BioControl
DOI 10.1007/s10526-010-9283-0

Toxicity of pesticides to *Tamarixia radiata*, a parasitoid of the Asian citrus psyllid

David G. Hall · Ru Nguyen

Received: 2 November 2009 / Accepted: 29 March 2010

Abstract Sixteen pesticides including two fungicides were evaluated for toxicity to adult *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae), a parasitoid of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). Percentage mortality data were evaluated to generally assess IPM-compatibility of the pesticides with adult parasitoids. The following were found to be least compatible with (most toxic to) adult *T. radiata* based on the toxicity of direct sprays and potential long residual life on leaves: carbaryl, chlorpyrifos, and fenpropathrin. Although highly toxic to the parasitoid as direct sprays or freshly dried residues, each of the following was more compatible with *T. radiata* because the toxicity of residues of these pesticides was either low at one to three days after application or relatively non-persistent: abamectin, chenopodium oil, fenpyroximate, and spirotetramat. Depending on environmental conditions, imidacloprid (foliar-applied), phosmet, pyridaben, sulfur and 435 spray oil might also be somewhat more compatible for the same reasons. The pesticides that consistently appeared to be most compatible with *T. radiata* were aluminum tris, copper hydroxide, diflubenzuron, and kaolin clay (Surround WP).

Keywords Biological control · Citrus greening disease · Eulophidae · Huanglongbing · Hymenoptera · *Diaphorina citri* · Asian citrus psyllid

Handling Editor: Torsten Meiners

D. G. Hall (✉)
USDA-ARS, 2001 South Rock Road, Fort Pierce, FL 34945, USA
e-mail: David.Hall@ars.usda.gov

R. Nguyen
Division of Plant Industry, Florida Department of Agriculture and Consumer Services, 1911 SW 34 Street, Gainesville, FL 32614, USA
e-mail: nguyenr@doacs.state.fl.us

Introduction

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), is an important pest of citrus primarily because it vectors the bacteria responsible for huanglongbing (sometimes referred to as yellow shoot or citrus greening disease). Huanglongbing is considered to be the world's most serious disease of citrus (Bové 2006). *D. citri* was first discovered in Florida, USA during 1998 (Tsai and Liu 2000), and huanglongbing was first discovered in Florida during 2005 (Bové 2006).

Tamarixia radiata (Waterston; Hymenoptera: Eulophidae) is a parasitoid of *D. citri* that was imported from Taiwan and Vietnam and released into Florida citrus shortly after the psyllid was first discovered in the state (Hoy et al. 1999; Michaud 2004; Halbert and Manjunath 2004). The classical

Published online: 23 May 2010

biological control effort was made in hopes of increasing biological control of the psyllid in order to reduce its population levels and damage, and to better prepare the Florida citrus industry for a possible introduction of huanglongbing. *T. radiata* had been credited with reducing infestations of *D. citri* sufficiently in Réunion Island to mitigate the impact of greening (Étienne et al. 2001; Chien and Chu 1996).

Tamarixia radiata is established in Florida (Hoy and Nguyen 2000) and is widespread (Qureshi et al. 2009), but parasitism rates of *D. citri* by the parasitoid in Florida (Tsai et al. 2002; Michaud 2004; Hall et al. 2008; Qureshi et al. 2009) have been lower and more sporadic than generally reported in Réunion Island, Guadeloupe, and some other areas (Étienne et al. 2001; Pluke et al. 2008). Reasons that *T. radiata* might be a less effective biological control agent of the psyllid in Florida than in some other areas could include environmental differences, interguild competition with other beneficial organisms, or genetic differences (Barr et al. 2009) among geographical populations of the parasitoid. Chemicals used in citrus such as insecticides, miticides, plant disease control chemicals, spray oils, and perhaps even nutritional sprays could negatively influence the effectiveness of biological control by *T. radiata*.

We report on the toxicity of some pesticides labeled for use in citrus to adult *T. radiata*. Thirteen pesticides with known activity against insect and mite pests were examined. Also, two fungicides and a petroleum oil commonly used in citrus were tested. The toxicity of these materials was tested as direct sprays and as residues on leaves over time.

Materials and methods

Tamarixia radiata adults used in the studies presented here were obtained from a colony maintained in Gainesville, FL, USA by the Florida Department of Agriculture and Consumer Services, Division of Plant Industry. Adults were aspirated when they were 1–3 days old, placed in vials with a small amount of honey as a food source, and sent in a cooler by overnight carrier to the U. S. Horticultural Research Laboratory in Fort Pierce, FL. The adults were subjected to toxicity experiments on the day they arrived in Fort Pierce and thus were 2–4 days old. The Gainesville lab shipped groups of 200–300 adult *T. radiata* per vial.

Pesticides tested for toxicity to *T. radiata* are presented in Table 1. The application rates of the pesticides were based on label rates for citrus in conjunction with rates presented in the 2007 Florida Citrus Pest Management Guide (Rogers and Timmer 2007). Insecticides and miticides were applied within the low to mid range of recommended rates for citrus (not specific for *D. citri*) except for spirotetramat which was inadvertently applied at the upper range of recommended rates, and for chenopodium oil of which a rate recommended by AgraQuest, Inc. was used. Fungicides were applied at the highest recommended rates. The commercially formulated products were diluted in tap water to the equivalent of 935 ha^{-1}. A new, fresh mix of each chemical in water was prepared for each test.

During all of the trials conducted on toxicity of the pesticides to *T. radiata*, adults exposed to direct sprays or residues were subsequently maintained in a laboratory fume hood [mean (SE) 23.0 (0.1)°C] and monitored for survival by examining the parasitoids under a dissecting microscope. A parasitoid was considered dead if it exhibited no movement during a 10–15 s examination period or when prodded.

