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TOXICITY OF DIFLUBENZURON, PYRIPROXYFEN, IMIDACLOPRID AND DIAFENTHIURON TO THE PREDATORY BUG *ORIUS LAEVIGATUS* (*HET.: ANTHOCORIDAE*)

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The susceptibility of the predatory bug *Orius laevigatus* (Fieber) to the insect growth regulators diflubenzuron, pyriproxyfen, the nitroguanidine insecticide imidacloprid and the thiourea compound diafenthiuron was investigated in the laboratory. Fifth-instar nymphs were exposed to formulated materials of each compound and adults were exposed to formulated materials of diafenthiuron and imidacloprid. In each case, exposure via ingestion and residual contact was tested. Pyriproxyfen was harmless to *O. laevigatus* nymphs by both ways of exposure. The respective LC_{50}-values of diflubenzuron via ingestion and residual contact were 229.9 and 391.1 mg a.i./l. Diafenthiuron did not cause significant mortality to fifth-instar nymphs and adults via ingestion but was toxic by residual contact with LC_{50}-values of 329.4 mg a.i./l and 125.9 mg a.i./l for nymphs and adults respectively. Imidacloprid proved to be the most toxic compound with LC_{50}-values of 1.1 and 0.04 mg a.i./l for nymphs and 2.1 and 0.3 mg a.i./l for adults, via ingestion and residual contact, respectively. The results suggest that use of pyriproxyfen in an integrated pest management programme will not cause any problems but that imidacloprid, and to a lesser extent, also diflubenzuron and diafenthiuron could be harmful to the predator.

KEY-WORDS: *Orius laevigatus*, diflubenzuron, pyriproxyfen, imidacloprid, diafenthiuron, side-effects.

The anthocorid predator *O. laevigatus* (Fieber) is one of the most promising natural enemies for the control of the western flower thrips *Frankliniella occidentalis* (Pergande). *F. occidentalis* has become an important pest in greenhouses and ornamental crops since its introduction from Columbia into Europe (Robb *et al.*, 1988; Brodsgaard, 1989). Since this pest is very difficult to control in a chemical way, it was imperative to develop an integrated pest management (IPM) programme (Van de Veire & Degheele, 1992). A key principle of IPM is to maximize pest control from natural mortality factors such as predators and parasitoids. These are supplemented where necessary with pesticides, which should be used in a way that minimizes disruption of the biological control agents of the pests. This requires knowledge about chemicals, spray thresholds and times of application that are compatible with the natural enemies. In the present study, the susceptibility of *O. laevigatus* to diflubenzuron, pyriproxyfen, imidacloprid and diafenthiuron was investigated. The benzoylphenylurea diflubenzuron inhibits chitin synthesis which results in a disruption of the moulting process; it has been widely used against dipteran and lepidopteran pests (Grosscurt, 1978; Soltani, 1983; Anonymous, 1994).

The second, pyriproxyfen, is a phenyl ether juvenile hormone mimic. It has ovicidal activity and suppresses embryogenesis and adult formation. The compound is used against homopteran (Peleg, 1988; Yatki & Poehling, 1988), heteropteran (Langley *et al.*, 1990), thysanopteran (Nagai, 1990a), dipteran (Kawada *et al.*, 1987; Langley *et al.*, 1988) and lepidopteran pests (Hatakoshi *et al.*, 1986; Yokoyama & Miller, 1991).

Imidacloprid is a chloronicotinyl insecticide with systemic activity acting through ingestion or contact (Leicht, 1993). The compound affects the nervous system of insects (Mullins, 1993) and exhibits excellent insecticidal activity against aphids, leafhoppers, planthoppers, thrips and whiteflies (Oetting, 1990; Elbert *et al.*, 1991; Woodford & Mann, 1992).

Finally, we investigated the effects of the thiourea compound diafenthiuron. In the presence of sunlight and oxygen, diafenthiuron is converted into the active compound 3-(2,6-diisopropyl-4-fenoxyfenyl)-1-tert. butylcarbodiimide (DFCD). This compound inhibits ATP-ase activity and ATP-synthesis (Ruder & Kayser, 1993). Diafenthiuron is recommended for use against mites (Anonymous, 1989) and sucking insects such as whiteflies (Ishaaya *et al.*, 1993).

The toxicity of these four compounds to fifth-instar nymphs and adults of *O. laevigatus* by ingestion and residual contact was investigated.

