 weeks before arrival of the pest are not negligible and perhaps sufficient in several fields with a low or moderate seed weevil pressure.

The effects of the products on adult parasitic wasps were high for most of the products, with a reduction of 60 to 70% of the populations. Plenum was the only product with limited effects when compared to the control, with 30% of reduction. When the hymenoptera family were identified and related to their expected hosts, the main effects were observed on adults of Tersilochinae, mostly related to pollen beetle. The effects on Pteromalidae, which are mainly related 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, Boravi WG, Mavrik 2F and Pyrinex, applied soon before flowering to control pollen beetle, had no impact on the main cabbage weevil parasitoid family. The only exception was Biscaya, with a significant impact on these insects despite of 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 if most of the products reduced the total number of pollen beetle larvae, the parasitism rates by Tersilochinae were not affected compared to the control with Boravi WG, Mavrik 2F and Plenum. These insecticides could be more or less evenly toxic for the pest than for the beneficial and in final, the long-term use of these compounds won't probably lead to beneficial control disruption when applied before flowering. Biscaya and, to a lesser extent, Pyrinex, had a different profile and reduced the parasitism rate of the pollen beetle larvae and the ratio beneficial/pest, with significant differences when compared to the control. The repeated use of these insecticides would probably affect the population of parasitic hymenoptera and are not recommended in the context of IPM.

These conclusions were however only based on one year trial and need to be replicate in the future. However, the fact that Biscaya significantly impacted the population of the main parasitic hymenoptera family related to a secondary pest, did not speak in favour of this kind of product in the context of sustainable agriculture.

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