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Abstract The effects of flonicamide and pymetrozine, on inert and natural substrates, on the rove beetle *Aleochara bilineata* (Gyll.), the parasitic wasp *Aphidius rhopalosiphi* (DeStefani-Perez), the ladybird *Adalia bipunctata* (L.), the carabid beetle *Bembidion lampros* (Herbst), and the hoverfly *Episyrphus balteatus* (DeGeer) were assessed in the laboratory. Deltamethrin and pirimicarb were also tested as toxic reference compounds. The results indicated high selectivity of flonicamide and pymetrozine for all the species tested. No significant effects on *B. lampros* and *A. bilineata* were recorded for sand or on *E. balteatus* for plants. Pymetrozine on inert substrates had no effects on *A. bipunctata* larvae, whereas flonicamid was slightly toxic on glass plates but harmless on plants. Both compounds were toxic to adult *A. rhopalosiphi* on glass plates and on plants in the laboratory, but no effects were observed on plants treated in the field. In comparison, the toxic reference products were always more toxic. Compared with classical insecticides tested on the same species using similar methods, flonicamide and pymetrozine seem to be promising insecticides for aphid control in terms of selectivity for aphid antagonists.

Keywords *Aleochara bilineata* · *Aphidius rhopalosiphi* · *Adalia bipunctata* · *Bembidion lampros* · *Episyrphus balteatus* · Pesticide side-effects

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Introduction

By eliminating aphid natural enemies, use of non-selective products has often led to severe aphid outbreaks (Ripper 1956; Pimentel 1961; Vickerman and Sunderland 1977; Horn 1983; Borgemeister and Poehling 1989). In this context, insecticides that are effective against aphids but have no or limited effects on aphids' natural enemies are needed for successful integrated pest-management (IPM) programs.

Among the products commercially available for control of aphids, pirimicarb has seemed the most selective, with limited or almost no effects on important beneficial aphid antagonist groups, for example parasitic hymenoptera (Borgemeister and Poehling 1989; Smart et al. 1989; Jansen 1996; Oakley et al. 1996), ladybirds and lacewings (Gräpel 1982; Hellpap 1982; Hassan et al. 1985; Schmuck et al. 1997; Rumpf et al. 1998, Jansen 2000), and carabid and staphylinid beetles (Unal and Jespon 1995). However, this product was particularly harmful to hoverfly larvae (Poehling and Dehne 1986;

Storck-Weyhermuller 1989; Niehoff and Poehling 1995; Jansen 2000) and was therefore of limited interest for IPM where hoverflies must be preserved. In addition, several economically important aphid species, for example *Aphis gossypii* Glover and *Myzus persicae* Sulzer, have developed resistance against this insecticide (Field et al. 1997; Barber et al. 1999; McLoon and Herron 2009).

The other insecticides used to control aphids, mainly pyrethroids and organophosphate compounds, were less selective than pirimicarb and were toxic to a wide variety of beneficial insects. Some had limited effects on a few groups of aphid antagonists (e.g., some pyrethroids on aphidiid wasps (Poehling 1987; Borgemeister and Poehling 1989; Oakley et al. 1996; Jansen 2001) and hoverflies (Jansen 2000)). The main conclusion of all this research was that insecticides that kill aphids without affecting the entire aphid natural enemy complex do not exist, with some being toxic to all beneficials and others being harmless for one or several groups of beneficials.

Pymetrozine and flonicamid are two new aphicides that have recently become available commercially. The mode of action of these systemic compounds differs completely from classical insecticides, inhibiting the feeding activity of the insect, which subsequently dies of starvation (Harrewijn and Kayser 1997; Morita et al. 2007). Thus, no effects on non plant-sucking insects, for example beneficials, would be expected. Initial tests conducted on beneficial arthropods to build a selectivity list for farmers confirm this behavior: no lethal effects on parasitic wasps, ladybirds, or hoverflies were observed (Van de Veire and Tirry 2003; Hautier et al. 2006). However, these tests did not include full assessment of the sub-lethal effects. The objective of this research was to assess the effects of these compounds on a variety of aphid natural enemies, using standard methods previously applied to the same species for classical products. The selected beneficial species were the ladybird, *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae), the parasitic wasp, *Aphidius rhopalosiphi* (DeStefani-Perez) (Hymenoptera: Aphidiidae), the two ground-dwelling predators, *Bembidion lampros* (Herbst) (Coleoptera: Carabidae) and *Aleochara bilineata* (Gyll) (Coleoptera: Staphylinidae), and the hoverfly, *Episyrphus balteatus* (DeGeer) (Diptera: Syrphidae).

