Effects of Biorational Pesticides on Four Coccinellid Species (Coleoptera: Coccinellidae) having Potential as Biological Control Agents in Interiorscapes

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ABSTRACT The direct toxicity of insecticidal soap, horticultural oil, Azatin, an extract from the Neem tree containing azadiractin, and BotainiGard, a commercial formulation of the entomopathogenic fungus *Beauveria bassiana*, was assessed on adults of four species of coccinellids—*Hippodamia convergens* (Guérin-Méneville), *Coleomegilla maculata* (DeGeer), *Harmonia axyridis* Pallas, and *Cryptolaemus montrouzieri* Mulsant. All biorationals caused less mortality than a conventional pesticide, carbaryl (Sevin). Horticultural oil (Sunspray ultrafine oil) consistently had no effect on beetle survivorship. Insecticidal soap (M-Pede) significantly reduced survival in all replicates for *C. maculata* and in at least one of the three replicates for the other three coccinellid species. *Beauveria bassiana* (BotaniGard) significantly reduced survival of *C. montrouzieri* at 72 h after spray in all three replicates. Azatin reduced survivorship in only one species, *C. maculata,* in only one of the three replicates.

KEY WORDS Coccinellidae, interiorscape pest management, biorational pesticides, biological control, nontarget effects.

HEAVY PUBLIC USE of interiorscapes, coupled with concerns about ecological and human health risks associated with synthetic pesticides, has contributed to increased interest in alternatives to conventional insecticides, including the use of biological control agents and biorational pesticides (Steiner and Elliot 1987, Stauffer and Rose 1997, Miller and Uetz 1998). The diverse plant species and microclimatic conditions typical of interiorscapes result in a great variety of pest species (Stauffer and Rose 1997). Although biological control agents are available for many of these pests (Steiner and Elliot 1987), managing the entire range of pests likely to be encountered in interiorscapes often requires integration of chemical and biological controls (Stauffer and Rose 1997). For these reasons, biorational pesticides that are compatible with natural enemies and present minimal risk to the environment and human health are preferred for interiorscape pest management (Steiner and Elliott 1987, Miller and Uetz 1998). Insecticidal soaps, horticultural oils, azadiractin, and *Beauveria bassiana* have been shown to be effective against the most common interiorscape pests, including the citrus mealybug, *Planococcus citri* (Risso); aphids such as *Myzus persicae* (Sulzer), *Aphis gossypii* (Glover) and *Chaetosiphon fragaefolii* (Cockerell); greenhouse whiteflies, *Trialeurodes vaporariorum* Westwood; and the twospotted spider mite, *Tetranychus urticae* Koch (Raupp et al. 1992, Lowery et al. 1993, Milner 1997, Miller and Uetz 1998). Although these biorational pesticides are touted as being compatible with natural enemies, few data are available documenting their effects on the biological control agents most commonly used in interiorscapes (Oetting and Latimer 1995).

For interiorscape pest management, inundative release of adults is the most common use of coccinellids. Because the life stages of a species can show differential response to the same pesticide (Banken and Stark 1998), we screened some commonly used biorational insecticides with adult coccinellids to best reflect current use. The International Organization for Biological Control has developed a procedure for assessing the impacts of pesticides on nontarget organisms (Hassan 1989). The first step is to determine the effect of the pesticide on the biological control agent in laboratory bioassays. If no harmful effects are observed, the pesticide can be considered compatible with the nontarget species. If harmful effects are observed, further testing in semi-field and field situations is required.

We conducted bioassays to test the compatibility of four common biorational pesticides (soap, oil, Azatin, and BotaniGard), with four commercially available adult coccinellid predators that are currently used, or have potential, as biological control agents for interiorscapes. *Hippodamia convergens* (Guérin-Ménéville), the convergent lady beetle, is often released for aphid control. *Coleomegilla maculata* (DeGeer) is a potentially effective biological control agent for interiorscapes because it consumes a variety of soft-bodied prey and can survive on plant pollen when prey numbers are low (Hodek and Honek 1997). *Harmonia axyridis* Pallas, the multicolored Asian lady beetle, is

Table 1. *F* and *P* values from two-way ANOVAs testing the effect of biorational pesticides on survival of four coccinellid species

