Effect of Insecticides on Mealybug Destroyer (Coleoptera: Coccinellidae) and Parasitoid *Leptomastix dactylopii* (Hymenoptera: Encyrtidae), Natural Enemies of Citrus Mealybug (Homoptera: Pseudococcidae)

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ABSTRACT In this study, we measured, under laboratory conditions, the direct and indirect effects of insecticides on mealybug destroyer, *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), and parasitoid *Leptomastix dactylopii* Howard (Hymenoptera: Encyrtidae), natural enemies of citrus mealybug, *Planococcus citri* (Risso) (Homoptera: Pseudococcidae). The adult stages of both natural enemies were exposed to sprays of the insecticides buprofezin, pyriproxyfen, flonicamid, acetamiprid, dinotefuran, and clothianidin at label-recommended rates to assess direct mortality after 24, 48, and 72 h, respectively. The effects of the insecticides on *L. dactylopii* parasitization rate and percentage of parasitoid emergence also were monitored using the label and 4× the recommended label rate. Dinotefuran was extremely detrimental to the adult parasitoid at the label rate with 100% mortality after 24 h. Buprofezin, pyriproxyfen, and flonicamid were not harmful to *L. dactylopii* when applied at the label rate. At 4× the recommended label rate, dinotefuran, acetamiprid, and clothianidin were all harmful to the parasitoid with 100% mortality 72 h after application. Both buprofezin and flonicamid were not toxic to *L. dactylopii* with 100% adult survival after 72 h. Pyriproxyfen and flonicamid, at both the label and 4× the recommended label rate, did not negatively affect *L. dactylopii* parasitization rate or percentage of parasitoid emergence. Acetamiprid, dinotefuran, and clothianidin were toxic to *C. montrouzieri* adults with 100% mortality after 48 h, whereas buprofezin, pyriproxyfen, and flonicamid demonstrated minimal (10–20% mortality after 48 h) harmful effects to the predator. Based on the results from our study, the indirect effects of the insect growth regulator (IGR) buprofezin were not decisive; however, the IGR pyriproxyfen and the insecticide flonicamid were not directly or indirectly harmful to the predator *C. montrouzieri* and parastioid *L. dactylopii*, indicating that these insecticides are compatible with both natural enemies when used together for control of citrus mealybug in greenhouses and conservatories.

KEY WORDS compatibility, insecticides, citrus mealybug, natural enemies, pest management

Pest management of phytophagous insects in greenhouses, conservatories, and interior plantscapes generally involves the use of insecticides, commonly in conjunction with natural enemies such as parasitoids, predators, or pathogens. The sole use of biological control may not always be sufficient to manage insect pest populations, particularly in greenhouses (van Lenteren 1987, Medina et al. 2003, Hassan and Van de Veire 2004). As a result, research has investigated the use of "biorational" or "reduced-risk" insecticides in combination with natural enemies (Cloyd 2005). Insecticides classified as biorational or reduced-risk include horticultural oils, insecticidal soaps, microbials, feeding inhibitors, and insect growth regulators (IGRs). These insecticides are generally considered to be less harmful to natural enemies compared with conventional insecticides (Croft 1990).

Studies have been conducted to assess the compatibility of biorational insecticides with predatory mites (Osborne and Petitt 1985; Oetting and Latimer 1995; Spollen and Isman 1996; Cabrera et al. 2004, 2005), predatory bugs (Nagai 1990, Delbeke et al. 1997, James 2004), and parasitoids (Lemma and Poe 1978, Gerling and Sinai 1994, Jones et al. 1998, Rothwangl et al. 2004). Additional studies have evaluated the compatibility of biorational insecticides with predatory insects, including the green lacewing, *Chrysoperla carnea* (Stephens) (Chrysopidae: Neuroptera) (Kiselek 1975, Niemczyk et al. 1985, Medina et al. 2003) and ladybird beetles (Mazzone and Viggiani 1980, Kismali and Erkin 1984, Smith and Papacek 1990, James and Lighthart 1994, Hattingh and Tate 1995). Most of these studies have been conducted under laboratory con-

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ditions, which provides a "worse-case scenario," indicating that if the insecticides demonstrate no harmful effects under these conditions, then in all probability they will not be detrimental when used in greenhouses, conservatories, or interior plantscapes (Cloyd 2005).

Most studies have primarily evaluated the lethal effects of biorational insecticides, that is, whether the natural enemy is killed immediately. However, it is just as important to assess the sublethal or long-term effects, which also may impact the feasibility of using natural enemies with pesticides. For example, the sublethal effects of biorational insecticides may involve inhibiting oviposition (Medina et al. 2003), reducing adult emergence (McNeil 1975, Lemma and Poe 1978), or sterilizing females (Hattingh and Tate 1995).

IGRs, which are most effective on the immature stages of plant-feeding insects (Staal 1975, Parrella and Murphy 1998), are commonly used in greenhouses, conservatories, and interior plantscapes. Buprofezin and pyriproxyfen are IGRs registered for control of aphids, whiteflies, and mealybugs. Buprofezin (Talus, SePro Corporation, Carmel, IN) is a chitin synthesis-inhibiting IGR (Shibuya 1984, Uchida et al. 1985, Gerling and Sinai 1994), whereas pyriproxyfen (Distance, Valent USA Corp., Walnut Creek, CA) is a juvenile hormone mimic (Ishaaya et al. 1994, Ware and Whitacre 2004) that also has ovicidal activity on whitefly eggs (Ascher and Eliyahu 1988). Feeding inhibitors or blockers are a relatively new class of insecticides that prevent insects from feeding by interfering with neural regulation of fluid intake into the mouthparts (Kayser et al. 1994; Fuog et al. 1995, 1998). The insecticide flonicamid (Aria, FMC Corporation, Philadelphia, PA), which was recently registered for use in greenhouses, is a feeding inhibitor for control of aphids and whiteflies.

