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EFFECTS OF REDUCED-RISK INSECTICIDES ON THREE ORCHID PESTS AND TWO PREDACIOUS NATURAL ENEMIES

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ABSTRACT

The toxicity of 2 reduced-risk pesticides, 2% horticultural oil with 0.05% Silwet L-77 and Grandevo (*Chromobacterium subtsugae* Strain PRAA4-1T), to 2 predators: *Metaseiulus occidentalis* (Nesbitt) (Acari: Phytoseiidae) and *Hemicheyletia wellsina* DeLeon (Acari: Cheyletidae), and 3 orchid pests: the two-spotted spider mite *Tetranychus urticae* Koch (Prostigmata: Tetranychidae), the phalaenopsis mite, *Tenuipalpus pacificus* Baker (Acari: Tenuipalpidae), and the longtailed mealybug *Pseudococcus longispinus* (Targioni-Tozzetti) (Hemiptera: Pseudococcidae) were tested. Grandevo did not kill either predator, and it failed to suppress populations of *T. urticae* and *P. longispinus*, but it reduced *T. pacificus* by 84%. The Silwet and horticultural oil mixture caused mortality of at least 82% in all 5 species tested.

Key Words: Grandevo, Silwet L-77, pesticide selectivity, horticultural oil, Orchidaceae

RESUMEN

Se probó la toxicidad de 2 plaguicidas de menor riesgo, 2% de aceite hortícola con 0.05% Silwet L-77 y Grandevo (*Chromobacterium subtsugae* Cepa PRAA4-1T), a 2 depredadores: *Metaseiulus occidentalis* (Nesbitt) (Acari: Phytoseiidae) y *Hemicheyletia wellsina* De Leon (Acari: Cheyletidae), y 3 plagas de orquídeas: la araña roja de dos manchas *Tetranychus urticae* Koch (Prostigmata: Tetranychidae), el ácaro de falenopsis *Tenuipalpus pacificus* Baker (Acari: Tenuipalidae), y la cochinilla harinosa con cola larga, *Pseudococcus longispinus* (Targioni-Tozzetti) (Hemiptera: Pseudococcidae). El Grandevo no mató los depredadores, y no pudo reprimir las poblaciones de *T. urticae* y *P. longispinus*, pero redujo el 84% de *T. pacificus.* La mezcla de Silwet con aceite hortícola causó una mortalidad de al menos 82% en todas las 5 especies probadas.

Palabras Clave: Grandevo, Silwet L-77, selectividad de plaguicidas, aceite hortícola, Orchidaceae

Orchid hobbyists are known to use toxic pesticides in an unsafe manner when trying to control pests (Cating et al. 2011). Three products, dimethoate, diazinon and chlorpyrifos, have previously been used but have been removed recently from sale for non-commercial use in the United States because of serious health and environmental problems (Johnson 2008). Orchids are susceptible to many types of arthropod pests, including scales, mealybugs, thrips, mites, aphids, and whiteflies (Bottom 2012). Of these, the most difficult to control are mites, which require a high rate of pesticide use on ornamental plants (Naher et al. 2005). Two types of mites, spider mites (Tetranychidae) and flat mites (Tenuipalpidae), are common orchid pests that can become problematic in greenhouses (Johnson 2008). The American Orchid Society recommends products for managing dense infestations of mites, most of which are toxic to both people and natural enemies (Johnson 2008). The goal of this study was to identify lower-risk products that are effective on key pests of orchids, while being less toxic to 2 natural enemies.

Grandevo is an organic insecticide meant to control a diverse range of pests while being soft on beneficial arthropods (Marrone Bio Innovations 2012). It is naturally derived from the bacterium *Chromobacterium subtsugae* Strain PRAA4-1T (Martin et al. 2007). Grandevo uses complex modes of action for control of pest insects and mites, including repellency, oral toxicity, reduced egg hatch, and reduced fecundity (Marrone Bio Innovations 2012). Martin et al. (2007) tested this product on the Colorado potato beetle (*Leptinotarsa decemlineata* (Say); Coleoptera: Chrysomelidae), Western and Southern corn rootworms (*Diabrotica virgifera* LeConte and *D. undecimpunctata howardi* Barber; Coleoptera: Chrysomelidae, respectively), small hive beetle (*Aethina tumida* (Murray); Coleoptera: Nitidulidae), diamondback moth (*Plutella xylostella* (L,);

