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Compatibility of a natural enemy, *Coleomegilla maculata lengi* (Col., Coccinellidae) and four insecticides used against the Colorado potato beetle (Col., Chrysomelidae)

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Abstract: The toxicity of four insecticides used to control the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), imidacloprid (Admire®), cryolite (Kryocide®), cyromazine (Trigard®), and *Bacillus thuringiensis* var. *tenebrionis* (Novodor®), to one of its natural enemies, the 12-spotted lady beetle, *Coleomegilla maculata lengi* Timberlake (Coleoptera: Coccinellidae) was determined in the laboratory. Toxicity assays against *C. maculata* adults and larvae consisted of (1) topical applications and (2) exposures to treated foliage and prey, using concentrations up to 10 times the manufacturer's recommendations. Over a 6-day period, cyromazine (insect growth regulator) and *B. t.* var. *tenebrionis* (microbial insecticide) had no lethal effects on first and third instars *C. maculata*. For both larval and adult stages, cryolite (inorganic insecticide) caused very low predator mortality when topically applied and moderate mortality when ingested through contaminated eggs of Colorado potato beetles. Imidacloprid (systemic organic insecticide) was highly toxic to adult and larval *C. maculata*. Its estimated LD_{50} at 6 days following treatment, corresponded to 0.02–0.09 times the recommended field concentration, depending on the developmental stage and mode of contamination. These results indicate that integrated pest management programmes for Colorado potato beetles using imidacloprid or, to a lesser degree, cryolite, would be detrimental to *C. maculata*. Cyromazine and *B. t.* var. *tenebrionis* seem to present a better compatibility with the protection of *C. maculata* populations.

Key words: imidacloprid, cryolite, cyromazine, *Bacillus thuringiensis*, *Coleomegilla maculata lengi*, *Leptinotarsa decemlineata*

1 Introduction

The 12-spotted ladybird beetle, *Coleomegilla maculata lengi* Timberlake (Col., Coccinellidae), is a nearctic polyphagous predator that attacks aphids (Coderre et al., 1987), mites (Putman, 1964), eggs of lepidopterans (Warren and Tadic, 1967), or immature stages of coleopteran species (Giles et al., 1994; Giroux et al., 1995). In potato fields, Hazzard et al. (1991) recorded an egg mortality of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Col., Chrysomelidae) up to 58%, because of predation by *C. maculata* natural populations. In semi-field studies, Groden et al. (1990) observed a significant reduction in the survival of Colorado potato beetle eggs and first instars in cages where adult *C. maculata* had been introduced. In laboratory studies, Giroux et al. (1995) showed that, like adults, *C. maculata* third and fourth instars could be of interest as a biological control agent, with predation rates being half and equal respectively to the predation by the adults (seven eggs or five first instars attacked in 24h at 20°C).

Since integrated pest management (IPM) gained acceptance as a preferred approach of pest control, many efforts have been initiated to protect natural or introduced enemies by choosing selective pesticides (Croft, 1990). To fully take advantage of beneficials as key components of the potato agroecosystem, potential effects of pesticides on natural enemies, such as *C. maculata*, should be assessed. Many studies have shown susceptibility of *C. maculata* to chemical insecticides such as malathion, cypermethrin, carbaryl, benomyl, methyl parathion, and methomyl (Johnson, 1974; Coats et al., 1979; Lecrone and Smilowitz, 1980; Scott et al., 1983; Weissling and Meinke, 1991; Roger et al., 1994). More recently, different insecticides have been introduced to control populations of Colorado potato beetles that have not been tested for non-target effects. Among them are imidacloprid (1-[(6-Chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolodinimine), a broad-spectrum systemic insecticide that belongs to a new class of insecticides, nitroguanidines, and used in soil applications or foliar treatments; cryolite (sodium aluminofluoride), an inorganic insecticide suitable for

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spray or dust foliar application; cyromazine, an insect growth regulator applied as a foliar spray; and *Bacillus tenebrionis* var. *tenebrionis*, a microbial insecticide, also applied as a foliar spray, and for which different formulations have been marketed. Little information is available on susceptibility of *C. maculata* to these insecticides.

