

A field study to assess the effects of insecticides used to control the Colorado beetle in potato on aphid antagonists

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Abstract: The effects of five insecticides used to control the Colorado beetle in Potato were assessed in a field trial on aphid-specific beneficial arthropod fauna, mainly parasitic hymenoptera, ladybirds, lacewings and hoverflies. The insecticides were applied at their commercial rate at the end of July, when beneficial arthropods and aphid population were at their maximum. The effects on aphid populations were followed by visual inspection up to 33 days after treatment and aphid natural enemies were collected by the beating methods 2, 7 and 18 days after treatment.

The results of the beatings showed that all insecticides had an impact on both ladybird and hoverfly larvae. The impact on ladybird was limited for azadirachtin, rynaxypyr and spynosin B while cypermethrin and thiametoxam were more toxic. Hoverfly were more sensitive to all the insecticides tested, with population reduction > 50% compared to the control. Spynosin B also reduced the parasitism of aphid by *Aphidius* wasps at DAT2, determined by dissection of aphid collected during the beatings, but the effects were limited in duration and no significant effects were observed later. The other product had no or limited effects on aphid parasitism.

The effects on aphid populations revealed that no insecticides were promoting aphid outbreak by directly impacting natural enemy's populations. However, the beneficial arthropods activity was so high that even high reductions of populations had no impact on aphid population in this particular field site.

These results are discussed in term on beneficial populations and IPM, taking into accounts both direct effects and long-term effects.

Introduction

The Colorado beetle *Leptinotarsa decemlineata* (Say) (Col.; Chrysomelidae) and several aphid species are the two main pest problem in potato produced for the fresh market and the industry in Belgium. The aphids are most of the time controlled by beneficial organisms naturally occurring in the environment, with parasitic hymenoptera, hoverfly, ladybird and lacewings larvae identified as the key beneficial arthropods (Jansen, 2002; Jansen & Warnier, 2004; Jansen, 2005). The activity of these insects limit the need for insecticide application, with a mean of one treatment recommended every 3 to 4 years during the period 1994-2013. When an insecticide application is needed, products that are selective for these beneficial are recommended in order to maintain this biological control effective at short and long term (Hautier *et al.*, 2006).

If the Colorado beetle is most of the time scarce in Belgium, it can be locally abundant and their population need to be controlled by insecticides. These treatments can interfered with the biological control of aphid and several non-selective products, as organophosphorous compounds are known to promote the aphids by negatively impacting hoverflies, ladybirds, lacewings and parasitic hymenoptera.

Since a few years, several newly registered insecticides as rynaxypyr, spinosad and neem extract are available to control the Colorado beetle. The effects of these products on aphid

natural enemies occurring in potato are however poorly known, with most of the results obtained in the laboratory. The aim of this study is to assess the effects of these insecticides in the field on potato aphid biological control. In addition to these products, a pyrethroid and a néonicotinoïde insecticide have been added to the study as reference products.

Material and methods

Test products

The insecticides tested were all registered in Belgium to control the Colorado beetle: Actara (Thiametoxam, WG, 250g a.i./kg) applied at 80 g of product/ha, Cytox (Cypermethrin, EC, 100 g/l) applied at 100ml/ha, Neemazal TS (Azadirachtine, EW, 10 g/l) applied at 2.5 l/ha, Tracer (Spinosad, SC, 480 g/l) applied at 50ml/ha and Coragen (Rynaxypyr, SC, 200 g/l) applied at 50 ml/ha. They were applied the 25th of July at their recommended field rate. The date of the treatment was selected to apply the products when the aphids and aphid natural enemy populations were at their maximum or close to. The products were applied with the help of a 11 m-wide sprayer with 110°-flat-fan nozzle at 50 cm spacing, with a spray volume of 300 l/ha.

Field site and test design

The test was implemented at Bierwart (Belgium, Namur), in a 4.50 ha commercial field (cv Elodie). The field, including the experimental plots, was repeatedly treated against the late blight (one fungicide application per week in average). The fungicides used (Fluazinam, propamocarb, mancozeb, ...) were all rated as green in the potato selectivity list and were selective against the aphid specific natural enemies. No insecticides were applied before the start of the experimentation. The test was performed on 1.32 ha, divided into 6 strips of 11 m x 200 m. 5 strips were treated with a test product and a strip was left untreated as control. Each strip was further divided into 4 plots of 50 m x 11 m and each plot was considered as a replicate. The samplings were performed in the 8 m x 40 m central part of each plot.