Neonicotinoid-based insecticides are a class of insecticides that have been commercially available since the mid-1990s, and several newly introduced materials that are registered for use in greenhouses include acetamiprid (TriStar, Cleary Chemical Co., Dayton, NJ), dinotefuran (Safari, Valent USA Corp.), and clothianidin (Celero, Arysta LifeScience North America Corp., San Francisco, CA). These insecticides are primarily used to control phloem-feeding insects such as aphids, whiteflies, and mealybugs (Tomizawa and Casida 2003).

There is relatively little information available on the compatibility of the IGRs buprofezin and pyriproxyfen, the insecticide flonicamid, and the neonicotinoid-based insecticides mentioned above with the mealybug destroyer, *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), and the parasitoid *Leptomastix dactylopii* Howard (Hymenoptera: Encyrtidae), which are commercially available natural enemies used in greenhouses and conservatories for control of citrus mealybug, *Planococcus citri* (Risso) (Homoptera: Pseudococcidae) (Kole and Hennekam 1990). In this study, we report on the compatibility of several IGRs and neonicotinoid-based insecticides, and an insecticide that acts as a feeding inhibitor on the natural enemies of citrus mealybug, including mealybug destroyer and *L. dactylopii* under laboratory conditions. We also make recommendations on how these insecticides can be used in a pest management program that includes the use of both natural enemies.

Materials and Methods

L. dactylopii

This study involved a series of experiments designed to evaluate the direct or indirect effects of selected insecticides on the parasitoid *L. dactylopii.* Direct refers to any lethal or immediate effects, whereas indirect refers to sublethal effects of the insecticides tested. The parasitoids used for the study were obtained from a laboratory colony reared on citrus mealybugs feeding on butternut squash, *Cucurbita maxima* (L.), maintained in an environmental chamber (model E7, Conviron, Winnipeg, Manitoba, Canada) with a temperature of 20 ± 2°C and a photoperiod of 14:10 (L:D) h. A honey–water solution [50:50 (vol:vol)] was freely available to *L. dactylopii.*

Experiment 1: Direct Effects of Insecticides Used at Labeled Rates. A 100- by 20-mm glass petri dish was inverted and Whatman No. 1 filter paper (Whatman, Maidstone, England) was placed in the bottom of the petri dish. A small amount (0.1 ml) of honey–water solution [50:50 (vol:vol)] was applied to the inside of the lid of each petri dish. The petri dish and filter paper were sprayed once with 0.8 ml of each treatment solution by using a 946-ml spray bottle calibrated so that the full spray trajectory thoroughly moistened the entire petri dish and filter paper. Deionized water was used as a solvent for all treatment applications. Newly emerged (<24-h-old) male and female *L. dactylopii* were collected from the laboratory colony by using an aspirator and allowed to mate for 24 h in 9-dram vials with a drop (0.05 ml) of honey–water solution [50:50 (vol:vol)] applied to the inside portion of the lid. A single-mated female parasitoid was then placed into each petri dish immediately after the treatments had been applied. Each female parasitoid was monitored after 24, 48, and 72 h, respectively, to assess whether they were alive or dead. After 48 h, a laboratory syringe was used to apply 0.8 ml of deionized water to the filter paper of each petri dish to ensure that the filter paper was constantly moist. The experiment was set up as a completely randomized design with eight treatments and five replications per treatment. The number of replications conducted per treatment was a result of the limited quantity of parasitoids (both female and male) that emerged simultaneously for use; however, we were still able to obtain a sufficient cohort of same-aged females and males for mated pairs. The insecticide rates used were based on the label recommendations from the manufacturer. The treatments and rates were buprofezin (Talus 40 SC, SePRO Corporation) at 1.3 ml/946 ml, acetamiprid (TriStar 30SG; Cleary Chemical Co.) at 0.18 g/946 ml, dinotefuran (Safari 20SG, Valent USA Corp.) at 0.56 g/946 ml, pyriproxyfen (Distance 0.86 EC; Valent USA

Corp.) at 0.88 ml/946 ml, clothianidin (Celero 16WSG; Arysta LifeScience North America Corp.) at 0.28 g/946 ml, and flonicamid (Aria 50SG; FMC Corporation) at 0.15 g/946 ml. There was also an untreated check (dried filter paper) and deionized water control.

Experiment 2: Direct Effects of Insecticides Used at Higher than Labeled Rates. The procedures used in this experiment were similar to those of experiment 1 (see above) except we used 4× the recommended label rate for all treatments, because it is not uncommon for rates, that are above the recommended label rate, to be inadvertently used (R.A.C., personal observation). There were eight treatments with 10 replications per treatment. The treatments and rates were buprofezin at 5.2 ml/946 ml, acetamiprid at 0.72 g/946 ml, dinotefuran at 2.24 g/946 ml, pyriproxyfen at 3.52 ml/946 ml, clothianidin at 1.12 g/946 ml, and flonicamid at 0.60 g/946 ml. There was also an untreated check (dried filter paper) and deionized water control.

Experiment 3: Indirect Effects of Insecticides Used at Labeled Rates on Parasitization Rate and Sex Ratio. We evaluated the indirect effects of the insecticides used in the previous two experiments, with the exception of dinotefuran, on the parasitization rate and sex ratio of *L. dactylopii.*