MATERIALS AND METHODS

INSECTS

A laboratory colony of *O. laevigatus* was established in 1994 with insects originating from Koppert B.V. (The Netherlands). The bugs were reared in small cylinder-shaped cages consisting of a Plexiglas frame (9 cm in diameter, 4.1 cm high) and Plexiglas plates (9 cm in diameter) using the methods described by Van de Veire (1995). These cages were placed on a plastic support which contained tap water. A small rooted Spanish pepper plant (*Capsicum annuum* L.) (4 to 6 leaves) was placed inside each container with its roots immersed in the water of the plastic support. *O. laevigatus* was fed with eggs of *Ephestia kuehniella* Zeller (Biobest, Westerlo, Belgium) and pollen (Weyn's Honingbedrijf, Gent, Belgium). The predators were kept in growth chambers at 24 ± 1°C, 65 ± 5% R.H. and a photoperiod of 16:8 (L:D)h.

CHEMICALS

Formulated materials were used in all experiments. Diflubenzuron WP 25 was supplied by Solvay-Duphar B.V., Weesp, The Netherlands. Pyriproxyfen 10% emulsifiable concentrate was obtained from Sumitomo Chemical, Osaka, Japan. Imidacloprid (Confidor 200 SL) was supplied by Bayer, Leverkusen, Germany and diafenthiuron (Polo 250 SC) was obtained from Ciba-Geigy, Basel, Switzerland.

EXPOSURE BY INGESTION

Newly moulted fifth-instars (< 24h old) and adults (< 2d old) were collected from the rearing unit. They were placed two by two in small plastic boxes (4.5 cm in diameter, 1.5 cm high) (Etablissements Caubère, Yebles, France). Each box was provided with a moisture source, consisting of a plug of cotton wool fitted into a plastic dish (0.75 cm in diameter). The cotton wool was saturated with the test compound in distilled water. Control

groups were supplied with distilled water alone. Twenty nymphs or adults were tested with six (diflubenzuron, pyriproxyfen) or eight (imidacloprid, diafenthiuron) concentrations. During the first 24h, the predators were not provided with food. From the second day on, *E. kuehniella* eggs were supplied *ad libitum*.The cotton wool plug was moistened again with distilled water if needed. Mortality counts were performed three days after adult emergence.

RESIDUAL EXPOSURE

Newly moulted fifth-instars (< 24h old) and adults (< 2d old) were collected from the rearing unit. They were placed in cages consisting of a cylinder-shaped round Plexiglas frame (9 cm in diameter, 2.2 cm high) and two glass plates (9 cm in diameter). The insecticides were diluted in distilled water and were applied to one side of the glass plate using a Cornelis spray chamber (Van Laecke & Degheele, 1993) for pyriproxyfen and diflubenzuron or a Burkard spraying apparatus (Vercruysse, 1996) for imidacloprid and diafenthiuron. Treated plates were left to dry and then connected to the frame with adhesive tape. Vercruysse (1996) proved that the results obtained with both spraying apparatuses are comparable. With the Cornelis spray chamber a homogeneous spray deposit (1 - 1.5 mg/cm^2) was obtained by spraying 0.5 ml of the test suspension on the plates for 2.5 s at a pressure of 1 bar. For the controls, plates were sprayed with 0.5 ml of distilled water. The Burkard spraying apparatus was set at 1.5 - 2 mg fluid per cm^2. Twenty nymphs (all compounds) and adults (diafenthiuron and imidacloprid) were tested with 5 concentrations. The insects were provided with *E. kuehniella* eggs and a plug of cotton wool was saturated with distilled water and fitted in a plastic cup (0.75 cm in diameter) as a moisture source. Mortality counts were performed three days after adult emergence.

RESULTS

Table 1 highlights the biological activity of diflubenzuron and pyriproxyfen against fifth-instar nymphs of *O. laevigatus*.

Predators treated with diflubenzuron failed to shed the exuvia; these insects remained alive after emergence but were not able to feed and died within 24 hours. Some of the emerging adults had incompletely formed or indented wings. LC_{50}-values via ingestion and residual contact were 229.9 and 391.1 mg a.i./l, respectively.

Pyriproxyfen was practically harmless to fifth instars of *O. laevigatus* at concentrations of 1,000 mg a.i./l via both ingestion and residual contact.

Toxicity of imidacloprid and diafenthiuron to fifth instars and adults of *O. laevigatus* is shown in table 2.

Imidacloprid proved to be the most toxic compound. Both ingestion and residual contact caused severe mortality. In general, fifth-instar nymphs are more sensitive to imidacloprid than adults. Ingestion caused less mortality than residual exposure. LC_{50}-values for exposure to imidacloprid via ingestion were 1.1 and 2.1 mg a.i./l, for nymphs and adults, respectively; exposure via residual contact yielded LC_{50}-values of 0.04 and 0.3 mg a.i./l respectively.

