Research Article

Received: 25 February 2009 Revised: 26 November 2009 Accepted: 20 December 2009 Published online in Wiley Interscience: 3 March 2010

(www.interscience.wiley.com) DOI 10.1002/ps.1924

Effects of organic-farming-compatible insecticides on four aphid natural enemy species

Jean P Jansen,* Thibaut Defrance and Anne M Warnier

Abstract

BACKGROUND: The toxicities of pyrethrins + rapeseed oil, pyrethrins + piperonyl butoxide (PBO), potassium salts of fatty acids and linseed oil were assessed in the laboratory on the parasitic wasp *Aphidius rhopalosiphi* (Destefani-Perez), the ladybird *Adalia bipunctata* (L.), the rove beetle *Aleochara bilineata* (Gyll.) and the carabid beetle *Bembidion lampros* (Herbst.). The methods selected were residual contact toxicity tests on inert and natural substrates.

RESULTS: Both the pyrethrin products led to 100% mortality in the adult parasitic wasps and ladybird larvae on glass plates and plants. The pyrethrins + PBO formulation was toxic for *B. lampros* on sand and natural soil, but the pyrethrins + rapeseed oil formulation was harmless for this species. Insecticidal soaps were harmless for all these beneficial species. None of the tested products significantly affected the parasitism of the onion fly pupae by *A. bilineata*.

CONCLUSION: The results indicated the potentially high toxicity of natural pyrethrins for beneficial arthropods. Although this toxicity needs to be confirmed in field conditions, the toxicity levels obtained in the laboratory were similar to or higher than those of several synthetic insecticides known to be toxic in the field. Insecticidal soaps could be considered as an alternative for aphid control in organic farming in terms of selectivity.

Keywords: pyrethrin; potassium soap; linseed oil; organic farming insecticides; aphid natural enemies; side effects

1 INTRODUCTION

Aphids are one of the main agricultural pest groups.[1] Considerable research has been done on their natural enemies, and it has been clearly established that aphid predators and parasites can limit the incidence of aphids and, in some cases, reduce insecticide use by varying degrees. Unfortunately, aphid natural enemies are directly exposed to pesticides, and the use of non-selective products can greatly affect their performance, resulting in rapid aphid outbreaks.[2–6] To derive the maximum benefit from the activity of aphid natural enemies and limit insecticide application, the use of selective products is recommended in the context of organic farming and the development of integrated pest management (IPM).

Products containing natural pyrethrins are one of the major insecticide groups used in organic farming and are widely used in aphid control.[7,8] However, various laboratory studies indicate that these insecticides are toxic to several species of beneficial arthropods.[8–15] Although the effects of these products in the field are probably limited over time owing to their instability in sunlight,[16] the question arises as to the impact of such toxic products on beneficial arthropods in organic farming. There is a paradox between on the one hand trying to maximise the activity of pests' natural enemies and on the other hand affecting their populations by non-selective products. However, this paradox probably exists because the range of products authorised for organic farming is relatively limited, and pyrethrins tend to be used by default in the absence of other compounds that have a better ecotoxicological profile and good aphicidal activity.

Insecticidal soaps could be an alternative for aphid control in organic farming. Their insecticidal action has long been known,[17,18] and various laboratory and field tests have shown the success of such products in controlling several species of economically important aphids,[19–22] although their effectiveness is sometimes more limited than that of conventional insecticides. Few data are available on the selectivity of these soaps on beneficials. In the laboratory, these products were found to be slightly toxic following topical application to *Harmonia axyridis* (Pallas) ladybird larvae,[15] but they had no significant effect on ladybird populations in peach orchards.[22] They also had no effect on *Chrysoperla carnea* (Stephens) lacewing larvae in the laboratory or on *Episyrphus balteatus* (Degeer) hoverfly larvae.[23,24] Initial research suggests that insecticidal soaps can be less toxic to aphid natural enemies than pyrethrins, but no clear comparison of the effects of the two categories of products, with the same methods and on the same species, is available. Furthermore, there are no data on the toxicity of these products on several groups of aphid natural enemies,

* *Correspondence to: Jean P Jansen, Walloon Agricultural Research Center, Life Science Department, Crop Protection and Ecotoxicology unit, Balachowsky building, Chemin de Liroux, 2, 5030 Gembloux, Belgium.*
E-mail: Labecotox@cra.wallonie.be

Centre Wallon de Recherches Agronomiques, Departement Lutte Biologique et Ressources Phytogénétiques, Gembloux, Belgium

Table 1. General information on tested products: commercial name, manufacturer/distributor, active ingredient identity and concentration, formulation type, tested rate and concentration of spray mixtures

Active ingredient	Commercial name (manufacturer/distributor)	AI content (g L^{-1})	Formulation type
Linseed oil	Stop Insect (Natura CJBC, Nevers, France)	Nearly pure	EC
Potassium salts of fatty acids	ECO-Insect (Ecostyle bvba, Geetbets, Belgium)	460	EC
Pyrethrum + rapeseed oil	Pyrethro-Pur Conc. (Ecostyle bvba, Geetbets, Belgium)	4.6 + 852.3	EC
Pyrethrum + piperonyl butoxide	Biopyretrex (Edialux-Formulex, Bornem, Belgium)	20 + 255	EC

such as parasitic hymenoptera and polyphagous ground-dwelling predators.