Insect sampling

The aphid populations were estimated by visual counting (50 full leaves/replicates, 4 x 50 leaves per object). Half of the leaves were taken in the low-mid part of the plant and the other half on the mid-upper part of the plant. The visual counts were performed shortly before the application of the product (one replicate/object) and 2 days, 14 days and 34 days after product application. All aphid natural enemies found during the visual counts (aphid parasite mummies, entomopathogenic fungi aphid mummies, eggs and larvae of ladybird, hoverfly and lacewings) were noted and these results were used to estimate the “Beneficial Arthropod Index” (BIx). This index give an indication on the importance of the aphid natural enemy population compared to the aphid population and is calculated as followed:

$$\text{BIx} = \left(\frac{\text{pondered sum of beneficial arthropods counted at one occasion}}{\text{the number of aphid count at the same occasion on the same leaves}} \right) * 100$$

The pondered sum of beneficial arthropods is obtained by giving a value of 1 to all aphid predator larvae counted and a value of 0.2 to all parasitized aphids (aphid mummies) and predator eggs.

This index has been developed in the context of aphid advisory systems and IPM in Belgium to estimate to possible biological control of aphids. An index < 2 is indicating an unfavourable balance for beneficial (increase of aphid population and possible risk to of aphid outbreak), an index between 2 and 10 is indicating of a equilibrium between the pest and the beneficial (aphid population stable or slow decrease) and an index > 10 is indicating a efficient aphid natural control by its enemies, leading to the crash of the aphid populations.

In addition to the visual counts, beatings were performed to collect predator larvae and aphids. A plastic device (50 cm x 30 cm x 10 cm) was placed under the row of potato and the plants below were shaken 5 to 10". The operation was performed 20 times (corresponding to the sampling of more or less 20 plants) in each plot. The insects collected were directly transferred into a plastic recipient filled with water and soap (1.0%). The samples were brought back in the laboratory and all predator larvae (hoverflies, ladybirds, lacewings), were isolated and counted. For each sample, when it was possible, 25 aphids were isolated and dissected under a binocular to estimate the parasitism rate. If less aphids were collected (e.g. due to lower aphid populations in several objects), the dissection was only performed if at least 10 aphids were isolated from the sample. The beatings were performed shortly before the application of the product (one replicate/object) and 2 days and 14 days after the application of the products.

Statistical analysis

The aphid population (aphid/leave), the GIB and the number of aphid mummies estimated by visual counts in the different object was analysed date by date with the help of an Anova (GLM, $p = 0.05$) followed by a Tukey test ($p = 0.05$) for pair-wise comparison. The RIB was analysed following the same procedure after Ln transformation to normalise the distribution.

The number of syrphid larvae, ladybird larvae and the sum of predator larvae (syrphids, ladybirds and the few lacewings collected) in the different object were analysed date by date with the help of an Anova (GLM, $p = 0.05$) followed by a Tukey test ($p = 0.05$) for pair-wise comparison.

The aphid parasitism estimated on basis of the dissection of aphids sampled during the beatings were also analysed following the same procedure after arcsin transformation of the percentages. All the analyses were performed using Minitab software.

Results

The number of ladybird larvae, hoverfly larvae and the sum of all aphid predator larvae (ladybird + hoverfly + lacewings) collected by beating after the application of the different insecticides are listed in Table 1, 2 and 3, respectively. An assessment performed just before the application of the products showed that there were no differences between the plots before the treatments (4.25-6.25 ladybird larvae/sample, 4.00 to 5.75 hoverfly larvae/sample, 8.25 to 11.75 total larvae/sample), with the lowest populations found most of the time in the control.

The main predator collected by beating were the ladybird larvae (mainly *Coccinella septempunctata*, *Propylea quatuordecimpunctata* and *Harmonia axyridis*). The hoverfly larvae population were at the same level than the ladybird before the treatments but rapidly decreased in the control. Only a few lacewing larvae (a maximum of 0.75 larvae/sample) were collected and the results were not analyzed separately for this beneficial. The ladybird and hoverfly populations were affected by all the products, with significant differences for all treatments. Cypermethrin and Thiametoxam were the most toxic products for ladybird larvae, while Azadirachtin, Spinosad and Rynaxypyr had only limited effects. Spinosad had severe

effects 2 days after the treatments, but the effects rapidly disappeared, indicating that the persistence of this insecticide was probably short in the field. Thiametoxam was the opposite, with no significant effects soon after product application and an important reduction of the captures 7 and 14 days after treatment.

Table 1. Ladybird larvae/sample at different date after the insecticide treatments. 4 x 20 plants sampled by beating at each assessment.

