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# Can predatory mites aid in the management of the citrus leprosis mite?

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**Introduction:** The flat mite *Brevipalpus yothersi* is the main vector of citrus leprosis in South and Central America and Mexico, where vector suppression using conventional acaricides is usually the only disease control method, leading to problems with acaricide resistance. This mite is present in Florida, where several viruses known to cause citrus leprosis were recently detected. This research builds preparedness for managing emerging viral diseases and mitigating potential problems associated with acaricide resistance by addressing the compatibility of chemical and biological control strategies against *B. yothersi*. The predatory mite *Amblyseius largoensis* has shown promise as a biological control agent of *B. yothersi*. However, the effects of acaricides used in citrus on this predator are unknown.

**Methods:** This study investigated the impact of different routes of acaricide exposure on *A. largoensis*, including direct contact, pesticide-contaminated prey, residual contact, and combined exposure routes in laboratory settings. Additionally, the efficacy of *A. largoensis* in controlling *B. yothersi*, alone and in combination with acaricide applications, was evaluated under greenhouse conditions.

**Results and discussion:** Commonly used acaricides like abamectin and spiroticlofen were highly effective in controlling *B. yothersi* and only slightly harmful to *A. largoensis*. Other acaricides were also effective against *B. yothersi* but were less compatible with the predatory mite. In the greenhouse trial, all acaricides, alone or in combination with the predators, and the predators alone, effectively suppressed *B. yothersi* one month after treatment application. However, *B. yothersi* populations significantly increased one month later in the abamectin-alone and predator-alone treatments. At the same time, the combination of abamectin and predators provided *B. yothersi* suppression throughout the experiment. The fenpyroximate-predator combination also provided better control than each treatment applied independently. All other acaricides provided prolonged *B. yothersi* control, hindering biological control

probably due to the lack of prey for *A. largoensis*. Research findings highlight the potential for enhancing the selectivity of acaricides towards *A. largoensis* by manipulating their exposure route. Combining predatory mites with acaricides shows promise in improving *B. yothersi* management.

#### KEYWORDS

acaricide selectivity, *Amblyseius largoensis*, biological control, *Brevipalpus yothersi*, exposure, integrated pest management

## 1 Introduction

*Brevipalpus*-transmitted diseases (BTDs) are caused by single-stranded RNA viruses belonging to the families *Kitaviridae* (genera *Cilevirus* and *Higrevirus*) and *Rhabdoviridae* (genus *Dichorhavirus*), transmitted exclusively by flat mites in the genus *Brevipalpus* (Locali-Fabris et al., 2006; Dietzgen et al., 2018; Quito-Avila et al., 2021). Recently, three of seven viruses known to cause citrus leprosis (CiL), one of the most damaging BTDs, were found infecting multiple ornamental plants in Florida, different species of orchids in California, and hibiscus, passionfruit, and citrus in Hawaii (Melzer et al., 2012; Roy et al., 2013; Roy et al., 2018a; Fife et al., 2021; Olmedo-Velarde et al., 2021a; Olmedo-Velarde et al., 2021b; Alvarez-Quinto et al., 2022; Dey et al., 2022). The flat mite, *Brevipalpus yothersi* Baker (Acari: Tenuipalpidae), is an important pest of citrus in South and Central America and Mexico (Kitajima et al., 2003; Rodrigues et al., 2003; Castillo et al., 2011; Roy et al., 2015; Ramos-González et al., 2018) due to its ability to transmit Citrus leprosis virus C (CiLV-C, *Cilevirus leprosis*) and citrus leprosis virus C2 (CiLV-C2, *Cilevirus colombiense*) which cause severe yield losses and a reduction in the lifespan of trees, especially in the sweet orange (*Citrus sinensis* L. Osbeck) (Bastianel et al., 2010; Moreira et al., 2022; Ramos-González et al., 2023). A strain of CiLV-C2 was recently detected in the US associated with *B. yothersi*, where proactive integrated pest management programs (IPM) are being developed to mitigate viral diseases transmitted by *Brevipalpus* mites (Peña et al., 2015; Revynthi et al., 2019). This research builds preparedness for managing BTDs by addressing the compatibility of chemical and biological control strategies against *B. yothersi*.

Acaricide sprays to suppress disease vectors are the main strategy for managing citrus leprosis in South and Central America (Amorim et al., 2006; Andrade et al., 2010; Silva et al., 2012; Della Vechia and Andrade, 2022). In fact, about 10% of the acaricide market worldwide is used to manage *Brevipalpus* spp. (Van Leeuwen et al., 2015). Given the importance of controlling *B. yothersi*, the high costs associated with acaricide applications (Bassanezi et al., 2019), and problems associated with acaricide resistance (Alves et al., 2000; Campos and Omoto, 2002; Rocha et al., 2021), an integrated approach combining chemical and biological control methods may be critical to managing these mites effectively (Bielza, 2016; Bielza et al., 2020; Leung et al., 2020). Compatibility

between biological and chemical control methods is usually overlooked but critical to the success of integrated pest management (Duso et al., 2020). Several active ingredients are used commercially to target *Brevipalpus* mites in citrus, including abamectin, bifenazate, cyflumetofen, etoxazole, fenpyroximate, hexythiazox, pyridaben, spirodiclofen, spirotetramat, and sulfur (Della Vechia et al., 2022). Some of these compounds are relatively selective to Phytoseiidae mites, killing the target pest without killing the predatory mites (Cote et al., 2022; Assis et al., 2013; Doker and Kazak, 2019b; Bajda et al., 2022; Barros et al., 2022).