Newly emerged male and female *L. dactylopii* were collected from the laboratory colony by using an aspirator and placed into 9-dram vials with a drop (0.05 ml) of honey-water [50:50 (vol:vol)] solution placed on the inside portion of the lid as a food source. Wasps were allowed to mate for 24 h. Glass petri dishes (100 by 20 mm) were inverted, lined with a Whatman No. 1 filter paper, and a small volume (0.1 ml) of honey-water solution was placed on the inside portion of the lid to provide a food source. The filter paper was moistened with 2.0 ml of deionized water by using a 946-ml spray bottle, and a green coleus, *Solenostemon scutellarioides* (L.) Codd, leaf obtained from a stock plant was positioned so that the leaf margins draped over the exterior of the petri dish. Twenty late second to early third instars of citrus mealybug were applied to the leaf surface with a small camel's-hair brush. Each petri dish containing the mealybugs was sprayed with 0.8 ml of each treatment solution by using a 946-ml spray bottle, which thoroughly moistened the mealybugs and coleus leaf. Deionized water was used for all treatment applications. The recommended label rates were used (refer to experiment 1). The petri dish lid was then inserted into the base, and a weight was placed on the petri dish to prevent the parasitoid and mealybugs from escaping. The petri dish lid was positioned such that mealybugs could not move to the underside of the leaf. This setup also prevented mealybugs from escaping attack by the parasitoid. The experiment was set up as a completely randomized design with seven treatments and five replications per treatment, per release time ($n = 2$), for a total of 70 replicates. In 35 of the replicates, the mated female *L. dactylopii* was placed into the petri dish containing citrus mealybugs immediately after the treatments had been applied, whereas in the remaining 35 replicates, newly mated *L. dactylopii* were released into the petri dishes 24 h after treatments had been applied. The parasitoids were allowed to remain in the petri dishes containing the citrus mealybugs for 24 h, after which time they were removed. The filter paper, underneath the coleus leaf, was moistened with 2.0 ml of deionized water by using a laboratory syringe, 7 d after the treatments had been applied to keep the leaves hydrated during the experiment. The petri dishes were checked daily for 2 wk after mealybugs had been parasitized (mummified), to assess adult parasitoid emergence. Parasitization rate denotes those parasitoids that survived to the mummy stage. Once emergence began, petri dishes were monitored daily, and the parasitoids were collected and sexed using antennal morphology. The experiment was terminated 2 d after no more adult parasitoids emerged from the parasitized mealybugs.

Experiment 4: Indirect Effects of Insecticides Used at Higher than Labeled Rates on Parasitization Rate and Sex Ratio. The procedures used in this experiment were similar to those in experiment 3 (see above). However, we used 4× the recommended label rate for the insecticides evaluated in experiment 3 with the exception of acetamiprid, which was lethal at the recommended label rate. We tested the 4× rate because it is not uncommon for rates, above the recommended label rate, to be inadvertently used (R.A.C., personal observation). There were six treatments with five replications per treatment, per release time ($n = 2$) for a total of 60 replicates. In 30 of the replicates, the mated female *L. dactylopii* was placed into the petri dish containing citrus mealybugs immediately after the treatments had been applied, whereas in the remaining 30 replicates, newly mated *L. dactylopii* were released into the petri dishes 24 h after treatments had been applied. The parasitoids were allowed to remain in the petri dishes containing citrus mealybugs for 24 h, after which they were removed. The filter paper, underneath the coleus leaf, was moistened with 2.0 ml of deionized water by using a laboratory syringe, 7 d after the treatments had been applied to keep the leaves hydrated during the experiment. The petri dishes were checked daily for 2 wk after mealybugs had been parasitized (mummified), to assess adult parasitoid emergence. Parasitization rate denotes those parasitoids that survived to the mummy stage. Once emergence had begun, petri dishes were monitored daily, and the parasitoids were collected and sexed using antennal morphology. The experiment was concluded 2 d after no more adult parasitoids emerged from the parasitized mealybugs.

C. montrouzieri

Direct Effects of Insecticides Used at Labeled Rates. This experiment was designed to evaluate the direct effects of the insecticides used in the *L. dactylopii* study (described previously) on the mealybug destroyer. Adult *C. montrouzieri* were obtained from a commercial supplier of biological control agents (IPM

Table 1. Percentage of mortality, *P* values, and likelihood of *L. dactylopii* adults being killed by the designated treatments 24, 48, and 72 h after insecticides had been applied using recommended labeled rates for experiment 1

Treatment	*n*	Rate (/946 ml)	% mortality			*P* value	Exp(beta)[a]
			24 h	48 h	72 h		
Buprofezin	5	1.30 ml	0	0	0	1.00	1.00
Acetamiprid	5	0.18 g	20	20	20	0.42	3.66
Dinotefuran	5	0.56 g	100	100	100	0.0018	120.99
Pyriproxyfen	5	0.88 ml	0	0	0	1.00	1.00
Clothianidin	5	0.28 g	20	20	20	0.42	3.66
Flonicamid	5	0.15 g	0	0	0	1.00	1.00
Untreated check	5		0	0	0		
Water control	5		0	0	0	1.00	1.00

[a] Exp(beta) indicates the likelihood of the parasitoid being killed by the treatments compared with the untreated check.

Laboratories, Inc., Locke, NY). Upon arrival, the adult beetles were processed immediately for the experiment. Fifteen citrus mealybugs of varying ages, from second to early fourth instar, were placed into an upside down glass petri dish (100 by 20 mm) containing a green coleus leaf and moistened Whatman No. 1 filter paper located underneath the leaf. One adult beetle was applied, with a moistened camel's-hair brush, to each petri dish. The beetles were then allowed to acclimate for 24 h. After 24 h, the treatments were applied using a 946-ml plastic spray bottle, which was calibrated to disperse 8.0 ml per 10 sprays. Each petri dish received five sprays (4.0 ml). This volume was enough to thoroughly moisten the adult *C. montrouzieri*, the mealybugs, and coleus leaf. Deionized water was used as a solvent for all treatment applications. Adult mortality was assessed after 24 and 48 h after application of the treatments. The experiment was set up as a completely randomized design. There were a total of eight treatments with 10 replications per treatment. The treatments and rates were the same as those used for experiment 1 in the *L. dactylopii* study.

Statistical Analysis

For experiments 1 and 2, all data were analyzed using a logistic regression repeated measures procedure, which combined both the 24- and 48-h time intervals. A PROC GENMOD program, which is a generalized linear model program, was used to analyze the data in a SAS Statistical Software Program, SAS Systems for Windows, version 9.1 (SAS Institute 2004) with treatment as the main effect. This program determines the likelihood of each treatment killing the parasitoid compared with the untreated check.