The objective of this study was to determine the toxicity of four insecticides used to control Colorado potato beetle populations, i.e. imidacloprid, cryolite, cyromazine, and *B. t.* var. *tenebrionis* to *C. maculata* adult and immature stages following direct contact or exposure to treated foliage and prey.

2 Material and Methods

2.1 Insects

Adult *C. maculata* were collected in May, from hibernation sites in Saint-Hyacinthe (72°56′W, 45°39′N), PQ, Canada. They were kept in an incubator on a wild flower pollen and liver-based artificial diet at 20°C, 70% RH, and a photoperiod of 16 : 8 h (L : D). Eggs were collected daily and put in Petri dishes for hatching. Larvae were reared under the same conditions and diet. These conditions were maintained throughout the experimental period. Eggs of the Colorado potato beetle were collected at L'Assomption (73°25′W, 45°50′N), PQ, Canada, in May. Beetles were reared on 'Kennebec' potato plants in the laboratory. All eggs used in the tests were < 24 h old.

2.2 Insecticides

The insecticides used in the bioassays were (a) imidacloprid [Admire® 240 water flowable solution (FS), Bayer, Etobicoke, ON] (240 g AI/l); (b) cryolite [Kryocide® water wettable powder (WP), Elf Atochem North America, Philadelphia, PA] (96% AI); (c) cyromazine (Trigard® 75WP Ciba-Geigy Canada, Mississauga, ON) (75% AI); and (d) *B. t.* var. *tenebrionis*, [Novodor® flowable concentrate (FC); Novo Nordisk, Danbury, CO] (3% coleopteran active toxin AI, i.e. 15 000 Colorado potato beetle international units per gram). Dilutions were prepared with distilled water in order to obtain solutions corresponding to 10, 1, 0.1, 0.01, and 0.001 times the manufacturer's recommended field rates for foliar application with 300 l/ha, hereafter referred to as '10×', '1×', '0.1×', '0.01×', and '0.001×'. These recommended field rates (1×) correspond to 200 ml for imidacloprid, 11 kg for cryolite, 373 g for cyromazine, and 6 l for *B. t.* var. *tenebrionis*. Control insects were treated with distilled water. For each insecticide, bioassays were conducted on two *C. maculata* developmental stages. Those stages were chosen on the basis of the mode of action of the insecticide and the developmental stages that are usually targeted or affected when used against Colorado potato beetles. Imidacloprid and cryolite were tested on *C. maculata* adults and third instars, while cyromazine and *B. t.* var. *tenebrionis* were tested on first and third instars.

2.3 Assessment of positive controls

To verify the quality of the insecticide samples, bioassays were conducted on early first-instar Colorado potato beetles. For each insecticide (water for the control) four young larvae (< 24-h old) were placed on a potato leaflet that had previously been dipped in the appropriate 1× solution and air-dried for 5 min. Leaflets were then placed in 50 mm Petri dishes. This procedure was repeated for nine replicates, for a total of 36 beetles per treatment. Mortality was recorded after 6 days. Differences in the proportion of dead larvae between the control and the other treatments were determined with a chi-squared test using the software Statview (version 1.03 for Macintosh computers) (Abacus Concepts, 1988).

2.4 Topical application

For each treatment, four coccinellids were treated on the ventral side with 1 μl of the appropriate solution using a Pipetman P20 micropipette (Gilson Medical Electronic, Villiers-le-Bel, France). In the fields, following a pesticide treatment, the insects may come into contact with the product either dorsally by the spray or ventrally by walking on treated plants. As predators are highly mobile, contact with the pesticide may occur mainly by the ventral side. Treated beetles were then placed in a single 50 mm Petri dish with excess water and pollen as food. This procedure was repeated for a minimum of four replicates, for a total of at least 16 beetles tested per concentration. Each insecticide was tested at concentrations of 0.1×, 1×, and 10×. A 0.01× treatment was added for imidacloprid. Mortality was noted every 24 h over a 6-day period. Data were corrected using Abbott's (1925) formula. For each product, probit analysis was carried out with the POLO-PC software (LeOra Software, 1987) for data sets corresponding to 2 and 6 days after treatment.