The aim of this study was to assess and compare the toxicity of two formulations containing natural pyrethrins (pyrethrins + piperonyl butoxide and pyrethrins + rapeseed oil) and two insecticidal soaps (linseed oil and potassium salts of fatty acids) used in organic farming on four species of aphid natural enemies, including species for which there was no or little information. The species used for the study were: the parasitic wasp *Aphidius rhopalosiphi* (Destefani-Perez), a specific cereal aphid parasite; the ladybird *Adalia bipunctata* (L.), an aphidophagous ladybird that is very common in agriculture and arboriculture; and two polyphagous ground-dwelling predators, the carabid beetle *Bembidion lampros* (Herbst) and the rove beetle *Aleochara bilineata* (Gyll.), both of which play an important role as aphid predators in the early stages of aphid infestation.[25–28] *A. rhopalosiphi* and *A. bilineata* are both indicator species used for ecotoxicological studies in the context of pesticide registration in Europe.[29,30] *A. bipunctata* and *B. lampros* were selected because of their smaller size and higher sensitivity to pesticides than the indicator species generally used for ladybird and carabid beetles.[31–34] The methods were based on the standard IOBC methods used for pesticide registration, with residual contact toxicity tests on a treated substrate. These methods have been used extensively with many products on the same species in previous research,[14,35] enabling results to be compared on the basis of the same methodology.

2 MATERIALS AND METHODS

2.1 Testing scheme

The insecticides were first tested on an inert substrate: glass plates for *A. bipunctata* and *A. rhopalosiphi*, and pure quartz sand for the ground-dwelling predators *B. lampros* and *A. bilineata*. The inert surface worked as a worst-case study, and the products that gave effects (mortality and/or fertility or parasitism rates) higher than 50% were further tested on plants for *A. rhopalosiphi* (barley seedlings) and *A. bipunctata* (French bean seedlings) and on natural soil for *B. lampros* and *A. bilineata*. A sandy loam agricultural soil was used (sand 72.7%, loam 18.9%, clay 8.3%, humus 2.8%, CEC 8.2 meq 100 g^{-1}).

2.2 Chemicals

Each product was tested at its maximum recommended field rate. Pyrethrins + piperonyl butoxide (PBO) formulation was also tested at 50% of its recommended field rate in order to enable a comparison to be made with pyrethrin + rapeseed oil, applied at the field rate, which has the same amount of natural pyrethrins per hectare. Details on products are given in Table 1.

For the glass plate tests, the spray solutions were applied on the substrate using a laboratory Burgerjon spray tower,[36] calibrated to deliver an application volume of 200 ± 20 L ha^{-1}, as required for standard tests on glass plates. Dilutions of the products were prepared shortly before application on the basis of this volume. Before being used for the test, the glass plates were left to dry for 1–2 h in ambient conditions.

For the tests performed on sand, soil and plants, the products were applied outdoors using a knapsack sprayer connected to a 2 m wide ramp with four Teejet flat-fan nozzles (Teejet XR 11 003, 110°, 50 cm spacing, 3.2 bar, speed 1 m s^{-1}). Nozzle type, pressure and speed of the sprayer were selected to deliver an application volume equivalent to 400 ± 40 L ha^{-1}, as required for the standard test on sand or natural soils. Before releasing the insects, the treated substrates were brought back into the laboratory and were left to dry for 1–2 h in ambient conditions.

2.3 *Adalia* test

2.3.1 *Insect origin and rearing*

The larvae used for this test were provided by mass rearing in the laboratory, established in 1996 from adults sampled outside from ornamental brushes. Each year, after a quarantine period, new field-collected adults were introduced into the rearing to renew a part of the genetic stock. The adults were kept in plexiglass cages and fed with aphids [*Acyrthosiphon pisum* (Harr.) reared on French beans and *Myzus persicae* (Sulzer) reared on sweet pepper] and collected honeybee pollen. The larvae were reared in plastic petri dishes and fed with the same aphids until pupation.

2.3.2 *Initial toxicity on glass plates*

The exposure units were similar to those described in a previous publication.[35] There were 4 × 10 units of one larva per product and 4 × 10 units for the water-treated control. Larval mortality was recorded until pupation, and the pupae were kept until adult emergence in order to determine pre-imaginal mortality. When the adults emerged, they were transferred to a plexiglass breeding cage (one per group). Food (living aphids offered on cut French bean plants, and honeybee pollen) and water were renewed 3 times a week. Crumpled filter paper was added to the cages to stimulate oviposition. During the second week of egg laying, five females were randomly selected in each breeding cage and confined for 24 h in plastic petri dishes with the bottom covered by a filter paper and with living aphids as food. After 24 h, they were transferred to a new petri dish with aphids for another 24 h of egg laying. This operation was repeated 5 times to obtain 5 × 5 females day^{-1} egg laying for each group. The eggs produced during each egg-laying period were counted and retained in order to check larval emergence and assess egg viability. Eggs that did not hatch after 1 week were considered to be unviable. The experiments were conducted at 20 ± 2 °C and 60–9% RH. The light was provided by sodium lamp on the basis of a 16 : 8 h light : dark photoperiod, with 7000–10 000 lux.

2.3.3 *Toxicity on plants*

The exposure units were similar to those described in a previous publication.[35] After a pesticide application with the knapsack sprayer, the pesticide residue was left to dry for 1–2 h at room temperature, and then two *A. bipunctata* larvae (2–3 days old) were released into each unit. There were four replicates of five plants and ten larvae for the control and for each tested product. Mortality was checked every 24 h. The exposure should have lasted for 7 days, after which an assessment of the fertility of the adults, as for the test on glass plates, was planned, but the test was stopped after 48 h owing to the high mortality in all treatments except the control.