*Amblyseius largoensis* (Muma) (Acari: Phytoseiidae) is a cosmopolitan and generalist predatory mite that inhabits citrus orchards and other fruit crops around the world (Moraes et al., 2004). This predator has been studied for its potential to control tenuipalpid, eriophyoid, and tarsonemid mites (Galvão et al., 2007; Rodríguez et al., 2011; Carrillo et al., 2012a; Carrillo et al., 2012b; Carrillo et al., 2014; Melo et al., 2015; Rodríguez et al., 2015) and has been reported as an efficient predator of *Brevipalpus* spp. (Haramoto, 1969; Kamburov, 1971; Galvão et al., 2008; Argolo et al., 2020).

A recent study suggests that *A. largoensis* could be an effective biocontrol agent of *B. yothersi* in Florida (Argolo et al., 2020), where this natural enemy has been the most abundant predator in backyard and varietal citrus in multiple surveys conducted in the last decades (Childers et al., 2022). This predator is also dominant in other agricultural systems in Florida (Carrillo et al., 2010; Carrillo et al., 2012), suggesting it must have been subject to continuous exposure to pesticides used in the region. Predators can be exposed to pesticides in different ways that can be more or less harmful (Pozzebon et al., 2011; Bajda et al., 2022). Routes of pesticide exposure include direct contact during spraying, contact with contaminated surfaces during foraging, or feeding on contaminated prey (Longley and Stark, 1996). Understanding the effects of different routes of exposure of *A. largoensis* to acaricides used to manage *B. yothersi* may be critical for its successful integration into biocontrol augmentation or conservation programs in the context of IPM.

We hypothesized that *A. largoensis* may be partially compatible with chemical controls available for *B. yothersi*. Therefore, the impact of multiple acaricides on *A. largoensis* administered via different exposure routes (direct contact, pesticide-laced diet (prey), residual contact, and a combination of all exposure routes) was

examined under laboratory conditions. In addition, the ability of *A. largoensis* to control *B. yothersi* alone and in combination with acaricide applications was assessed under greenhouse conditions.

## 2 Materials and methods

### 2.1 *Brevipalpus yothersi* colony

A pesticide susceptible strain of *B. yothersi* obtained from citrus plants has been maintained in the Tropical Fruit Entomology laboratory (UF-TREC) for more than five years without pesticide exposure. The mites were reared on common bean seedlings (*Phaseolus vulgaris* L.) grown on floral foam, following the methods described by Groot and Breeuwer (2006). The colony was kept in environmental chambers (Panasonic MLR-352H-PA, PHC Corporation of North America, Wood Dale, IL) at  $27 \pm 1^\circ\text{C}$ ,  $70 \pm 5\%$  RH, and 12:12 h (L:D).

### 2.2 Predatory mite colony

A colony of *A. largoensis* was established with individuals collected from coconut fronds in the spring of 2018. Standard methods for rearing phytoseiid mites, described by Overmeer (1985), were used to rear *A. largoensis*. *Typha* spp. pollen (Nutrimite™, Biobest), *Tetranychus urticae* Koch (Acari: Tetranychidae), and *B. yothersi* mites were provided thrice weekly

as food for the predatory mites. The colony was kept in an environmental chamber under controlled conditions of  $25 \pm 1^\circ\text{C}$ ,  $80 \pm 5\%$  RH, and 12:12 h (L:D).

### 2.3 Laboratory assays

#### 2.3.1 Screening test

##### 2.3.1.1 Screening adulticide and ovicide treatments against *Brevipalpus yothersi*

Two separate trials were conducted to evaluate ovicides and adulticides on *B. yothersi* using the bioassays described in Rodrigues et al. (2007). The experimental units were prepared using lime fruit collected from unsprayed trees at UF-TREC. Fruits were submerged in paraffin, leaving a 5.0 cm diameter area without paraffin, representing the experimental arena. The arenas were also “ringed” with entomological glue (Tree Tanglefoot Insect Barrier®, The Tanglefoot Company) to prevent mites from escaping.

For the ovicide assay, 15 *B. yothersi* adult females (2-3 days old) were transferred to each arena and allowed to oviposit. After 48 h, females were removed, and the number of eggs was adjusted to 15 per arena. Then, the part of the fruit with the experimental arena was submerged in the pesticide (ovicide) solutions described in (Table 1) or in water (control) for two seconds. Nine replicates (fruit) were used for each treatment in the ovicide assay. Egg mortality was determined by counting the number of larvae that

TABLE 1 Ovicide and adulticide treatments used against *Brevipalpus yothersi*.

Treatment	Commercial name	Rate (L ha <sup>-1</sup> )	Rate (g a.i. L <sup>-1</sup> ) <sup>1</sup>	IRAC <sup>2</sup>
Egg assay				
Water	-	-	-	-
Etoxazole	Zeal	2.5	4.5	10B
Hexythiazox	Ax-hexythiazox	1.8	1.5	10A
Spirodiclofen	Envidor	1.5	0.7	23
Spirotetramat	Movento	0.7	0.3	23
Adult assay				
Water	-	-	-	-
Abamectin	Reaper	0.1	0.9	6
Bifenazate	Acramite	0.1	0.2	25
Cyflumetofen	Nealta	1.0	0.5	25
Fenpyroximate	Portal	2.3	5.2	21A
Pyridaben	Nexter	1.2	0.3	21A
Sulfur	Microthiol Dispers	1.5	3.0	UN
Tolfenpyrad	Apta	2.0	1.3	21A
Spirodiclofen	Envidor	1.5	0.7	23
Spirotetramat	Movento	0.7	0.3	23

<sup>1</sup> g a.i.L<sup>-1</sup> – grams of active ingredient per liter of water. <sup>2</sup>IRAC - Insecticide Resistance Action Committee.

emerged daily for ten consecutive days after treatment application. Eggs that did not hatch were considered dead.

New fruits were used to prepare additional experimental arenas with ten newly emerged adult *B. yothersi* females for the adulticide trial. The infested arenas were dipped in the pesticide solutions described in Table 1 for two seconds, using water as a control. The number of live and dead adults was recorded daily for seven consecutive days after treatment application. Twelve replicates were used for each treatment in the adulticide trial.

