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Toxicity of selected insecticides to the two-spot ladybird *Adalia bipunctata*

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Abstract The toxicity of pirimicarb, imidacloprid, dimethoate, lambda-cyhalothrin, flonicamid and spinosad to the two-spot ladybird, *Adalia bipunctata,* was evaluated in a laboratory study. Susceptibility of fourth instars and female adults was assessed by measuring toxicity via residual contact and ingestion through feeding on contaminated green peach aphids (*Myzus persicae*). Flonicamid and spinosad had no lethal effects on larvae and female adults. Pirimicarb was harmless to the predator by ingestion exposure but showed some residual toxicity at high concentrations to both larval and adult stages. Imidacloprid was highly toxic to the larval stage by residual and ingestion exposure but caused very low adult mortality when ingested through contaminated prey. Dimethoate and lambda-cyhalothrin were highly toxic to both the larval and adult stages of the ladybird. Our findings indicate that pest management programs in agricultural crops using dimethoate, lambda-cyhalothrin and, to a lesser degree, imidacloprid, are detrimental to *A. bipunctata*, whereas pirimicarb, flonicamid and spinosad are more compatible with the use of this predator.

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The two-spot ladybird *Adalia bipunctata* (L.) is a polyphagous predator native to Europe, Central Asia and North America (Hodek 1973). It has played a major role in the natural control of aphid pests in various habitats and has also been commercialized for augmentative biological control of aphids in Europe since 1999 (De Clercq et al. 2005; Omkar and Pervez 2005). The sole use of biological control may not always be sufficient to manage insect pest populations and corrective insecticide treatments may be needed, particularly in greenhouses. Furthermore, chemical control may be required to suppress pests that have no effective biological control agents. Hence, compatibility between natural enemies and pesticides is a primary concern in programs of integrated pest management (IPM) (Hassan and Van de Veire 2004).

In the current study, the compatibility of two biorational insecticides (spinosad and flonicamid) and four conventional insecticides (pirimicarb, dimethoate, lambda-cyhalothrin and imidacloprid) with fourth instars and female adults of *A. bipunctata* was assessed, following residual contact or ingestion of treated prey. These compounds are routinely used against aphid and lepidopteran pests in protected cultivation in Europe, where *A. bipunctata* may be an additional asset in IPM programs.

Larvae and adults of *A. bipunctata* were maintained in growth chambers at 23±1°C, 65±5% r.h., and a 16:8 h (L:D) photoperiod and provided with an ad libitum supply of a 50/50 w/w mixture of frozen *Ephestia kuehniella* Zeller eggs and fresh bee pollen (De Clercq et al. 2005), supplied by Koppert BV, Berkel en Rodenrijs, the Netherlands. Green peach aphid, *Myzus persicae* (Sulzer), used for the ingestion assay, was reared on broad bean, *Vicia faba* L. var. *thalia,* at 26±2°C, 60±20% r.h. and a 16:8 h (L:D) photoperiod. The insecticides pirimicarb (Pirimor®, 50% water dispersible granular), dimethoate (Perfekthion S®, 500 g l^{-1} emulsifiable concentrate), lambda-cyhalothrin (Karate Zeon®, 100 g l^{-1} capsule suspension), imidacloprid (Confidor®, 200 g l^{-1} suspension concentrate), flonicamid (Teppeki®, 50% water dispersable granular) and spinosad (Tracer®, 480 g l^{-1} suspension concentrate) were selected for the experiments. Newly moulted fourth instars (12–24 h old) and freshly emerged female adults (12–48 h old, 12.5–14 mg) of *A. bipunctata* were exposed to different concentrations of the insecticides by residual contact and by ingestion. Fourth instars were selected because of their superior predation capacity to the younger instars (Jalali et al. 2009) and hence their greater expected contribution to biological control.