For experiments 3 and 4, all data were analyzed using a one-way analysis of variance (SAS Institute 2002) with treatment as the main effect. Significant treatment means were separated using a Fisher's protected least significant difference (LSD) test at $P \leq 0.05$.

For the *C. montrouzieri* experiment, all data were analyzed using a logistic regression repeated measures procedure. A PROC GENMOD program, which is a generalized linear model program, was used to analyze the data in a SAS Statistical Software Program, SAS Systems for Windows, version 9.1 (SAS Institute 2004) with treatment as the main effect. This program determines the likelihood of each treatment killing the predator compared with the untreated check.

Results

L. dactylopii

Experiment 1: Direct Effects of Insecticides Used at Labeled Rates. The treatments buprofezin, pyriproxyfen, and flonicamid were not toxic to *L. dactylopii* adults based on percentage of mortality (Table 1). The neonicotinoid-based insecticide dinotefuran was very harmful to the parasitoid with a percentage of mortality value of 100% after 24 h. In addition, dinotefuran had a significantly higher likelihood of killing *L. dactylopii* compared with the untreated check and other treatments, based on the exp(beta) value (Table 1). For example, the probability of killing the parasitoid was 120 times greater for dinotefuran compared with the untreated check and other treatments (Table 1). The other neonicotinoid-based insecticides, acetamiprid and clothianidin were less toxic to the parasitoid with mortality values of 20% after 24, 48, and 72 h, respectively (Table 1).

Experiment 2: Direct Effects of Insecticides Used Higher than Labeled Rates. At 4× the recommended label rate, the treatments buprofezin and flonicamid were not lethal to *L. dactylopii* adults based on percentage of mortality (Table 2). The neonicotinoid-based insecticides acetamiprid and dinotefuran were very harmful to the parasitoid at the higher rates with mortality values of 100% after 24 h (Table 2). The neonicotinoid-based insecticide clothianidin was also toxic to *L. dactylopii* with mortality values of 50, 60, and 100% after 24, 48, and 72 h, respectively. In addition, all three insecticides had a significantly higher probability of killing *L. dactylopii* compared with the untreated check and other treatments, based on the exp(beta) values. The probability of killing the parasitoid was 121 times greater for both acetamiprid and dinotefuran and 33 times greater for clothianidin, compared with the untreated check and other treatments. Pyriproxyfen, at 4× the recommended label rate, was more toxic to *L. dactylopii* than in the first experiment with a 50% mortality value after 72 h;

Table 2. Percentage of mortality, *P* values, and likelihood of *L. dactylopii* adults being killed by the designated treatments 24, 48, and 72 h after insecticides had been applied using 4× the recommended labeled rates for experiment 2

Treatment	*n*	Rate (/946 ml)	% mortality			*P* value	Exp(beta)[a]
			24 h	48 h	72 h		
Buprofezin	5	5.20 ml	0	0	0	1.00	1.00
Acetamiprid	5	0.72 g	100	100	100	0.0018	121.00
Dinotefuran	5	2.24 g	100	100	100	0.0018	121.00
Pyriproxyfen	5	3.52 ml	10	10	50	0.73	1.70
Clothianidin	5	1.12 g	50	60	100	0.013	33.00
Flonicamid	5	0.60 g	0	0	0	1.00	1.00
Untreated check	5		0	0	0		
Water control	5		0	0	0	1.00	1.00

[a] Exp(beta) indicates the likelihood of the parasitoid being killed by the treatments compared with the untreated check.

however, overall, pyriproxyfen was not significantly toxic to *L. dactylopii* and was not statistically different from the untreated check and water control (Table 2).

Experiment 3: Indirect Effects of Insecticides Used at Labeled Rates on Parasitization Rate and Sex Ratio. Treatment was significant for parasitization rate (=number of mummies per parasitoid) ($F = 37.88$; df = 6, 34; $P < 0.0001$) and percentage of parasitoid emergence ($F = 29.65$; df = 6, 34; $P < 0.0001$) when *L. dactylopii* were released immediately after the treatments had been applied. However, sex ratio did not significantly differ among the treatments ($F = 0.96$; df = 4, 24; $P = 0.45$). Both the pyriproxyfen and flonicamid treatments had parasitization rates that were similar to the water control and were significantly higher than the other treatments except for the untreated check (Table 3). Flonicamid had the highest percentage of parasitoid emergence (60 ± 7; mean ± SE) of all the treatments with the exception of the untreated check (73 ± 3) (Table 3).

Treatment was significant for the parasitization rate ($F = 10.18$; df = 6, 34; $P < 0.0001$) and percentage of parasitoid emergence ($F = 9.13$; df = 6, 34; $P < 0.0001$) when *L. dactylopii* were released 24 h after the treatments had been applied. However, sex ratios were not significantly different among the treatments ($F = 1.93$; df = 5, 29; $P = 0.12$). Similar to the immediate release part of the experiment, both the pyriproxyfen and flonicamid treatments had parasitization rates that were not statistically different from the water control and untreated check (Table 3). In addition, both treatments had percentage of parasitoid emergence values that were significantly higher than the other treatments but not statistically different from the untreated check and water control (Table 3).

Experiment 4: Indirect Effects of Insecticides Used at Higher than Labeled Rates on Parasitization Rate and Sex Ratio. At 4× the recommended label rate, treatment was significant for parasitization rate ($F = 4.51$; df = 5, 29; $P = 0.0049$) and percentage of parasitoid emergence ($F = 5.66$; df = 5, 29; $P = 0.0014$) when *L. dactylopii* were released immediately after the treatments had been applied. Sex ratio was not significant ($F = 1.86$; df = 3, 19; $P = 0.17$). As in experiment 3, both the pyriproxyfen and flonicamid treatments, even at 4× the recommended label rate, had parasitization rates that were statistically similar to the water control and untreated check (Table 4). The flonicamid treatment had a percentage of parasitoid emergence value (42 ± 8; mean ± SE) that was not statistically different from the untreated check and water control (Table 4). In addition, flonicamid, at 4× the recommended label rate had a percentage of parasitoid emergence value not significantly different from pyriproxyfen (Table 4).