### 2.3.1.2 Mortality of *Amblyseius largoensis* exposed to acaricides via direct contact

The experimental arenas consisted of 2.5 cm diameter x 1.5 cm height plastic cages with an opening in the lid covered with mite-proof mesh (mesh opening: 0.0385 mm) to allow ventilation. A thin layer of 1% agarose (Fisher Scientific) was placed on the bottom and a bean leaf disk (~ 2.5 cm diam.) was placed on the agar. One *A. largoensis* adult female (3-4 days old) was released onto the leaf disk and treatments were sprayed directly onto the mites on the arenas before placement of the lids. Acaricide solutions were applied with a manual pressurized sprayer (Porter Cable Air Compressor), delivering 0.8 mL of spray solution per leaf disk at the rates included in Table 1; water was used as a control treatment. A total of 15 replicates were used for each treatment. Predatory mite mortality was recorded daily for seven consecutive days after treatment. Every two days, mites were offered *B. yothersi* (eggs, immatures, and adults) and *Typha* spp. pollen *ad libitum* as food.

Pesticide compatibility with *A. largoensis* was based on predator mortality on the seventh day after application according to the IOBC (International Organization for Biological and Integrated Control) toxicity categories i.e., class 1 =  $E < 25\%$  (innocuous); class 2 =  $25\% < E < 50\%$  (slightly harmful); class 3 =  $51\% < E < 75\%$  (moderately harmful) e class 4 =  $E > 75\%$  (harmful) (Hassan et al., 1994).

### 2.3.2 Effect of acaricide exposure route on predator survival

Abamectin, cyflumetofen, fenpyroximate, and spiroadiclofen are among the most used acaricides worldwide (Van Leeuwen et al., 2015). These acaricides provided effective *B. yothersi* adult control and were either innocuous (Cyflumetofen) or slightly harmful to *A. largoensis* (abamectin, fenpyroximate, and spiroadiclofen) in the screening tests (Sect 2.3.1). Therefore, these acaricides were selected to investigate *A. largoensis*'s survival after exposure to them via four delivery routes: (i) residual contact, (ii) direct contact, (iii) contaminated prey, and (iv) a combination of residual contact, direct contact, and contaminated prey. Acaricide solutions were applied at the rates in Table 1 and using the same experimental arenas (plastic cages) and spray volumes as in the previous assay.

In the residual contact treatment (i), experimental arenas were constructed with bean leaf disks sprayed with individual acaricides and allowed to dry for 30 minutes before one *A. largoensis* female (3-4 days old) was released per arena. In addition, 50 *B. yothersi*

eggs (less than 5 days old) were transferred from the stock colony to each arena as a food source for the predators, which were replenished every other day. For direct contact (ii), individual predatory mite females were placed on untreated leaf disks and then sprayed with an acaricide. Treated predators were also offered 50 *B. yothersi* eggs from the colony, which were replenished every other day. For the acaricide-contaminated prey treatment (iii), *B. yothersi* infested bean leaves from the colony were sprayed with acaricides and allowed to dry for 30 minutes before transferring groups of 50 treated eggs (less than 5 days old) to untreated arenas. Then, one *A. largoensis* female was released per arena. Treated *B. yothersi* eggs offered as food source were replenished every other day. The combined effect of direct contact, residual contact, and acaricide-laced prey (iv) was investigated by first releasing *A. largoensis* on bean leaf disks arenas with 50 *B. yothersi* eggs, and then spraying the leaf disks containing predators and prey. The control treatment consisted of an arena with one *A. largoensis* female and 50 *B. yothersi* eggs (replenished as before), which were treated with water on the bean leaf disk. The experiment was kept in a climate-controlled chamber at  $25 \pm 1^\circ\text{C}$ ,  $80 \pm 5\%$  relative humidity (RH), and a 12:12 h (L:D) photoperiod. Predator survival was recorded daily for five days. Predatory mite eggs were counted and removed from the leaf disk daily. Four assays were performed, each with one acaricide. Each experiment comprised five exposure routes (i, ii, iii, iv, and control) and 20 replicates, totaling 100 predatory mites per acaricide.

## 2.4 Greenhouse assay

The experiment was conducted in a glasshouse with controlled temperature and relative humidity ( $25 \pm 4^\circ\text{C}$  and  $75 \pm 15\%$  RH) at UF-TREC from March to July 2022. Sixty orange plants (Valencia variety, seeded 1 year before use, approximately 30 cm tall, unsprayed, and pest-free) were used. Each plant was artificially infested with one orange leaf containing 75 *B. yothersi* adults taken from the colony. The infested leaf was attached to the abaxial surface of a leaf in the center of the experimental plant using a paper clip. Infested plants were then placed in individual cages (62.0 cm x 23.5 cm, 160  $\mu\text{m}$  mesh size) and held for 45 days before being randomly assigned to one of the following treatments: 1) Control (water), 2) Water + predatory mite (*A. largoensis*), 3) Abamectin ( $0.9 \text{ g a.i.L}^{-1}$ ), 4) Abamectin + predatory mite, 5) Cyflumetofen ( $0.5 \text{ g a.i.L}^{-1}$ ), 6) Cyflumetofen + predatory mite, 7) Fenpyroximate ( $5.2 \text{ g a.i.L}^{-1}$ ), 8) Fenpyroximate + predatory mite, 9) Spiroadiclofen ( $0.7 \text{ g a.i.L}^{-1}$ ), 10) Spiroadiclofen + predatory mite. For the treatments receiving predatory mites, five adult *A. largoensis* females were transferred from the stock colony into Eppendorf tubes (1.5 mL) that were hung to the experimental plants using a hair clip. This predator-prey ratio effectively suppressed pestiferous tenuipalpid mites in previous research with *A. largoensis* (Carillo et al., 2014; Argolo et al., 2020). The tubes were then opened, and the predators were allowed to colonize the plant freely. The acaricide applications were made 15 days after the predators were released. Each treatment was replicated on six plants.