Toxicity of direct sprays

Two trials were conducted to assess the toxicity to *T. radiata* of direct sprays of the pesticides presented in Table 1, the first trial with 11 of the pesticides and the second with six (one of the pesticides was studied in both trials; a water control was included in each trial). The procedures used to conduct the trials were the same. Briefly, adult *T. radiata* parasitoids were sprayed with a pesticide, transferred to a citrus leaf disk, and thereafter monitored for survival. Specific details were as follows.

Individual fresh citrus leaves (‘Duncan’ grapefruit, *Citrus paradisi* Macf.) were excised from young trees maintained in a greenhouse that were periodically treated for general pest control with an insecticidal soap based on potassium salts of fatty acids (49% active ingredient; M-Pede, Dow AgroSciences LLC, Indianapolis, IN, USA) at a 2% v/v rate in water. The leaves had not been sprayed for at least ten days before the trials, and before the leaves were used they were thoroughly washed with tap water to eliminate any soap residues. The leaf disks were prepared using a 2.34 cm diameter copper pipe with sharpened edges

Table 1 Pesticides screened for toxicity to *Tamarixia radiata*

Trade name	Class	Active ingredient	Application rate (product/1 liter water)	Manufacturer
AgriMek 0.15EC	Glycoside	Abamectin (2%)	0.8 ml	Syngenta
Aliette WDG	Phosphonate	Aluminum tris (80%)	6.0 g	Bayer CropScience
Citrus Soluble Oil	Petroleum oil	Petroleum oil 435 (99.3%)	30.0 ml	Loveland Products Inc.
Danitol 2.4EC	Pyrethroid	Fenpropathrin (30.9%)	1.3 ml	Valent USA Corp.
Imidan 70 W	Organophosphate	Phosmet (70%)	1.5 g[a]	Gowan Company
Kocide 101WP	Copper fungicide	Copper hydroxide (77%)	14.4 g	Griffin L.L.C.
Lorsban 4E	Organophosphate	Chlorpyrifos (44.9%)	6.3 ml	Dow AgroSciences
Microfine Sulfur	Elemental Sulfur	Elemental Sulfur (90%)	6.0 g	Diamond R. Fertilizer Co.
Micromite 80WGS	IGR	Diflubenzuron (80%)	0.5 g	Crompton Manufacturing Company, Inc.
Movento 240SC	Tetramic acid	Spirotetramat (22.4%)	0.8 ml[b]	Bayer
Nexter	Pyridazinone	Pyridaben (75%)	0.5 g	Gowan
Portal	METI[c]	Fenpyroximate (5%)	5.0 ml	Nichino America, Inc.
Provado 1.6	Neonicotinoid	Imidacloprid (17.4%)	0.8 ml	Bayer CropScience
Requiem 25EC	Not yet specified	Chenopodium oil (25%)[d]	9.4 ml	AgraQuest, Inc.
Sevin XRL	Carbamate	Carbaryl (44.1%)	12.5 ml	Bayer CropScience
Surround WP	Kaolin clay	Kaolin clay (95%)	30.0 g	Engelhard Company

[a] Buffered to pH 5.5

[b] With 3% X-77 Spreader per liter

[c] Mitochondrial electron transport inhibitor

[d] Extract from *Chenopodium ambrosioides* near *ambrosioides*

similar to a cork borer. The pipe was used to cut each leaf disk. Each leaf disk (23.4 mm in diameter) was then embedded on agar (7 g/500 ml water) in a small Petri dish (suspension culture dish, 35 mm × 10 mm, non-treated polystyrene, #430588, Corning Inc., Corning, NY, USA). The vials containing adults were briefly flooded with CO_2 and the anesthetized adults were quickly divided (by tapping the vial) without regard to sex among Petri dishes lined with filter paper. Adults were then misted directly with a chemical until each insect was visibly wet. One dish containing adults was thoroughly misted with plain tap water as a control. A separate 180 ml Nalgene aerosol spray bottle (#2430-0200, Fisher Scientific, Pittsburgh, PA, USA) was used to apply each chemical. Immediately following the spray, the parasitoids were transferred to a leaf disk using a very fine camel hair brush (one brush for each chemical). After all the adults were transferred, the Petri dish lid was put into place and secured with a rubber band. Small drops of honey applied to the inside of the Petri dish lid served as a food source. The dishes with treated parasitoids were placed into the fume hood and examined to count numbers of live and dead parasitoids after 24, 48, and 72 h. There were three replications of each of the two trials over time. Numbers of adults sprayed during each repetition varied according to numbers available for testing. The target sample size per treatment per replication was 15 and 20 for the first and second trials, respectively, but some variability in the actual number of adults per treatment per replication was expected using the tapping method of subdividing adults. Across all three repetitions, means (SE) of 15.0 (0.6) and 19.4 (0.9) adults were exposed to each chemical during the first and second trials, respectively. The number of males and females tested was determined at the end of each trial.

Toxicity of fresh, dried residues on leaves

Two trials were conducted to assess the toxicity to *T. radiata* of fresh residues of the pesticides presented in Table 1, the first trial with 11 of the pesticides and the second with six (one of the 16 pesticides was studied in both trials; a water control was included in each trial). The procedures used to conduct the trials were the same. Briefly, a citrus leaf disk was treated with a pesticide and the pesticide was allowed to dry. Adult *T. radiata* parasitoids were then placed onto the leaf disk and monitored for survival. Specific details were as follows.