Treatment was significant for parasitization rate ($F = 8.32$; df = 5, 29; $P = 0.0001$) and for percentage of parasitoid emergence ($F = 7.54$; df = 5, 29; $P = 0.0002$) when *L. dactylopii* were released 24 h after the treatments had been applied. Sex ratios were not significantly different among the treatments ($F = 2.12$; df = 5, 29; $P = 0.09$). Only the pyriproxyfen treatment parasitization rate, which was the highest among the treatments (15.4 ± 1.3; mean ± SE), was significantly

Table 3. Mean ± SE number of citrus mealybug mummies per *L. dactylopii* from 20 citrus mealybugs per petri dish, sex ratio (female:male), and percentage of parasitoid progeny that emerged from mummies when the parasitoid was released immediately and 24 h after treatments had been applied, using recommended label rates, for experiment 3

Treatment	*n*	No. mummies/parasitoid		Sex ratio		% parasitoid emergence	
		Immediate	24 h	Immediate	24 h	Immediate	24 h
Buprofezin	5	8.4 ± 1.0c	3.0 ± 1.4b	1.4:1a	0.3:1a	21 ± 4d	8 ± 5b
Acetamiprid	5	0.0 ± 0.0d	0.2 ± 0.2b			0 ± 0e	1 ± 1b
Pyriproxyfen	5	12.4 ± 1.2b	10.0 ± 1.8a	0.9:1a	1.3:1a	45 ± 8c	41 ± 11a
Clothianidin	5	0.2 ± 0.2d	2.4 ± 0.2d		0.8:1a	1 ± 1e	10 ± 4b
Flonicamid	5	12.0 ± 1.4b	9.8 ± 0.5a	1.2:1a	2.1:1a	60 ± 7ab	48 ± 3a
Untreated check	5	15.6 ± 0.6a	12.6 ± 0.9a	1.6:1a	1.5:1a	73 ± 3a	63 ± 5a
Water control	5	12.2 ± 1.3b	10.8 ± 2.9a	3.2:1a	1.4:1a	59 ± 6bc	54 ± 15a

Means within a column not followed by a common letter are significantly different ($P = 0.05$) as determined by Fisher's protected LSD mean separation test.

Table 4. Mean ± SE number of citrus mealybug mummies per *L. dactylopii* from 20 citrus mealybugs per petri dish, sex ratio (female:male), and percentage of parasitoid progeny that emerged from mummies when the parasitoid was released immediately and 24 h after treatments had been applied, using 4× the recommended label rates, for experiment 4

Treatment	*n*	No. mummies/parasitoid		Sex ratio		% parasitoid emergence	
		Immediate	24 h	Immediate	24 h	Immediate	24 h
Buprofezin	5	3.0 ± 1.3bc	9.4 ± 2.2b		2.6:1a	8 ± 4cd	21 ± 6bc
Pyriproxyfen	5	6.8 ± 2.5ab	15.4 ± 1.3a	0.7:1a	1.9:1a	28 ± 11bc	62 ± 6a
Clothianidin	5	0.2 ± 0.2c	1.8 ± 0.6c		0.2:1a	1 ± 1d	9 ± 3c
Flonicamid	5	8.4 ± 1.6a	8.8 ± 1.1b	1.8:1a	1.3:1a	42 ± 8ab	44 ± 6ab
Untreated check	5	10.4 ± 1.2a	10.2 ± 0.9b	3.5:1a	1.1:1a	52 ± 6a	51 ± 5a
Water control	5	5.4 ± 2.3ab	11.8 ± 2.2ab	0.7:1a	1.9:1a	27 ± 12bc	58 ± 11a

Means within a column not followed by a common letter are significantly different ($P = 0.05$) as determined by Fisher's protected LSD mean separation test.

different from the other treatments, with the exception of the water control (Table 4). At 4× the recommended label rate, both the pyriproxyfen and flonicamid treatments had parasitization rates and percentage of parasitoid emergence values that were not statistically different from the untreated check and water control (Table 4).

C. montrouzieri

The treatments buprofezin, pyriproxyfen, and flonicamid were less toxic to *C. montrouzieri* adults based on percentage of mortality after 24 and 48 h than the neonicotinoid-based insecticides, acetamiprid, dinotefuran, and clothianidin (Table 5). Indeed, both acetamiprid and clothianidin were highly toxic to the adults after 24 h with 70% mortality values. The buprofezin, pyriproxyfen, and flonicamid treatments resulted in mortality values of 20% or less after 48 h compared with all the neonicotinoid-based insecticide treatments, which had mortality values of 100% after 48 h (Table 5).

All the neonicotinoid-based insecticides had significantly higher probabilities of killing, *C. montrouzieri* adults based on the exp(beta) values compared with the untreated check (Table 5). For example, the probability of killing the beetle was 32 times greater for both acetamiprid and clothianidin and 13 times greater for dinotefuran compared with the untreated check (Table 5).

Discussion

The experiments, for both studies, were conducted in a laboratory to simulate a worse case scenario to demonstrate that those insecticides that were determined to be nontoxic to the natural enemies are likely to be not harmful in a greenhouse, conservatory, or interiorscape. The IGRs buprofezin and pyriproxyfen and the insecticide flonicamid demonstrated minimal, if any, direct and indirect effects to mealybug destroyer and the parasitoid *L. dactylopii*, whereas the neonicotinoid-based insecticides tested were all directly and indirectly harmful to both natural enemies.