Aphidius rhopalosiphi is a cereal aphid-specific parasitic hymenoptera. This insect was selected as one of the two standard species to be tested in the context of product registration at the European level because of its high sensitivity to pesticides in general (Candolfi et al. 1999, 2001). This is also typical of species belonging to the Aphidiidae, all of which are aphid-specific parasitoids. *Adalia bipunctata* is an aphidophagous ladybird commonly found in agricultural ecosystems. It has been selected as an indicator species of ladybirds, an important group of aphid predators, because of its greater sensitivity to pesticides than larger species (Jansen and Hautier 2006). *Aleochara bilineata* and *B. lampros* are soil-dwelling predatory beetles feeding on small insects, including aphids. They are regarded as important aphid predators at the start of infestations, especially of cereals (Sunderland 1975; Sunderland et al. 1987; Chiverton 1987; Holland and Thomas 1997). *Aleochara bilineata* is the indicative species for rove beetles in the registration of pesticides, and *B. lampros* was preferred to the larger carabid beetle species used in the past for assessing the side-effects of pesticides on this family, because of its greater sensitivity to pesticides (Hassan et al. 1987, 1988, 1991). *Episyrphus balteatus* is the most common syrphid species found in arable crops in several central and north European countries (Niehoff and Poehling 1995; Jansen 2000). The larvae are aphid-specific predators that can be very effective in open fields and orchards (Chambers et al. 1983; Chambers 1992; Wyss et al. 1999; Freier et al. 2007).

Materials and methods

Testing scheme

The products were assessed by following a typical IOBC standard sequential testing scheme for beneficial insects (Hassan et al. 1991). The initial toxicity of the insecticides was 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.* Products with effects (mortality and/or fertility or incidence of parasitism) higher than 50% were further tested, in the laboratory, on natural substrates, for example plants for *A. rhopalosiphi, A. bipunctata*, and *E. balteatus* and natural soil for *B. lampros* and *A. bilineata*. The tests with *E. balteatus* larvae started directly on plants

because tests with this species on glass plates often led to unusually high control mortality. For *A. rhopalosiphi*, because of the effects observed on plants in the laboratory, an additional test was also performed with winter wheat (*Triticum aestivum* L.) plants treated in the field under conditions similar to conventional practice.

Chemicals

For each commercial product, the maximum recommended field rate was tested, diluted in 200 l spray mixture ha^{-1} for glass plate tests and 400 l ha^{-1} for tests on plants or sand. Because of its high toxicity to beneficial organisms, deltamethrin was used as a toxic reference, except for *E. balteatus.* Because this species is less sensitive to pyrethroids (Hautier et al. 2006; Jansen et al. 2008), deltamethrin was replaced by pirimicarb, known to be toxic to hoverflies in the field (Jansen 2000). Details of products are given in Table 1. For the glass plate tests, the spray solutions were applied to the substrate by use of a Laboratory Burgerjon spray tower (Burgerjon 1956) calibrated to deliver an application volume of 200 l ± 10% ha^{-1}. For tests performed on sand 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 series, 110°). The apparatus was calibrated to deliver an application volume of 400 l ± 10% ha^{-1}. When not specifically referred to in the description of the methods used, the exposure phase in the experiments started 1–2 h after product application, working with freshly applied, but dried, pesticide residues.

Aphidius rhopalosiphi tests

The insects used for these tests were obtained by mass rearing in the laboratory, established in 1994 from aphid mummies collected in winter wheat fields. The genetic stock used for rearing was renewed regularly. The parasitoid was produced using the cereal grain aphid *Sitobion avenae* (F.) as the host aphid and barley seedlings (*Hordeum vulgare* L.) as the host plants.

For the glass plate test, adult wasps were confined in groups of ten in test units made of two treated glass plates (10 × 10 cm) held apart by an untreated metal frame (10 × 10 × 2 cm), with the treated face of the glass plates turned inside the units. The metallic frame was pierced with several holes and covered with nylon gauze for ventilation. Two holes were used to provide food for the wasps (honey/water solution) via cotton pads. A third hole was used to connect the units to a peristaltic pump to renew the air in the unit every 1–2 min, to prevent pesticide vapour accumulating, and over-estimation of toxicity by inhalation. There were five replicates of ten wasps each (five males, five females, 0–48 h old) for each product and the water-treated control. After 48 h of exposure, the units were dismantled and mortality was checked. After exposure of the wasps to the insecticides, 15 female wasps were assessed for fecundity. When fewer than 15 survived the exposure, all surviving females were assessed for fertility. The female wasps were individually confined for 24 h on barley seedlings infested with 60–80 grain cereal aphids. Parasitised aphids were left to develop for 10–12 days and then counted when aphid mummies appeared. The tests were performed in a climatic chamber at 20 ± 2°C, 60–90% RH, and 16:8 L:D using a sodium lamp. Light intensity was 1,000–2,000 lux for the 48 h of exposure and 7,000–10,000 lux during fertility assessment. These methods were based on existing guidelines for the registration of pesticides (Mead-Briggs et al. 2000).