2.4 *Aleochara* test

The methods used were similar to those used for the registration of pesticides,[37] except for some small details. A stock of *A. bilineata* adults was provided by a commercial supplier (De Groene Vlieg, Nieuwe Tonghe, The Netherlands) in the form of parasitised onion fly pupae. The pupae were left to hatch, and the new rove beetles that emerged were transferred into plastic cages filled with wet sand. They were fed 2–3 times a week with frozen mosquito flies (Discus fish food). The exposure units were similar to those used for the *Bembidion* test. Between 1 and 2 h after product application, 20 rove beetles (3–7 days old) were released into each unit. Frozen mosquito larvae were provided as food. On days 7, 14 and 21, 500 onion fly pupae provided by the same commercial supplier, and kept at 4–8 °C before being used, were carefully introduced into each unit. Food was replaced at the same time. On day 28, the units were carefully dismantled and the substrate gently rinsed through a sieve to separate the sand and onion fly pupae. The fly pupae were left to dry at ambient temperature and transferred to Berlese funnels. Rove beetle emergence was assessed over a period of 6–8 weeks, until no beetles had emerged for a week. The test conditions were similar to those for the tests with *Adalia*, except for temperature during beetle emergence (15–25 °C) and light. As the insects are mostly active at night, the light intensity during exposure was reduced to 1000–2000 lux, and the emergence was done in the dark, except for counts. There were four replicates of 20 rove beetles per treatment. The parasitism rate was calculated for each treatment, and the reduction was compared with the control to assess the effects of each product.

2.5 *Aphidius* tests

2.5.1 *Insect origin and rearing*

The insects used for this test were provided by mass rearing in the laboratory, established in 1994 from aphid mummies collected in winter wheat fields. The rearing process is described in a previous publication.[38] Each year, after a quarantine period, new field-collected adults were introduced into the rearing to renew a part of the genetic stock.

2.5.2 *Initial toxicity on glass plates*

The initial toxicity of the products was assessed in two stages: first, an assessment of residual toxicity of pesticide residue applied to the glass plates; second, an evaluation of the reproduction performance of females that survived the exposure. The exposure units were similar to those used for registration tests.[39] Between 1 and 2 h after the pesticide application, the units were assembled, and parasitoid wasps (five males, five females, all 0–48 h old), slightly anaesthetised with carbon dioxide, were released into them. Mortality was recorded after 48 h exposure. There were five units of ten wasps each for each treatment. The surviving females were individually confined for 24 h on potted barley seedlings infested with at least 60 *Sitobium avenae* F. aphids. The barley seedlings were surrounded by a perspex cage covered with nylon gauze for ventilation. The aphid mummies that developed 10–12 days later were counted in each unit. For each product, the fertility performance of 15 females was assessed, if this number had survived the exposure. If not, all surviving females were assessed for fertility. Temperature and relative humidity were similar to that for the *A. bipunctata* test, except for lighting during 48 h of exposure. As adult wasps were attracted by light sources, the light intensity was limited to 1000–2000 lux (diffused light) instead of 7000–10 000 lux so as not to disturb the exposure.

2.5.3 *Toxicity on plants*

The exposure of adult wasps to the insecticides was made on eight barley seedlings (8–10 cm high, with 2–3 leaves) cultured in plastic pots. These plants were surrounded by a perspex cage with the top and two lateral cutaways covered with nylon netting for ventilation. Between 2 and 3 days before product application, about 60 aphids were released into each unit to produce honeydew to attract the wasps onto the treated plants and feed them. The perspex cages were removed just before applying the pesticide with the knapsack sprayer. When the pesticide residues were dry, ten adult wasps (five males, five females), slightly anaesthetised with carbon dioxide, were released into each unit, and the plants were then surrounded by the perspex cages. The units were dismantled and mortality was recorded 48 h after treatment. The surviving females were assessed for fertility in the same way as for the glass plate test. There were six replicates of ten wasps for the control and each product. Fertility was assessed on 15 females per treatment, if this number had survived the exposure. Test conditions were similar to those for the *A. bipunctata* test.

2.6 *Bembidion* test

2.6.1 *Insect origin and rearing*

A stock of *B. lampros* adults were caught in July–August in cereal field margins using pitfall traps and a small aspirator. They were kept in large units on natural soils for 2–8 weeks at 20 ± 2 °C before being used for the tests. They were fed to excess with *Ephestia kuehniella* (Keller) eggs (Nutrimac®), aphids and onion fly pupae.

2.6.2 *Initial toxicity on sand*

A bioassay toxicity test with *B. lampros* was based on methods developed to test the toxicity of products on the carabid beetle, *Poecilus cupreus* L., in registration studies in Europe.[40] The exposure unit consisted of a plastic box (17 × 12 × 6 cm) filled with 500 g of sand. Three days before the test product was applied, 90 mL of water was added to each unit to attain a water-holding capacity of about 70% in the sand, and six beetles from the laboratory stock were introduced into each unit, along with *Ephestia* eggs as food. There were five replicates of six beetles for each treatment. After product application, the mortality was checked on days 1, 2, 4, 7, 11 and 14. Final mortality was recorded on day 14. Food was renewed at each mortality assessment. The test conditions were similar to those for the first part of the *A. bilineata* test.

2.6.3 *Toxicity on sandy soil*

For products where the effect was >50% on sand, the same experiments were repeated using the sandy loam soil instead of pure sand. The amount of water added to the units was adapted to reach about 70% of the water-holding capacity.