Minimal research has been conducted on the direct and indirect effects of IGRs on *L. dactylopii.* Rothwangl et al. (2004) demonstrated that pyriproxyfen was generally not harmful to *L. dactylopii* when released immediately, 24 h, and 48 h after treatment of insecticides. This is consistent with our results in which pyriproxyfen was not harmful to the parasitoid based on measurement of mortality (Table 1). At 4× the recommended label rate, pyriproxyfen did exhibit toxic effects after 72 h with 50% mortality, although this was still much lower than the direct effects of the neonicotinoid-based insecticides (Table 1). Moreover, pyriproxyfen, even at 4× the recommended label rate, did not affect the parasitization rate of *L. dactylopii.* The results of our study were also consistent with Rothwangl et al. (2004) in that the sex ratio of *L. dactylopii* was not affected by any of the insecticide treatments. Hoddle et al. (2001) indicated that pyriproxyfen was highly repellent to *Eretmocerus eremicus* Rose & Zolnerowich (Hymenoptera: Aphelinidae) adult females and that females failed to forage on silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring (Homoptera: Aleyrodidae), nymphs treated with pyriproxyfen. However, in our study, pyriproxyfen did not demonstrate any repellent affects, because the

Table 5. Percentage of mortality, *P* values, and likelihood of mealybug destroyer adults being killed by the designated treatments 24 and 48 h after insecticides had been applied using the recommended label rates

Treatment	*n*	Rate (/946 ml)	% mortality		*P* value	Exp(beta)[a]
			24 h	48 h		
Buprofezin	10	1.30 ml	0	10	0.34	0.29
Acetamiprid	10	0.18 g	70	100	0.0004	32.11
Dinotefuran	10	0.56 g	40	100	0.0032	13.22
Pyriproxyfen	10	0.88 ml	20	20	0.75	1.41
Clothianidin	10	0.28 g	70	100	0.0004	32.11
Flonicamid	10	0.15 g	10	10	0.72	0.62
Untreated check	10		10	2		
Water control	10		10	20	1.00	1.00

parasitization rate, even at 4× the recommended label rate, was comparable to the untreated check and water control (Tables 3 and 4).

Hymenopteran parasitoids, including *Encarsia luteola* Howard (Hymenoptera: Aphelinidae) (Gerling and Sinai 1994), *E. eremicus* (Hoddle et al. 2001), *Eretmocerus tejanus* Rose & Zolnerowich (Hymenoptera: Aphelinidae), and *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) (Jones et al. 1998) have been shown to be generally compatible with buprofezin. Smith and Papacek (1990) tested the effects of buprofezin on *L. dactylopii* and found that the IGR was not toxic to the parasitoid based on percentage of adult mortality after 48 h. We found that buprofezin was not directly toxic to *L. dactylopii* even 72 h after exposure to spray applications (Tables 1 and 2). Although Smith and Papacek (1990) did not assess parasitization rate and percentage of parasitoid emergence of *L. dactylopii*, in our study, we found that buprofezin seemed to be less compatible with *L. dactylopii* than pyriproxyfen and flonicamid based on parasitization rate (=number of mummies per parasitoid) and percentage of parasitoid emergence regardless of release time (Table 3). Buprofezin has been shown to have vapor activity (De Cock et al. 1990) and may have directly affected the foraging behavior of *L. dactylopii* or inhibited acceptance by reducing the quality of the citrus mealybugs. This may explain the lower parasitization rate and percentage of parasitoid emergence observed for buprofezin when labeled rates were used in experiment three, whether the parasitoid was released immediately or 24 h after application. In all cases, the parasitization rate and percentage of parasitoid emergence were significantly lower than pyriproxyfen, flonicamid, the untreated check, and water control (Table 3). However, the influence of vapor activity was negligible when 4× the recommended label rate was used (Table 4). The reason for the response at the 4× rate is not known at this time.

IGRs have been demonstrated to be toxic to certain coccinellids (Peleg 1983, Biddinger and Hull 1995). Indeed, buprofezin is not compatible with the vedalia beetle, *Rodolia cardinalis* (Mulsant) (Coleoptera: Coccinellidae) (Grafton-Cardwell and Gu 2003), or *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) (James 2004). However, these studies primarily tested the larval stage, which may be a more susceptible life stage compared with the adults (Croft 1990). In our study, we found that buprofezin was not directly toxic to the adult stage of *C. montrouzieri* even after 48-h exposure to spray residues, which is consistent with Smith and Papacek (1990). Pyriproxyfen also has been shown to negatively affect coccinellids including *C. montrouzieri* (Hattingh and Tate 1995) and the vedalia beetle, *R. cardinalis* (Grafton-Cardwell and Gu 2003). However, as with the studies mentioned above with buprofezin, these studies mainly used the larval stage. We found that pyriproxyfen was not significantly harmful to the adult stage of *C. montrouzieri* after 48 h (Table 5). This suggests that differences in susceptibility to IGRs are dependent on the life stage.

Pymetrozine, which has a similar mode of action as flonicamid, has been shown to be nontoxic to the larval stages of the ladybird beetle, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) (Sechser et al. 2002), and the parasitoids *E. eremicus* (Hoddle et al. 2001) and *E. formosa* (Hassan and Van de Veire 2004). Of all the insecticides we tested in this study, flonicamid was the least toxic, both directly and indirectly to *C. montrouzieri* and *L. dactylopii.* This study is the first to quantitatively demonstrate that flonicamid is compatible with the commercially available natural enemies of the citrus mealybug.

All three of the neonicotinoid-based insecticides tested were either directly or indirectly harmful to both *C. montrouzieri* and *L. dactylopii*, and as such they are not compatible with these natural enemies. However, both dinotefuran and clothianidan are labeled for applications to the growing medium, which may negate their negative effects to both natural enemies, whereas acetamiprid is only labeled for foliar applications indicating that this insecticide is less likely to be compatible with the natural enemies of citrus mealybug than dinotefuran and clothianidin. Our study is the first to assess the impact of acetamiprid, dinotefuran, and clothianidin, on the natural enemies of citrus mealybug. However, further studies are needed, particularly under greenhouse conditions, to assess the relative toxic effect of dry residues of the neonicotinoid-based insecticides on both natural enemies.