2.5 Exposure to treated foliage and prey

For each treatment, potato leaflets holding a Colorado potato beetle egg mass, were dipped in the appropriate solution, air-dried for 5 min, and then placed in a 50 mm Petri dish with four coccinellids, for a period of 6 days. Following pre-tests, eggs were provided *ad libitum* > 90 eggs per Petri dish. The number of Colorado potato beetle eggs consumed by the coccinellids was not recorded, however, in all cases some eggs (> 10) still remained at the end of the test. The procedure was repeated for a minimum of 10 replicates, for a total of at least 40 beetles tested per concentration. Each insecticide was tested at concentrations of 0.1×, 1×, and 10×. Treatments of 0.01× and 0.001× were added for imidacloprid. Mortality was noted every 24 h. For each product, a probit analysis was carried out as in the previous experiment (2 and 6 days after treatment).

3 Results

3.1 Assessment of positive controls

Bioassays conducted with Colorado potato beetle larvae confirmed the toxicity of all insecticides. After 6 days, the mortality of first instars fed on treated potato foliage was 100% for imidacloprid, cryolite, and *B. t.* var. *tenebrionis*, and 95.0% for cyromazine. These percentages differed significantly from the 5.0% mortality obtained in the control ($\chi^2 = 36.2$ and 32.4; d.f. = 3; $P < 0.0001$).

3.2 Topical application

Daily mortality rates observed in imidacloprid treatments (fig. 1) indicated a quick dose–response effect. Over 80% mortality was observed after 48 h, in 1× and