Table 2. Toxicity of insecticides to larvae of the ladybird *Adalia bipunctata* on glass plates and on plants in the laboratory. Larval and pre-imaginal mortality and fertility of adult females obtained from larvae exposed to the products[a]

	Tested rate (AI ha^{-1})	Larval mortality (± SD) (%)	Pre-imaginal mortality (± SD) (%)	Eggs female^{-1} (± SD)	Viable eggs female^{-1} (± SD)
		Test on glass plates			
Control		12.5(±8.3) a	20.0(±7.1) a	19.9(±4.5) a	14.6 (±2.9) a
Linseed oil	12.0 L	15.0(±11.2) a	17.5(±8.3) a	25.4(±3.9) a	15.4 (±3.8) a
Potassium salts of fatty acids	4.0 L	40.0(±10.0) b	42.5(±8.3) b	20.3(±3.1) a	17.1 (±2.4) a
Pyrethrin + rapeseed oil	18.4 g + 3.4 L	100.0(±0.0) c	100.0(±0.0) c	–	–
Pyrethrin + piperonyl butoxide	10 g + 128 g	100.0(±0.0) c	100.0(±0.0) c	–	–
Pyrethrin + piperonyl butoxide	20 g + 256 g	100.0(±0.0) c	100.0(±0.0) c	–	–
		$H_5 = 21.81, P = 0.001$	$H_5 = 21.81, P = 0.001$	$F_{2,14} = 2.31, P = 0.162$	$F_{2,14} = 0.60, P = 0.573$
		Test on plants			
Control		7.5(±8.3) a	10.0(±7.1) a	Not assessed	Not assessed
Pyrethrin + rapeseed oil	18.4 g + 3.4 L	100.0(±0.0) b	100.0(±0.0) b	–	–
Pyrethrin + piperonyl butoxide	10 g + 128 g	100.0(±0.0) b	100.0(±0.0) b	–	–
Pyrethrin + piperonyl butoxide	20 g + 255 g	100.0(±0.0) b	100.0(±0.0) b	–	–
		$H_3 = 14.66, P = 0.002$	$H_3 = 14.66, P = 0.002$		

[a] Means followed by the same letter are not significantly different; Kruskal–Wallis at $P = 0.05$ for percentage data; LSD at $P = 0.05$ for other data.

Table 3. Toxicity of insecticides to the rove beetle *Aleochara bilineata* on sand in the laboratory. Mean number of progeny (parasitised onion fly pupae/unit) and reduction in parasitism rate compared with the control[a]

	Tested rate (AI ha^{-1})	Progeny (± SD)	Reduction in parasitism (%)
Control		326.3(±26.2) ab	
Linseed oil	12.0 L	306.0(±24.5) ab	6.2
Potassium salts of fatty acids	4.0 L	358.0(±37.1) a	−9.7
Pyrethrin + rapeseed oil	18.4 g + 3.4 L	352.0(±32.7) a	−7.9
Pyrethrin + piperonyl butoxide	10 g + 128 g	344.3(±28.8) a	−5.5
Pyrethrin + piperonyl butoxide	20 g + 255 g	252.0(±27.8) b	22.8
		$F_{5,23} = 5.00, P = 0.007$	

[a] Means followed by the same letter are not significantly different; LSD, $P = 0.05$.

2.7 Experimental design and statistical analysis

All the replicates of the same experiment were conducted at the same time and with the same set of test organisms. The replicates were randomly distributed in the climatic chamber. The results of the tests were analysed using Statistical Minitab software. For mortality percentages, non-parametric Kruskal–Wallis tests ($P = 0.05$) were used, because percentages did not follow a normal distribution, even after arcsine transformation. For the other set of data, a one-way ANOVA test (LSD) for variance analysis was performed, followed by Tukey tests for multiple comparisons between treatments ($P = 0.05$). For the ANOVA, the homogeneity of variance was previously checked using Bartlett tests ($P = 0.05$).

3 RESULTS

The results of the tests performed with *A. bipunctata* larvae are given in Table 2. On glass plates, linseed oil was the only test insecticide that did not differ significantly from the control. The potassium salts of fatty acids formulation was slightly more toxic than the control, with 40% larval mortality ($P = 0.019$), but less toxic than the three natural pyrethrin treatments ($P = 0.013$) which all led to 100% mortality. This high mortality was obtained after less than 2 days of exposure, indicating the drastic action of natural pyrethrins on *A. bipunctata* larvae on glass plates. Potassium salts and linseed oil did not significantly reduce the egg production of the ladybird females obtained from larvae that had survived the exposure, or their viability. On plants, 100% mortality was obtained with all natural-based pyrethrin products after only 2 days of exposure, and the test was then stopped. High mortality was also observed after 24 h, with several larvae still alive but greatly affected by the products.

The results with *A. bilineata* on sand are given in Table 3. There were significant differences between the treatments, but no product significantly reduced the onion fly pupae parasitism rate by rove beetles compared with the control. The only significant difference was observed between pyrethrins + PBO formulation at 2.0 L ha^{-1}, which had the lowest parasitism rate, and potassium salt of fatty acids, linseed oil and pyrethrins + PBO formulation at 1.0 L ha^{-1}, which had parasitism rates that were slightly higher than the control, indicating that the differences between pyrethrins + PBO formulation at 2.0 L ha^{-1} and the control were small enough to be significant if the parasitism rate in the control was just a little higher or the variability of the data was just a little lower.