To evaluate residual contact activity, the predators were placed in a cage consisting of a Plexiglas ring (9 cm diam, 3.5 cm high) sandwiched between two glass plates (9×9 cm). Insecticide solution (600 µl) was sprayed on one side of the glass plates (constituting the inner walls of the cage) using a Cornelis spray tower, at 1 bar pressure (1.5 mg aqueous insecticide deposit cm^{-2}). For the controls, the plates were sprayed with 600 µl of distilled water. Predators were placed individually (larvae) or 3–4 per cage (adults) approximately 1 h after application of the insecticide. The ranges of concentrations tested were: 75–750 mg a.i. l^{-1} (pirimicarb), 0.78–6.25 mg a.i. l^{-1} (dimethoate, fourth instars), 0.78–12.5 mg a.i. l^{-1} (dimethoate, adults), 0.5–3 mg a.i. l^{-1} (lambda-cyhalothrin, fourth instars), 1–15 mg a.i. l^{-1} (lambda-cyhalothrin, adults), 1.04–6.25 mg a.i. l^{-1} (imidacloprid, fourth instars), 3.125–100 mg a.i. l^{-1} (imidacloprid, adults), 1.56–1000 mg a.i. l^{-1} (flonicamid), and 1.56–2000 mg a.i. l^{-1} (spinosad). Ten and three replications were done per concentration for larval and adult stages, respectively. The same numbers of replications were prepared for the controls. *Ephestia kuehniella* eggs on rectangular pieces of paper (3×5 cm with 200–300 eggs cm^{-2}) were provided as food every day and old eggs were removed. An Eppendorf tube stoppered with a moist piece of cotton was used as a water source. Survival was monitored during 3 days in the case of adults or throughout the stadium for fourth instars (4–6 days). For the ingestion exposure, 2000–2500 *M. persicae* individuals (mixed instars) were placed in a cylindrical glass container (12.5 cm diam×10 cm high) and sprayed with 1160 µl of each compound at different concentrations under 0.5 bar pressure resulting in a 1.50±0.06 mg aqueous insecticide deposit cm^{-2}. The ranges of concentrations tested were: 75–2500 mg a.i. l^{-1} (pirimicarb), 10–200 mg a.i. l^{-1} (dimethoate), 0.5–8 mg a.i. l^{-1} (lambda-cyhalothrin, fourth instars), 1–20 mg a.i. l^{-1} (lambda-cyhalothrin, adults), 20–100 mg a.i. l^{-1} (imidacloprid, fourth instars), 80–600 mg a.i. l^{-1} (imidacloprid, adults), 1.56–1000 mg a.i. l^{-1} (flonicamid), and 1.56–2000 mg a.i. l^{-1} (spinosad). For the control, aphids were sprayed with distilled water. Treated aphids were transferred to a clean container for a few minutes before being introduced to the predator cages. Fourth instars of the predator were provided daily with an *ad libitum* supply of freshly sprayed aphids during the 4–6 day period. Adults were supplied *ad libitum* with freshly sprayed aphids for three consecutive days. Replication and survival assessment were performed as described for the residual contact test. Probit regression lines were generated using POLO-PC (LeOra Software 1987), to estimate lethal concentration (LC) values (mg a.i. l^{-1}) and their 95% confidence limits.

Dimethoate, lambda-cyhalothrin and imidacloprid were consistently the most toxic of the tested insecticides when *A. bipunctata* was exposed by residual contact (Table 1). Based on LC_{50} values, adults were three and six times more susceptible to lambda-cyhalothrin and dimethoate than to imidacloprid. Larvae, however, were equally susceptible to these insecticides, with LC_{50} values ranging from 0.8 to 1.7 mg a.i. l^{-1}. Residual contact toxicity to pirimicarb was low for both larvae and adults, with LC_{50} values exceeding 400 mg a.i. l^{-1}. After exposure to dimethoate, lambda-cyhalothrin or imidacloprid by residual contact, larvae of *A. bipunctata* were more susceptible than adults based on the calculated LC_{50} values. Among the insecticides tested, lambda-cyhalothrin caused the highest mortal-

Table 1 Toxicity of different insecticides to fourth instars (L4) and female adults of *Adalia bipunctata* by residual contact and ingestion exposure[z]

Insecticide	Stage	Residual contact				Ingestion			
		n	Slope±SE	LC_{50} [95% CL]	χ 2 (df)	n	Slope±SE	LC_{50} [95% CL]	χ 2 (df)
Dimethoate	L4	60	5.3±2.2	0.8 [0.5–1.0]	32 (48)	70	3.2±0.7	37.8 [25.1–51.4]	45 (58)
	Adult	70	2.6±0.5	2.3 [1.7–3.1]	15 (16)	70	3.5±0.8	50.3 [35.6–66.8]	8 (16)
Lambda-cyhalothrin	L4	70	3.0±0.7	1.1[0.7–1.5]	58 (58)	60	2.7±0.6	1.5 [1.0–2.2]	44 (48)
	Adult	80	2.5±0.6	4.7 [2.9–7.0]	20 (26)	70	2.4±0.5	6.1 [4.1–9.3]	9 (16)
Pirimicarb	L4	70	4.4±1.1	454.0 [351.2–594.7]	87 (58)	70	/	/	/
	Adult	70	9.0±2.4	527.5 [463.1–590.3]	11 (22)	70	/	/	/
Imidacloprid	L4	59	1.8±0.7	1.7 [0.7–2.7]	49 (47)	70	3.4±0.9	35.0 [21.6–46.3]	47 (48)
	Adult	118	1.4±0.3	13.8 [9.8–20.8]	30 (44)	70	4.7±1.1	245.5 [190.9–300.3]	8 (16)

[z] Survival was monitored for 4–6 days for fourth instars or for 3 days for adults; n: total number of insects tested; LC: lethal concentration (mg a.i. l^{-1}); CL: confidence limits