	DAT2	DAT7	DAT14	Sum DAT2-14	IOBC classification
Control	11.50 a	7.75 a	3.50 a	22.75 a	-
Cypermethrin	3.50 ab	1.75 c	0.75 bc	6.00 c	3
Azadirachtin	9.00 ab	3.50 bc	3.25 ab	15.75 b	2
Spinosad	2.25 b	9.00 a	4.50 a	15.75 b	2
Thiametoxam	4.25 ab	0.75 c	0.75 bc	5.75 c	3
Rynaxypyr	8.75 ab	6.50 ab	3.25 ab	18.50 b	1

Anova followed by Tukey pair-wise comparison. At each date, results followed by the same letter are not statistically different ($p = 0.05$). IOBC classification (field test): 1 = $E < 25\%$, 2 = $25 < E < 50\%$, 3 = $50 < E < 75\%$; 4 = $E > 75\%$.

The hoverfly larvae populations were in general more affected by the insecticides than the ladybirds. No clear differences between products were observed and Azadirachtin, Spinosad and Rynaxypyr, that only slightly affected the ladybird larvae populations, were at the same level of toxicity for hoverfly larvae than products as Thiametoxam and cypermethrin.

Table 2. Syrphid larvae/sample at different date after the insecticide treatments. 4 x 20 plants sampled by beating at each assessment.

	DAT2	DAT7	DAT14	Sum DAT2-14	IOBC classification
Control	6.25 a	4.25 a	1.75 a	12.25 a	-
Cypermethrin	2.75 ab	0.00 c	0.50 ab	3.25 b	3
Azadirachtin	3.75 ab	1.25 bc	0.50 ab	5.50 b	3
Spinosad	2.75 ab	0.00 c	0.00 b	2.75 b	4
Thiametoxam	1.00 b	2.50 ab	0.25 b	3.75 b	3
Rynaxypyr	3.75 ab	0.50 c	0.00 b	4.25 b	3

Anova followed by Tukey pair-wise comparison. At each date, results followed by the same letter are not statistically different ($p = 0.05$). IOBC classification (field test): 1 = $E < 25\%$, 2 = $25 < E < 50\%$, 3 = $50 < E < 75\%$; 4 = $E > 75\%$.

When all the predator larvae sampled (ladybirds, hoverflies and the few lacewings) are pooled, the 5 insecticides tested can be classified in two categories. The first grouped products with significant but limited effects, as Azadirachtin, Spinosad and Rynaxypyr, all rated as slightly harmless, and the second grouped products with more severe effects, as Cypermethrin and Thiametoxam, classified as moderately harmless. It must be stressed that these products, with respectively 72.3% and 73.0% less larvae sampled after treatment than in the control, were very close to be rated as harmful (limit class category: 75%).

Table 3. Aphid predator larvae (hoverfly, ladybird and lacewing)/sample at different date after the insecticide treatments. 4 x 20 plants sampled by beating at each assessment.

	DAT2	DAT7	DAT14	Sum DAT2-14	IOBC classification
Control	17.75 a	12.00 a	5.50 a	35.25 a	-
Cypermethrin	6.75 b	1.75 d	1.25 bc	9.75 c	3
Azadirachtin	13.00 ab	5.00 bc	4.00 ab	22.00 b	2
Spinosad	5.75 b	9.00 ab	4.50 ab	19.25 bc	2
Thiametoxam	5.25 b	3.25 cd	1.00 bc	9.50 c	3
Rynaxypyr	13.00 ab	7.00 ab	3.25 abc	23.25 b	2

Anova followed by Tukey pair-wise comparison. At each date, results followed by the same letter are not statistically different ($p = 0.05$). IOBC classification (field test): 1 = $E < 25\%$, 2 = $25 < E < 50\%$, 3 = $50 < E < 75\%$; 4 = $E > 75\%$.

The possible effects of the insecticides on aphid parasitic wasps, determined by the dissection of the aphids collected during the beating samples, are listed in Table 4. Before the application of the products, the parasitism rates were comprised between 24.0% and 36.0% and were not statistically different. No significant differences were detected 2 days and 7 days after treatments with the insecticides, except with Spinosad, with a significant reduction of the aphid parasitism at DAT2. However, the parasitism rates were similar than in the control at DAT7.

The evolution of aphid population just before and after the application of the insecticides is illustrated in Figure 1. The only counts that were significantly different than the control were Thiametoxam (DAT2 only, lower populations) and Cypermethrin (DAT7 and 104, higher populations). If for Thiametoxam, this lower aphid population can be attributed to the direct effects of the product on the aphids, the higher populations observed for Cypermethrin is probably the consequence of the toxicity of the product on aphid predator larvae (reduction of 73% of the larvae compared to the control, see Table 3) linked with a limited efficacy of this insecticide on aphid one or two week after the application.