Source of variation	*Hippodamia convergens*				*Coleomegilla maculata*				*Harmonia axyridis*				*Cryptolaemus montrouzieri*			
	8 h		72 h		8 h		72 h		8 h		72 h		8 h		72 h	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Treatment[a]	10.12	0.001	7.72	0.001	80.31	0.001	69.45	0.001	13.32	0.001	8.95	0.001	28.90	0.001	115.34	0.001
Replicate[b]	2.12	0.12	10.75	0.001	50.51	0.001	51.85	0.001	15.11	0.001	11.72	0.001	23.65	0.001	9.72	0.001

[a] df = 4,293. The conventional pesticide treatment Sevin was omitted from this analysis because it violated the assumption of constant variance.
[b] df = 2,293.

a generalist known to feed on aphids and scale insects (Hodek and Honek 1997). *Cryptolaemus montrouzieri* Mulsant, the mealybug destroyer, is widely used to control the citrus mealybug, *P. citri*, in greenhouses and interiorscapes (Steiner and Elliot 1987).

Materials and Methods

Organisms Tested. Adult *C. maculata, C. montrouzieri,* and *H. convergens* were purchased from ARBICO (Tucson, AZ). *Harmonia axyridis* adults were purchased from The Green Spot (Nottingham, NH). Upon receipt, each beetle species was held for at least 24 h in rearing boxes in an incubator under a photoperiod of 16:8 (L:D) h, 25 ± 1°C, and 55–75% RH. They consumed honey and water ad libitum.

Pesticides Tested. Sunspray Ultrafine Oil (parrafinic oil, Sun, Philadelphia, PA), M-Pede (soap, potassium salts of fatty acids, Mycogen, San Diego, CA), Azatin (azadiractin, Olympic Horticultural Products, Mainland, PA), BotaniGard (*B. bassiana,* strain GHA, Mycotech, Butte, MT), and Sevin (carbaryl, Dexol Industries, Torrance, CA) were mixed at the labeled rate and applied with 16-oz hand-held trigger sprayers. The rates tested were: Sunspray Ultrafine oil, 20 ml/l; M-Pede insecticidal soap, 20 ml/l; Azatin, 1.25 ml/l; Botainigard, 7.5 ml/l; and Sevin, 5.21 ml/l. For comparisons to the biorationals, we included Sevin as a conventional pesticide and water as a control treatment.

Bioassay Designs. A group of four beetles were placed into petri dishes (100 x 15 mm) containing a piece of medium porosity filter paper. There were 20 petri dishes in each treatment for a total of 80 beetles per treatment. Each dish was treated with 0.8 ± 0.2 ml of solution, sprayed as a mist over the beetles. Beetle survival was checked at 8, 24, 48, 72, and 80 h. For the duration of the bioassays, dishes were kept in an incubator under a photoperiod of 16:8 (L:D) h, 25 ± 1°C, and 55–75% RH. This design was replicated three times for each species, with all six treatments performed simultaneously.

Statistical Analyses. The percentage of beetles surviving per dish was transformed to the arcsine of the square root. For each species, the effect of replicate and treatment was analyzed with a two-way analysis of variance (ANOVA) using the percentage surviving per dish as the response. Sevin killed all adults within 24 h, so it was omitted from this analysis to satisfy the assumption of homogeneous variances. Survival for each species was analyzed at 8 and 72 h. When analyzed by two-way ANOVA, the replicate term was significant in all but one case. Therefore, each replicate for every species was analyzed independently, with Sevin returned to the analysis. When each replicate was analyzed independently, the constant variance assumption of ANOVA was repeatedly violated; some treatments had 100% survival or, in the case of Sevin, 0% survival. Therefore, Wilcoxon rank-sum tests were used to compare survival in each treatment to survival in the control within the replicate. Following the Bonnferroni method, a significance level of $\alpha = 0.01$ was used for these tests, which kept an experiment-wide significance level of $\alpha = 0.05$ within each replicate (Sokal and Rohlf 1995, SAS Institute 1998).

Results

Survival of beetles exposed to biorational pesticides varied among the replicate bioassays for all four species tested, as indicated by a significant replicate effect in the ANOVA (Table 1). Survival in the controls was >98% after 8 h for all species, and ranged from 81.3 to 100% after 72 h (Tables 2–5). Sevin caused 100% mortality for all species in all replicate bioassays, except one by 8 h and in all replicates by 72 h (Tables 2–5).