The results of the *A. rhopalosiphi* toxicity test are given in Table 4. On glass plates, the test products significantly affected adult wasp survival. Control mortality reached 2.0% and was lower

Table 4. Toxicity of insecticide to adults of *Aphidius rhopalosiphi* on glass plates and on plants. Mortality (48 h exposure) and number of aphid mummies produced by female wasps surviving the exposure[a]

	Tested rate (AI ha^{-1})	Mortality (± SD) (%)	Aphid mummies female^{-1} (± SD)
		Test on glass plates	
Control		2.0 (±4.0) a	31.1(±13.4) a
Linseed oil	12.0 L	36.0(±15.0) b	28.5(±12.3) a
Potassium salts of fatty acids	4.0 L	22.0(±7.5) b	31.7(±14.8) a
Pyrethrin + rapeseed oil	18.4 g + 3.4 L	100.0(±0.0) c	–
Pyrethrin + piperonyl butoxide	10 g + 128 g	100.0(±0.0) c	–
Pyrethrin + piperonyl butoxide	20 g + 255 g	100.0(±0.0) c	–
		$H_5 = 27.85, P < 0.001$	$F_{2,44} = 0.18, P = 0.836$
		Test on plants	
Control		8.3 (±10.7) a	43.1(±23.4)
Pyrethrin + rapeseed oil	18.4 g + 3.4 L	100.0(±0.0) b	
Pyrethrin + piperonyl butoxide	10 g + 128 g	100.0(±0.0) b	
Pyrethrin + piperonyl butoxide	20 g + 255 g	100.0(±0.0) b	–
		$H_5 = 22.48, P < 0.001$	

[a] Means followed by the same letter are not significantly different; Kruskal–Wallis at $P = 0.05$ for percentage data; LSD at $P = 0.05$ for other data.

Table 5. Toxicity of insecticides to the ground beetle *Bembidion lampros* on sand and on soil in the laboratory; observed mortality after 14 days of exposure[a]

		Mortality (± SD) (%)	
	Tested rate (AI ha^{-1})	Sand	Natural soil
Control		0.0 a	0.0
Linseed oil	12.0 L	0.0 a	
Potassium salts	4.0 L	0.0 a	
Pyrethrin + rapeseed oil	18.4 g + 3.4 L	3.3 (±6.7) a	
Pyrethrin + piperonyl butoxide	10 g + 128 g	40.0 (±17.0) b	
Pyrethrin + piperonyl butoxide	20 g + 255 g	86.7 (±19.4) c	100.0 (±0.0)
		$H_5 = 26.89, P < 0.001$	

[a] Means followed by the same letter are not significantly different; Kruskal–Wallis at $P = 0.05$.

than the other test products, with 22% for potassium salts of fatty acids ($P = 0.009$), 36% for linseed oil ($P = 0.009$) and 100% for the three treatments based on natural pyrethrins ($P = 0.004$). Linseed oil and potassium salts did not differ from each other, but were statistically different from the three pyrethrin treatments ($P = 0.005$). The test products that enabled an assessment to be made of fertility performance did not significantly reduce aphid mummy production. With the pyrethrin treatments, no females survived and fertility assessment was possible. When the three natural pyrethrins were tested on plants, observed mortality still reached 100% and was statistically different from the control ($P = 0.013$).

The results of the toxicity test with *B. lampros* are given in Table 5. The mortalities obtained with pyrethrins + PBO at 1.0 L ha^{-1} (40.0%) and 2.0 L ha^{-1} (86.7%) were significantly higher than the control ($P = 0.005$ at 1.0 and 2.0 L ha^{-1}). There was higher mortality at 2.0 L ha^{-1} than at 1.0 L ha^{-1} with pyrethrins + PBO ($P = 0.019$). On natural soil, pyrethrins + PBO applied at the maximum recommended field rate led to 100% mortality, but no mortality was recorded for the control. As all the replicates reached 0% mortality in the control and 100% with pyrethrins + PBO, there was no variance and statistical analysis was not possible.

4 DISCUSSION

The results of this study indicated that, in general, the two formulations containing natural pyrethrins were very toxic in the laboratory, with mortalities of more than 50% for three of the four beneficial species. The most frequently observed effect was direct mortality after contact of the test organism with surfaces, glass plates, plants, sand or soil treated with the insecticide. No sublethal effects on reproduction were observed when this parameter was assessed. The rove beetle, *A. bilineata*, was the only insect that was unaffected by the pyrethrins, with no reduction significantly below the control. As rove beetles are burrower insects, they probably have limited contact with surface insecticides. *B. lampros* was affected by the pyrethrin + PBO formulation, with 86.7 and 100% mortality at the recommended field rate on an inert substrate and on soil respectively, and 40% on an inert substrate at the same field rate of pyrethrin as that for the pyrethrin + rapeseed oil formulation. At the same time, this latter formulation had no effects on the beetles, indicating a possible synergistic role of PBO on carabid beetles. Apart from this result, no conclusion could be drawn about the possible difference in toxicity between the two formulations, because toxicity levels were either very low (*Aleochara*) or 100% (*Aphidius, Adalia*) for both products. A

multirate approach, independent of the recommended field rate, should be adopted in order to work with concentration ranges that can produce differentiated results.

The toxicity of the two formulations containing pyrethrins on *A. rhopalosiphi* and *A. bipunctata* was very marked, with 100% mortality on an inert substrate and on plants. In the case of *A. bipunctata*, mortality occurred after only 24–48 h exposure. These results confirm the very high toxicity of pyrethrins reported in other species of parasitic hymenoptera and ladybirds.[8,9,12,14,15,41–47] The impact of these products on parasitic hymenoptera and ladybirds remains to be confirmed in the field.