*Brevipalpus yothersi* populations were evaluated four times: 1) before the pesticide application and predatory mite release, 2) 30 days after treatment (acaricide) application (DAT), 3) 62 DAT, and 4) 104 DAT. In the first evaluation, two leaves were randomly removed from each plant, placed in a paper bag, and immediately inspected under a stereomicroscope in the laboratory to record the number of *B. yothersi* adults, immatures, and eggs. Four and six leaves were collected for the second and third evaluations. The last evaluation comprised the entire plant, which was destructively sampled to record all *B. yothersi* individuals on the plant.

## 2.5 Statistical analysis

Abbott's transformation was used to correct control mortality (Abbott, 1925) in the ovicide and adulticide assays, which was less than 10%. Egg and adult mortality after acaricide applications in the screening test was analyzed using generalized logistic regression techniques implemented in the JMP Fit Model Platform (JMP Statistical Discovery LLC, Cary, NC) using a binomial data distribution. Treatment least squares means were compared using simple t-tests at  $\alpha = 0.05$  based on the recommendations by Milliken and Johnson (2009) and Saville (2015). Least squares means and confidence limits were back-transformed to the data scale.

Survival data in the lab study were analyzed using generalized linear model procedures as implemented in SAS<sup>®</sup> PROC GLIMMIX (SAS/STAT 15.2; SAS Institute, Cary, NC) using a binomial distribution function and the canonical logit link. Because the control treatment (water) was the same for all acaricides tested, we analyzed it separately to obtain the best estimate for this treatment across all acaricides. The remaining treatments were analyzed using the model  $\text{Survived/Total} = \text{Acaricides} + \text{Treatment} + \text{Acaricides} * \text{Treatment}$ . Interaction least squares means plus 95% confidence limits were calculated and treatments were compared within acaricide using a simple t-test (LSD) based on the recommendations by Milliken and Johnson (2009) and Saville (2015). Least squares means and confidence limits were back transformed to the data scale.

For the greenhouse experiment, count data were analyzed using generalized linear model methodology procedures as implemented in SAS<sup>®</sup> PROC GLIMMIX (SAS/STAT 15.2; SAS Institute, Cary, NC) with a negative binomial distribution function and the canonical log link function. This factorial experiment consisted of factors treatment (5 levels), predator (2 levels) and DAA (4 levels); the generalized linear model contained all three main effects, all possible two-way interaction effects, and the three-way interaction effect. Least squares means comparisons followed the same approach as described earlier.

## 3 Results

### 3.1 Screening adulticide and ovicide treatments against *Brevipalpus yothersi*

The effects of ovicides and adulticides on *B. yothersi* varied among the acaricides tested (Tables 2, 3). Etoxazole and spirodiclofen had the highest acute ovicidal activity, causing 100% egg mortality six days after application (DAA). Hexythiazox was also highly effective, reaching 99% mortality 10 DAA. Spirotetramat was the least effective ovicide, reaching approximately 70% egg mortality at the end of the experiment.

Cyflumetofen, abamectin, fenpyroxymate, pyridaben, and sulfur showed high acute activity, causing 100% *B. yothersi* adult mortality 1-2 days after application. Spirotetramat, spirodiclofen, and tolfenpyrad had a slower activity, reaching 100% mortality 4-7 days after application. Bifenazate had the lowest activity on *B. yothersi*, causing significantly less mortality (~80% at the end of the experiment) than other acaricides tested on every evaluation date.

### 3.2 Mortality of *Amblyseius largoensis* exposed to conventional acaricides via direct contact

*Amblyseius largoensis* survival decreased slightly after direct contact with the tested acaricides relative to the water control

TABLE 2 *Brevipalpus yothersi* egg mortality after acaricide exposure.

Treatment	Rate g a.i.L <sup>-1</sup>	Days after application		
		6 DAA	8 DAA	10 DAA
Etoxazole	4.5	1.00 [1.000,1.000] a	1.00 [1.000,1.000] a	1.00 [1.000,1.000] a
Hexythiazox	1.5	0.98 [0.937,0.996] b	0.98 [0.937,0.996] b	0.99 [0.957,0.997] b
Spirodiclofen	0.7	1.00 [1.000,1.000] a	1.00 [1.000,1.000] a	1.00 [0.975,0.999] ab
Spirotetramat	0.3	0.69 [0.451,0.863] c	0.69 [0.451,0.863] c	0.68 [0.426,0.859] c
Probability > $\chi^2$		< 0.0001	< 0.0001	< 0.0001
Wald $\chi^2$		388.1	280.1	302.8
Denominator degree of freedom		3	3	3

Least squares means [95% confidence limits in brackets] within each date, followed by the same letter, are not statistically meaningfully different at  $\alpha = 0.05$ . Data were analyzed using generalized logistic regression techniques implemented in the JMP Fit Model Platform (JMP Statistical Discovery LLC, Cary, NC).



TABLE 3 *Brevipalpus yothersi* adult mortality after acaricide exposure.