Lepidoptera: Plutellidae), gypsy moth (*Lymantria dispar* (L.); Lepidoptera: Erebidae), tobacco hornworm (*Manduca sexta* (L.); Lepidoptera: Sphingidae), sweet potato whitefly (*Bemisia tabaci* (Gennadius); Hemiptera: Aleyrodidae), southern green stink bug (*Nezara viridula* (Linnaeus); Hemiptera: Pentatomidae), and a mosquito (*Culex pipiens* L.; Diptera: Culicidae). It has been tested as well on the black pecan aphid (*Melanocallis caryaefoliae* (Davis); Hemiptera: Aphididae) and pecan weevil (*Curculio caryae* (Horn); Coleoptera: Curculionidae) (Shapiro-Ilan et al. 2013).

Silwet L-77 is an organosilicone surfactant that can be used as an adjuvant to enhance coverage of pesticides (Foy 1989). Silwet L-77 and horticultural oil have been shown to reduce populations of pest mites (*Tenuipalpus pacificus* Baker; Acari: Tenuipalpidae) and scales (*Diaspis boisduvalii* Signoret; Hemiptera: Diaspididae) on orchids in the greenhouse, although complete coverage of the plants is necessary and there is no residual toxicity (Cating et al. 2010). Silwet L-77 also was tested on the Asian citrus psyllid (*Diaphorina citri* Kuwayama; Hemiptera: Liviidae) and its parasitoid (*Tamarixia radiata* (Waterston); Hymenoptera: Eulophidae) (Cocco & Hoy 2008; Srinivasan et al. 2008), citrus leafminer (*Phyllocnistis citrella* Stainton; Lepidoptera: Gracillariidae) (Shapiro et al. 1998). It was tested by Tipping et al. (2003) on pests of table grapes (*Vitis* spp.; Vitales: Vitaceae) including the grape mealybug (*Pseudococcus maritimus* (Ehrhorn); Hemiptera: Pseudococcidae), omnivorous leafroller (*Platynota stultana* Walsingham; Lepidoptera: Tortricidae), Pacific spider mite (*Tetranychus pacificus* McGregor; Acari: Tetranychidae), cotton aphid (*Aphis gossypii* Glover; Hemiptera: Aphididae), and western flower thrips (*Frankliniella occidentalis* (Pergande); Thysanoptera: Thripidae).

Five species were tested with Silwet L-77 plus oil and Grandevo in the laboratory, including 2 predatory mites, *Metaseiulus* (=*Typhlodromus* or *Galendromus*) *occidentalis* (Nesbitt) (Acari: Phytoseiidae) and *Hemicheyletia wellsina* De Leon (Acari: Cheyletidae), 2 phytophagous mites: the two-spotted spider mite [*Tetranychus urticae* (Koch) (Acari: Tetranychidae)] and *Tenuipalpus pacificus* Baker (Acari: Tenuipalpidae), and the long-tailed mealybug, *Pseudococcus longispinus* (Targioni-Tozzetti) (Hemiptera: Pseudococcidae).

Materials and Methods

Pesticides

Grandevo (Marrone Bio Innovations, Davis, California) was applied at the suggested high label rate of 3.6 g/1000 mL (3 lbs/100 gallons). Silwet L-77 at 0.05% (Helena Chemical Company, Collierville, Tennessee) was applied in combination with 2% of Prescription Treatment® Ultra-Pure™ horticultural oil (BASF Chemical Company, Triangle Park, North Carolina) diluted with tap water. Only 40 ml of the pesticides were made daily, and all pesticide concentrations were made fresh daily.

Arthropod Colonies

All colonies in this experiment were established at the University of Florida Department of Entomology and Nematology, except the *M. occidentalis* colony, which was developed in 1984 and is resistant to carbaryl, sulfur and organophosphates (Hoy 1984). It is kept on paraffin-coated paper squares resting on water-soaked cotton and fed all life stages of *T. urticae*. The *H. wellsina* colonies were established in November 2012 after being discovered in a greenhouse on orchids in August 2012, and were fed all life stages of *T. urticae*. *Hemicheyletia wellsina* were maintained on *Phalaenopsis* orchid leaf squares on water-soaked cotton. *Metaseiulus occidentalis, T. urticae,* and *H. wellsina* were maintained in the laboratory at 25 °C, 45-55% RH and a 16:8 L:D photoperiod. The *T. urticae* colony has been reared on pinto beans, *Phaseoulus vulgaris* L. (Fabales: Fabaceae), in a greenhouse since 1992. The *Phalaenopsis* mite and long-tailed mealybug colonies were established in March 2012 from infested *Phalaenopsis* plants in a greenhouse, and were held in a growth chamber at 27 °C, 65-70% RH and a 16:8 L:D photoperiod on *Phalaenopsis* leaves.