No mortality above control was observed at the highest concentration tested for flonicamid (1000 mg a.i. l^{-1}) and spinosad (2000 mg a.i. l^{-1}) by either exposure route, and for pirimicarb (2500 mg a.i. l^{-1}) by ingestion exposure

ity by consumption of treated aphids. The toxicity of this compound was 25 and 23 times higher (LC_{50}) than that of dimethoate and imidacloprid, respectively, in the case of fourth instars, and eight and 40 times higher in the case of adults. Dimethoate was more toxic by ingestion to adults of *A. bipunctata* than imidacloprid, but larvae were equally susceptible to either insecticide. Although imidacloprid was toxic to larvae (LC_{50}: 35.0 mg a.i. l^{-1}), it had low impact on adults at up to double the recommended field dose (*ca* 100 mg a.i. l^{-1}). Pirimicarb caused no mortality by ingestion within the range of examined concentrations. For both larvae and adults, spinosad and flonicamid did not differ in terms of mortality from the control after residual contact or ingestion exposure.

The study demonstrated differences between the insecticides evaluated in terms of their toxicity to *A. bipunctata*. Dimethoate and lambda-cyhalothrin were the most toxic insecticides both by residual contact and ingestion. Many studies have reported toxic effects of these two pesticides to predatory coccinellids (e.g. Galvan et al. 2005; James 2003; Singh et al. 2004; Tillman and Mulrooney 2000). In a study by Olszak (1999), 30 pesticides (including insecticides, acaricides and fungicides) were tested on different stages of *A. bipunctata*; pyrethroids (alpha-cypermethrin, fenpropathrin, esfenvalerate, acrinathrin) and an organophosphate (phosalone) proved to be the most toxic compounds. In our study, *A. bipunctata* was highly susceptible to imidacloprid, especially by residual contact. Bozsik (2006) found imidacloprid to be harmless to *Coccinella septempunctata* L. adults. Michaud (2002) showed that imidacloprid was toxic to larvae of *Harmonia axyridis* (Pallas) and *Cycloneda sanguinea* L. using a similar method of exposure. In our laboratory study, pirimicarb was demonstrated to be harmless to *A. bipunctata*. The selectivity of this insecticide has been demonstrated for many coccinellid species (e.g. Cabral et al. 2008; James 2003; Jansen 2000). The biorational insecticides flonicamid and spinosad did not induce mortality in fourth instar and adult *A. bipunctata* at up to ten times the recommended field concentrations (*ca* 100 mg a.i. l^{-1} for both insecticides). Spinosad has been reported to be harmless to coccinellids (e.g. Elzen and James 2002; Galvan et al. 2005; Tillman and Mulrooney 2000). There is little information on the effects of flonicamid on *A. bipunctata* or other natural enemies. According to Cloyd and Dickinson (2006), direct sprays of flonicamid on adults of the coccinellid predator *Cryptolaemus montrouzieri* Mulsant at label-recommended rates did not reduce survival after 72 h.

We observed a different response of *A. bipunctata* when exposed by tarsal contact with dry spray residues or when fed on surface-contaminated aphids. Pirimicarb, dimethoate and imidacloprid exposure by ingestion resulted in higher LC_{50} values than residual exposure for both life stages, whereas LC_{50} values were similar for lambda-cyhalothrin. Better survival of the predator in the ingestion assay may in part be

related to a lower consumption of aphids that had died as a result of the insecticide treatment. Based on LC_{50} values, imidacloprid was highly toxic to *A. bipunctata* adults in the residual contact bioassay, but was harmless via ingestion, with less than 10% expected mortality at the recommended field concentration (100 mg a.i. l^{-1}). There was a clear difference between the susceptibility of fourth instars and female adults of *A. bipunctata* to the tested insecticides. This difference was distinct for imidacloprid, which was seven to eight times more toxic to larvae than to adults for both exposure methods. Because of their closer adhesion to the substrate using the anal pads and their higher foraging activity, larvae may be more likely to be in contact with a contaminated surface (dry spray deposit on glass plates). Furthermore, the exposure time might also be important: in the present study adults were exposed to the insecticides for 3 days, compared with 4–6 days for larvae. Earlier instars of the predator may be more susceptible to toxic materials than the fourth instars tested here.

In conclusion, our laboratory experiments show that the level of toxicity of the tested insecticides to *A. bipunctata* may be highly influenced by the exposure method and life stage. Flonicamid and spinosad were non-toxic to fourth instars and adults of *A. bipunctata*. These insecticides may be incorporated into IPM programs for the control of certain greenhouse pests, such as aphids, caterpillars, and thrips. Pirimicarb also was minimally toxic to *A. bipunctata*. In contrast, dimethoate, lambda-cyhalothrin and imidacloprid were highly toxic to the ladybird. Additional laboratory studies assessing sub-lethal effects and field studies are needed to understand fully the selectivity to *A. bipunctata* of the evaluated insecticides.

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