M-Pede was the only biorational pesticide to affect survival of all four species. The survival of *C. maculata* (Table 3) was reduced by soap in all three replicates by 8 h. M-Pede reduced survival in one of three replicates for *H. axyridis* and *C. montrouzieri,* and two of three replicates for *H. convergens* (Tables 2–5).

BotaniGard had no effect on *H. convergens* or *H. axyridis,* but dramatically decreased survival of *C. montrouzieri* in all three replicates by 72 h (Table 5). BotaniGard reduced *C. maculata* survival after 8 h in two of three replicates. Azatin affected survival of one species, *C. maculata,* in only one replicate. Sunspray oil had no effect on any of the species.

Discussion

All biorational pesticides tested had less of an effect on survival of the coccinellids than Sevin, which resulted in 100% mortality. Of the biorationl pesticides tested, M-Pede, an insecticidal soap, had the most consistent negative effects. Soap reduced survival at 72 h in two of three replicates for *H. convergens,* all three replicates for *C.*

Table 2. Percentage survival of *H. convergens* exposed to biorational pesticides and a conventional pesticide (Sevin) in laboratory bioassays

Treatment	Replicate 1		Replicate 2		Replicate 3	
	8 h	72 h	8 h	72 h	8 h	72 h
Control	98.8 ± 1.25	97.5 ± 1.72	100	100	100	97.5 ± 1.72
Azatin	100	96.3 ± 2.74	100	93.8 ± 2.48	100	96.3 ± 2.05
Sunspray	98.8 ± 1.25	88.8 ± 5.28	100	97.5 ± 1.72	100	98.0 ± 2.00
Botanigard	100	95.0 ± 2.29	100	98.8 ± 1.25	100	97.5 ± 1.72
M-Pede	91.3 ± 3.75	69.6 ± 5.00*	95.0 ± 2.29	92.5 ± 2.63*	97.5 ± 1.72	97.5 ± 1.72
Sevin	0*	0*	0*	0*	0*	0*

Means followed by an asterisk are statistically different from controls within a column ($\alpha = 0.01$, Wilcoxon rank sum test).

maculata, one of three replicates for *H.axyridis,* and one of three replicates for *C. montrouzieri* (Tables 2–5). Insecticidal soap works on contact by disrupting cell membranes (Hough-Goldstein and Keil 1991). Although our laboratory bioassays indicate that insecticidal soap may cause mortality in coccinellids, further testing may reveal that in interiorscapes, the use of insecticidal soap is compatible with the release of predatory coccinellids. Because direct contact is required for soap to take effect, the high mobility of adult coccinellids may reduce their exposure to insecticidal soap in interiorscapes. For example, Osborne and Pettit (1985) found in laboratory tests of direct toxicity that insecticidal soap decreased survival of the predaceous mite *Phytoseiulus persimilis* Athias-Henriot, which is used for control of *T. urticae* in the greenhouse. However, greenhouse tests showed that predatory mites did not suffer increased mortality when infested plants were sprayed with soap (Osborne and Pettit 1985).

BotaniGard, the commercial formulation of the entomopathogenic fungus *B. bassiana,* reduced survival of *C. montrouzieri* at 72 h after spray in all three replicates. It reduced survival of *C. maculata* in two of three replicates at 8 h after spray. BotaniGard did not significantly reduce the survival of *H convergens* or *H. axyridis* to levels statistically different from controls. However, given the high mortality of *C. montrouzieri* caused by this formulation of *B. bassiana,* further testing in the field is warranted to determine if BotaniGard is compatible with augmentative releases of *C. montrouzieri* adults. Although Goettel et al. (1990) lists *C. montrouzieri, C. maculata,* and *Hippodamia* spp. among the many nontarget hosts for *B. bassiana,* the authors' caution that entries on the list are based on single specimens and identifications of host and pathogen may be questionable. Furthermore, they argue that entomopathogenic fungi may be more specific under field conditions and during epizootics than in lab studies. James et al. (1998) found that *H. convergens* larvae pick up three times more *B. bassiana* conidia in laboratory exposures than in greenhouse sprays. In addition, small differences in environmental conditions can have a large effect on the virulence of entomopathogenic fungi (James et al. 1998).