The insecticidal soaps were noticeably less toxic to the beneficials tested than the pyrethrin-based formulations. The highest mortality rates occurred on glass, with 35% mortality for *A. rhopalosiphi* adults with linseed oil and 28% pre-imaginal mortality for *A. bipunctata* larvae with potassium salts of fatty acids, where mortalities were corrected according to Abbott.[48] The two insecticidal soaps had no significant effect on the two ground-dwelling predators, *B. lampros* and *A. bilineata*. Compared with products containing natural pyrethrins, both insecticidal soap formulations have a positive ecotoxicological profile in the laboratory, and, even if some effects differed significantly from the control, no adverse effects are expected in the field. However, some authors indicate that these products do not always provide a level of control that compares favourably with conventional insecticides, or are not sufficiently effective against some aphid species.[21,22,49] They are not, therefore, a completely satisfactory alternative to natural pyrethrins in organic farming, and other solutions will need to be found, at least for certain situations.

When the results of this study were compared with those from previous studies conducted using the same methods and on the same species and strains of test organisms, the high toxicity of the formulations containing natural pyrethrins raised some questions, at least with regard to parasitic hymenoptera and ladybirds. In the case of *A. rhopalosiphi*, the toxicity levels observed were equivalent to those for insecticides considered to be non-selective, such as dimethoate and methiocarb (100% mortality on plants for both products, at 250 g and 750 g AI ha^{-1} respectively), and were noticeably higher than several synthetic insecticides that are effective against aphids, such as pirimicarb (12% of corrected mortality on plants at 200 g AI ha^{-1}) and pymetrozine (4% of corrected mortality on glass plates at 150 g AI ha^{-1}).[14,29] Several formulations containing synthetic pyrethrins were even less toxic to the parasite than natural pyrethrins, such as lambda-cyhalothrin (1% of corrected mortality at 10 g AI ha^{-1} on plants) and alpha-cypermethrin (38% of corrected mortality at 12.5 g AI ha^{-1} on plants).[14,35] For *A. bipunctata* larvae, pirimicarb and pymetrozine were far less toxic than natural pyrethrins on glass plates, with 21 and 0% mortality respectively, after 2 days of exposure, at the same rates as for *Aphidius*.[14,35] The higher toxicity could be explained by the presence of a synergist such as PBO or a coformulant such as rapeseed oil, which does not occur in the formulations containing synthetic pyrethrins. Another explanation might be the higher rate of pyrethrins tested, with at least 20 g of natural pyrethrins in the mixture per hectare, compared with the lower rates of synthetic pyrethrins per hectare generally applied. It must also be noted that the synthetic products were not tested at the same time as organic farming insecticides, and that small differences due to the variability of the data and/or reproducibility of the experiments could have occurred but were probably insignificant.

In conclusion, the results of this study show that natural pyrethrins could potentially be highly toxic to various species of beneficial insects, whereas insecticidal soaps had no major effects. The toxicity of natural pyrethrin formulations needs to be confirmed in field conditions, but it was similar in the laboratory to that obtained with the synthetic products considered to be non-selective, and in some cases higher than synthetic pyrethrinoids and aphicides considered to be selective for beneficials, such as pirimicarb and pymetrozine. The difference in toxicity between natural pyrethrin formulations and synthetic insecticides will perhaps lie in the duration of harmful activity of natural pyrethrin extracts in the field, which can be relatively short[8,9,50] Insecticidal soaps emerge as a possible alternative to pyrethrins, at least in terms of selectivity, but there are some limitations with regard to their limited effectiveness on some aphid species.[21,22,49] Several synthetic insecticides that are more effective than insecticidal soaps and far more selective than pyrethrins could be a possible alternative if they were to be allowed in organic farming.

REFERENCES

1 Aeschlimann JP, Horn D and Mackauer M, Augmentation and enhancement of aphidophaga – introduction. *Agr Ecosyst Environ* **52**:1–2 (1995).
2 Ripper WE, Effect of pesticides on balance of arthropod populations. *Annu Rev Entomol* **1**:403–438 (1956).
3 Pimentel D, An ecological approach to the insecticide problem. *J Econ Entomol* **54**:108–114 (1961).
4 Vickerman GP and Sunderland KD, Some effects of dimethoate on arthropods in winter wheat. *J Appl Ecol* **14**:767–777 (1977).
5 Horn DJ, Selective mortality of parasitoids and predators of *Myzus persicae* on collards treated with malathion, carbaryl, or *Bacillus thuringiensis*. *Entomol Exp Appl* **34**:208–211 (1983).
6 Borgemeister C and Poehling HM, The impact of insecticide treatments on the population dynamics of cereal aphids and their parasitoids. *IOBC/WPRS Bull* **12**:122–132 (1989).
7 Isman MB, Botanical insecticides, deterrents, and repellents in modern agriculture and increasingly regulated world. *Annu Rev Entomol* **51**:45–66 (2006).
8 Ledieu MS, Helyer NL and Derbyshire DM, Pests and diseases of protected crops, in *Pest and Disease Control Handbook*, ed. by Scopes N and Stables L. BCPC Publications, Thornton Heath, Surrey, UK, pp. 405–511 (1989).
9 Franz JM, Bogenschütz H, Hassan SA, Huang P, Naton E, Suter H, *et al*, Results of a joint pesticide testing programme by the IOBC/WPRS working group: pesticides and beneficial arthropods. *Entomophaga* **25**:231–236 (1980).
10 Hassan SA, Relative tolerance of three different strains of the predatory mite *Phytoseiulus persimils* A.-H. (Acari, Phytoseiidae) to 11 pesticides used on glasshouse crops. *J Appl Entomol* **93**:55–63 (1982).
11 Castagnoli M, Liguori M, Simoni S and Duso C, Toxicity of some insecticides to *Tetranychus urticae, Neoseiulus californicus* and *Tydeus californicus*. *Biocontrol* **50**:611–622 (2005).
12 Medina P, Morales JJ, Budia F, Adan A, Del Estal P and Vinuela E, Compatibility of endoparasitoid *Hyposoter didymator* (Hymenoptera: Ichneumonidae) protected stages with five selected insecticides. *J Econ Entomol* **100**:1789–1796 (2007).
13 Duso C, Malagnini V, Pozzebon A, Castagnoli M, Liguori M and Simoni S, Comparative toxicity of botanical and reduced-risk insecticides to Mediterranean populations of *Tetranychus urticae* and *Phytoseiulus persimilis* (Acari Tetranychidae, Phytoseiidae). *Biol Cont* **47**:16–21 (2008).
14 Jansen JP, Hautier L, Mabon N and Schiffers B, Pesticide selectivity list to beneficial arthropods in four field vegetable crops. *IOBC/WPRS Bull* **35**:66–77 (2008).
15 Kraiss H and Cullen EM, Efficacy and nontarget effects of reduced-risk insecticides on *Aphis glycines* (Hemiptera: Aphidiidae) and its biological control agent *Harmonia axyridis* (Coleoptera: Coccinellidae). *J Econ Entomol* **101**:391–398 (2008).
16 Crosby DG, Environmental fate of pyrethrins, in *Pyrethrum Flowers: Production, Chemistry, Toxicology, and Uses*, ed. by Casida JE and Quistad GB. Oxford University Press, New York, NY, pp. 194–213 (1995).