For the test on laboratory-treated plants, the wasps were confined for 48 h on treated barley seedlings (groups of 5–8 plants, 8–10 cm high, with two or

Table 1 Commercial name, active substance (a.s.) identity, concentration, formulation type, and amount of product applied

Commercial name	Active substance (a.s.)	a.s. Content	Formulation type	Amount applied (a.s. ha^{-1})
Plenum	Pymetrozine	50% w/w	WG	150 g
Teppeki	Flonicamid	50% w/w	WG	80 g
Decis Micro	Deltamethrin	6.25% w/w	WG	5 g
Pirimor G	Pirimicarb	50% w/w	WG	125 g

WG: water dispersible granules

three leaves) grown in plastic pots. These plants were surrounded by a perspex cage (12 cm ∅; 20 cm high) and infested with 60–80 grain cereal aphids to produce honeydew to attract the wasps to the treated plants and to feed them. There were six replicates of ten wasps (five males, five females) for each product and control. After 48 h of exposure, mortality was recorded and the surviving females were assessed for fertility in a same way as for the glass plate test. The test conditions were similar to those during the glass plate test, except that the light intensity was 7,000–10,000 lux during the exposure and fertility assessments.

For tests performed with field-treated plants, the plants originated from a 1.5 ha winter wheat field (cv "Centenaire") cultivated using normal agricultural practices in terms of fertilisation and herbicides. The field received only one fungicide treatment (Fandago Pro, 50 g l^{-1} fluoxastrobine + 100 g l^{-1} prothioconazole, 2.0 l ha^{-1}) applied between the first and second node stage (Zadoks GS31–32) on the whole field, at least six weeks before the start of the experiments. The test products were applied at the end of June after wheat flowering (Zadoks GS65–68) on small plots (3 × 10 m). One hour after application, tillers used for the test were sampled in the 2 × 8 m central part of these plots and tillers used for the control were sampled in an untreated 3 × 10 m plot. No rain occurred between treatments and sampling, and the mean temperature was between 15°C and 20°C. The test was then performed in the laboratory using field-treated plants as the exposure substrate, following the method described by Jansen (2001) and using a similar approach to that used in the first test performed with laboratory-treated plants, with assessment of lethal effects after 48 h of exposure and evaluation of the reproduction capacity of surviving females. The test conditions were similar to those during the first test performed on plants in the laboratory.

Adalia bipunctata test

The larvae used for this test were obtained by mass rearing in the laboratory, established in 1996 from adults sampled outside on ornamental bushes. The adults were kept in Plexiglass cages and fed with an excess of aphids (a mixture of pea aphids, *Acyrtosiphon pisum* (Harris) reared on French beans (*Vicia fabae* L.) and green peach aphids, *Myzus persicae* (Sulzer) reared on sweet pepper (*Capsicum annuum* L.)) and honeybee-collected pollen. The larvae were reared in plastic petri dishes and fed with the same aphid species until pupation.

For the initial toxicity test on glass plates, 2–3 day old *A. bipunctata* larvae were individually confined until pupation in units consisting of a small treated glass disc (5 cm ∅) surrounded by a plastic ring coated with Fluon GP1 to prevent the larvae from escaping. The larvae were fed daily with an excess of pea aphids. Pre-imaginal mortality was calculated when all surviving larvae pupated. There were four replicates of ten larvae per product and for the control. Adult ladybirds, when they emerged, were collected and the mean development time to the adult stage was calculated for each replicate. For fertility assessment, all adult ladybirds of the same treatment group obtained from the exposure period were grouped in plastic rearing cages and fed with an excess of pea and green peach aphids, water and honeybee-collected pollen. The dates of first egg-laying were noted, and in the second week of egg-laying five females per tested product, if this number survived, or all surviving females if fewer than five survived, were individually confined on plastic petri dishes. The adults were transferred daily into new, clean petri dishes and egg-laying was assessed over five consecutive 24-h periods. Pea aphids were offered in excess. Eggs were counted per female and retained to assess larval emergence and egg viability. The tests were performed in a climatic chamber at 20 ± 2°C, 60–90% RH, and 16:8 L:D using a sodium lamp (7,000–10,000 lux).

For the toxicity test on plants, exposure units consisted of young French bean seedlings treated with insecticides, or water for the control. Between 1 h and 2 h after product application, two *A. bipunctata* larvae (2–3 days old) were released on each individual plant and kept there until pupation. There were four replicates of ten larvae (five plants) for the control and for each tested product. Pea aphids were provided as food. Mortality was checked every 24 h until pupation. The pupae were harvested and left to emerge. Development time and fertility performance were recorded using the same approach as for the initial toxicity on glass plates. The test conditions were similar to those for the initial toxicity on glass plates.

Bembidion lampros test

A stock of *B. lampros* adults used for the test was caught in July and 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 in excess with *Ephestia kuehniella* eggs (Nutri-mac®), aphids and onion fly pupae.