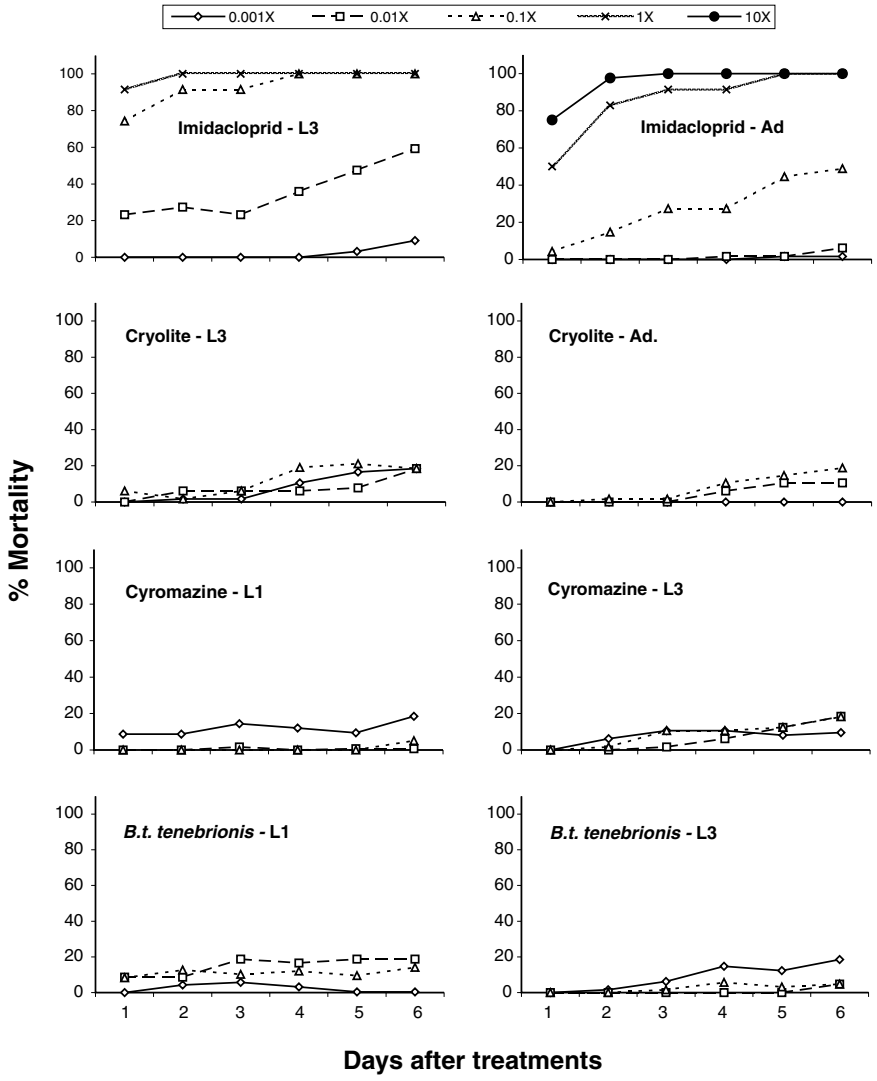

Fig. 1. *Cumulative mortality of* Coleomegilla maculata *adults (Ad), third (L3), and first instars (L1) (mean ± SE) following topical application of imidacloprid (broad-spectrum systemic insecticide), cryolite (inorganic insecticide), cyromazine (insect growth regulator), and* Bacillus thuringiensis *var.* tenebrionis *(microbial insecticide). 1× corresponds to the manufacturer's recommended field concentrations of 200 ml Admire®, 11 kg Kryocide®, 373 g Trigard® and 6 l Novodor® per hectare in 300 l of water, respectively*

Table 1. *Toxicity of imidacloprid after ventral application to different* Coleomegilla maculata *developmental stages*

			After 2 days			After 6 days		
Insecticide	St.[a]	*n*	Slope ± SE	LD_{50}[b] (95% CL)	χ^2	Slope ± SE	LD_{50} (95% CL)	χ^2
Imidacloprid	Ad	20	290.2 ± 58.0	0.074 μg (0.027–0.270)	13.9 (10)	329.4 ± 97.0	0.013 μg (0.005–0.027)	2.1 (10)
Imidacloprid	L3	16	386.6 ± 131.0	0.034 μg (0.015–0.078)	7.0 (10)	247.4 ± 66.6	0.008 μg (0.002–0.019)	2.0 (10)

Ad, adult; L3, third instar larva; CL, confidence limits; AI, active ingredient.
[a] Stage of *C. maculata.*
[b] LD_{50}, μg AI/insect.

10× treatments, for both adult and third instar *C. maculata*. Imidacloprid was very toxic with an estimated LD_{50} respectively for adults and third instars of 0.074 and 0.034 μg AI/insect (0.46× and 0.21×) after 2 days, and, of 0.013 and 0.008 μg AI/insect (0.08× and 0.05×) after 6 days (table 1).

Very low mortality was observed in cryolite topical treatments for both adult and third instar *C. maculata*. With the range of concentrations tested, cryolite did not cause sufficient mortality to allow LD_{50} estimations for neither third nor first instar *C. maculata*. This was also the case for cyromazine and *B. t.* var. *tenebrionis*.

3.3 Exposure to treated foliage and prey

High mortality rates were observed with imidacloprid, reaching 100% 2 days after the 10× treatment, for both adults and third instar *C. maculata* (fig. 2). At the recommended field concentration (1×), mortality rates at 6 days after treatment were 78.2 and 95.1% for adults and third instars, respectively. Toxicity of imidacloprid to *C. maculata* was very high with an estimated LD_{50} of 60.8 and 14.4 mg AI/l (0.38× and 0.09×) for adults, and 12.8 and 3.2 mg AI/l (0.08× and 0.02×) for third instar, after 2 and 6 days, respectively (table 2). Third instars were significantly more

