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Author: Michaud, J. P.

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# NON-TARGET IMPACTS OF ACARICIDES ON LADYBEETLES IN CITRUS: A LABORATORY STUDY

J. P. MICHAUD
University of Florida, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33881

ABSTRACT

Two ladybeetles, *Cycloneda sanguinea* (L) and *Harmonia axyridis* Pallas, were exposed to leaf residues and topical applications of five acaricide formulations commonly used in citrus production in Florida. Dicofol was the only compound demonstrating no toxicity to larvae of either species, even at twice the recommended rate. Abamectin + petrolium oil was the most toxic material; leaf residues applied at the recommended rate caused 100% mortality of both species. Diflubenzuron was toxic to larvae of both species as a leaf residue applied at twice the recommended rate, and to larvae of *C. sanguinea* when topically applied at the recommended rate. Fenbutatin oxide was toxic to larvae of *C. sanguinea* when topically applied at the recommended rate, and as a leaf residue applied at twice the recommended rate, but not to larvae of *H. axyridis*. Pyridaben was toxic to larvae of both species as a leaf residue applied at twice the recommended rate, and in topical application at the recommended rate. Adult beetles of both species survived topical applications of all materials without sustaining significant mortality over a 48-h period. Larvae of *C. sanguinea* surviving exposure to fenbutatin-oxide and diflubenzuron exhibited slower development than control larvae. Larvae of *H. axyridis* surviving exposure to abamectin + oil at 1/10 the recommended rate developed significantly faster than their control counterparts.

Key Words: Acaricides, *Cycloneda sanguinea*, development, *Harmonia axyridis*, repellency, toxicity

RESUMEN

Dos especies de Coccinellidae, *Cycloneda sanguinea* (L) y *Harmonia axyridis* Pallas, fueron expuestas a cinco acaricidas de uso comun en citricultura en Florida en forma residual en hojas y en aplicaciones directas. Dicofol fue el unico compuesto que no mostro toxicidad alguna en larvas de ambas especies, inclusive al doble de la concentracion recomendada. Abamectin + aceite de petroleo fue el material mas toxico; residuos en hoja aplicadas a la dosis recomendada causaron 100% de mortalidad en larvas de ambas especies. Diflubenzuron fue toxico a larvas de ambas especies como residuo en hoja aplicado al doble de la concentracion recomendada, y a larvas de *C. sanguinea* cuando fue aplicado a la dosis recomendada en forma directo. Fenbutatin oxide fue toxico a larvas de *C. sanguinea* a la dosis recomendada en aplicacion directa, y como residuo en hojas aplicadas al doble de la concentracion recomendada, pero no a larvas de *H. axyridis*. Pyridaben fue toxico a larvas de ambas especies como residuo en hoja aplicado al doble de la concentracion recomendada, y en aplicacion directa a la dosis recomendada. Adultos de ambas especies sobreviviron aplicacions directas de todas las materiales sin mortalidad significativa sobre un periodo de 48-h. Larvas de *C. sanguinea* que sobrevivieron a fenbutatin-oxide y diflubenzuron mostraron un desarollo significativamente mas lento que larvas del testigo. Larvas de *H. axyridis* que sobrevivieron abamectin + oil a 1/10 el dosis recomendado mostraron un desarollo significativamente mas rapido que los testigos.

Large volumes of acaricides are applied to Florida citrus every year to control mite pests such as the citrus red mite, *Panonychus citri* (McGregor), the rust mite, *Phylocoptruta oleivora* (Ashmead), and the broad mite, *Polyphagotarsonemus latus* (Banks). All citrus varieties produced for fresh market in Florida require protection from rust mites that cause russeting of the peel, and more pesticides are applied against the rust mite than against all other pests of citrus in Florida combined. For example, in 1993, 1995 and 1997 abamectin was applied on 34, 49, and 35% of the orange-growing acreage respectively, and 78, 83, and 65% of the grapefruit-growing acreage (Florida Agricultural Statistics Service, 1998). Similarly, the statistics for use of fenbutatin-oxide over the same period are 30, 7, and 7% of total orange acreage and 43, 21, and 13% of total grapefruit acreage.

Some previous studies have examined the nontarget effects of some acaricides on beneficial species such as spiders (Amalin et al. 2000), scale parasitoids (Rehman et al. 1999), and leaf miner parasitoids (Villanueva-Jimenez and Hoy, 1998). However, generalist predators also contribute to biological control of pests and are especially important mortality factors for aphids, psyllids and other Homoptera that currently lack effective

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parasitoids in Florida. This work was initiated to assess the toxicity of a series of acaricides to the generalist predators *Cycloneda sanguinea* L. and *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) using standardized laboratory assays.