General Experimental Conditions

Assays were conducted in the laboratory between May and Aug 2013. *Phalaenopsis* orchids (Kerry's Orchids, Apopka, Florida) were used as substrates for *H. wellsina, T. pacificus* and *P. longispinus,* and pinto beans (*P. vulgaris*) were used for *M. occidentalis* and *T. urticae*. Plants were grown in a greenhouse at the University of Florida, Gainesville. All species were placed on the abaxial side of the orchid or pinto bean leaf discs, which were kept on wet cotton to discourage run off. Sprays were applied using aerosol sprayers (Preval, Chicago Aerosol, Coal City, Illinois) under a fume hood. Leaf discs were sprayed to drip then allowed to dry for 1 h. Twenty adult *T. urticae* were added daily after treatment to leaf discs containing *H. wellsina* and *M. occidentalis* so that a lack of food would not affect survival. Each product was tested on at least 2 dates for each species. Each species was tested with the high label rate of Grandevo, 0.05% Silwet L-77 in combination with 2% horticultural oil, and a tap water control. *Metaseiulus occidentalis* and *T. urticae* were also tested with 0.05% Silwet and 1% horticultural oil.

Metaseiulus occidentalis Adult Females

Five 20-mm diam pinto bean leaf discs that had been naturally infested with abundant *T. urticae* prey were made, and 5 newly mated *M. occidentalis* females were added to each leaf disc. The leaf discs were sprayed with each pesticide and allowed to dry. Leaf discs were then held in the laboratory at 25 ± 1 °C with 45-55% RH. Leaf discs were scored after 24, 48, and 72 h to record mortality of *M. occidentalis.* This was repeated for a total of *n* = 50 mites for each treatment.

Another experiment was conducted with a total *n* = 25 mites using 1% horticultural oil with 0.05% Silwet L-77 after preliminary results indicated that not all *M. occidentalis* were killed with 2% oil and 0.05% Silwet L-77.

Hemicheyletia wellsina Adult Females

Five 20-mm diam *Phalaenopsis* orchid leaf discs with all stages of *T. urticae* prey were made and 5 newly emerged and recently mated *H. wellsina* females were added to each leaf disc. The leaf discs were sprayed with each treatment and allowed to dry. Once removed from the fume hood they were held at 25 ± 1 °C, 45-55% RH and a 16:8 L:D photoperiod. Leaf discs were scored after 24, 48, and 72 h to record mortality of *H. wellsina.* This was repeated for a total of *n* = 50 for each treatment.

Tenuipalpus pacificus Adult Females

Leaf discs were made from *Phalaenopsis* leaves for *T. pacificus* because it is one of the few host plants for this mite. Preliminary studies showed that *T. pacificus* preferred to settle on the edge of the leaf disc, often resulting in accidental death from run off. To reduce the likelihood that this would affect the results, melted paraffin wax was painted around the edge of the disc to form a barrier and this kept 94% of the *Phalaenopsis* mites on the leaf. Five 20-mm leaf discs were placed on wet cotton and 5 adult female *T. pacificus* were added to each. The leaf discs were sprayed with each pesticide and allowed to dry. Because this species requires a higher temperature and RH, the leaf discs were held in a growth chamber at 27 °C, 65-70% RH and a 16:8 L:D photoperiod. Total mortality was recorded after 24, 48, and 72 h. A total of *n* = 50 for each treatment was evaluated.

Pseudococcus longispinus Adults

Because *P. longispinus* is larger than the mites tested, *Phalaenopsis* leaf squares measuring approximately 60 × 60 mm were made for each treatment. To each leaf square, 15 *P. longispinus* adults (mixture of males and females) were added, and sprayed with either a water control, Grandevo, or 2% horticultural oil with 0.05% Silwet. The long-tailed mealybugs were held in a growth chamber at 27 °C and 65-70% RH. Mortality was recorded after 24, 48, and 72 h. This was repeated for a total of *n* = 60 mealybugs for each treatment.