17 van der Meulen PA and van Leeuwen ER, A study of the insecticidal properties of soap against the Japanese beetle. *J Econ Entomol* **22**:812–814 (1923).
18 Fulton BB, The relation of evaporation to killing efficiency of soap solutions on the harlequin bug and other insects. *J Econ Entomol* **23**:625–630 (1930).
19 Pinnock DE, Brand RJ, Milstead JE and Coe NF, Suppression of populations of *Aphis gossypii* and *A. spiraecola* by soap sprays. *J Econ Entomol* **67**:783–784 (1974).
20 Lawson DS and Weires RW, Management of European red mite (Acari: Tetranychidae) and several aphid species on apple with petroleum oils and an insecticidal soap. *J Econ Entomol* **84**:1550–1557 (1991).
21 Fournier V and Brodeur J, Dose–response susceptibility of pest aphids (Homoptera: Aphididae) and their control on hydroponically grown lettuce with the Entomopathogenic fungus *Verticillium lecanii*, azadirachtin and insecticidal soap. *Environ Entomol* **29**:568–578 (2000).
22 Karagounis C, Kourdoumbalos AK, Margaritopoulos JT, Nanos GD and Tsitsipis JA, Organic farming-compatible insecticides against the aphid *Myzus persicae* (Sulzer) in peach orchards. *J Appl Entomol* **130**:150–154 (2006).
23 Bigler F and Waldburger M, Effects of pesticides on *Chrysoperla carnea* Steph. (Neuroptera, Chrysopidae) in the laboratory and semi-field results, in *Side Effects of Pesticides on Beneficial Organisms, Comparison of Laboratory, Semi-field and Field Results*, ed by Vogt H. *IOBC/WPRS Bull* **17**:55–70 (1994).
24 Pineda A, Marcos-Garcia MA and Jansen JP, Lethal and sublethal effects of four organic farming-compatible insecticides on the aphidophagous hoverfly *Episyrphus balteatus*, in *Los sirfidos (Diptera, Syrphidae) en el control integrado de plagas de pulgon en cultivos de pimiento de invernadero*, Tesis Doctoral (Pineda A.), CIBIO, Universidad de Alicante, pp. 129–148 (2008).
25 Sunderland KD and Vickerman GP, Aphid feeding by some polyphagous predators in relation to aphid density in cereal fields. *J Appl Ecol* **17**:389–396 (1980).
26 Sunderland KD, Crook NE, Staceyn DL and Fuller BT, A study of feeding by polyphagous predators on cereal aphids using Elisa and gut dissection. *J Appl Ecol* **24**:907–934 (1987).
27 Chiverton PA, Predation of *Rhopalosiphum padi* (Homoptera: Aphidiidae) by polyphagous predatory arthropods during the aphids' pre-peak period in spring barley. *Ann Appl Biol* **111**:257–269 (1987).
28 Holland JM and Thomas SR, Quantifying the impact of polyphagous invertebrate predators in controlling cereal aphids and in preventing wheat yield and quality reductions. *Ann Appl Biol* **131**:375–397 (1997).
29 Candolfi M, Bakker F, Cañez V, Miles M, Neumann C, Pilling E, *et al*, Sensitivity of non-target arthropods to plant protection products: could *Typhlodromus pyri* and *Aphidius* spp. be used as indicator species? *Chemosphere* **39**:1357–1370 (1999).
30 Candolfi MP, Barrett KL, Campbell PJ, Forster R, Grandy N, Huet MC, *et al*, Guidance document on regulatory testing procedures for pesticides with non-target arthropods, in *Workshop on European Standard Characteristics of Beneficial Regulatory Testing (ESCORT 2), 21–23 March 2000*, ed. Candolfi MP, Barrett KL, Campbell PJ, Forster R, Grandy N, Huet MC, *et al*. Society of Environmental Toxicology and Chemistry – Europe (2000).
31 Jansen JP and Hautier L, Comparative sensitivity of four ladybird species to five pesticides. *IOBC/WPRS Bull* **29**:95–104 (2006).
32 Hassan SA, Albert R, Bigler F, Blaisinger P, Bogenschütz H, Boller E, *et al*, Results of the third joint pesticide testing programme of the IOBC/WPRS working group 'Pesticides and Beneficial Organisms'. *J Appl Entomol* **103**:92–107 (1987).
33 Hassan SA, Bigler F, Bogenschütz H, Boller E, Brun J, Chiverton P, *et al*, Results of the fourth joint pesticide testing programme of the IOBC/WPRS working group 'Pesticides and Beneficial Arthropods'. *J Appl Entomol* **105**:321–329 (1988).