The ladybeetles *Cycloneda sanguinea* L. and *Harmonia axyridis* Pallas are both beneficial insects in Florida citrus where they prey on a range of soft-bodied insect pests. In particular, both these beetles attack the brown citrus aphid, *Toxoptera citricida* (Kirkaldy) (Michaud 1999) and the Asian citrus psyllid, *Diaphorini citri* Kuwayama (Michaud 2000a), two recently arrived invasive species. *Cycloneda sanguinea* is an indigenous species and primarily an aphid feeder, although it has also been observed to consume Florida red scale crawlers, *Chrysomphalus aonidum* (L.) (van Brussel & Bhola 1970), Australian red scale, *Aonidiella aurantii* (Maskell) (de Crouzel et al. 1979), green scale, *Coccus viridis* (Green) (Sousa & Perez 1977), whitefly, *Bemisia tabaci* (Gennadius) (Link et al. 1980), citrus whitefly, *Dialeurodes citri* (Ashmead) (Morrill & Back 1911), eggs of *Heliothis virescens* (F.) (McDaniel & Sterling 1979), *Empoasca* sp. (Cotte & Cruz 1989) and eggs of the phytophagous coccinellid *Epilachna varivestis* Mulsant (Chicas & Maes 1993). The multi-colored Asian ladybeetle, *H. axyridis*, originates in China, although material released in the USA was imported from Japan (McClure 1986a). *Harmonia axyridis* is also primarily an aphid feeder but its full range of prey in Florida citrus has yet to be determined. It is reported to prey on various scale families (MacClure 1983; 1986b; Shi et al. 1997), Lepidoptera (Kim & Noh 1968) Heteroptera (Fujisaki 1975) and even mites (Lucas et al. 1997). Together, *H. axyridis* and *C. sanguinea* constitute a significant component of biological control in the citrus ecosystem.

In this study, I examined the toxicity of five acaricides frequently employed in Florida citriculture to larvae of *C. sanguinea* and *H. axyridis* using topical applications and timed exposures to leaf residues.

## Materials and Methods

### Stock Colonies

Larvae of *C. sanguinea* and *H. axyridis* were reared on a diet of frozen *Ephestia* eggs, bee pollen, water and a liquid diet formulation (Entomos Inc., Gainesville, FL) in individual plastic Petri dishes (5.5 cm dia × 1.0 cm). The water and liquid diet were encapsulated in polymer beads that permitted both larvae and adults to access the contents, while maintaining a low relative humidity in the dishes. Adult beetles of both species were maintained in 1 L ventilated glass mason jars (~50 -90 /jar) filled with strips of shredded wax paper for their first 9-12 d of life following eclosion. The jars were kept in a greenhouse maintained at 24 ± 2°C, 60 ± 5% RH, with natural lighting and provisioned with water (on a wick), the liquid diet (presented in stretched Parafilm® domes), bee pollen, and frozen *Ephestia* eggs on a daily basis. Adult females were transferred to individual plastic petri dishes (as above) for oviposition. Ovipositing females were provided with water beads, diet beads, frozen *Ephestia* eggs, and bee pollen as necessary. Eggs were harvested daily and held in an incubator at 24°C, 60 ± 5% RH under fluorescent light (P:S-16:8) and hatched ca. 3.5 ± 0.5 d later under these conditions. Larvae for each experiment were selected from hatching egg clusters derived from different females, provided with *Ephestia* eggs for their first 24 h of life, and were used for experiments on the morning of the second day when they were 24 ± 6 h old.

### Acaricides

Five different miticidal formulations were selected for testing against both coccinellid species. These were: abamectin (Agrimek®, Merck & Co. Inc., Rahway, NJ, 07065) in combination with 1% petroleum oil, diflubenzuron (Micromite® 4L, Uniroyal Chemical Co. Inc., Middlebury, CT, 06749), fenbutatin-oxide (Vendex® 50WP, Griffin L.L.C., Valdosta, GA, 31601), dicofol (Kelthane® MF, Rhom & Haas Co., 100 Independence Mall W., Philadelphia, PA, 19106) and pyridaben (Nexter®, BASF Corporation, Research Triangle Park, NC, 27709). The acaricide concentrations tested initially approximated field rates as recommended in the 2000 Florida Citrus Pest Management Guide (J. Knapp, ed.) assuming application in 935 L of water per ha (100 gallons of water per acre). Since materials are typically applied on citrus trees in volumes ranging from 1000 to 3000 L per ha, depending on available equipment and the nature of tank mixes, the concentrations used in these experiments represented the 'high end' of the concentration range that beetles would likely be exposed to under field conditions.