Treatment	Rate g a.i.L <sup>-1</sup>	Days after application					
		1 DAA	2 DAA	3 DAA	4 DAA	6 DAA	7 DAA
Abamectin	0.9	1.00 [1.000,1.000] a	0.99 [0.980,0.994] a	0.98 [0.950,0.989] a	1.00 [1.000,1.000] a	0.99 [0.988,0.998] a	1.00 [1.000,1.000] a
Bifenazate	0.2	0.18 [0.116,0.267] d	0.39 [0.267,0.535] c	0.52 [0.369,0.673] c	0.62 [0.478,0.748] c	0.77 [0.619,0.874] b	0.80 [0.660,0.892] b
Cyflumetofen	0.5	1.00 [1.000,1.000] a	0.99 [0.980,0.994] a	0.98 [0.950,0.989] a	1.00 [1.000,1.000] a	0.99 [0.988,0.998] a	1.00 [1.000,1.000] a
Fenpyroximate	5.2	1.00 [1.000,1.000] a	0.99 [0.980,0.994] a	0.98 [0.950,0.989] a	1.00 [1.000,1.000] a	0.99 [0.988,0.998] a	1.00 [1.000,1.000] a
Pyridaben	0.3	1.00 [1.000,1.000] a	0.99 [0.980,0.994] a	0.98 [0.950,0.989] a	1.00 [1.000,1.000] a	0.99 [0.988,0.998] a	1.00 [1.000,1.000] a
Spirotetramat	0.3	0.48 [0.392,0.570] c	0.66 [0.550,0.761] b	0.76 [0.648,0.842] b	0.92 [0.830,0.964] b	0.99 [0.988,0.998] a	1.00 [1.000,1.000] a
Spirodiclofen	0.7	0.52 [0.346,0.693] c	0.80 [0.694,0.873] b	0.88 [0.723,0.950] b	0.95 [0.875,0.978] b	0.99 [0.988,0.998] a	1.00 [1.000,1.000] a
Sulfur	3.0	0.98 [0.932,0.992] b	0.99 [0.980,0.994] a	0.98 [0.950,0.989] a	1.00 [1.000,1.000] a	0.99 [0.988,0.998] a	1.00 [1.000,1.000] a
Tolfenpyrad	1.3	0.56 [0.382,0.727] c	0.80 [0.694,0.873] b	0.98 [0.950,0.989] a	1.00 [1.000,1.000] a	0.99 [0.988,0.998] a	1.00 [1.000,1.000] a
Probability > $\chi^2$		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Wald $\chi^2$		2770.2	392.4	54.5	1480.9	53.9	776.1
Denominator degree of freedom		8	8	8	8	8	8

Least squares means [95% confidence limits in brackets] within each date, followed by the same letter, are not statistically meaningful different at  $\alpha = 0.05$ . Data were analyzed using generalized logistic regression techniques implemented in the JMP Fit Model Platform (JMP Statistical Discovery LLC, Cary, NC).

(Figure 1). Abamectin, cyflumetofen, etoxazole, spiroadiclofen, and sulfur did not differ significantly from the control (water). Fenpyroximate and pyridaben were also slightly harmful to *A. largoensis* but caused significantly more mortality than the

control. Tolfenpyrad and hexythiazox were the most harmful acaricides, causing more than 50% of *A. largoensis* mortality. According to the IOBC toxicity categories, cyflumetofen (21.4% mortality seven days after application) can be considered innocuous

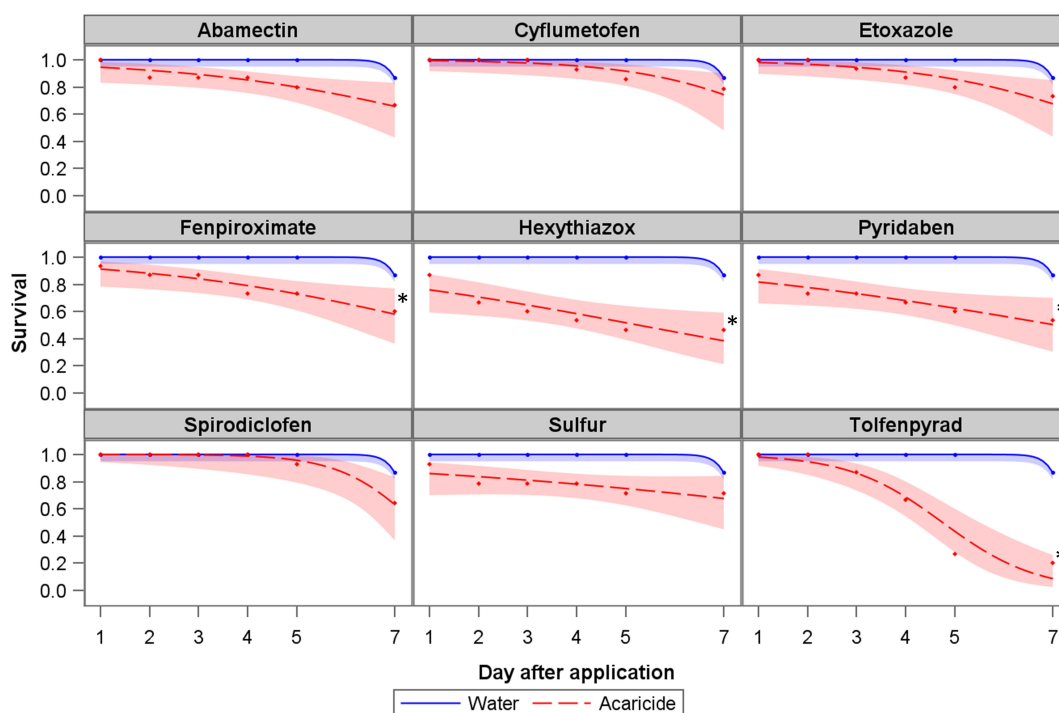


FIGURE 1 Survival of *Amblyseius largoensis* exposed to acaricides spiroadiclofen (0.7 g a.i.L<sup>-1</sup>), cyflumetofen (0.5 g a.i.L<sup>-1</sup>), abamectin (0.9 g a.i.L<sup>-1</sup>), tolfenpyrad (1.3 g a.i.L<sup>-1</sup>), fenpyroximate (5.2 g a.i.L<sup>-1</sup>), pyridaben (0.3 g a.i.L<sup>-1</sup>), sulfur (3.0 g a.i.L<sup>-1</sup>), etoxazole (4.5 g a.i.L<sup>-1</sup>), and hexythiazox (1.5 g a.i.L<sup>-1</sup>). The shaded areas represent the 95% prediction interval. The asterisk (\*) represents a statistically meaningful difference between water and acaricide treatment ( $\alpha = 0.05$ ).