In any treatment, there was an aphid outbreak that required additional insecticide application due to the toxic effects on natural enemies, while a slight trend to promote the aphids was observed with Cypermethrin.

Table 4. Aphid parasitism before and after product application, determined by aphid dissection. 4 x 25 aphids, sampled by beating at each assessment.

	DAT0	DAT2	DAT7
Control	32.0% a	40.0% a	35.0% a
Cypermethrin	28.0% a	31.0% ab	23.0% a
Azadirachtin	36.0% a	25.0% ab	25.0% a
Spinosad	32.0% a	19.0% b	35.0% a
Thiametoxam	28.0% a	27.0% ab	NA
Rynaxypyr	24.0% a	32.0% a	32.0% a

NA = not assessed (not enough aphids available).

Anova followed by Tukey pair-wise comparison, after asin transformation of percentages. At each date, results followed by the same letter are not statistically different ($p = 0.05$).

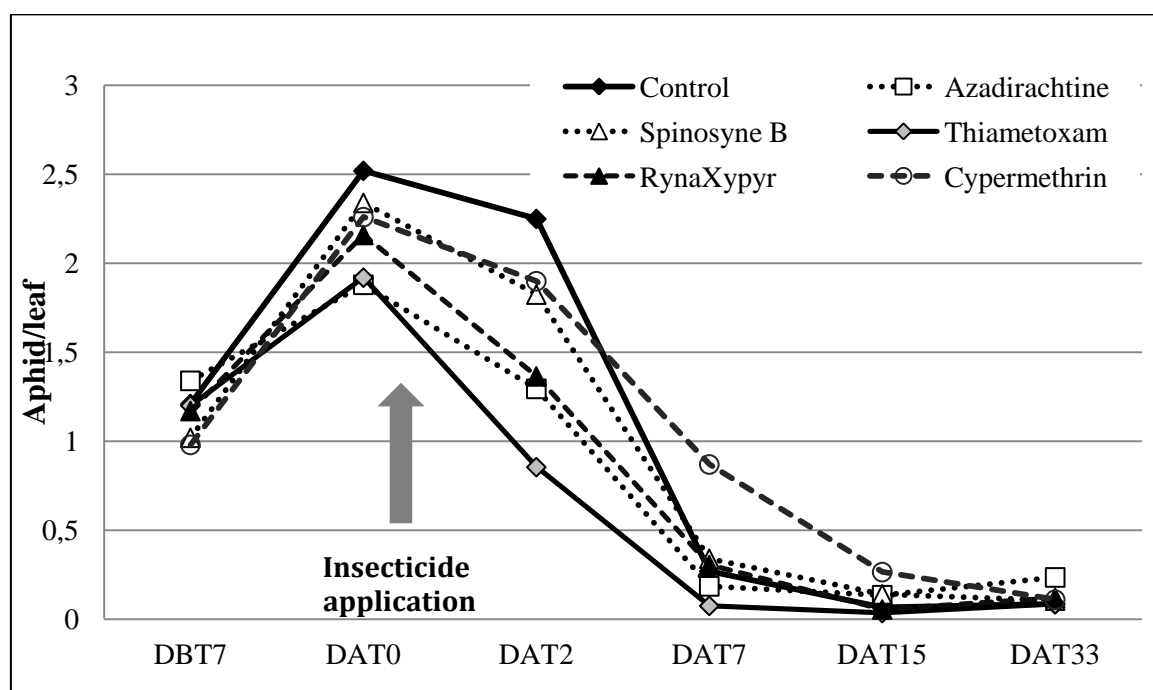


Figure 1. Evolution of the aphid populations before and after the application of the insecticides.

The relative abundance of the aphid natural enemies, compared to the aphid population, estimated both by visual inspection, are illustrated in Figure 2. Thiametoxam was the only insecticide that reduced the relative abundance of beneficial arthropods compared to the control, with significant differences 7 and 14 days after treatment. All the other insecticides gave results close to the control, with a similar balance between the aphids and their natural enemies.