The methods used were based on those developed to test the toxicity of products on the carabid beetle, *Poecilus cupreus* L., in registration studies at the European level (Heimbach et al. 2000). The exposure units consisted of a plastic box (17 × 12 × 6 cm) filled with 500 g sand. Water was added to each unit to attain 70% water-holding capacity of the sand. Three days before product application, six beetles from the laboratory stock were introduced into each unit, with *Ephestia* eggs as food. There were five replicates of six beetles for each treatment. After product application, mortality was checked on days 1, 2, 4, 7, 11 and 14. The final mortality was recorded on day 14. Food was renewed at each mortality assessment. The tests were performed in climatic chambers at 20 ± 2°C, 60–90% RH, and 16:8 L:D using a sodium lamp (1,000–2,000 lux).

Aleochara bilineata test

The methods used were based on those used for the registration of pesticides (Grimm et al. 2000). The effects of the product on *A. bilineata* were assessed by measuring the incidence of parasitism of onion fly pupae provided to adult rove beetles in a treated arena over a 28-day exposure period and by comparing this incidence with that for a water-treated control.

The *A. bilineata* adults used for the test were provided by a commercial supplier (De Groene Vlieg, Nieuwe Tonghe, The Netherlands) in the form of parasitised onion fly pupae. The parasitised onion fly pupae were left to hatch and the young rove beetles that emerged were transferred into plastic cages filled with wet sand and fed with frozen mosquito flies (Discus fish food) until they were used for the tests. The exposure units consisted of a plastic box (17 × 12 × 6 cm) filled with 500 g sand wetted at 70% of its water-holding capacity. The units were treated with the insecticides and twenty 3–7 day old rove beetles were released into each unit for a 28-day exposure period. On days 7, 14 and 21, 400 unparasitised onion fly pupae were carefully introduced into each unit. Frozen mosquito larvae were provided as food on days 0, 7, 14 and 21. On day 28, the units were carefully dismantled and the substrate gently rinsed through a sieve to separate sand, surviving beetles, and onion fly pupae added to the units during exposure. The fly pupae were left to dry at ambient temperature. Rove beetle emergence from the onion fly pupae harvested at the end of the exposure phase was assessed over a 6–8 week period, using modified Berlese units. During the exposure phase, the test conditions were similar to those for the *B. lampros* toxicity assessments. The 6–8 week emergence assessment was conducted in the laboratory at 15–25°C in the dark. There were four replicates of 20 rove beetles per treatment and 1,200 onion fly pupae added to each unit during the exposure period.

Episyrphus balteatus test

The larvae used for this test were obtained by mass rearing in the laboratory. The adults were kept in wooden-frame cages and fed with sugar and frozen honeybee-collected pollen. The larvae were kept on French bean seedlings and fed with pea aphids until pupation.

The exposure units consisted of five or six French bean plants, each 8–10 cm high and grown in plastic pots (20 cm ∅) filled with a mixture of gardening compost and vermiculite. The surface of the units was covered with fine sand to form a standard surface for observation. The plants were infested with an excess of pea aphids and then treated outside using a knapsack sprayer. The plants were left to dry under ambient conditions for 1–2 h. Ten larvae were then released into each unit and the units were covered with a perspex cylinder (20 cm ∅; 30 cm high) with the top closed by nylon gauze for ventilation. There were five replicates of ten larvae for the control and for each tested product. The units were kept in climatic chambers at 22–25°C, 50–90% RH, and 16:8 L:D using a sodium lamp (7,000–10,000 lux). Pea aphids were added on days three and six of the exposure phase. The units were dismantled after eight days of exposure, when the first pupae were observed in the control. Hoverfly pupae and the remaining larvae were collected in each unit and mortality was

calculated for each replicate. The surviving larvae were fed with pea aphids until pupation, and the pupae were kept until adult emergence. For the pupae in each treatment, mean development time was calculated on the basis of the development time obtained for each replicate. The adults were transferred into breeding cages (Jansen 1998) and fed with sugar and pollen. Water was provided on a piece of cotton wool. There was one breeding cage per test product and one for the control. The test conditions were similar to those used for larvae exposure, except for the lighting (neon tubes, 5,000–8,000 lux) because high adult mortality had been observed with the sodium lamp (Jansen, personal observation). In the second week after adult emergence, French beans infested with aphids were introduced into each cage to stimulate oviposition. The plants were renewed 2–3 times a week so as not to restrict egg production. Eight days after the first egg-laying, five females were randomly selected from each breeding cage and individually transferred into individual Plexiglas cylinders (20 cm ∅; 30 cm high) with a screen mesh top. An aphid-infested broad bean plant, sugar, pollen, and water were then introduced into each cylinder. Syrphids were left to lay their eggs for 24 h and the eggs were counted on the following day. The plants were renewed four times to obtain a total of five successive 24-h egg-laying periods. At each egg-laying, a subsample of at least 25 eggs, or all the eggs if fewer than 25 had been produced, were kept to check larval emergence. The total number of viable eggs per female was thus calculated.