34 Hassan SA, Bigler F, Bogenschütz H, Boller E, Brun J, Calis JN, *et al*, Results of the fifth joint pesticide testing programme of the IOBC/WPRS working group 'Pesticides and Beneficial Arthropods'. *Entomophaga* **36**:55–67 (1991).
35 Hautier L, Jansen JP, Mabon N and Schiffers B, Building a selectivity list of plant protection products on beneficial arthropods in open field: a clear example with potato crop. *IOBC/WPRS Bull* **29**:21–32 (2006).
36 Burgerjon A, Pulvérisation et poudrage au laboratoire par des préparations pathogènes insecticides. *Annales Epiphytes* **IV**: 675–684 (1956).
37 Grimm C, Reber B, Barth M, Candolfi MP, Drexler A, Maus C, *et al*, A test for evaluating the chronic effects of plant protection products on the rove beetle *Aleochara bilineata* Gyll. (Coleoptera: Staphylinidae) under laboratory and extended laboratory conditions, in *Guidelines to Evaluate Side-effects of Plant Protection Products to Non-target Arthropods, an IOBC, BART and EPPO Joint Initiative*, ed. by Candolfi MP, Blumel S, Forster R, Bakker F, Grimm C, Hassan SA, *et al*, pp. 1–12 (2000).
38 Jansen JP, Side effects of insecticides on *Aphidius rhopalosiphi* (Hym. Aphidiidae) in laboratory. *Entomophaga* **41**:37–43 (1996).
39 Mead-Briggs MA, Brown K, Candolfi MP, Coulson MJ, Miles M, Moll M, *et al*, A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (DeStefani-Perez) (Hymenoptera: Braconidae), in *Guidelines to Evaluate Side-effects of Plant Protection Products to Non-target Arthropods, an IOBC, BART and EPPO Joint Initiative*, ed. by Candolfi MP, Blumel S, Forster R, Bakker F, Grimm C, Hassan SA, *et al*, pp 13–26 (2001).
40 Heimbach U, Dohmen P, Barett KL, Candolfi MP, Jackel B, Kenedy PJ, *et al*, A method for testing effects of plant protection products on the carabid beetle *Poecilus cupreus* (Coleoptera, Carabidae) under laboratory and semi-field conditions, in *Guidelines to Evaluate Side-effects of Plant Protection Products to Non-target Arthropods, an IOBC, BART and EPPO Joint Initiative*, ed. by Candolfi MP, Blumel S, Forster R, Bakker F, Grimm C, Hassan SA, *et al*, pp. 87–106 (2001).
41 Kalushkov P, The effect of aphid prey quality on searching behaviour of *Adalia bipunctata* and its susceptibility to insecticides. *Entomol Exp Appl* **92**:277–282 (1999).
42 Bayoun IM, Plapp FW, Gilstrap FE and Michels GJ, Toxicity of selected insecticides to *Diuraphis noxia* (Homoptera: Aphidiidae) and its natural enemies. *J Econ Entomol* **88**:1177–1185 (1995).
43 Olszak RW, Influence of some pesticides on mortality and fecundity of the aphidophagous coccinellid *Adalia bipunctata* L. (Col., Coccinellidae). *J Appl Entomol* **123**:41–45 (1999).
44 Wiles JA and Jepson PC, Dosage reduction to improve the selectivity of deltamethrin between aphids and coccinellids in cereals. *Entomol Exp Appl* **76**:83–96 (1995).
45 Longley M, Jepson PC, Izquierdo J and Sotherton N, Temporal and spatial changes in aphid and parasitoid populations following applications of deltamethrin in winter wheat. *Entomol Exp Appl* **83**:41–52 (1997).
46 Jansen JP, A three-year field study on the short-term effects on insecticides used to control cereal aphids on plant-dwelling aphid predators in winter wheat. *Pest Manag Sci* **56**:533–539 (2001).
47 Jansen JP, Toxicity of insecticides used in wheat to adults of *Aphidius rhopalosiphi* DeStefani-Perez (Hymenoptera: Aphidiidae) with field treated plants. *IOBC/WPRS Bull* **24**:17–24 (2001).
48 Abbott WS, A method of computing the effectiveness of an insecticide. *J Econ Entomol* **18**:265–267 (1925).
49 Edelson JV, Duthie J and Roberts W, Toxicity of biorational insecticides: activity against the green peach aphid, *Myzus persicae* (Sulzer). *Pest Manag Sci* **58**:255–260 (2002).
50 Vinuela E, Handel U and Vogt H, Evaluacion en campo de los efectos secundarios de dos plaguicidas de origen botanico, una piretrina natural y un extracto de neem, sobre *Chrysoperla carnea* Steph. (Neuroptera; Chrysopidae). *Bol San Veg Plagas* **22**:97–106 (1996).