Diafenthiuron did not cause significant mortality to fifth-instar nymphs and adults of *O. laevigatus* via ingestion at concentrations of respectively 2,000 and 1,000 mg a.i./l. The compound showed, however, some toxicity by residual contact. In the latter case, diafenthiuron was two to three times as toxic to adults as it was to nymphs. Fifty percent of nymphs and adults were killed after residual treatment with respectively 329.4 and 125.9 mg a.i./l.

TABLE 1

Toxicity of diflubenzuron and pyriproxyfen by residual contact and ingestion to fifth-instar nymphs of O. laevigatus[a]

Compound	n	Slope ± SE[b]	LC_{10} (95% FL)	LC_{50} (95% FL)	LC_{90} (95% FL)
Diflubenzuron					
Residual contact	20	1.6 ± 0.5	58.5 (1.2-155.1)	391.1 (140.5-815.6)	2615.5 (1121.0-62628.9)
Ingestion	20	2.0 ± 0.7	52.3 (2.4-110.2)	229.9 (108.0-397.3)	1010.5 (525.9-13246.5)
Pyriproxyfen					
Residual contact	20	-[c]	-	-	-
Ingestion	20	-[c]	-	-	-

[a] Based on mortality 3 days after adult emergence
[b] LC-values and slopes (in mg a.i./l) were calculated with POLO PC (LeOra Software, 1987)
[c] No mortality above control was noted at highest dose tested (1,000 mg a.i./l)

DISCUSSION

Predatory insects may be exposed to a pesticide via direct contact, residual contact or by feeding on contaminated prey. Moreover, anthocorids may also be poisoned when taking up contaminated free water or plant juices, since they need moisture to survive. They may acquire this by feeding on plants (Askari & Stern, 1972).

Pyriproxyfen did not show any harmful effect on nymphs of *O. laevigatus* by both ways of exposure. Nagai (1990a, b, c, 1991) came to the same conclusions when testing *Orius* spp. on eggplant treated with 100 mg a.i./l. However, effects of pyriproxyfen on heteropterans appear to vary between species. Langley *et al.* (1990) reported an ED_{50}-value of 0.15 µg/insect following topical application of pyriproxyfen to fifth-instar nymphs of *Rhodnius prolixus* Stål. Doses of 0.2 µg pyriproxyfen applied topically to fifth instars of this triatomine bug were needed to induce sterility of eggs deposited by attained adults; doses of around LD_{25} were not observed to induce egg sterility. De Clercq *et al.* (1995) showed that pyriproxyfen was highly toxic to the predatory bug *Podisus maculiventris* (Say) with LC_{50}-values of 0.3 and 15 µg/ml by residual contact and ingestion, respectively, and an LD_{50}-value of 27 ng/insect by topical application. However, latter workers showed that female adults emerging from fifth instars treated with concentrations around LC_{10} by ingestion (5 µg/ml) produced viable eggs and had similar oviposition rates as control bugs. Since pyriproxyfen had no harmful effect on *O. laevigatus* at concentrations of 1,000 mg a.i./l, which is well above field concentrations, we do not expect substantial problems with sterility of adults exposed to field concentrations. Findings of these and other studies suggest that use of pyriproxyfen in an integrated pest management programme that includes *Orius* bugs, will not cause any problems provided that pyriproxyfen is used at a field dose of 100 mg a.i./l. Nevertheless, further (semi-)field trials are needed to test the hypotheses made from the data of the present laboratory study. Evidence is accumulating that pyriproxyfen is harmful to some other insect predators. Yakti & Poehling (1988) reported that the predatory insects *Coccinella septempunctata* L. and, to a lesser extent, *Chrysoperla carnea* (Stephens), were affected by pyriproxyfen when young eggs, older larval instars and

pupae were treated topically or when adults or larvae were fed with treated prey at a concentration of 0.02%. Hattingh & Tate (1995) noted that applications of pyriproxyfen in citrus orchards in southern Africa adversely affected populations of the coccinellids *Rodolia cardinalis* (Mulsant) and *Chilocorus nigrata* (F.).