'Duncan' grapefruit leaves were excised from the young trees maintained in the greenhouse. After each leaf was thoroughly washed in tap water, leaf disks (23.4 mm diameter) were prepared (as described under 'Toxicity of direct sprays') and dipped into each chemical solution, placed on a sheet of paper toweling, and allowed to air dry for approximately 3 h in a fume hood. An exception to the dipping procedure was that, for the kaolin clay and sulfur treatments, leaves were thoroughly misted with each chemical using the before-mentioned aerosol spray bottles. These two treatments were misted rather than dipped because dipping resulted in a visibly uneven distribution of these chemicals on the leaves. A control leaf (dipped in tap water) was included in each trial. After drying, each treated leaf disk was embedded on agar in a Petri dish as described under 'Toxicity of direct sprays'. Anesthetized adult *T. radiata* were tapped in groups of 15–20 without regard to sex onto each treated leaf disk and lids were secured. Again, honey was supplied as a food source. The dishes with parasitoids were held in the fume hood and examined to count numbers of live and dead parasitoids after 24, 48, and 72 h of exposure to the treated leaves. Each trial was repeated three times. Across all three repetitions, means (SE) of 18.5 (0.6) and 17.6 (0.9) adults were exposed to each chemical during the first and second trials, respectively. The number of males and females tested was determined at the end of each trial.

Toxicity of residues on leaves over time

Four trials were conducted to evaluate the toxicity to adult parasitoids of pesticide residues on citrus leaves over a 3-week period after application. The procedures used to conduct the trials were the same. Briefly, leaves on outdoor potted citrus plants were individually treated with a pesticide, and subsamples of these leaves were excised and evaluated for toxicity one day later and thereafter at 3–5 days intervals over a 3-week period after the treatment. A different set of trees were used for each trial. Specific details were as follows.

Potted citrus trees ('Valencia' sweet orange, approximately 0.9 m tall) maintained outdoors at the US Horticultural Research Laboratory in Fort Pierce, FL were treated individually with the chemicals listed in Table 1 (some trees were treated with plain water for control purposes). Three trees were treated with each chemical or water. For each tree, ten leaves were selected, marked, and sprayed to run off using the before-mentioned aerosol spray bottles. On each sample day during the three weeks following treatment applications, one randomly selected leaf treated on each tree was excised, placed into an individual zip lock bag, and transported to the laboratory for processing. A leaf disk from each leaf was prepared by cutting the whole leaf with a sharpened copper tube as described under 'Toxicity of direct sprays'. After each leaf disk was cut, the copper tube was washed with soap and water and then dried with a disposable paper towel to avoid cross contamination. Each leaf disk was individually embedded on agar in a Petri dish as described under 'Toxicity of direct sprays'. Vials containing adult *T. radiata* were briefly flooded with CO_2 and the anesthetized adults were divided among the Petri dishes (ten to 12 adults per dish without regard to sex) by quickly tapping them onto the leaf disks. Means (SE) of 10.6 (0.2), 10.8 (0.2), 10.0 (0.2), and 10.1 (0.2) adults per replication were exposed to each chemical during the four trials. Lids with honey drops were then placed onto the culture dishes and secured with rubber bands. The dishes with parasitoids were held in the fume hood and examined to count numbers of live and dead parasitoids after 72 h. The number of males and females tested was determined at the end of each trial.

The four trials differed with respect to when they took place and, consequently, environmental conditions differed. The first trial was initiated (leaves were sprayed) on 29 September 2008; the second was initiated on 27 October 2008; the third was initiated on 6 April 2009; and the fourth was initiated on 8 June 2009. The trials also differed with respect to the pesticides tested. Each of the pesticides listed in Table 1 (along with water controls) was included in each of two of the four trials. Temperature and rainfall data were obtained over each 3-week period following treatment applications from a weather station (WatchDog Data Logger, Spectrum Technologies, Inc., Plainfield, IL, USA) operated at the outdoor study site.

Statistical analyses

For each experiment, percentage mortality data for adults exposed to each chemical were adjusted for mortality under the control treatment using Abbott's formula (1925). *F*-protected one-way analyses of variance on arcsine-transformed data were conducted using PROC ANOVA (SAS Institute 2008), and treatment means were compared using Tukey's test. For each of the four residual toxicity trials, two repeated measurements ANOVAs were conducted on percent mortality of *T. radiata* adults exposed to residues on leaves, one on data for 1- to 3-day-old residues (two sample dates) and one on data for 8- to 22 day-old residues (three or four sample dates).

Results

Toxicity of direct sprays

Direct sprays of the pesticides were made to means (SE) of 4.0 (0.3) males and 12.7 (0.5) females per treatment per replication over both trials. In the first trial, a mean (SE) of 11.8 (9.6)% adults treated with water (controls) were dead at 24 h and 48 h after treatment, and 13.8 (8.5)% were dead after 72 h. Abamectin, carbaryl, chenopodium oil, chlorpyrifos, fenpropathrin, imidacloprid, and 435 spray oil were each highly toxic to the parasitoid when applied as a direct spray, with 88–100% mortality occurring within 24 h and 100% by 72 h (Table 2). Aluminum tris, copper hydroxide, diflubenzuron, and kaolin clay were each relatively nontoxic when applied as a direct spray. In the second trial, the percentage of adults sprayed with water that were dead at 24, 48 and 72 h after treatment was 0.0 (0.0), 1.7 (1.7) and 10.0 (10.0)%, respectively. Direct sprays of fenpropathrin, fenpyroximate, phosmet, pyridaben, spirotetramat, or sulfur were each highly toxic to adults (Table 3).