Tetranychus urticae Adults and Larvae

Experiment One. Five pinto bean leaf discs were used for each of the 3 treatments, and 5 newly emerged and mated *T. urticae* females were placed on each disc and sprayed. After drying, the leaf discs were kept at 25 ± 1 °C, 45-55% RH and a 16:8 L:D photoperiod, and scored after 24, 48, and 72 h. This was repeated for a total of *n* = 50 mites for each treatment. An additional spray was completed using 0.05% Silwet with 1% horticultural oil, for a total of *n* = 25 mites.

Experiment Two. An additional experiment was conducted using a total of *n* = 25 *T. urticae* larvae in which the high label rate of Grandevo was applied using distilled water to dilute the product to rule out the possibility of water quality being a factor in the effectiveness of Grandevo.

Experiment Three. Five recently hatched *T. urticae* larvae were added to leaf discs and sprayed with the high label rate of Grandevo. Mortality was assessed after 24, 48, and 72 h, and surviving mites were allowed to develop to adulthood on the residues. Once these mites became adults, new leaf discs were made and 5 recently emerged and mated *T. urticae* females that had been treated with Grandevo as larvae were added to each. An additional treatment was established to assess recently emerged and mated spider mites that had not been previously exposed to Grandevo, as well as a tap water control using unexposed mites. The spider mite adults that had been previously sprayed with Grandevo as larvae, as well as 1 treatment that had not been exposed previously, were sprayed with the high label rate of Grandevo. After 24, 48, and 72 h mortality and the total cumulative number of eggs laid was recorded at each time interval.

Statistical Analysis

All data were arcsine-square root transformed and analyzed by one-way analysis of variance (ANOVA) with JMP 9.0.2 (SAS Institute 2010,) but untransformed means are presented. A Tukey-Kramer test was used to detect significant differences of the means ($\alpha < 0.05$).

RESULTS

Metaseiulus occidentalis

The toxicities of Grandevo and 0.05% Silwet with 2% horticultural oil to *M. occidentalis* adult females were tested by direct application. Grandevo had no negative effect on *M. occidentalis*, resulting in a 100,100, and 96% survival rates of the adult females after 24, 48, and 72 h, respectively, ($P = 0.885$) (Fig. 1) compared with 100% survival of the water control.

The 0.05% Silwet and 2% horticultural oil resulted in 18% survival compared with the water control ($P < 0.0001$) (Fig. 2). Survival from 0.05% Silwet and 2% horticultural oil was consistent after 24, 48, and 72 h. After reducing the horticultural oil to a 1% concentration, 64% of *M. occidentalis* adult females survived after 24, 48, and 72 h, which was significantly different from the water control ($P = 0.0002$).

Hemicheyletia wellsina

Grandevo did not affect the survival of *H. wellsina* adult females, resulting in a 92, 90, 90% survival rates ($P = 0.0901$) after 24, 48, and 72 h (Fig. 1) compared with the water control. The direct spray of 0.05% Silwet and 2% horticultural oil resulted in a 10% survival rate that was consistent after 24, 48, and 72 h (Fig. 2), and was significantly different from the water control ($P < 0.0001$).

Tenuipalpus pacificus

There was a significant decrease in survival of *T. pacificus* adult females after 72 h compared with the water control when exposed to Grandevo ($P < 0.0001$). Only 30, 24, and 16% of 50 *T. pacificus* survived after 24, 48, and 72 h, respectively (Fig. 1). The 0.05% Silwet and 2% horticultural oil caused 0% survival of *T. pacificus* after 72 h ($P < 0.0001$) (Fig. 2).

Pseudococcus longispinus

Pseudococcus longispinus exhibited a 92, 87, and 85% survival rate after 24, 48, and 72 h when exposed to Grandevo (Fig. 1), which was not significantly different from the 98% survival rate of the water control ($P = 0.204$). However, when *P. longispinus* was sprayed with 0.05% Silwet and 2% horticultural oil, survival fell to 18% after 24 h and remained at that after 72 h (Fig. 2), which was significantly different from the water control ($P < 0.0001$). Silwet and oil appeared to cause a loss of the white, waxy coating of the mealybugs.