### Topical Assays

All larvae were sprayed in a Potter Precision Spray Tower (Burkard Munfacturing Co. Ltd., Rickmansworth Herts, UK) with a nozzle aperture of 0.6985 mm at an operating pressure of 1.12 kg/cm$^2$. Coccinellid larvae 24 (±6) h-old were selected from a series of hatching egg clusters, one from each cluster. Treatment larvae (n = 16) were placed into two plastic Petri dishes (5.5 cm dia × 1.0 cm), eight per dish, and sprayed with 1 ml aqueous solution of the test material. Larvae (n = 16) in control groups were placed into two plastic Petri dishes (as above), eight per dish, and sprayed with 1 ml of distilled water. Larvae were then individually transferred to clean plastic Petri dishes

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(as above), one per dish, and provisioned with *Ephestia* eggs, water beads, diet beads and bee pollen. Dishes were checked for mortality at 4 h, 24 h, and daily thereafter until eclosion of adults. Larvae were provided with fresh food every 3 d until pupation or death. Dates of pupation and adult eclosion were recorded for each replicate. Mortality data were analyzed using a Chi-square Goodness-of-fit test and data for larval developmental time were analyzed by one-way ANOVA (SPSS 1998). When a topical assay resulted in significant larval mortality, an additional trial was performed with adult beetles, identical in every way to the larval assay with the exception that beetles were monitored for mortality for only 48 hours. Data was corrected for control mortality using Abbott's formula (Abbott 1925).

### Leaf Residue Assays

Freshly picked grapefruit leaves were washed in a 0.005% solution of Chlorox® bleach, rinsed in distilled water, and dried on paper towels. Leaf disks 3 cm in diameter were punched from the leaves using a sharpened piece of steel pipe. Leaf disks in treatment series (n = 16) were sprayed with 1 ml of aqueous solution of the test material in a Potter Precision Spray Tower; leaf disks in control series (n = 16) were sprayed with 1 ml distilled water. Both treated and control leaf disks were placed into numbered plastic Petri dishes (3.5 cm dia × 1.0 cm) and a small measure of *Ephestia* eggs was placed in the center of each disk. Single, 24 (±6) h-old first-instar coccinellid larvae, each taken from different egg clusters, were then transferred to each dish. Larvae were transferred to clean plastic Petri dishes (5.5 cm dia × 1.0 cm) after 24 h exposure to residues and provisioned with *Ephestia* eggs, bee pollen, diet beads and water beads. All replicates were checked for mortality at 4 h, 24 h, and daily thereafter. Larvae were provided with fresh food every 3 d until pupation or death. Dates of pupation and adult eclosion were recorded for each replicate. Mortality data were analyzed using a Chi-square Goodness-of-fit test and data for larval developmental time were analyzed by one-way ANOVA (SPSS 1998). When a residue assay resulted in significant larval mortality (after correction for control mortality using Abbott's formula, Abbott 1925), an additional trial was performed with adult beetles, identical in every way to the larval assay with the exception that beetles were monitored for mortality for a period of 48 h.

## Results and Discussion

The combination of abamectin and petrolium oil (Concentration in Fig. 1 given as abamectin + oil) appeared to be the most toxic of all formulations to both coccinellid species, producing 100% mortality when larvae were exposed to leaf residues applied at the recommended field rate (Fig. 1a). Larvae of *C. sanguinea* experienced significant mortality when exposed to leaf residues corresponding to 1/10 of recommended field rates. Larvae of *H. axyridis* surviving leaf residues of abamectin + oil at 1/10 the recommended rate exhibited significantly faster development than their control counterparts (Mean ± SEM = 12.4 ± 0.51 d vs. 11.1 ± 0.33 d; $F$ = 4.237; 1,17 df; $P$ = 0.05). An increase in developmental rate is typically a benefit of improved diet and/or growing conditions, but it is difficult to intrepret such an increase as beneficial in the context of chemical exposure. Previously, Villanueva-Jimenez and Hoy (1998) reported that abamectin + oil was toxic to parasitoids of citrus leaf miner and concluded it was not compatible with IPM programs for this pest. The present results indicate that this material is likely harmful to generalist predators as well.