Toxicity of fresh, dried residues on leaves

Means (SE) of 4.8 (0.3) males and 13.6 (0.6) females were exposed to fresh dry residues of the pesticides over both trials. In the first trial, 1.5 (1.5)% of the adults exposed to leaves treated with water (controls) were dead at 24 h and 48 h after treatment, and 6.3

Table 2 Percent mortality of adult *Tamarixia radiata* exposed to direct sprays (trial 1) and fresh dry residues of various pesticides (trial 1)

Chemical	Mean (SE) percent mortality after the indicated period of time[a]					
	Direct sprays[b]			Fresh dry residues[c]		
	24 h	48 h	72 h	24 h	48 h	72 h
Abamectin	100.0 (0.0)a	100.0 (0.0)a	100.0 (0.0)a	100.0 (0.0)a	100.0 (0.0) a	100.0 (0.0) a
Aluminum tris	9.9 (13.3)b	12.7 (14.2)b	15.4 (16.0)b	5.4 (6.0)b	32.0 (17.4)bc	54.5 (21.5)abc
Carbaryl	88.1 (5.9)a	100.0 (0.0)a	100.0 (0.0)a	100.0 (0.0)a	100.0 (0.0)a	100.0 (0.0)a
Chenopodium oil	88.9 (2.8)a	97.4 (2.6)a	94.6 (5.4)a	5.8 (11.0)b	19.4 (18.1)c	19.9 (18.9)c
Chlorpyrifos	95.6 (4.4)a	100.0 (0.0)a	100.0 (0.0)a	100.0 (0.0)a	100.0 (0.0)a	100.0 (0.0)a
Copper hydroxide	−16.5 (14.1)b	−16.5 (14.1)b	−18.6 (13.0)b	4.7 (7.2)b	18.9 (18.9)c	18.6 (18.2)c
Diflubenzuron	4.8 (5.3)b	8.1 (8.1)b	6.1 (8.6)b	14.4 (6.5)b	32.4 (24.3)bc	42.5 (28.3)bc
Fenpropathrin	91.3 (4.8)a	100.0 (0.0)a	100.0 (0.0)a	97.4 (2.6)a	100.0 (0.0)a	100.0 (0.0)a
Imidacloprid	88.2 (6.5)a	90.7 (9.3)a	100.0 (0.0)a	92.0 (1.6)a	100.0 (0.0)a	100.0 (0.0)a
Kaolin clay	−4.5 (2.4)b	−2.1 (4.7)b	−4.0 (2.8)b	−1.4 (2.1)b	2.5 (2.5)c	8.5 (14.1)c
435 oil	100.0 (0.0)a	100.0 (0.0)a	100.0 (0.0)a	82.7 (9.5)a	88.7 (5.9)ab	92.9 (4.3)ab

[a] Raw mean percentages corrected for percentage mortality of untreated parasitoids presented. Analyses on arcsine-transformed mean percentages. Means in the same column followed by the same letter are not significantly different ($P = 0.05$), Tukey's test

[b] Results of ANOVA: for 24 h—$F = 37.3$, $P = < 0.0001$; for 48 h—$F = 42.6$, $P = < 0.0001$; for 72 h—$F = 53.3$, $P = < 0.0001$ ($df = 10{,}20$ for each analysis)

[c] Results of ANOVA: for 24 h—$F = 35.6$, $P = < 0.0001$; for 48 h—$F = 13.2$, $P = < 0.0001$; for 72 h—$F = 10.8$, $P = < 0.0001$ ($df = 10{,}20$ for each analysis)

Table 3 Percent mortality of adult *Tamarixia radiata* exposed to direct sprays (trial 2) and fresh dry residues of various pesticides (trial 2)

Chemical	Mean (SE) percent mortality after the indicated period of time[a]					
	Direct sprays[b]			Fresh dry residues[c]		
	24 h	48 h	72 h	24 h	48 h	72 h
Fenpropathrin	100.0 (0.0)a	100.0 (0.0)a	100.0 (0.0)a	100.0 (0.0)a	100.0 (0.0)a	100.0 (0.0)a
Fenpyroximate	100.0 (0.0)a	100.0 (0.0)a	100.0 (0.0)a	79.6 (10.6)abc	93.6 (4.2)ab	93.6 (4.2)ab
Phosmet	100.0 (0.0)a	100.0 (0.0)a	100.0 (0.0)a	92.5 (4.4)ab	100.0 (0.0)a	100.0 (0.0)a
Pyridaben	83.8 (8.1)a	83.4 (8.3)a	85.2 (7.9)a	46.5 (12.2)bcd	51.3 (8.1)abc	68.8 (8.9)ab
Spirotetramat	68.1 (23.5)a	66.8 (24.9)ab	70.2 (21.5)a	37.3 (19.3)cd	53.1 (27.2)bc	58.5 (26.3)ab
Sulfur	0.0 (0.0)b	14.4 (3.5)b	62.0 (22.6)a	−5.6 (5.6)d	5.3 (11.5)c	37.7 (28.1)b

[a] Raw mean percentages corrected for percentage mortality of untreated parasitoids presented. Analyses on arcsine-transformed mean percentages. Means in the same column followed by the same letter are not significantly different ($P = 0.05$), Tukey's test

[b] Results of ANOVA: for 24 h—$F = 14.7$, $P = 0.0002$; for 48 h—$F = 7.8$, $P = 0.0022$; for 72 h—$F = 1.5$, $P = 0.26$ ($df = 5,10$ for each analysis)

[c] Results of ANOVA: for 24 h—$F = 10.7$, $P = 0.0006$; for 48 h—$F = 9.5$, $P = 0.001$; for 72 h—$F = 4.0$, $P = 0.02$ ($df = 5,10$ for each analysis)

(2.1)% were dead at 72 h. From 83% to 100% mortality of parasitoids occurred within 24 h on leaves treated with abamectin, carbaryl, chlorpyrifos, fenpropathrin, imidacloprid, or 435 spray oil (Table 2). Fresh dry residues of diflubenzuron were relatively nontoxic based on 24 h of exposure but moderately toxic based on 72 h of exposure. Aluminum tris was moderately toxic based on mortality of adults after 72 h of exposure. Chenopodium oil and copper hydroxide were slightly toxic, with about 19% mortality occurring within 72 h. Fresh residues of kaolin clay were nontoxic. In the second trial, 4.8 (4.8), 7.5 (4.1) and 7.5 (4.1)% adults exposed to leaves treated with water were dead after 24, 48 and 72 h, respectively. Fresh dry residues of fenpropathrin, fenpyroximate, and phosmet were each highly toxic to adult *T. radiata* (Table 3). Fresh residues of pyridaben or spirotetramat were highly toxic based on 48 h and 72 h observations. Fresh residues of sulfur appeared slightly to nontoxic based on 24 h and 48 h observations but moderately toxic based on 72 h observations.