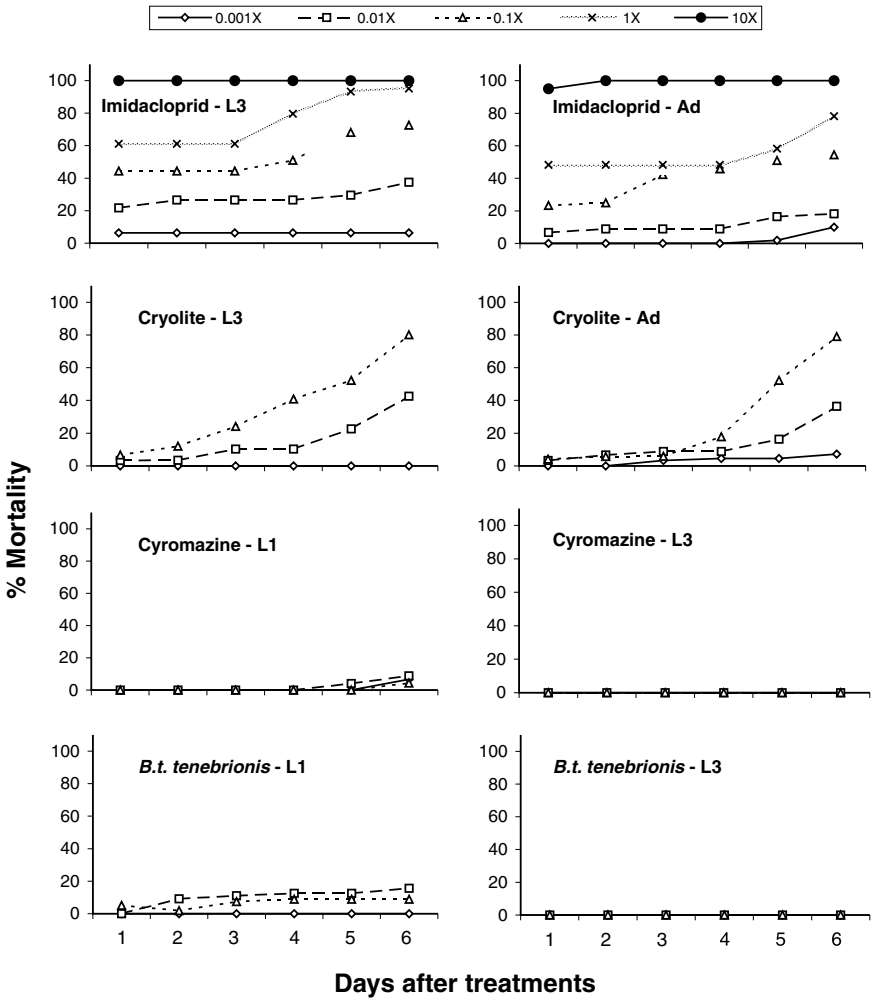

Fig. 2. *Cumulative mortality of* Coleomegilla maculata *adults (Ad), third (L3), and first instars (L1) (mean ± SE) following exposure to potato foliage and* Leptinotarsa decemlineata *eggs contaminated with imidacloprid (broad-spectrum systemic insecticide), cryolite (inorganic insecticide), cyromazine (insect growth regulator), and* Bacillus thuringiensis *var.* tenebrionis *(microbial insecticide). 1× corresponds to the manufacturer's recommended field concentrations of 200 ml Admire®, 11 kg Kryocide®, 373 g Trigard®, and 6 l Novodor® per hectare in 300 l of water, respectively*

Table 2. *Toxicity of foliage and* Leptinotarsa decemlineata *eggs treated with insecticides to* Coleomegilla maculata

			After 2 days			After 6 days		
Insecticide	St.[a]	*n*	Slope ± SE	LD_{50}[b] (95% CL)	χ^2	Slope ± SE	LD_{50} (95% CL)	χ^2
Imidacloprid	Ad	60	177.4 ± 21.4	60.8 (33.6–126.1)	10.4 (10)	171.8 ± 29.6	14.4 (2.45–41.36)	18.1 (10)
Imidacloprid	L3	60	107.0 ± 16.8	12.8 (4.4–35.3)	10.8 (10)	200.8 ± 39.0	3.2 (0.8–7.4)	5.7 (10)
Cryolite	Ad	36	[c]			10.99 ± 2.37	83424 (23459–302672)	8.6 (6)
Cryolite	L3	45	[c]			13.32 ± 2.85	58784 (22119–117823)	6.7 (7)

Ad, adult; L3, third instar larva; CL, confidence limits; AI, active ingredient.
[a] Stage of *C. maculata*.
[b] LD_{50}, mg AI/l.
[c] No significant mortality above control could be detected.

susceptible than adults after 2 days (confident limits, table 2) but no difference was recorded after 6 days. At high and moderate concentrations (10×, 1× and 0.1×), intoxication symptoms were observed almost immediately. In many cases, very few of the contaminated eggs were consumed.