The negative effects of the insecticides tested may be due to direct exposure of the natural enemies to sprays or indirectly when the natural enemies ingest spray droplets to obtain moisture. The consumption of these droplets, which typically contain the insecticide active ingredient and inert ingredients such as carriers and/or adjuvants, may lead to lethal or sublethal effects.

The IGRs buprofezin and pyriproxyfen and the insecticide flonicamid, which is a feeding inhibitor, had minimal direct effects and were not harmful to *C. montrouzieri* and *L. dactylopii.* In addition, both flonicamid and pyriproxyfen had minimal indirect effects, based on parasitization rates and percentage of parasitoid emergence, on *L. dactylopii*, indicating that these insecticides are most compatible with the predator and parasitoid when used together for control of citrus mealybug in greenhouses and conservatories. Based on the results of this study, greenhouse producers and conservatory curators may use the insecticides buprofezin, pyriproxyfen, and flonicamid with releases of *C. montrouzieri* and *L. dactylopii* without forfeiting efficacy because of mortality.

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References Cited

Ascher, K.R.S., and M. Eliyahu. 1988. The ovicidal properties of the juvenile hormone mimic Sumitomo S-31183 (SK-591) to insects. Phytoparasitica 16: 15–21.

Biddinger, D. J., and L. A. Hull. 1995. Effects of several types of insecticides on the mite predator, *Stethorus punctum* (Coleoptera: Coccinellidae) including insect growth regulators and abamectin. J. Econ. Entomol. 88: 358–366.

Cabrera, A. R., R. A. Cloyd, and E. R. Zaborski. 2005. Lethal and sub-lethal effects of novaluron (Pedestal) on the soil-dwelling predatory mite *Stratiolaelaps scimitus* (Acari: Mesostigmata: Laelapidae), under laboratory conditions. J. Entomol. Sci. 40: 47–53.

Cabrera, A. R., R. A. Cloyd, and E. R. Zaborski. 2004. Effects of greenhouse pesticides on the soil-dwelling predatory mite *Stratiolaelaps scimitus* (Acari: Mesostigmata: Laelapidae) under laboratory conditions. J. Econ. Entomol. 97: 793–799.

Cloyd, R. 2005. Compatibility conflict: is the use of biological control agents with pesticides a viable management strategy?, pp. 546–554. *In* Proceedings of the International Sympsoim on Biological Control of Arthropods. United State Department of Agriculture Forest Service, Publication FHTET-2005-08 Vol. II.

Croft, B. A. 1990. Arthropod biological control agents and pesticides. Wiley, New York.

De Cock, A., I. Ishaaya, D. Degheele, and D. Veierov. 1990. Vapor toxicity and concentration-dependent persistence of buprofezin applied to cotton foliage for controlling the sweetpotato whitefly (Homoptera: Aleyrodidae). J. Econ. Entomol. 83: 1254–1260.

Delbeke, T.S.L. Vercruysse, L. Tirry, P. DeClerq, and D. Degheele. 1997. Toxicity of diflubenzuron, pyriproxyfen, imidacloprid and diafenthiuron to the predatory bug *Orius laevigatus* (Het.: Anthoocoridae). Entomophaga 42: 349–358.

Fuog, D., S. Fergusson, and C. Fluckiger. 1995. Reduction of virus transmission after application of pymetrozine. *In* Proceedings of the XIII International Plant Protection Congress, 2–7 July 1995, The Hague, The Netherlands.

Fuog, D., S. J. Fergusson, and C. Fluckiger. 1998. Pymetrozine: a novel insecticide affecting aphids and whiteflies, pp. 40–49. *In* I. Ishaaya and D. Degheele [eds.], Insecticides with novel modes of action. Springer-Verlag, New York.

Gerling, D., and P. Sinai. 1994. Buprofezin effects on two parasitoid species of whitefly (Homoptera: Aleyrodidae). J. Econ. Entomol. 87: 842–846.

Grafton-Cardwell, E., and P. Gu. 2003. Conserving vedalia beetle, *Rodolia cardinalis* (Mulsant) (Coleoptera: Coccinellidae) in citrus: a continuing challenge as new insecticides gain registration. J. Econ. Entomol. 96: 1388–1398.

Hassan, S. A., and M. Van de Veire. 2004. Compatibility of pesticides with biological control agents, pp. 129–147. *In* K. M. Heinz, R. G. Van Driesche, and M. P. Parrella [eds.], Biocontrol in protected culture. Ball Publishing, Batavia, IL.

Hattingh, V., and B. Tate. 1995. Effects of field-weathered residues of insect growth regulators on some Coccinellidae (Coleoptera) of economic importance as biocontrol agents. Bull. Entomol. Res. 85: 489–493.

Hoddle, M. S., R. G. Van Driesche, S. M. Lyon, and J. P. Sanderson. 2001. Compatibility of insect growth regulators with *Eretmocerus eremicus* (Hymenoptera: Aphelinidae) for whitefly (Homoptera: Aleyrodidae) control on poinsettias. I. Laboratory assays. Biol. Control 20: 122–131.

Ishaaya, I., D. De Cock, and D. Degheele. 1994. Pyriproxyfen, a potent suppressor of egg hatch and adult formation of the greenhouse whitefly (Homoptera: Aleyrodidae). J. Econ. Entomol. 87: 1185–1189.

James, D. G. 2004. Effect of buprofezin on survival of immature stages of *Harmonia axyridis*, *Stethorus punctum picipes* (Coleoptera: Coccinellidae), *Orius tristicolor* (Hemiptera: Anthocoridae), and *Geocoris* spp. (Hemiptera: Geocoridae). J. Econ. Entomol. 97: 900–904.