Toxicity of residues on leaves over time

Counts at the end of the trials indicated that, for each replication of each treatment, means (SE) of 3.0 (0.2) males and 7.5 (0.2) females were screened during the first trial; 3.5 (0.2) males and 7.3 (0.2) females were screened during the second trial; 3.3 (0.1) males and 6.7 (0.2) females were screened during the third trial; and 2.7 (0.1) males and 7.4 (0.2) females were screened during the fourth trial.

A total of 156 mm of rainfall occurred during the first trial on residual toxicity of pesticides to adult *T. radiata*, and 34 mm of rainfall occurred within the first 24 h after the pesticides were applied (Fig. 1a). Appreciable (>10 mm) rainfall occurred on days 7–13 after treatments were applied. The mean (SE) air temperature during the 22 days experiment was 25.7 (0.1)°C. Among adults exposed to leaves treated with water (controls), over all exposure dates a mean (SE) of 3.1 (1.3)% adults died. Chlorpyrifos was the most toxic pesticide in this 22 days residue trial, and carbaryl was the second most toxic (Table 4, Trial 1). One- to 3-day-old residues of chlorpyrifos, carbaryl or fenpropathrin were highly toxic to *T. radiata*, and these residues were significantly more toxic than the residues of the three other pesticides. Abamectin, diflubenzuron and imidacloprid were each relatively nontoxic to adults. Although initially highly toxic, the toxicity of fenpropathrin residues was not persistent.

A total of 63 mm of rainfall occurred during the second trial (Fig. 1b). Although rain events occurred on ten of the 22 days of the trial, most of these events occurred 16–22 days after treatment applications, and appreciable rainfall occurred on only two days (trial days six and seven). The mean (SE) air temperature during the 22 days experiment was 19.7 (0.2)°C.

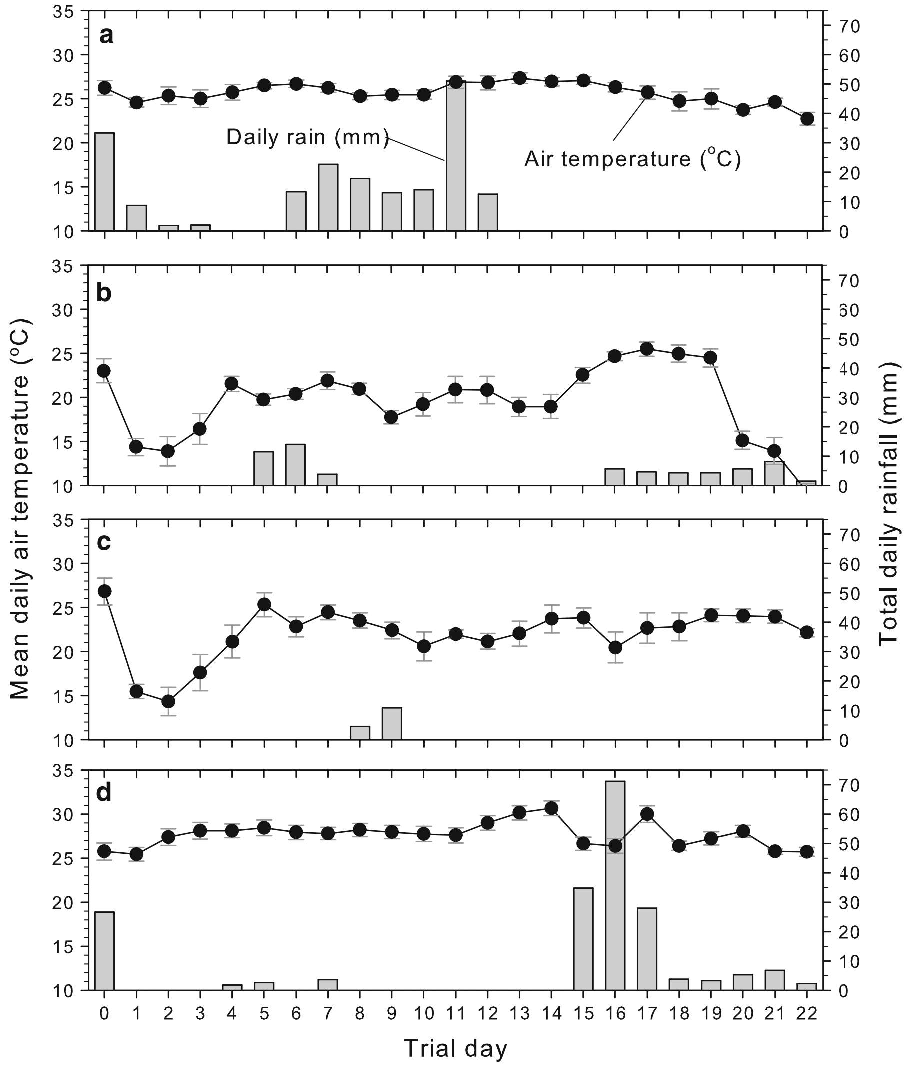

Fig. 1 Daily rainfall (vertical bars) and mean (±SE) daily temperature (solid data point) during the four trials on residual toxicity of pesticides to adult *Tamarixia radiata*. **a**—trial one, **b**—trial two, **c**—trial three, **d**—trial four

Among adults exposed to leaves treated with water (controls), over all exposure dates a mean (SE) of 4.5 (2.6)% adults died. Spirotetramat and 435 spray oil residues were each initially moderately toxic to *T. radiata*, but the residual toxicity of these pesticides was not persistent (Table 4, Trial 2). Residues of each of the other four pesticides were relatively nontoxic throughout the trial.