(class 1) to *A. largoensis*, while abamectin (33.3%), etoxazole (26.7%), fenpyroximate (40.0%), pyridaben (46.7%), spiroadiclofen (35.7%) and sulfur (28.6%) can be considered class 2, slightly harmful acaricides. Hexythiazox (53.3%) and tolfenpyrad (80.0%) can be regarded as class 3 (moderately harmful) and (class 4, harmful), respectively.

### 3.3 Effect of acaricide exposure route on predator survival

All the acaricides, independent of the exposure route, reduced the mite survival rate relative to the water control (Figure 2). *Amblyseius largoensis* survival decreased between 16% and 48% due to abamectin exposure. This pesticide was significantly more harmful via ingestion of contaminated prey and the combination exposure routes (D+R+I) than through direct or residual contact (Figure 2). Predatory mite survival was 45% to 51% after cyflumetofen exposure, and no differences were observed among the different exposure routes (Figure 2). By contrast, the route of exposure to fenpyroximate significantly affected predator survival. The combination of exposure routes was most harmful to *A. largoensis*, causing nearly 100% mortality, followed by ingesting fenpyroximate contaminated prey, causing a 93% reduction in predator survival. Direct contact with fenpyroximate was significantly less harmful than the combination of exposure routes or ingestion of contaminated prey, but residual contact was the least harmful of all exposure routes (Figure 2).

Spiroadiclofen had a low impact on predator survival rates via ingestion exposure and the combination of exposure routes

followed by residual contact. Survival rates of predators subject to the combination of exposures were similar to those exposed via residual contact. Unexpectedly, direct exposure to spiroadiclofen resulted in the lowest survival rate of *A. largoensis*.

### 3.4 Greenhouse assay

Before treatment, all experimental plants had similar *B. yothersi* infestation levels. Thirty days after treatment application, *B. yothersi* populations had increased by approximately 5-fold in the water control treatment (Table 4). By contrast, all acaricide treatments alone or in combination with the predators, and the predators alone, significantly suppressed *B. yothersi*. However, among the acaricide-only treatments, abamectin and cyflumetofen provided a faster knockdown effect than spiroadiclofen and fenpyroximate thirty days after treatment application (Table 4). The predators alone caused a significant reduction in *B. yothersi* populations relative to the water control. However, this reduction was significantly lower than when predators were combined with any of the acaricides, resulting in nearly 100% control.

Sixty-two days after treatment application, *B. yothersi* populations continued to increase in plants subject to the water control treatment. Mite populations on abamectin-alone treated plants also increased at a lower extent than in those treated with water, but significantly more than when other acaricides were applied alone. The predators alone suppressed *B. yothersi* populations relative to the water control, but *B. yothersi* suppression by the predators was significantly low compared to treatments that combined predators and acaricides. The abamectin-

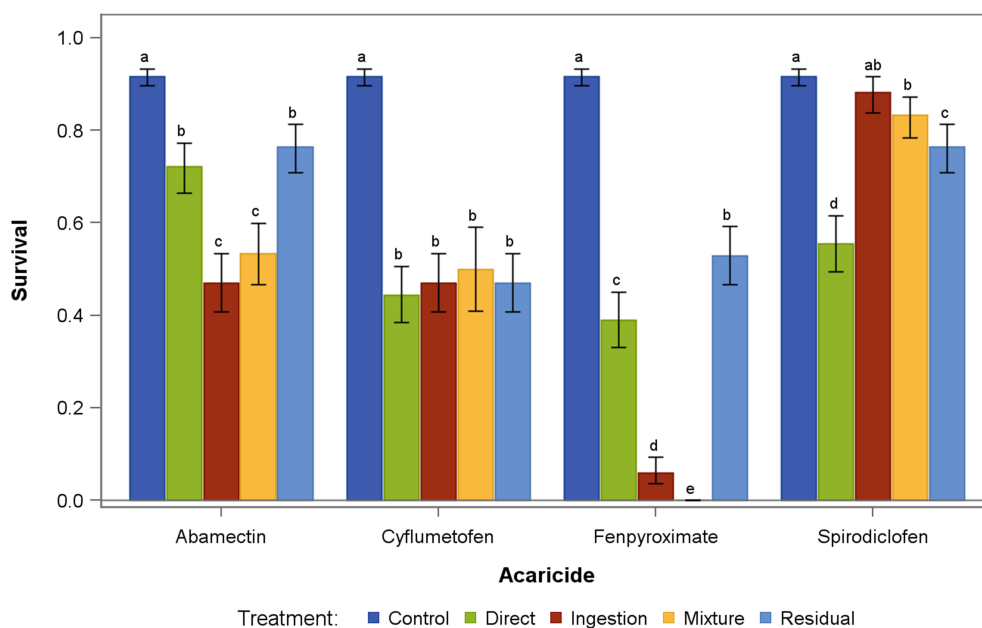


FIGURE 2

Mean survival of *Amblyseius largoensis* five days after exposure to four acaricides via different routes (direct contact, acaricide-contaminated prey (ingestion), a combination of exposure routes(D+R+I), and residual contact). Error bars represent 95% confidence intervals. Within acaricide, bars sharing letters are not statistically meaningful different at  $\alpha = 0.05$  based on simple tests, following the recommendations by Milliken and Johnson (2009) and Saville (2015).

TABLE 4 Influence of acaricides and their synergy with *Amblyseius largoensis* on controlling *Brevipalpus yothersi* infestations in greenhouse citrus plants.