James, R. R., and B. Lighthart. 1994. Susceptibility of the convergent lady beetle (Coleoptera: Coccinellidae) to four entomogenous fungi. Environ. Entomol. 23: 190–192.

Jones, W. A., M. A. Ciomperlik, D. A. Wolfenbarger. 1998. Lethal and sublethal effects of insecticides on two parasitoids attacking *Bemisia argentifolii* (Homoptera: Aleyrodidae). Biol. Control 11: 70–76.

Kayser, H., L. Kaufmann, F. Schurmann, and P. Harrewijn. 1994. Pymetrozine (CGA 215′944): a novel compound for aphid and whitefly control. An overview of its mode of action. Proceedings of the 1994 Brighton Crop Protection Conference—Pests and Diseases 2: 737–742.

Kiselek, E. V. 1975. The effect of biopreparations on insect enemies. Zash. Rast. 12: 23.

Kismali, S., and E. Erkin. 1984. Effects of juvenile hormone analogues of the development of some useful insects. I. Effects on egg hatch in *Coccinella septempunctata* L. Turkiye Bitki Koruma Dergisi. 8: 99–104.

Kole, M., and M. Hennekam. 1990. Update: six years of successful biological control in interior plantscapes in The Netherlands. IPM Pract. 12: 1–4.

Lemma, K. M., and S. L. Poe. 1978. Juvenile hormone analogues: effects of ZR-777 on *Liromyza sativae* and its endoparasite. Fla. Entomol. 61: 67–68.

Mazzone, P., and G. Viggiani. 1980. Effects of diflubenzuron (Dimilin) on the larval stages of the predator *Cryptolaemus montrouzieri* Muls. (Col. Coccinellidae). Bull. Entomol. Agraria "Filippo Silvestri," Portici 37: 17–21.

Medina, P., G. Smagghe, F. Budia, L. Tirry, and E. Vinuela. 2003. Toxicity and absorption of azadirachtin, diflubenzuron, pyriproxyfen, and tebufenozide after topical application in predatory larvae of *Chrysoperla carnea* (Neuroptera: Chrysopidae). Environ. Entomol. 32: 196–203.

McNeil, J. 1975. Juvenile hormone analogs: detrimental effects on the development of an endoparasitoid. Science (Wash., D.C.) 189: 640–642.

Nagai, K. 1990. Effects of a juvenile hormone mimic material, 4-phenoxyphenyl (RS)-2-(2-pyridyloxy) propyl ether, on *Thrips palmi* Karny (Thysanoptera: Thripidae) and its predator *Orius* sp. (Hemiptera: Anthocoridae). Appl. Entomol. Zool. 25: 199–204.

Niemczyk, E., M. Pruska, and M. Miszczak. 1985. Toxicity of diflubenzuron to predacious and parasitic insects. Rocniki Nauk Rolniczych, E. (Ochrona Roslin) 11: 181–191.

Oetting, R. D., and J. G. Latimer. 1995. Effects of soaps, oils, and plant growth regulators (PGRs) on *Neoseiulus cucumeris* (Oudemans) and PGRs on *Orius insidiosus* (Say). J. Agric. Entomol. 12: 101–109.

Osborne, L. S., and F. L. Petitt. 1985. Insecticidal soap and predatory mite, *Phytoseiulus persimilis* (Acari: Phytoseiidae), used in management of the twospotted spider mite (Acari: Tetranychidae) on greenhouse grown foliage plants. J. Econ. Entomol. 78: 687–691.

Parrella, M. P., and B. C. Murphy. 1998. Insect growth regulators. GrowerTalks 62: 86–89.

Peleg, B. A. 1983. Effect of three insect growth regulators on larval development, fecundity and egg viability of the coccinellid, *Chilocorus bipustulatus* (Col., Coccinellidae). Entomophaga 28: 117–121.

Rothwangl, K. B., R. A. Cloyd, and R. N. Wiedenmann. 2004. Effects of insect growth regulators on citrus mealybug parasitoid *Leptomastix dactylopii* (Hymenoptera: Encyrtidae). J. Econ. Entomol. 97: 1239–1244.

SAS Institute. 2002. SAS/STAT user's guide, version 8.2. SAS Institute, Cary, NC.

SAS Institute. 2004. SAS/STAT user's guide, version 9.1. SAS Institute, Cary, NC.

Sechser, B., B. Reber, and F. Bourgeois. 2002. Pymetrozine: selectivity spectrum to beneficial arthropods and fitness for integrated pest management. J. Pestic. Sci. 75: 72–77.

Shibuya, M. 1984. Applaud, a new selective insecticide. Jpn. Pest Inform. 44: 17–21.

Smith, D., and D. F. Papacek. 1990. Buprofezin: an effective and selective insect growth regulator against *Unaspis citri* (Hemiptera: Diaspididae) on citrus on south-east Queensland. Gen. Appl. Entomol. 22: 25–28.

Spollen, K. M., and M. B. Isman. 1996. Acute and sublethal effects of a neem insecticide on the commercial biological control agents *Phytoseiulus persimilis* and *Amblyseius cucumeris* (Acari: Phytoseiidae) and *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae). J. Econ. Entomol. 89: 1379–1386.

Staal, G. B. 1975. Insect growth regulators with juvenile hormone activity. Annu. Rev. Entomol. 20: 417–460.

Tomizawa, M., and J. E. Casida. 2003. Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. Annu. Rev. Entomol. 48: 339–364.

Uchida, M., T. Asai, and T. Sugimoto. 1985. Inhibition of cuticle deposition and chitin synthesis by a new IGR, buprofezin, in *Nicaparvata lugens*. Stat. Agric. Biol. Chem. 49: 1233–1234.

van Lenteren, J. C. 1987. World situation of biological control in greenhouses and factors limiting use of biological control. IOBC/WPRS Bull. 10: 78–81.

Ware, G. W., and D. M. Whitacre. 2004. The pesticide book, 6th ed. MeisterPro Information Resources, Willoughby, OH.

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