Tetranychus urticae

Experiment 1. *Tetranychus urticae* exhibited no significant difference in mortality compared to the water control, with a 94, 94, and 90% survival rate of Grandevo diluted with tap water after 24, 48, and 72 h ($P = 0.163$). When sprayed with 0.05% Silwet and 2% horticultural oil, 0% survival occurred by 24 h ($P < 0.0001$) (Fig. 2). The 0.05% Silwet plus 1% horticultural oil resulted in a 28% survival rate of *T. urticae*, consistent after 24, 48, and 72 h ($P < 0.0001$).

Experiment 2. When treated with Grandevo diluted with distilled water, 96, 92, and 88% survived ($P = 0.178$) after 24, 48, and 72 h, respectively (Fig. 1). There was no significant differ-

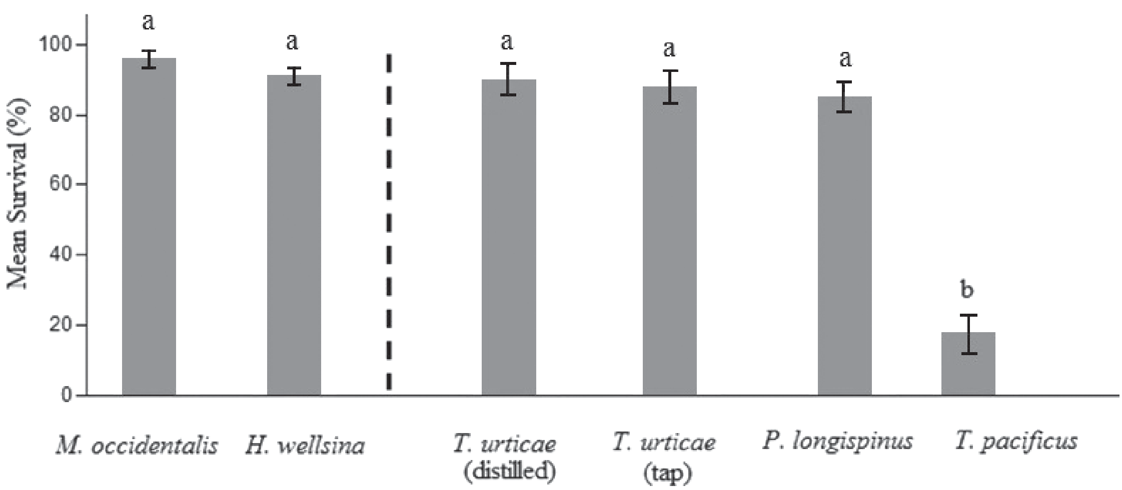

Fig. 1. Percentage survival at the highest label rate of Grandevo by 2 predators (*Metaseiulus occidentalis* and *Hemicheyletia wellsina*) and 3 arthropod pests (*Tetranychus urticae*, *Pseudococcus longispinus*, and *Tenuipalpus pacificus*) after 72 hours. SEM bars with the same letter are not different by Tukey-Kramer test ($\alpha < 0.05$). Both predators and *T. urticae* were held at 25 °C, 45-55% RH and a 16:8 h L:D photoperiod, and *T. pacificus* and *P. longispinus* were held at 27 °C, 65-70% RH and a 16:8 h L:D photoperiod.