A total of 16 mm of rainfall occurred during the third trial (Fig. 1c). Rain events occurred on two days of the trial (days 8 and 9 after treatment applications). The mean (SE) air temperature during the 22 days experiment was 22.0 (0.3)°C. Among adults exposed to leaves treated with water (controls), over all exposure dates a mean (SE) of 3.8 (1.5)% adults died. One- to three-day residues of carbaryl, chlorpyrifos, fenpropathrin, imidacloprid and phosmet were each highly toxic (Table 4, Trial 3). Among these five pesticides, residues of carbaryl, chlorpyrifos, fenpropathrin and phosmet remained highly toxic to adults over the entire 22 days trial. Residues of sulfur were at least moderately toxic throughout the trial. Residues of abamectin, diflubenzuron, fenpyroximate and pyridaben were moderately toxic for 1–3 days but thereafter relatively nontoxic.

The fourth trial was subjected to the greatest amount of rainfall, a total of 190 mm (Fig. 1d). An appreciable amount of rainfall (27 mm) occurred during the first 24 h after treatments were applied. Rainfall occurred on 11 other days during the trial,

Table 4 Repeated measurements analyses of variance on residual toxicity to adult *Tamarixia radiata* of pesticides on leaves, percent mortality corrected for mortality of adults exposed to leaves treated with water

Pesticide	Mean (SE) percent mortality of adults exposed to residues	
	1 and 3 days residues	8–22 days residues
Trial 1[a]		
Abamectin	0.0 (3.1)b	4.6 (4.4)c
Carbaryl	80.9 (15.0)a	50.8 (12.9)b
Chlorpyrifos	100.0 (0.0)a	92.7 (4.7)a
Diflubenzuron	1.9 (3.5)b	−0.6 (2.2)c
Fenpropathrin	55.1 (19.6)a	13.3 (7.8)c
Imidacloprid	0.1 (2.0)b	3.3 (4.2)c
Trial 2[b]		
Aluminum tris	5.5 (6.0)ab	−1.2 (2.0)a
Chenopodium	2.2 (7.0)ab	−0.7 (1.9)a
Copper hydroxide	−8.3 (3.7)b	−0.2 (2.1)a
Kaolin clay	4.6 (9.7)ab	1.0 (2.8)a
Spirotetramat	26.1 (12.9)ab	5.3 (2.9)a
435 spray oil	33.3 (16.2)a	−1.5 (1.0)a
Trial 3[c]		
Abamectin	54.6 (8.9)b	4.6 (2.7)c
Carbaryl	100.0 (0.0)a	63.8 (15.0)ab
Chlorpyrifos	100.0 (0.0)a	78.4 (11.4)a
Diflubenzuron	11.9 (17.7)b	0.6 (0.9)c
Fenpropathrin	100.0 (0.0)a	64.4 (9.6)ab
Fenpyroximate	10.8 (10.4)b	−0.3 (1.3)c
Imidacloprid	90.6 (7.3)a	3.7 (3.0)c
Phosmet	93.3 (4.8)a	55.5 (10.6)ab
Pyridaben	46.4 (8.2)b	3.8 (2.8)c
Sulfur	48.9 (8.0)b	24.6 (12.1)bc
Trial 4[d]		
Aluminum tris	6.1 (5.3)b	−1.8 (1.9)a
Chenopodium	2.2 (3.7)b	−3.4 (1.2)a
Copper hydroxide	11.4 (4.4)b	−2.3 (1.6)a
Fenpyroximate	−0.5 (2.3)b	−0.7 (2.5)a
Kaolin clay	6.8 (4.0)b	−2.8 (1.5)a
Phosmet	97.9 (2.1)a	6.5 (8.6)a
Pyridaben	17.4 (6.5)b	−0.4 (3.1)a
Spirotetramat	5.5 (4.4)b	−2.5 (1.5)a
Sulfur	2.0 (3.5)b	−0.7 (1.9)a
435 spray oil	1.1 (3.9)b	−2.5 (1.7)a

For each trial, means in the same column followed by the same letter are not significantly different ($P = 0.05$), Tukey's test. Analyses on arcsine-transformed mean percentages. Raw mean percentages corrected for percentage mortality of control parasitoids presented

[a] Adults exposed to residues 1, 3, 8, 15 and 22 days after pesticide sprays. For 1 and 3 days residue ANOVA, $F = 5.6$, $P = 0.0004$, $df = 5,18$; for 8–22 days residue ANOVA, $F = 7.2$, $P = < 0.0001$, $df = 5,54$

[b] Adults exposed to residues 1, 3, 8, 10, 16 and 22 days after pesticide sprays. For 1 and 3 days residue ANOVA, $F = 2.4$, $P = 0.04$, $df = 5,18$; for 8–22 days residue ANOVA, $F = 1.0$, $P = 0.46$, $df = 5,54$

[c] Adults exposed to residues 1, 3, 8, 15 and 22 days after pesticide sprays. For 1 and 3 days residue ANOVA, $F = 7.0$, $P = < 0.0001$, $df = 9,30$; for 8–22 days residue ANOVA, $F = 4.6$, $P = < 0.0001$, $df = 9,60$

[d] Adults exposed to residues 1, 3, 8, 10, 16 and 22 days after pesticide sprays. For 1 and 3 days residue ANOVA, $F = 11.1$, $P = < 0.0001$, $df = 9,30$; for 8–22 days residue ANOVA, $F = 0.8$, $P = 0.79$, $df = 9,90$

with appreciable rainfall on days 15, 16, and 17. The mean (SE) air temperature during the 22 days experiment was 27.7 (0.2)°C. Among adults exposed to leaves treated with water (controls), over all exposure dates a mean (SE) of 5.6 (3.5)% adults died. Phosmet residues were highly toxic to adults for at least three days but were thereafter relatively non-toxic (Table 4, Trial 4). Residues of the other pesticides were slightly toxic to nontoxic during the first three days and basically nontoxic by eight days after application.