Experimental design and statistical analysis

All replicates of the same experiment were carried out at the same time and with the same set of test organisms. The replicates were randomly distributed in the climatic chamber. As required, treatment mortality was corrected for observed control mortality using Abott's formula (Abott 1925). The results of the tests were analysed using Statistical Minitab software. A one-way ANOVA test (LSD) for variance analysis was performed, followed by Tukey tests for multiple comparisons between treatments ($P = 0.05$). Before analysis percentage observed mortality was transformed using the Arcsin method. The results of the ANOVA test (F, df, and P value) are given in the tables. The results of Tukey tests (t, df, and P value) are indicated in the text. Means that did not differ significantly from each other are followed by the same letter in the tables.

Results

The results of tests performed on glass plates and on plants with *A. bipunctata* larvae are given in Table 2. On glass plates, significant differences between treatments were detected for pre-imaginal mortality and fertility, but not for developmental time to the adult stage. Pre-imaginal mortality was significantly higher with flonicamide (77.5%) and deltamethrin (100%) than in the control group (20%) ($t = 6.025$ and 12.995, df = 6, $P = 0.0003$ and <0.0001). Mortality recorded with pymetrozine (35%) was not significantly higher than for the control, but was lower than with flonicamide ($t = 4.774$, df = 6, $P = 0.022$) and Decis ($t = 11.75$, df = 6, $P < 0.0001$). Flonicamide was also less toxic than deltamethrin ($t = 6.97$, df = 6, $P = 0.0001$). In terms of development time to the adult stage, control, pymetrozine and flonicamide were not significantly different. Females obtained from larvae treated with flonicamide, pymetrozine or water (control) produced means of 7.4, 13.6, and 14.4 viable eggs day^{-1} over a five-day period. The analysis revealed significant differences between flonicamide and the control and between flonicamide and pymetrozine ($t = 3.318$, df = 8, $P = 0.0157$ and $t = 2.973$, df = 8, $P = 0.0291$, respectively). Pymetrozine and control female egg production were similar ($t = 0.345$, df = 8, $P = 0.9368$).

When flonicamide was tested on plants in the laboratory, mortality and fertility did not differ from the control values. Development time to adult emergence was significantly longer in the flonicamide group than in the control group, with 21.1 days instead of 19.3 days ($t = 4.802$, df = 6, $P = 0.0172$) from egg to adult emergence. Apparent differences in development time between the tests performed on glass plates and on plants could be explained by the 1–2°C difference in mean temperature.

The results of the test with *A. bilineata* on sand in the laboratory are given in Table 3. Differences between treatments were detected. Of 1,200 onion fly pupae offered, rove beetles from the control units parasitised a mean of 678.5 pupae, and the incidence of parasitism reached 56.5%. This was significantly higher than the 5.9% parasitism observed for deltamethrin ($t = 8.258$,

Table 2 Toxicity of insecticides to larvae of the ladybird species *A. bipunctata* on glass plates and on plants in the laboratory

	Pre-imaginal mortality (mean ± SE)	Development time to adult stage (mean ± SE)	Viable eggs/female/day (mean ± SE)
Test on glass plates			
Control	20.0 ± 8.2 a	23.3 ± 0.16 a	14.4 ± 2.5 a
Pymetrozine	35.0 ± 12.6 a	22.9 ± 0.17 a	13.6 ± 3.2 a
Flonicamide	77.5 ± 17.1 b	23.4 ± 0.44 a	7.4 ± 4.0 b
Deltamethrin	100.0 ± 0.0 c	–	–
F-value	$F_{3,12} = 69.35$, $P < 0.001$	$F_{2,6} = 2.79$, $P = 0.139$	$F_{2,12} = 6.65$, $P = 0.011$
Test on plants			
Control	18.3 ± 11.0 a	19.3 ± 0.8 a	10.3 ± 2.7 a
Flonicamide	18.3 ± 8.0 a	21.1 ± 0.15 b	11.2 ± 2.0 a
F-value	$F_{1,6} = 0.00$, $P = 0.994$	$F_{1,3} = 23.06$, $P = 0.017$	$F_{1,8} = 0.32$, $P = 0.588$

Pre-imaginal mortality (%), development time to adult stage (days), and fertility of adult female obtained from the larvae exposed to the test products

One-way ANOVA followed by Tukey test for multiple comparison. Arcsin transformation for percentage. Results followed by different letters are significantly different ($P = 0.05$)

Table 3 Toxicity of insecticides to the rove beetle species *A. bilineata* on sand in the laboratory