Diflubenzuron was only toxic to coccinellid larvae as a leaf residue when applied at a concentration corresponding to twice the recommended field rate (Fig. 1b). Topical application of diflubenzuron at the recommended rate resulted in significant mortality of *C. sanguinea* larvae, but not *H. axyridis* larvae. Sundari et al. (1998) reported that late instars of *Cryptolaemus montrouzieri* fed mealybugs treated with diflubenzuron experienced some decrease in protein assimilation but were increasingly able to compensate over time. Exposure of *C. sanguinea* larvae to leaf residues of diflubenzuron applied at twice the recommended field rate significantly increased developmental time for surviving larvae (Mean ± SEM = 11.3 ± 0.59 d vs. 9.7 ± 0.41 d; $F$ = 4.845; 1,17 df; $P$ = 0.042). Hassan et al. (1994) rated diflubenzuron as 'slightly harmful' to larvae of *Coccinella septempunctata* L. and 'moderately harmful' to larvae of *Coccinella novemnotata* Herbst. Diflubenzuron is an insect growth regulator known to induce sterility in some insects (Schroeder 1996; Ji et al. 1999; Park et al. 1999) and we did not test for such effects in this study. Mani et al. (1997) found adverse effects on the fertility of *C. montrouzieri* and did not recommend it for IPM programs. Nevertheless, Chakraborty & Chatterjee (1999) concluded that diflubenzuron was compatible with ladybeetles for control of safflower aphids and Villanueva-Jimenez and Hoy (1998) concluded that diflubenzuron was an IPM-compatible pesticide for control of citrus leaf miner.

Fenbutatin-oxide was toxic to larvae of *C. sanguinea*, either as a topical application at the recommended rate, or as a leaf residue at twice the recommended rate (Fig. 1c) and larvae surviving the residue treatment experienced an extended developmental time relative to control larvae (Mean ± SEM = 12.4 ± 0.51 d vs. 11.1 ± 0.33 d; $F$ = 4.237; 1,17 df; $P$ = 0.050). Fenbutatin oxide did

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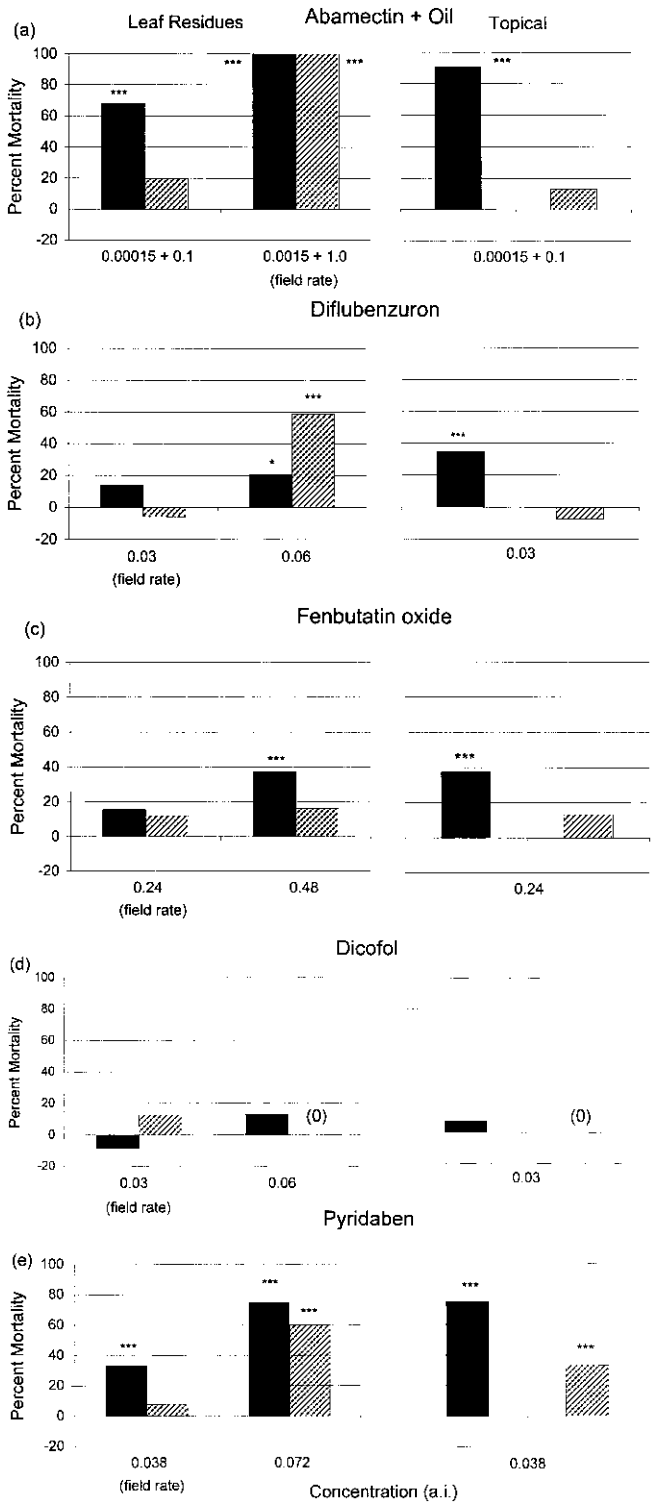