Discussion

An average of 24.9% of the adult *T. radiata* subjected to these toxicity experiments were males. This percentage of males was more similar to percentages reported by Chien (1995) for field populations

(23.8%) than percentages reported by Skelley and Hoy (2004) for laboratory colonies (33.3–35.7%). Whether differences exist between the sexes with respect to susceptibility to the toxins studied was not addressed but could occur. For example, males are smaller in size than females and thus might be more sensitive to some rates of a pesticide than females.

The residual toxicity of pesticide residues to adult *T. radiata* over a 3-week period after application was evaluated for each pesticide twice, and for some pesticides there were differences between these two evaluations with respect to how toxic the residues were and how long they remained toxic. We attributed these differences to rainfall and possibly temperature. For example, 24 h residues of imidacloprid or sulfur were slightly toxic or nontoxic in a study during which considerable rainfall occurred (190 mm total rain) and air temperatures were warm (27.7°C), but residues of these pesticides were high in toxicity in a study during which little rainfall occurred (16 mm total rain) and air temperatures were cooler (22.0°C).

The research results indicated that, among the pesticides and rates studied, the following were least IPM-compatible with adult *T. radiata* based on the toxicity of direct sprays and potential long residual life on leaves: carbaryl, chlorpyrifos, and fenpropathrin. Although highly toxic to the parasitoid as direct sprays or freshly dried residues, each of the following pesticides may be somewhat more compatible with *T. radiata* because the toxicity of residues of these pesticides was either low 1–3 days after application or sharply decreased thereafter: abamectin, chenopodium oil, fenpyroximate, and spirotetramat. Depending on environmental conditions, imidacloprid, sulfur, phosmet, pyridaben, and 435 spray oil might also be somewhat more compatible for the same reasons. A direct spray of spirotetramat was highly toxic to adult *T. radiata* (this pesticide might have been less toxic as a direct spray if we had studied the lowest label rate), but the material appeared to be more compatible than some of the other pesticides because fresh dry residues of the high rate were only moderately toxic and field residues were either moderate or low in toxicity 1–3 days after application. The pesticides that consistently appeared to be most IPM-compatible with *T. radiata* at the rates tested were aluminum tris, copper hydroxide, diflubenzuron, and kaolin clay (all either nontoxic or short lived).

Kaolin clay (Surround WP) was nearly nontoxic to adults in all of the experiments. In retrospect, the rate we studied was low and higher rates might have been toxic. Also, kaolin clay might have a negative influence on parasitism rates by *T. radiata* as deposits of this pesticide on leaves have been shown to negatively affect insect movement and activity (Hall et al. 2007). Dust particles are well-known to be disruptive of some natural enemies (DeBach 1979) and may inflate problems with some pest species that are regulated by natural enemies (Knight et al. 2000).

Some of the chemicals we studied had previously been tested for toxicity to adult *T. radiata*. Direct sprays of abamectin and fresh dry residues of imidacloprid were previously reported to be highly toxic to female *T. radiata* (Cocco and Hoy 2008). Copper hydroxide was previously reported to be relatively nontoxic to *T. radiata* (Cocco and Hoy 2008). In contrast to the results of our study, Cocco and Hoy (2008) reported that 435 petroleum oil at 2% (v/v) was relatively nontoxic to *T. radiata* as either direct sprays or fresh residues. Our rate (equivalent to 3%) was higher and might explain the toxicity difference. Although our study indicated direct sprays and fresh dry residues of 435 spray oil were highly toxic, older oil residues varied in toxicity depending on rainfall and air temperature but were consistently nontoxic by eight days after application in the two studies presented here.

Chenopodium oil, an extract from the plant *Chenopodium ambrosioides* near *ambrosioides* (Kiuchi et al. 2002), was highly toxic to adult *T. radiata* as a direct spray, but field residues of this pesticide were basically nontoxic. Chiasson et al. (2004) suggested that this botanical pesticide would be effective for greenhouse infestations of whiteflies, thrips, and aphids with negligible effect on biological control agents based on research with the whitefly parasitoid *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae).

Adult *T. radiata* sometimes exhibited signs of poisoning (incapable of standing, lying on their side with one or more body parts twitching) within 24 h after exposure to a toxin. In contrast, adults exposed to sulfur simply became lethargic with their antenna pointing downward. Regardless of the particular signs of poisoning, in our investigations parasitoids that appeared affected by a toxin did not recover and were dead by 48–72 h later.

Not presented in this report were comparisons conducted on percent mortality of adult *T. radiata* on individual sample dates during the four trials on toxicity of pesticide residues over time. This was because, in some trials, large but insignificant differences in percentage mortality were observed among some treatments on some sample dates. In these cases, variability in percentage mortality of adults was attributed to differences among individual leaves with respect to their exposure to rain, sunlight and temperature and, consequently, the amount of a toxin remaining on the leaves. Significant differences likely would have been found using a sample size larger than three leaves per replication per treatment. The repeated measurements ANOVAs compensated for data variability to some extent, facilitating an assessment of the average residual toxicity of each pesticide. Future investigations into the residual toxicity of pesticides to adult *T. radiata* should benefit from using sample sizes larger than studied here and could take into account the probability that some toxins may be degraded or washed off at different rates depending on leaf location and environmental conditions. Of interest would be comparisons of the residual toxicity of pesticides on interior versus exterior canopy leaves. As an alternative to working with leaves exposed to weather, Ulmer et al. (2006) assessed residual toxicity of a number of pesticides to the eulophid *Aprostocetus vacquitarum* Wolcott by treating filter paper strips with various treatments and allowing the strips to weather outdoors without exposure to sunlight or precipitation. This assay approach provided information on maximum potential residual activity.