Azatin reduced survival to 10% compared with 92.5% for controls at 72 h in replicate 2, whereas survival in replicates 1 and 3 was not different from controls (91.3 and 97.5% survival, respectively, at 72 h) in *C. maculata.* It is possible that beetles used in replicate 2were of lower quality than beetles used in replicates 1 and 3, which was responsible for their increased mortality from Azatin. The beetles used in each replicate came from separate shipments. When averaged across all treatments, beetles in replicate 2 had lower overall survival (47.7% survival with Sevin omitted) than those in replicates 1 (81.0% survival with Sevin omitted) and 3 (65.8% survival with Sevin omitted). Variation in the quality of commercially available coccinellids is well documented and could account for this variation in survival among replicates (O'Neil et al. 1998). Beetle age and previous nutrition can greatly affect bioassay results.

Past research did not find direct toxic effects of formulations of azadiractin on adult coccinellids. Banken and Stark (1998) found that a commercial product containing azadiractin did not cause mortality of adults of *Coccinella septempunctata* L., but that it did reduce oviposition by these beetles and significantly delayed larval development. The best-documented effects of azadiractin involve insect growth regulation and feeding deterrency (Banken and Stark 1998, Koul et al. 1990).

Table 3. Percentage survival of *C. maculata* exposed to biorational pesticides and a conventional pesticide (Sevin) in laboratory bioassays

Treatment	Replicate 1		Replicate 2		Replicate 3	
	8 h	72 h	8 h	72 h	8 h	72 h
Control	100	98.8 ± 1.25	100	92.5 ± 2.62	98.8 ± 1.25	96.3 ± 2.05
Azatin	97.5 ± 1.72	91.3 ± 2.73	11.3 ± 5.58*	10.0 ± 4.59*	100	97.5 ± 1.72
Sunspray	100	92.5 ± 3.19	98.8 ± 1.25	90.0 ± 0.03	100	100
Botanigard	98.8 ± 1.25	90.0 ± 4.21	63.8 ± 8.79*	47.5 ± 8.09*	81.3 ± 6.25*	70.0 ± 7.39*
M-Pede	66.3 ± 6.09*	57.5 ± 7.04*	12.5 ± 0.04*	10.0 ± 3.80*	15.0 ± 4.9*	15.0 ± 4.9*
Sevin	9.4 ± 3.13*	0*	0*	0*	0*	0*

Means followed by an asterisk are statistically different from controls within a column ($\alpha = 0.01$, Wilcoxon rank sum test).

Table 4. Percentage survival of *H. axyridis* exposed to biorational pesticides and a conventional pesticide (Sevin) in laboratory bioassays

Treatment	Replicate 1		Replicate 2		Replicate 3	
	8 h	72 h	8 h	72 h	8 h	72 h
Control	100	81.3 ± 5.09	98.8 ± 1.25	97.5 ± 1.72	100	100
Azatin	100	96.3 ± 3.75*	96.3 ± 2.05	96.3 ± 2.05	100	100
Sunspray	100	95.0 ± 2.29	100	98.8 ± 1.25	100	100
Botanigard	100	92.5 ± 3.19	93.8 ± 3.08	86.3 ± 5.28	100	100
M-Pede	97.5 ± 1.72	87.5 ± 3.39	73.8 ± 6.14*	67.5 ± 7.50*	93.8 ± 4.00	92.5 ± 4.48
Sevin	0*	0*	0*	0*	0*	0*

Means followed by an asterisk are statistically different from controls within a column ($\alpha = 0.01$, Wilcoxon rank sum test).

Although this study was concerned with the effects of biorationals on predators, several studies have illustrated the effectiveness of biorational pesticides on pests commonly found in interiorscapes. Puritch et al. (1982) found that a commercial insecticidal soap, at a concentration of 0.5%, caused >94% mortality for larval and adult life stages of *T. vaporariorum* and more that 82% mortality for pupae. Miller and Uetz (1998) found that oil and soap were as effective as Orthene (acephate) in reducing *M. persicae* and *A. gossypii* numbers on ivy. Miller and Uetz (1998) also found that commercial neem extract reduced aphid numbers on ivy and chrysanthemums. Oil, soap, and neem were as effective as cyfluthrin (pyrethroid) at controlling citrus mealybugs on coleus (Miller and Uetz 1998). In addition, Miller and Uetz (1998) found no phytotoxic effects of repeated sprays of soap, oil, or neem on 52 species of bedding plants.