Fig. 1. Percent mortality (corrected using Abbott's formula) of coccinellid larvae (n = 16 in all trails; solid bars, *C. sanguinea*; hatched bars, *H. axyridis*) exposed to different concentrations of acaricides as leaf residues and in topical application. The calculated field rates assume application in 935 L water per ha. '(0)' indicates identical survival between treatment and controls. for each concentration of a material, treatment mortality for each species was compared to control mortality using a Chi-Square Goodness-of-fit text (*, $P < 0.05$; ** $P < 0.01$; ***, $P < 0.0001$).

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not result in measurable toxicity to larvae of *H. axyridis* in any trial.

Leaf residues of dicofol produced no measurable toxicity to larvae of either species, even when applied at twice the recommended rate, and neither did topical applications (Fig 1d). Similarly, Bartlett (1963) found that contact toxicity of dicofol was low to seven species of coccinellid. There were no significant differences in developmental time between treatment and control larvae in any trial.

Pyridaben was toxic to *C. sanguinea* larvae as a leaf residue at the recommended rate, but twice the recommended rate was required to produce significant mortality in *H. axyridis* larva, unless the material was topically applied (Fig. 1e). Shipp et al. (2000) found pyridaben to be more toxic than abamectin + oil to a series of beneficial insects and mites when applied under greenhouse conditions. The present study indicates that abamectin + oil is more toxic to these two coccinellid species than is pyridaben when both are applied at the recommended rate.

Adult *C. sanguinea* receiving topical applications of all materials applied at recommended field rates had 100% survival in all trials (n = 16 in each trial, data corrected using Abbott's formula). Adult *H. axyridis* experienced 6.25% mortality in the abamectin + oil treatment (Chi-square = 0.391, n.s.) and 12.5% mortality in the pyridaben trial (Chi-square = 1.562, n.s.); all other trials yielded 100% survival over the 48 hr period. Differential susceptibility of life stages to pesticides is well recognized (Hassan et al. 1994), and adults of both these species are evidently far less sensitive to compounds that appeared significantly toxic to larvae in these trials.

Two of the five compounds tested as leaf residues at recommended field rates caused significant mortality to larvae of *C. sanguinea* (abamectin and pyridaben) whereas only one produced significant toxicity in larvae of *H. axyridis* (abamectin). When materials were applied topically at recommended rates, four out of five were toxic to larvae of *C. sanguinea* whereas only one (pyridaben) was toxic to larvae of *H. axyridis*. The only compound without significant toxicity to *C. sanguinea* was dicofol, a compound that is no longer recommended for control of rust mite due to development of widespread resistance (Omoto et al. 1995). It should also be noted that diet can influence coccinellid susceptibility to insecticides and that many coccinellids readily consume prey items that do not support their development or reproduction (Michaud 2000b) which may increase their susceptibility to toxins (Kalushkov 1999). The standardized diet employed in these experiments yields excellent survival of both species and may therefore underestimate the sensitivity of these species under field conditions when diet may be less optimal.

Prior to 1998, *C. sanguinea* was the most abundant aphidophagous coccinellid in Florida citrus state-wide (Michaud, 2000b) but recent observations (Michaud, unpublished) indicate that its abundance is declining while that of *H. axyridis* is increasing. It seems reasonable to expect that widespread use of acaricides in citrus destined for fresh market will differentially impact *C. sanguinea* as *H. axyridis* appears to be more resistant to many of these materials.

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