Toxicity of cryolite was lower and took longer than imidacloprid to manifest. At the recommended field concentration (1×), observed mortality rates for adults and third instar *C. maculata*, 6 days after treatment, were 36.4 and 42.6%, respectively (fig. 2). After 48 h, low mortality rates did not allow estimations of LD_{50}. However, 6 days after treatment, LD_{50} values were estimated for adults and third instars at 83424 and 58784 mg AI/l (2.37× and 1.67×), respectively (table 2).

It was not possible to estimate LD_{50} for cyromazine and *B. t.* var. *tenebrionis*, as they did not cause any significant mortality, for neither third nor first instar *C. maculata*. In both treatments, most of the contaminated eggs were consumed at the end of the tests.

4 Discussion

Drastic differences were observed in the toxicity of the different products against the 12-spotted ladybeetle.

The more toxic insecticide to *C. maculata* was imidacloprid confirming its very broad-spectrum activity among arthropods. Although a certain level of specificity can be observed, this specificity is based on the fact that this compound acts only on the nicotinergic type of acetylcholine receptors (Abbink, 1991). However, these receptors, when compared with the muscarinergic receptors, are found in most insects (Breer and Sattelle 1987, in Abbink, 1991). Susceptibility to imidacloprid has been shown for many arthropod species among mites (Bullock and Pelosi, 1993), aphids (Palumbo and Kerns, 1994; Stark et al., 1995), whiteflies (Ernst, 1994), noctuids (Lagadic et al., 1993), plant bugs (Mizell and Sconyers, 1992), lacewings (Mizell and Sconyers, 1992), parasitoids (Stark et al., 1995), weevils (Marco and Castanera, 1994), scarabs (Drinkwater and Groenewald, 1994), bees (Stark et al., 1995) and wireworms (Drinkwater, 1994). On insect predators, Pflüger and Schmuck (1991) stated that imidacloprid has limited effects because of its predominantly systemic action. That is the case for different species of predatory mites for which only low toxicity was observed (Mizell and Sconyers, 1992). However, detrimental effects have been observed on predatory bugs (De Cock et al., 1996) and ladybeetles (Mizell and Sconyers, 1992; Stark et al., 1995; Kaakeh et al., 1996). In addition, Smith and Krischik (1999) showed that *C. maculata* adults, confined with imidacloprid-treated flower inflorescences, had a lower mobility and took longer to produce their first egg than beetles from untreated controls. The results of the present study indicate that, if sprayed on foliage, imidacloprid could affect natural enemies. Observations on beetle mortality during the first days following exposure to imidacloprid-treated foliage and prey indicate a possible 'knockdown effect' of the compound on the insects, as several beetles, apparently dead during 1 or 2 days, revived. Roger et al. (1991) described a similar phenomenon with *C. maculata* following contact with cypermethrin or benomyl, suggesting the possibility of overestimating the effect. This points to the importance of looking at the subjects longer than a 24- or 48-h period in laboratory studies.

Although mortality did not occur as quickly, nor as strongly as with imidacloprid, cryolite was toxic to *C. maculata*, particularly following exposure to contaminated eggs. As cryolite acts as a 'stomach insecticide', apparently by releasing fluoride ions and simultaneously inhibiting several enzymatic processes (Corbett et al., 1984), toxicity via ingestion was expected to be higher. The susceptibility to cryolite of *Perillus bioculatus* (Hem., Pentatomidae), another predator of Colorado potato beetles, has been studied by Hough-Goldstein and Keil (1991). Their results indicated no or very low toxicity 2 days after topical and residual contact, or ingestion of contaminated prey. After 48 h, our results also indicate very low toxicity of this compound. However, after 6 days, mortality of L3/adults was significantly greater, with estimated LD_{50} corresponding to about twice the recommended concentrations. Again, these results underline the importance of longer observation periods in such bioassays.