TABLE 2

Toxicity of imidacloprid and diafenthiuron by residual contact and ingestion to fifth-instar nymphs (N5) and adults of O. laevigatus[a]

Compound	n	Stage	Slope ± SE[b]	LC_{10} (95% FL)	LC_{50} (95% FL)	LC_{90} (95% FL)
Imidacloprid						
Residual contact	20	N5	1.2 ± 0.5	0.004 (0.0-0.03)	0.04 (0.0002-1.2)	0.5 (0.2-2.1)
	20	adult	2.2 ± 0.4	0.07 (0.02-0.1)	0.3 (0.2-0.4)	1.1 (0.7-2.4)
Ingestion	20	N5	0.5 ± 0.1	0.003 (0.0-0.04)	1.1 (0.1-2.9)	365.6 (95.5-9939.0)
	20	adult	0.8 ± 0.1	0.05 (0.01-0.2)	2.1 (1.0-3.8)	85.3 (34.7-380.9)
Diafenthiuron						
Residual contact	20	N5	2.2 ± 0.6	85.1 (9.5-188.2)	329.4 (125.3-533.7)	1275.6 (793.6-3146.1)
	20	adult	2.4 ± 0.5	36.7 (13.1-63.0)	125.9 (76.5-190.8)	432.2 (273.9-936.5)
Ingestion	20	N5	-[c]	-	-	-
	20	adult	-[d]	-	-	-

[a] Based on mortality 3 days after adult emergence
[b] LC-values and slopes (in mg a.i./l) were calculated with POLO PC (LeOra Software, 1987)
[c] No mortality above control was noted at highest dose tested (2,000 mg a.i./l)
[d] No mortality above control was noted at highest dose tested (1,000 mg a.s./l)

Diflubenzuron was more toxic to *O. laevigatus* when ingested via drinking water than by residual contact. In general, BPU's have been shown to be more effective by ingestion than by contact, because their cuticular absorption is relatively low; this phenomenon has often been claimed to be the reason for their selectivity towards beneficials (Retnakaran & Wright, 1987). Symptoms induced by diflubenzuron, such as the inability to shed the exuvia, were similar to those reported for the predatory bug *P. maculiventris* when diflubenzuron was applied topically or by ingestion. Fifth-instar nymphs of *P. maculiventris* were not sensitive to diflubenzuron by residual contact (De Clercq *et al.*, 1995). When applied in this way, the compound was harmless to *Aphidoletes aphidimyza* (Rondani) (Helyer, 1991) and *Acholla multispinosa* DeGeer (Broadbent & Pree, 1984). On the other hand, it was toxic to *C. carnea* (Vogt, 1992), *Ceraeochrysa cubana* (Hagen) (Mattioli *et al.*, 1992) and *Coccinella undecimpunctata* L. (Fayad & Ibrahim, 1988). Adults of *Geocoris punctipes* (Say) and adults and nymphs of *Hippodamia convergens* (Guérin-Méneville) were not sensitive to a topical treatment of diflubenzuron at 10,000 mg a.i./l (Wilkinson *et al.*, 1978). Application of diflubenzuron in orchards caused severe mortality to nymphs of *Forficula*

auricularia L. (Sauphanor *et al.*, 1993) but not to *Anthocoris nemorum* (L.) (Celli & Nicoli, 1989). The predatory mite *Amblyseius longispinosus* (Evans) was not sensitive when the compound was used at field doses (Clement & David, 1988). Treated full-grown larvae of *Chrysopa septempunctata* Wesmael died during the pre-pupal stage (Kawashima, 1988). Application of diflubenzuron in combination with *O. laevigatus* should be carefully considered; field doses range from 500 to 750 mg a.i./l and semi-field and field experiments are necessary to verify the results from our laboratory experiments.

Both ways of exposure to imidacloprid caused significant mortality in nymphs and adults. Our results indicate that nymphal and adult populations of *O. laevigatus* may be suppressed by foliar applications of imidacloprid at field rates (100 mg a.i./l). In both ways of exposure, nymphs were more sensitive to the compound than adults. De Cock *et al.* (1996) concluded that imidacloprid was harmful to *P. maculiventris* via both ingestion, with LC_{50}-values of 20.3 and 2.5 mg a.i/.l and residual contact, with LC_{50}-values of 4.15 and 0.46 mg a.i./l for nymphs and adults, respectively. Hough-Goldstein & Whalen (1993) could not observe significant mortality of the predator *Perillus bioculatus* (F.) after 24 h of contact with potato foliage recently sprayed with imidacloprid. Laboratory experiments showed that imidacloprid was harmless to the predatory insects *Deraeocoris nebulosus* (Uhler), *Olla v-nigrum* (Say), *Chrysoperla rufilabris* (Burmeister), and predatory mites *Neoseiulus collegae* (De Leon), *Phytoseiulus macropilis* (Banks) and *Proprioseiopsis mexacanus* (Mizell & Sconyers, 1992). Pflüger & Schmuck (1991) likewise concluded that the compound was not harmful to predatory mites.