	Parasitized onion fly pupae/unit (mean ± SE)	Incidence of parasitism	Parasitism reduction
Control	678.5 ± 83.0 a	56.5	
Pymetrozine	580.3 ± 77.5 ab	48.4	16.3
Flonicamide	438.8 ± 127.6 b	36.6	36.7
Deltamethrin	70.5 ± 43.8 c	5.9	89.8
F-value	$F_{3,12} = 27.41$, $P < 0.001$		

Mean number of parasitisized onion fly pupae, incidence of parasitism (%), and reduction in parasitism compared with control (%)

One-way ANOVA (LSD) followed by Tukey test for multiple comparison. Results followed by different letters are significantly different ($P = 0.05$)

df = 6, $P < 0.001$) and the 36.6% observed for flonicamide ($t = 3.377$, df = 6, $P = 0.025$), but did not differ from that observed for pymetrozine, 48.4% ($t = 1.501$, df = 6, $P = 0.4665$). No insecticides, except for deltamethrin used as the toxic standard, led to reduction in the incidence of parasitism of more than 50% compared with the control.

The results of the *A. rhopalosiphi* toxicity test on glass plates and on plants are given in Table 4. Differences between treatments were observed for mortality and fertility. No control mortality was observed and mortality was significantly higher for all the test products: 44.0% for flonicamide ($t = 3.018$, df = 8, $P = 0.0369$), 68.0% for pymetrozine ($t = 5.457$, df = 8, $P = 0.0003$), and 100% for deltamethrin ($t = 10.232$, df = 8, $P < 0.0001$). Deltamethrin was significantly more toxic than flonicamide and pymetrozine ($t = 7.213$ and 4.775, df = 8, $P < 0.0001$ and 0.0011, respectively). No differences were detected between flonicamide and pymetrozine ($t = 2.439$, df = 8, $P = 0.109$). Females that survived exposure to flonicamide and pymetrozine produced 83.0% and 91.0%, respectively, fewer aphid mummies than the control females. These values differed from the control ($t = 3.337$, df = 24, $P = 0.0063$ for flonicamide, $t = 3.171$, df = 20, $P = 0.0095$ for pymetrozine). The high variability of the data was partly explained by the null value observed in several replicates in the control (one female), flonicamide (six females) and pymetrozine groups (four females).

Table 4 Effects of insecticide on *A. rhopalosiphi* adults on glass plates and on plants

	48 h mortality (mean ± SE)	Mummies/female (mean ± SE) (number of females)	Reduction in fertility
Test on glass plates			
Control	0.0 ± 0.0 a	22.8 ± 20.0 a (15)	–
Pymetrozine	68.0 ± 22.0% b	2.0 ± 3.1 b (7)	91.0
Flonicamide	44.0 ± 18.2% b	3.8 ± 7.1 b (11)	83.0
Deltamethrin	100.0 ± 0.0 c	–	–
F-value	$F_{3,16} = 37.39$, $P < 0.001$	$F_{2,30} = 7.76$, $P = 0.002$	
Test on laboratory-treated plants			
Control	1.7 ± 4.1 a	21.9 ± 21.3 a (15)	
Pymetrozine	1.7 ± 4.1 a	6.1 ± 5.7 b (15)	72.0
Flonicamide	5.0 ± 8.4 a	9.6 ± 6.3 b (15)	56.1
Deltamethrin	31.7 ± 11.7 b	–	–
F-value	$F_{3,20} = 21.12$, $P < 0.001$	$F_{2,42} = 5.87$, $P = 0.006$	
Test on field-treated plants			
Control	6.0 ± 8.0 a	17.3 ± 9.4 a (15)	–
Pymetrozine	18.0 ± 7.5 a	18.3 ± 10.9 a (15)	−5.8
Flonicamide	14.0 ± 10.2 a	16.0 ± 8.7 a (15)	7.3
Deltamethrin	54.0 ± 8.0 b	15.3 ± 5.0 a (12)	11.2
F-value	$F_{3,20} = 14.48$, $P < 0.001$	$F_{3,53} = 0.28$, $P = 0.843$	

Mortality after 48 h exposure (%), number of aphid mummies produced by females that survived the exposure (number of females assessed), and reduction of fertility compared with control (%)

One-way ANOVA followed by Tukey test for multiple comparison. Arcsin transformation for percentage. Results followed by different letters are significantly different ($P = 0.05$)

When the products were applied to plants in the laboratory, toxicity was lower than on glass plates. Deltamethrin was the only product significantly different from the control, with 31.7% mortality ($t = 6.707$, df = 10, $P < 0.0001$). Although flonicamide and pymetrozine did not have significant lethal effects, aphid parasitization was affected by these products, with 56.1% reduction of aphid mummies produced per female for flonicamide and 72.0% for pymetrozine, compared with the control. Although these reductions were lower on plants than on glass plates, they were still significantly different from the control ($t = 2.541$, df = 28, $P = 0.0386$ and $t = 3.26$, df = 28, $P = 0.0062$, respectively).