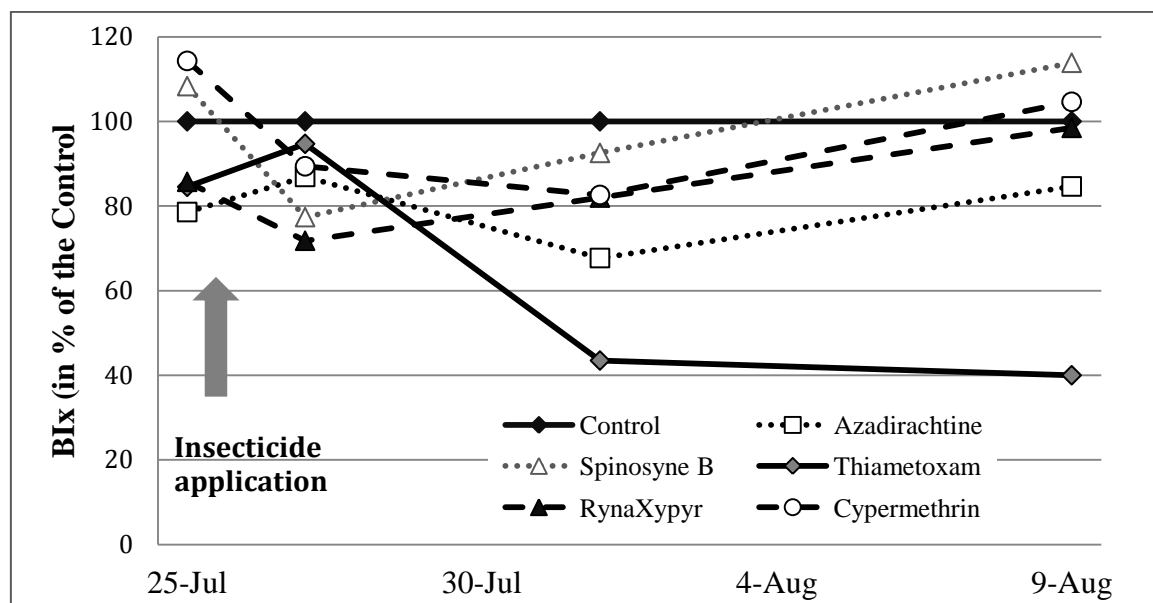


Figure 2. Abundance of beneficial arthropods in the treated plots corrected by the aphid density (Beneficial arthropod Index – BIX) and expressed in % of the control.

Discussion

The results obtained during this field trial showed that Azadirachtin, Spinosad and Rynaxypyr used to control the Colorado beetle in potato had not or limited effects on the biological control of potato aphids by their key natural enemies, as parasitic wasps, ladybird and hoverfly larvae. Even if the ladybird and hoverfly larvae sampled by beating were slightly reduced compare to the control, no aphid population increase or possible outbreak were detected. In the test conditions, the effects of Thiametoxam and Cypermethrin were higher than for the other products, with an important reduction of the ladybird and hoverfly larvae captures for both products and, for Cypermethrin, a significant increase of the aphid population soon after the application of the product. With Thiametoxam, the non-promotion of aphid population was probably linked to the high efficacy of this product in controlling aphid, compared to Cypermethrin, known to be effective soon after application but with a short persistence due to the photo-instability of pyrethroid product outdoor.

One possible explanation of the reduction of the aphid specific predator larval abundance could be the decline in aphid abundance, but this hypothesis is not confirmed by the visual counting and the comparison of the beneficial index that gave the relative abundance of the predator larvae in relation with the aphid densities, with a relative abundance of beneficial about 60% lower than in the control 2 weeks after treatment.

The results present in this publication were obtained one year and in one field. For an extrapolation of the results, two points in particular need to be taken into account. The first one is that the products were applied late in the aphid season, close to the aphid peak, when beneficial arthropods populations were important to facilitate insect sampling, counting and analysis. At this moment, the balance beneficial/aphid was clearly in favor of the beneficial, with an index of 12.5-17.0 in the plots 3 days before treatment. Indexes below 2 are considered as critical and 10.0 as clearly positive in term of aphid control. Thus, a moderate reduction of the beneficial arthropod population at this moment would probably be without negative consequence for aphid control, the surviving predator larval being sufficient to

prevent aphid outbreak. An application in a field with a lower index (close to 2) or an application earlier in the season, when the beneficial populations are lower and the presence of all predator larvae critical for aphid control success would probably had a more important impact on the promotion of aphid population.

The second point is that ladybird larvae were the dominant aphid specific predator during this study. The results obtained on hoverfly larvae is indicating that this beneficial is probably more sensitive to the insecticides tested and the negative impact in term of aphid control would be more important in fields where hoverflies were the key species, as its currently occurring in several fields.

Thus, a repetition of this study in other fields, with other timing of application of the products and/or other beneficial arthropods population composition and abundance need to be undertaken before going to a final conclusion.

References

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