Our results also confirm the higher specificity of insecticides such as cyromazine or *B. t.* var. *tenebrionis*. As this insect growth regulator has proved effective against the Colorado potato beetle (Sirota and Grafius, 1994), many might expect potential negative effects on other coleopteran insects such as coccinellids. Such variability in the susceptibility to cyromazine among species of the same order has also been observed with Lepidoptera (Miller et al., 1981). It is also the case with *B. t.* strains, where susceptibility among coleopteran insects varies considerably according to species and developmental stages (Herrnstadt et al., 1986, 1987; Krieg et al., 1987; Cranshaw et al., 1989; Bauer, 1990; Hough-Goldstein and Keil, 1991). In field experiments using *B. t.* var. *tenebrionis*, 30% mortality of adult of the ladybeetle *Coccinella septempunctata* L. was observed, 5 days following treatment (Mateeva et al., 1991), while no significant reduction of predators was noted by Krieg et al. (1984). In laboratory tests carried out with *B. t.* var. *tenebrionis*, no mortality was observed either on larvae of the phytophagous ladybeetle *Epilachna varivestis* Muls. (Krieg et al., 1987). Giroux et al. (1994a,b) showed that *B. t.* var. *san diego* was not lethal to *C. maculata* adults and larvae. As *B. t.* var. *san diego* and *B. t.* var. *tenebrionis* have been found to be identical strains (Krieg et al., 1987), similar results between this study and Giroux et al. (1994a,b) were expected. However, different levels of susceptibility may be due to the two different commercial formulations used. The absence of significant mortality caused by cyromazine and *B. t.* var. *tenebrionis* on *C. maculata* third and even first instars, with concentrations up to 10 times the manufacturer's recommendations, are conclusive regarding their lethal potency on this natural enemy as exposure to toxicants was maximized through ingestion. However, in order to evaluate their innocuity towards *C. maculata*, sub-lethal effects of these two compounds should be studied. Such effects have already been described by Dogan (1994) who observed a reduction in larval development, pupal weight, and ovipositional rate of *Hippodamia convergens* Guérin with *B. t.* var. *tenebrionis*. In addition, Giroux et al. (1994a) observed a reduction in predation efficiency of *C. maculata* adults upon prey contaminated with *B. t.* var. *san diego*. Other biorational pesticides including neem extracts and a commercial formulation of the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin, caused less mortality to *C. maculata* adults than a conventional pesticide, carbaryl (Smith and Krischik, 2000).

Finally, concerning the method of insecticide application, the estimated toxicity was lower following topical applications than after exposure to treated eggs and foliage. Despite the fact that this second protocol was designed mostly to have beetles ingesting the compound, mortality may have been caused by contact effects (walking on contaminated foliage), by gas phase effects (in closed Petri dishes), or by both. Furthermore, intoxication symptoms were observed very quickly, often before any consumption of eggs. In addition, dipping leaves into insecticides may lead to higher amounts of insecticides on the plant compared

with standard spraying methods. Then, conditions of the second experiment should be considered as extremes.

These preliminary results suggest that IPM programmes against Colorado potato beetles using foliar applications of imidacloprid may not be compatible with the protection of *C. maculata* natural populations nor with augmentative releases of this predator. Imidacloprid may be more compatible with biocontrol when used as soil applications in the field, as beneficial insects may not come in contact with the pesticide. Cryolite showed a much lower toxicity than imidacloprid, but its use could also have negative effects on natural or introduced populations of the predator. Nevertheless, cryolite could represent an interesting alternative to be used later in the season, if necessary, when mostly old larvae of Colorado potato beetles are present, and against which, *C. maculata*, or bacterial insecticides and growth regulators are less effective. Its impact on *C. maculata* populations could then be minimized as very few egg masses are still present, and therefore most *C. maculata* gone. Although sub-lethal effects of cyromazine or *B. t.* var. *tenebrionis* have not been investigated in this study, these compounds seem to present the greater compatibility with *C. maculata*, and therefore should be more suitable in IPM programmes against Colorado potato beetles. Finally, laboratory data may be of limited value to predict compatibility of insecticides and natural enemies *in situ* (see Stark et al., 1995) and further field or semi-field studies are needed to confirm the present results under real-world conditions.

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