Treatment	Before application		Contrast	30 DAA		Contrast
	No Predator	Predator		No Predator	Predator	
Water	24.8 [9.4,65.6] a	23.7 [8.9,62.6] a	ns	187.2 [71.6,489.0] a	15.0 [5.6,40.0] a	*
Abamectin	13.8 [5.2,37.0] a	11.5 [4.3,30.9] a	ns	0.0 [0.0,0.0] c	0.0 [0.0,0.0] b	ns
Cyflumetofen	18.7 [7.0,49.5] a	13.5 [5.1,36.1] a	ns	0.0 [0.0,0.0] c	0.0 [0.0,0.0] b	ns
Fenpyroximate	17.7 [6.6,47.0] a	13.8 [5.2,37.0] a	ns	0.3 [0.1,1.9] b	0.0 [0.0,0.0] b	ns
Spirodiclofen	17.7 [6.6,46.9] a	31.3 [11.9,82.6] a	ns	0.5 [0.1,2.4] b	0.0 [0.0,0.0] b	ns
	62 DAA			104 DAA		
	No Predator	Predator		No Predator	Predator	
Water	314.5 [120.5,821.1] a	72.8 [27.8,190.8] a	*	273.7 [104.8,714.6] a	353.0 [135.2,921.5] a	ns
Abamectin	58.7 [22.2,155.1] b	0.2 [0.0,1.4] b	*	415.3 [157.8,1093] a	2.0 [0.7,6.1] b	*
Cyflumetofen	0.0 [0.0,0.0] c	0.0 [0.0,0.0] c	ns	0.0 [0.0,0.0] c	0.0 [0.0,0.0] c	ns
Fenpyroximate	0.0 [0.0,0.0] c	0.0 [0.0,0.0] c	ns	29.5 [11.1,78.7] b	1.3 [0.4,4.4] b	*
Spirodiclofen	0.0 [0.0,0.0] c	0.0 [0.0,0.0] c	ns	0.0 [0.0,0.0] c	0.0 [0.0,0.0] c	ns

Means [95% confidence limits in brackets] within a column and date followed by the same letter are not statistically meaningful different ( $\alpha = 0.05$ ). The contrast column presents the results from the contrast comparing predator vs. non-predator within each treatment, where an \* represents a statistically meaningful difference ( $\alpha = 0.05$ ).

predator combination reduced *B. yothersi* populations significantly more than when each treatment was applied separately. When fenpyroximate, cyflumetofen, and spirodiclofen were combined with predators, suppression was almost complete; however, suppression can be attributed to the acaricides because the same levels of *B. yothersi* suppression were recorded when these acaricides were applied alone.

In the last evaluation, 104 days after treatment application, *B. yothersi* populations in the water control and predator-alone treatments were similar. Among the treatments involving acaricides only, abamectin-treated plants had similar *B. yothersi* numbers as the water control, and a slight but significant increase in *B. yothersi* populations was recorded on fenpyroximate-treated plants. By contrast, the complete *B. yothersi* suppression by cyflumetofen and spirodiclofen persisted. In plants treated with abamectin and fenpyroximate, the combination with predators resulted in a significantly greater reduction of *B. yothersi* than the acaricides alone. Treatments that combined cyflumetofen or spirodiclofen with predators provided complete control. However, suppression can be attributed to the acaricides rather than the predators, as in the previous evaluation period.

## 4 Discussion

Our results suggest that chemical control could be a highly effective tactic for managing viruliferous *B. yothersi* in the U.S.A. Eggs of Florida populations of *B. yothersi* were highly susceptible to common mite ovicides used in citrus, such as etoxazole, hexythiazox, spirodiclofen, and spirotetramat. Moreover, abamectin, bifentazate, cyflumetofen, fenpyroximate, pyridaben,

sulfur, spirotetramat, spirodiclofen, and tolfenpyrad showed high efficacy in controlling *B. yothersi* adults. However, overreliance on chemical pesticides may lead to the selection of *B. yothersi* resistant populations, and the integration of nonchemical strategies like biological control could be fundamental to resistance management (Omoto et al., 2000; Campos and Omoto, 2002; Rocha et al., 2021). One acaricide classified as innocuous (cyflumetofen) and three acaricides classified as slightly harmful to *A. largoensis* (abamectin, fenpyroximate, and spirodiclofen) were used to study the effects of different pesticide exposure routes on *A. largoensis* (Hassan et al., 1994). These acaricides are the most commercialized worldwide and have different modes of action (Van Leeuwen et al., 2015). Cyflumetofen showed low to moderate toxicity to *A. largoensis* regardless of the exposure route. The available literature suggests that cyflumetofen has a low impact through direct and residual contact on several phytoseiids including *Neoseiulus fallacis* German, *Neoseiulus californicus* McGregor, *Phytoseiulus persimilis* Athias-Henriot, *Amblyseius eharai* Amitai and Swirski, *Amblyseius tsugawai* Ehara, *Euseius sojaensis* Ehara, and *Typhlodromus vulgaris* Ehara (Lee and Kim, 2015; Kishimoto et al., 2018; Bergeron and Schmidt-Jeffris, 2020).

Fenpyroximate has been reported as selective to populations of *A. largoensis* on coconut (Assis et al., 2013; Barros et al., 2022) and to other phytoseiid mites in other systems (Kim and Paik, 1996; Ghasemzadeh and Qhreshi, 2018). However, we found that this selectivity can vary depending on the exposure route. Direct contact, feeding on fenpyroximate-laced prey, and the combination of exposure routes were highly toxic to *A. largoensis* (61.1% to 100.0% mortality). Surprisingly, residual contact with fenpyroximate was only slightly harmful to *A. largoensis* (47.1%



mortality). Similarly, Sato et al. (2002) recorded a high survival in *N. californicus* released on fenpyroximate-contaminated leaves. On the other hand, direct contact with fenpyroximate resulted in 100% *Galendromus occidentalis* (Nesbitt) mortality (Irigaray and Zalom, 2007). These differences in toxicity among fenpyroximate exposure routes can be related to the amount of pesticide uptake by the predators (Van Leeuwen and Dermauw, 2016). The higher toxicity of fenpyroximate through direct exposure suggests a role of integumental absorption of this pesticide by *A. largoensis*.