Use of imidacloprid in integrated pest management programmes cannot be recommended given its high toxicity for *O. laevigatus*. It is advisable to limit its use as much as possible to soil applications or seed dressing, thus reducing the chances of predators being affected by the compound. However, since imidacloprid is a systemic insecticide, the drinking of contaminated plant juices might still have an effect on *O. laevigatus*.

Treatment with diafenthiuron induced symptoms such as frequent tumbling, that were similar to those reported for the spined soldier bug *P. maculiventris* (De Cock *et al.*, 1996), diamondback moth, *Plutella xylostella* (L.), twospotted spider mite *Tetranychus urticae* Koch, and bulb mite *Rizoglyphus echinopus* (Fumouze & Rabin) (Kadir & Knowles, 1991). Morphological abnormalities similar to those observed in *P. maculiventris* (De Cock *et al.*, 1996) did not occur in *O. laevigatus*. The compound did not cause any mortality when it was ingested with drinking water. De Cock *et al.* (1996) found that, when applied via ingestion, 50% of the nymphs and adults of *P. maculiventris* died at 169.1 and 31.4 mg a.i./l, respectively.

Diafenthiuron showed some toxicity when tested by residual contact; adults were more sensitive than nymphs with respective LC_{50}-values of 125.9 and 329.4 mg a.i./l. Our results suggest that *O. laevigatus* may be harmed by applying diafenthiuron at a recommended field dose of 300 mg a.i./l. Diafenthiuron was practically non toxic to *P. maculiventris* when applied topically with LC_{50}-values of 100,000 and 48,000 mg a.i./l for nymphs and adults, respectively. Both ingestion and residual contact caused severe mortality (De Cock *et al.*, 1996). Van de Veire & Degheele (1993), in contrast, classified diafenthiuron as harmless for both nymphs and adults of *Orius niger* Wolff according to IOBC-standards. The compound reduced populations of *Amblyseius fallacis* Garman and *Typhlodromus occidentalis* (Nesbitt) immediately after spraying but 4-day-old spray residues did not affect these predatory mites (Anonymous, 1989). El-Sayad & El-Ghar (1992) observed a strong reduction of the populations of the predators *C. carnea, C. septempunctata* and *Syrphus* spp. after treatment with diafenthiuron. This is in contrast to the findings of Streibert *et al.* (1988), who observed a temporary reduction of the populations of juvenile stages of heteropterous predators (Anthocoridae and Miridae) in field trials with diafenthiuron;

however, recovery was reported to take place within 10 days. In latter study, adults of Heteroptera, *Coccinella*, and *Chrysoperla* were not found to be affected by the compound.

Our results indicate that integrated pest management programmes involving diflubenzuron, diafenthiuron and imidacloprid should regard possible side-effects to *O. laevigatus*.

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RÉSUMÉ

La toxicité du diflubenzuron, pyriproxyfen, imidacloprid et diafenthiuron sur le prédateur *Orius laevigatus* (Fieber) (Heteroptera : Anthocoridae)

La sensibilité du prédateur *O. laevigatus* envers les insecticides diflubenzuron, pyriproxyfen, imidacloprid et diafenthiuron a été déterminée au laboratoire. Les larves du cinquième stade ont été exposées aux matériaux formulés de chaque produit et les adultes ont été exposés aux matériaux formulés de diafenthiuron et imidacloprid. Dans tous les cas, le traitement par ingestion et par contact résiduel a été examiné.

Le pyriproxyfen s'est avéré sans danger pour les larves de *O. laevigatus* selon les deux manières d'exposition. Les valeurs CL_{50} du diflubenzuron par ingestion et contact résiduel étaient de 229.9 et 391.1 mg m.a./l., respectivement. Le diafenthiuron n'a pas provoqué de mortalité significative par ingestion chez les larves et les adultes. Par contre, le diafenthiuron s'est montré toxique par contact résiduel, avec des valeurs CL_{50} de 329.4 et 125.9 mg m.a./l pour les larves et les adultes, respectivement. L'imidacloprid était le produit le plus toxique avec des valeurs CL_{50} de 1.1 et 0.04 mg m.a./l pour les larves et 2.1 et 0.3 mg m.a./l pour les adultes, par ingestion et par contact résiduel, respectivement.

Les données suggèrent que l'usage du pyriproxyfen ne causera pas de problèmes dans un programme de lutte intégrée. Cependant l'usage de l'imidacloprid et, à un degré moindre, du diflubenzuron et du diafenthiuron en combinaison avec *O. laevigatus* doit être considéré avec prudence.

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