On wheat plants treated outside and brought back into the laboratory for toxicity assessment, flonicamide and pymetrozine did not have lethal effects on adult survival and did not reduce the females' ability to parasitise aphids, compared with the control. Deltamethrin led to observed mortality of 54.0% and was still significantly more toxic than the control ($t = 6.33$, df = 8, $P < 0.0001$), flonicamide ($t = 4.75$, df = 8, $P = 0.0007$), and pymetrozine ($t = 3.68$, df = 8, $P = 0.0074$). This product did not reduce the ability of female wasps to parasitise aphids, compared with the control.

The carabid beetle, *B. lampros*, was not affected by either flonicamide or pymetrozine after being confined for 14 days on sand treated with these products (Table 5). Mortality was below 10.0% and did not differ from the control. Under the same conditions, deltamethrin was slightly more toxic, with 43.3% mortality. This differed significantly from the control ($t = 3.436$, df = 8, $P = 0.0161$), flonicamide ($t = 3.144$, df = 8, $P = 0.0288$), and pymetrozine ($t = 3.436$, df = 8, $P = 0.0161$).

The tested insecticides significantly affected the survival of *E. balteatus* larvae (Table 6). Pirimicarb was the most toxic product, with 84.0% observed mortality, and it differed significantly from all the other treatments, including the control ($t = 8.64$, df = 8, $P < 0.0001$). With 26.0% mortality, pymetrozine differed significantly from the control ($t = 3.35$, df = 8, $P = 0.0259$) but not from flonicamide. Flonicamide was the least toxic tested product and observed mortality did not differ from that for the control. No tested product affected development time to adult stage or fertility performance of the female.

Table 5 Toxicity of insecticides to the ground beetle species *B. lampros* on sand in the laboratory

	Observed mortality (mean ± SE)	Corrected mortality
Control	6.7 ± 9.1 a	
Pymetrozine	6.7 ± 9.1 a	0.0
Flonicamide	10.0 ± 14.3 a	3.4
Deltamethrin	43.3 ± 25.9 b	38.0
F-value	$F_{3,16} = 5.61$, $P = 0.008$	

Observed and corrected mortality (%) after 14 days of exposure

One-way ANOVA followed by Tukey test for multiple comparison. Arcsin transformation for percentage. Results followed by different letters are significantly different ($P = 0.05$)

Discussion

The results obtained in this study are indicative of the low toxicity of flonicamide and pymetrozine, in the laboratory, to several beneficial insect species representative of four of the main natural enemies of aphids: parasitic hymenoptera, ladybirds, hoverflies, and generalist ground-dwelling predators, for example carabid and rove beetles. The methods used met IOBC standards for laboratory tests on inert and natural substrates. As these standards are considered to be the worst-case scenario for exposure (maximum recommended field rate, forced exposure during long periods, most susceptible life stage tested when possible, etc.), no significant effects could be expected under field conditions when the products are harmless with these methods. Thus, flonicamide and pymetrozine could be highly selective aphid antagonists under field conditions.

Among products tested on aphid natural enemies by IOBC standard methods, very few were rated as harmless after laboratory exposure and most of the insecticides resulted in 100% mortality on glass plates and effects >50% on natural substrates (Hassan et al. 1987, 1988, 1991). Most of the harmless products were very specific compounds, for example IGR, *Bacillus thuringiensis*, insect virus-based insecticides, or specific acaricides, and none except pirimicarb was potentially useful for aphid control.

Pymetrozine on glass plates was not toxic to larvae of the two-spotted ladybird, *A. bipunctata*. Flonicamide was slightly more toxic to this species, with significant effects on larvae survival and adult egg production on glass plates. However, no effects were recorded on plants, indicating that the product would probably be harmless under field conditions. Compared with other insecticides, pymetrozine and flonicamide were far less toxic than insecticides such as synthetic pyrethroids, organophosphate, and neonicotinoids tested using similar methods (Hautier et al. 2006; Jansen and Hautier 2006; Jansen et al. 2008) and the level of effects observed in the laboratory with these two new compounds were similar to those with pirimicarb. The harmlessness of pirimicarb for ladybirds and the toxicity of classical insecticides in the laboratory were consistent with results obtained under field conditions (Hellpap 1982; Wiles and Jepson 1995; Jansen 2000).