Abamectin was only slightly harmful to *A. largoensis* via residual or direct contact. Feeding upon abamectin-contaminated prey was more harmful but still allowed more than 50% survival of the predator populations. Abamectin is regarded as harmful to predatory mites of the family Phytoseiidae (Lima et al., 2013; Fernández et al., 2017; Doker and Kazak, 2019a). However, in Florida abamectin is commonly used in citrus and other tropical and subtropical fruit production systems. Therefore, *A. largoensis* populations may have repeated exposure to this pesticide and likely developed some tolerance/resistance. Our results suggest that *A. largoensis* can survive on plants treated with abamectin or colonize plants recently treated with this pesticide. In addition, *A. largoensis* releases after abamectin applications could help maintain *B. yothersi* populations suppressed longer than when the pesticide is used alone.

Spirodiclofen showed low toxicity to *A. largoensis* independently of the delivery route, corroborating the results obtained by Assis et al. (2013) on predator populations associated with another tenuipalpid mite, *Raoiella indica* (Acari: Tenipalpidae). Other phytoseiid species (i.e., *Amblyseius swirskii* Athias-Henriot and *N. californicus*) also have reported tolerance to spirodiclofen (Kaplan et al., 2012; Doker and Kazak, 2019a). Our results suggest that spirodiclofen is compatible with *A. largoensis* and useful for the integrated management of *B. yothersi* because it effectively controls the pest mite and shows low toxicity to its biocontrol agent. However, direct contact with spirodiclofen resulted in significantly more mortality than ingestion of contaminated prey and the combination of exposure routes, which suggests that spirodiclofen detoxification enzymes or other unknown mechanisms may be present in *A. largoensis*.

In the greenhouse trial, all acaricides, alone or in combination with the predators, and the predators alone, effectively suppressed *B. yothersi* one month after treatment application. Abamectin alone showed the shortest control period among the acaricides (approximately 30 days). However, when combined with *A. largoensis*, the pest mite control was extended throughout the experimental period. Combining abamectin and *A. largoensis* provided better control (99.9% *B. yothersi* population reduction) than each control method alone (76.8% and 81.3% *B. yothersi* population reduction, respectively). Furthermore, plants treated with fenpyroximate in combination with *A. largoensis* exhibited fewer mites at 104 DAA than plants treated only with this acaricide. Our results suggest that *A. largoensis* can improve *B. yothersi* control after abamectin and fenpyroximate applications. All other acaricides were highly effective and provided prolonged *B. yothersi* control throughout the experiment, hindering biological control by *A. largoensis*, probably due to the lack of prey. In the predator-alone

treatment, *A. largoensis* initially suppressed *B. yothersi*, but after 62 days, the pest population increased. This result suggests that periodic releases may be necessary for effective and prolonged biological control by this predatory mite. An alternative explanation relies on the controlled experimental conditions without access to alternative prey. Under natural conditions, *A. largoensis* utilizes multiple arthropod prey, including other mites and insects inhabiting citrus plants and other tropical and subtropical plants. This predator is classified as a type III generalist phytoseiid mite that feeds on insects and mites as well as alternative food sources like pollen and nectar, allowing them to survive in the field even when pest populations are low (McMurtry and Croft, 1997; Reis and Alves, 1997; Sarmiento et al., 2011; Carrillo and Peña, 2012; Gerson and Weintraub, 2012). The presence of alternative prey, or/and the provision of alternative foods (i.e., pollen) in the context of conservation biological control, may play an important role in the ability of *A. largoensis* to persist on citrus plants and provide long-term *B. yothersi* suppression.

Research findings highlight the potential for enhancing the selectivity of acaricides towards *A. largoensis* by manipulating the way predators are exposed to them. For instance, direct and residual contact with abamectin had little effect on *A. largoensis*; therefore, the predator can be released before or after applications with this pesticide. By contrast, direct contact was the most harmful exposure route for *A. largoensis* to spirodiclofen. Therefore, predator releases should be done after the spirodiclofen applications. In addition, residual contact with fenpyroximate was slightly harmful to *A. largoensis*, suggesting that predators can be released safely after fenpyroximate application. The exposure route appears less relevant when applying cyflumetofen, which was moderately harmful to *A. largoensis* via the different exposure routes.

The results of this study provided an outline to design conservation biocontrol strategies in areas infested with *B. yothersi* and its associated viruses. In Florida, a strain of citrus leprosis virus C2 infecting hibiscus is highly associated with *B. yothersi* (Roy et al., 2018b; Roda et al., 2022). In the same area, *A. largoensis* is the most common predatory mite in backyard-grown citrus, hibiscus, and other plants in the landscape infested with *B. yothersi*, making it a promising biological control strategy for managing this pest (Argolo et al., 2020; Childers et al., 2022). The present study suggests potential compatibility between acaricides and *A. largoensis* depending on the route of exposure. Spirodiclofen and abamectin appear safer for *A. largoensis* than fenpyroximate and cyflumetofen. However, it is essential to highlight that the effects of sublethal concentrations of pesticides can be significant (Desneux et al., 2007), and these are unknown for *A. largoensis*. Assessing sublethal effects of acaricides on the physiology and behavior of *A. largoensis* may be crucial for establishing IPM programs combining chemical and biological control strategies to manage citrus leprosis vectors.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Author contributions

JD: Conceptualization, Investigation, Writing original draft, Writing – review & editing. DA: Conceptualization, Investigation, Writing – review & editing. AT: Investigation, Writing – review & editing. AR: Funding acquisition, Writing – review & editing. ES: Formal Analysis, Writing – review & editing. DC: Conceptualization, Funding acquisition, Investigation, Resources, Supervision, Writing – original draft, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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