Acknowledgements The authors thank Matt Hentz, Kathy Moulton, and Anthea Diamondis (USDA-ARS) for their invaluable assistance during this research. Daniel Flores (USDA-APHIS) critically reviewed an early draft of the manuscript. This research was funded in-part by a grant from the Florida Citrus Production Research Advisory Committee. This article reports the results of research only. Mention of a trademark or proprietary product is solely for the purpose of providing specific information and does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

References

Abbott W (1925) A method for computing the effectiveness of an insecticide. J Econ Entomol 18:265–267

Barr NB, Hall DG, Weathersbee AA, Nguyen R, Stansly P, Qureshi JA, Flores D (2009) Comparison of laboratory colonies and field populations of *Tamarixia radiata*, an ecto-parasitoid of the Asian citrus psyllid, using international transcribed spacer and cytochrome oxidase subunit 1 DNA sequences. J Econ Entomol 102:2325–2332

Bové JM (2006) Huanglongbing: a destructive, newly-emerging, century-old disease of citrus. J Plant Pathol 88:7–37

Chiasson H, Vincent C, Bostanian NJ (2004) Insecticidal properties of a *Chenopodium*-based botanical. J Econ Entomol 97:1378–1383

Chien CC (1995) The role of parasitoids in the pest management of citrus psyllid. In: Proceedings of the Symposium on Research and Development of Citrus in Taiwan, Taichung, Taiwan, pp 245–261

Chien CC, Chu YI (1996) Biological control of citrus psyllid, *Diaphorina citri* in Taiwan. Biological Pest Control in Systems of Integrated Pest Management: 1996. Reprinted from Food and Fertilizer Technology Center Book Series No. 47, Taipei, Taiwan, pp 93–104

Cocco A, Hoy MA (2008) Toxicity of organosilicone adjuvants and selected pesticides to the Asian citrus psyllid (Hemiptera: Psyllidae) and its parasitoid *Tamarixia radiata* (Hymenoptera: Eulophidae). Fla Entomol 91:610–620

DeBach P (1979) Biological control by natural enemies. Cambridge University Press, New York, p 323

Étienne J, Quilici S, Marival D, Franck A (2001) Biological control of *Diaphorina citri* (Hemiptera: Psyllidae) in Guadeloupe by imported *Tamarixia radiata* (Hymenoptera: Eulophidae). Fruits 56:307–315

Halbert SE, Manjunath KL (2004) Asian citrus psyllids (Sternorrhyncha: Psyllidae) and greening disease of citrus: a literature review and assessment of risk in Florida. Fla Entomol 87:330–353

Hall DG, Lapointe SL, Wenninger EJ (2007) Effects of a particle film on biology and behavior of *Diaphorina citri* (Hemiptera: Psyllidae) and its infestations in citrus. J Econ Entomol 100:847–854

Hall DG, Hentz MG, Adair RC (2008) Population ecology and phenology of *Diaphorina citri* in two Florida citrus groves. Environ Entomol 37:914–924

Hoy MA, Nguyen R (2000) Classical biological control of Asian citrus psylla. Citrus Ind 81(12):48–50

Hoy MA, Nguyen R, Jeyaprakash A (1999) Classical biological control of the Asian citrus psylla-release of *Tamarixia radiata*. Citrus Ind 80(9):20–22

Kiuchi F, Itano Y, Uchiyama N, Honda G, Tsubouchi A, Nakajima-Shimada J, Aoki T (2002) Monoterpene hycroperoxides with trypanocidal activity from *Chenopodium ambrosioides*. J Nat Prod 65:509–512

Knight AL, Unruh TR, Christianson BA, Puterka GJ, Glenn DM (2000) Effects of a kaolin-based particle film on obliquebanded leafroller (Lepidoptera: Tortricidae). J Econ Entomol 93:744–749

Michaud JP (2004) Natural mortality of Asian citrus psyllid (Homoptera: Psyllidae) in central Florida. Biol Control 29:260–269

Pluke RWH, Qureshi JA, Stansly PA (2008) Citrus flushing patterns, *Diaphorina citri* (Homoptera: Psyllidae) populations and parasitism by *Tamarixia radiata* (Hymenoptera: Eulophidae) in Puerto Rico. Fla Entomol 91:36–42

Qureshi JA, Rogers ME, Hall DG, Stansly PA (2009) Incidence of invasive Asian citrus psyllid, *Diaphorina citri*

(Hemiptera: Psyllidae) and its introduced parasitoid *Tamarixia radiata* (Hymenoptera: Eulophidae) in Florida citrus. J Econ Entomol 102:247–256

Rogers ME, Timmer LW (2007) Florida citrus pest management guide. University of Florida Cooperative Extension Service, Institute of Food and Agricultural Services. SP-43. Download at http://edis.ifas.ufl.edu/features/handbooks/CPMG.html

SAS Institute (2008) SAS Procedures Guide, Version 9.1. SAS Institute, Cary, NC, USA

Skelley LH, Hoy MA (2004) A synchronous rearing method for the Asian citrus psyllid and its parasitoids in quarantine. Biol Control 29:14–23

Tsai JH, Liu YH (2000) Biology of *Diaphorina citri* (Homoptera: Psyllidae) on four host plants. J Econ Entomol 93:1721–1725

Tsai JH, Wang JJ, Liu YH (2002) Seasonal abundance of the Asian citrus psyllid, *Diaphorina citri* (Homoptera: Psyllidae) in southern Florida. Fla Entomol 85:446–451

Ulmer BJ, Lapointe SL, Peña JE, Duncan RE (2006) Toxicity of pesticides used in citrus to *Aprostocetus vaquitarum* (Hymenoptera: Eulophidae), an egg parasitoid of *Diaprepes abbreviatus* (Coleoptera: Curculionidae). Fla Entomol 89:10–19