Whereas pymetrozine had no significant effect on onion fly pupae parasitism by *A. bilineata*, flonicamide reduced it by 37% compared with the control. Although this reduction was significant, the effects were low compared with deltamethrin tested at the

Table 6 Toxicity of insecticides to larvae of the hoverfly species *E. balteatus* on plants in the laboratory

	Pre-imaginal mortality (mean ± SE)	Development time to adult stage (mean ± SE)	Viable eggs/female/day (mean ± SE)
Control	4.0 ± 8.0 a	16.6 ± 0.3 a	38.1 ± 15.8 a
Pymetrozine	26.0 ± 12.0 b	17.1 ± 0.5 a	38.9 ± 18.8 a
Flonicamide	12.0 ± 7.5 ab	17.5 ± 0.4 a	28.2 ± 10.7 a
Pirimicarb	84.0 ± 10.2 c	17.3 ± 0.6 a	54.1 ± 10.6 a
F-value	$F_{3,16} = 27.87$, $P < 0.001$	$F_{3,10} = 2.05$, $P = 0.170$	$F_{3,10} = 1.08$, $P = 0.401$

Pre-imaginal mortality (%), development time to adult stage (days), and fertility of adult females obtained from the larvae exposed to the test products

One-way ANOVA followed by Tukey test for multiple comparison. Arcsin transformation for percentage. Results followed by different letters are significantly different ($P = 0.05$)

same time. Other insecticides, for example carbosulfan, deltamethrin, lambdacyhalothrine, methiocarb and natural pyrethrins extracts, previously tested under the same conditions all gave 100% parasitism reduction on sand, although the toxicity of some was reduced to acceptable effects on natural soils (Jansen et al. 2008). The effects of deltamethrin were of the same magnitude in the two studies (90% and 100% parasitism reduction). Some 45 insecticides have also been assessed using different methodology but also based on IOBC standards, and none that could be used for aphid control, except pirimicarb, was harmless to this rove beetle (Samsoe-Petersen 1993). Thus, the addition of flonicamide and pymetrozine to this positive list could be helpful for IPM.

Among the beneficial arthropod species used for this study, *A. rhopalosiphi* seemed to be the most sensitive. High mortality was observed on glass plates and the ability of surviving females to parasitise aphids was reduced by more than 80%. On barley seedlings in the laboratory, the effects were reduced, but even if no significant mortality was recorded, effects on aphid parasitism ability of >50% were still detected. These results emphasized the need to assess both lethal and sub-lethal effects because if the assessment was based on survival only, these products on plants in the laboratory would have been rated as harmless. Because of these effects on plants in the laboratory, a higher-tier test was conducted with winter wheat tillers treated in the field and brought back to the laboratory. When these plants treated under conditions resembling conventional practice were used for exposure and toxicity assessment, no significant effects of pymetrozine and flonicamide were observed. Deltamethrin was still toxic, with 54% mortality. The results indicate that flonicamide and pymetrozine would probably be harmless to *A. rhopalosiphi* adults in the field. Compared with products tested using similar methods (Jansen 2001), these two products were less toxic than pyrethroids such as bifenthrin, deltamethrin, and esfenvalerate. The effects of deltamethrin assessed in the two studies were of a similar order (46.4% and 51.1% corrected mortality, respectively).

Adults of the carabid beetle, *B. lampros*, were not affected by flonicamide and pymetrozine on sand. At the same time, mortality caused by deltamethrin reached 43.9%. Although carabid beetles seem to be less sensitive than parasitic wasps or ladybirds to plant-protection products, very few insecticides on inert substrates were found to be harmless in previous studies in the laboratory. Among 32 insecticides used, only fenoxycarb and hexythiazox, an IGR and an acaricide, resulted in mortality lower than 50%. All pyrethroids and organophosphates tested caused 100% mortality or were close to this level (Hassan et al. 1987, 1988, 1991; Jansen et al. 2008).

Flonicamide and pymetrozine were not toxic for hoverfly larvae on plants in the laboratory. No difference in mortality from the control was observed and development time to adult stage and egg production was not affected. In the same experiment, pirimicarb was highly toxic to larvae, with 83.3% corrected mortality, but development time and egg production was not affected. These results confirm the toxicity generally observed for hoverflies with pirimicarb in the laboratory (Jansen 1998; Hautier et al. 2006) and in the field (Poehling and Dehne 1986; Storck-Weyhermuller 1989; Niehoff and Poehling 1995; Jansen 2001).

In the context of aphid control and selective products, the harmlessness of pymetrozine and flonicamide both to hoverflies and ladybirds is particularly interesting. Because ladybirds and hoverflies are often associated and exploit the same aphid resources, products that do not affect either predator could be interesting for IPM because classical insecticides that are harmless for hoverflies (e.g., some pyrethroids) are toxic to ladybirds, whereas pirimicarb, which is not toxic to ladybirds, is toxic to hoverflies.

In conclusion, flonicamide and pymetrozine seem to be very promising insecticides in the context of aphid control because they are highly selective for a range of beneficial arthropods related to aphid control. Toxicity observed in the laboratory was mostly negligible, even on glass plates, and lower than or equivalent to that of classical products used for aphid control. Pymetrozine and flonicamide are the only commercially available insecticides that are not toxic to hoverflies, ladybirds, parasitic hymenoptera, carabid and rove beetles at the same time. In addition, because they have been used for a few years only, no aphid resistance problems have yet been recorded for these compounds, although this could occur in the future.

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