The use of adult coccinellids in landscape pest management, including interiorscape pest management, is widespread. It has been estimated that several billion *H. convergens* are collected from overwintering sites in California each year and released throughout North America for biological control of aphids in a wide variety of settings, including interiorscapes (Dreistadt and Flint 1996). Although the effectiveness of this tactic has been questioned (Obrycki and Kring 1998), Dreistadt and Flint (1996) found that inundative releases of *H. convergens* adults significantly reduced *A. gossypi* numbers on potted chrysanthemums. Aphid numbers decreased 3 d after release, despite the observed dispersal of the beetles from the release site (Dreistadt and Flint 1996).

In this research, we used two species, *H. axyridis* and *C. maculata*, which are possible alternatives to *H. convergens* for aphid control in interiorscapes. *C. maculata* supplements its diet with plant pollen and can complete its life cycle on pollen alone (Hodek and Honek 1997). This beetle is a potentially important biological control agent for interiorscapes because it can maintain its populations in times of low prey density by feeding on plant pollen. *H. axyridis* was introduced from East Asia for control of the pear psylla, *Psylla pyricola* (Förster), and several other arboreal homopteran species, including scales (LaMana and Miller 1996), and is currently spreading throughout North America (Nalepa et al. 1996). It is commercially available and can be used for the management of various aphids and scale insects in interiorscapes. In interiorscapes, using a single species for a number of target pests may lower overall pest management costs (Gurney and Hussey 1970). Therefore, predators such as *C. maculata* and *H. axyridis*, more generalist feeders compared with *H. convergens*, are potentially better control agents in interiorscapes.

The use of *C. montrouzieri* for control of *P. citri* in interiorscapes has historically been successful (Steiner and Eliot 1987). All life stages of *P. citri*, including egg masses, are covered with wax that appears to stimulate oviposition by *C. montrouzieri* (Merlin et al. 1996). However, *C. montrouzieri* is a less effective predator of the long-tailed mealybug, *Pseudococcus longispinus* (Targioni-Tozzetti), which is also common in interiorscapes, often in association with *P. citri. Pseudococcus longispinus* is less waxy and does not lay egg masses because it is viviparous. Because *C. montrouzieri* may require mealybug eggs and the waxy filaments to induce oviposition (Williams 1985), the beetle does not reproduce as well on *P. longispinus.* Consequently, *P. longispinus* needs to be controlled with biorational pesticides. If both mealybug species are present, then biorationals other than *B. bassiana* need to be used to conserve *C. montrouzieri.*

Table 5. Percentage survival for *C. montrouzieri* exposed to biorational pesticides and a conventional pesticide (Sevin) in laboratory bioassays

Treatment	Replicate 1		Replicate 2		Replicate 3	
	8 h	72 h	8 h	72 h	8 h	72 h
Control	100	90.0 ± 3.34	100	85.0 ± 3.80	100	86.3 ± 4.24
Azatin	100	98.8 ± 1.25	98.8 ± 1.25	96.3 ± 2.05	98.8 ± 1.25	85.0 ± 3.34
Sunspray	98.8 ± 1.25	73.8 ± 6.41	100	82.9 ± 4.67	100	90.0 ± 3.34
Botanigard	100	10.0 ± 3.34*	100	10.0 ± 3.34*	96.3 ± 2.73	7.5 ± 4.10*
M-Pede	23.3 ± 6.24*	14.6 ± 4.68*	96.3 ± 2.05	88.8 ± 3.83	100	81.3 ± 4.76
Sevin	0*	0*	0*	0*	0*	0*

Means followed by an asterisk are statistically different from controls within a column ($\alpha = 0.01$, Wilcoxon rank sum test).

The wide variety of plant species and microclimatic conditions found in interiorscapes can lead to a wide variety of pest species in a small area. Often, several pest management tools must be used simultaneously to deal with such a situation. Biorational pesticides and biological control agents are two such tools that are used simultaneouslybecausae of their effectiveness, potential compatibility, and minimal risk to the environment and human health. Our results indicate that biorational pesticides conserve four coccinellid predators better than the conventional pesticide Sevin (carbaryl). However, BotaniGard (*B. bassiana,* strain GHA) caused consistent mortality for *C. montrouzieri,* suggesting that these two tools may not be compatible for interiorscape pest management. Furthermore, data suggest that insecticidal soap may not conserve adult coccinellids as well as formulations of azadiractin and horticultural oil.

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