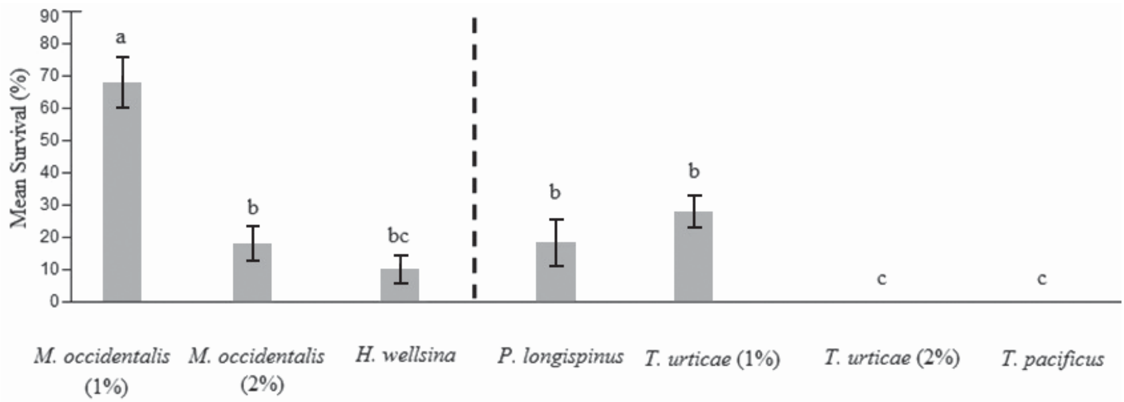

Fig. 2. Percentage survival of 2 predators (*Metaseiulus occidentalis* and *Hemicheyletia wellsina*) and 3 arthropod pests (*Tetranychus urticae*, *Pseudococcus longispinus*, and *Tenuipalpus pacificus*) treated with 0.05% Silwet and 1% or 2% horticultural oil after 72 hours. SEM bars with the same letter are not significantly different by the Tukey-Kramer test ($\alpha < 0.05$). Both predators and *T. urticae* were held at 25 °C, 45-55% RH and a 16:8 h L:D photoperiod, and *T. pacificus* and *P. longispinus* were held at 27 °C, of 65-70% RH and a 16:8 h L:D photoperiod .

ence between the results of Grandevo tested with tap or distilled water ($P = 0.995$).

Experiment 3. The fecundity of *T. urticae* after being exposed once as adults to Grandevo was reduced approximately 26.5% compared to the water control, but was not significantly different after 72 h ($P = 0.1965$) (Fig. 3). There was also no significant difference between the *T. urticae* that were exposed once as adults and those exposed as both adults and larvae after 72 h ($P = 0.1451$).Those sprayed as larvae with Grandevo and again as adults had a 35% reduction in egg production after 72 h compared to the water control, and according to the Tukey-Kramer test ($\alpha < 0.05$), the number of eggs produced in the multiple-exposure treatment was significantly different from the number of eggs in the water control for each 24-h period ($P = 0.0133$).

Discussion

One goal of integrated pest management (IPM) is to preserve natural enemies while still

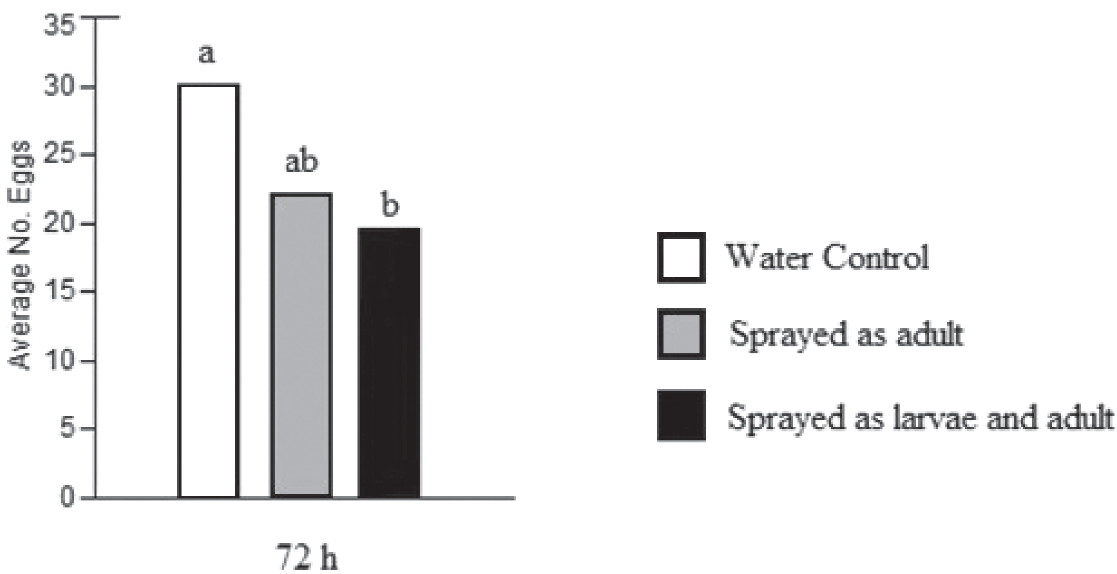

Fig. 3. Average cumulative fecundity of *Tetranychus urticae* adult females after exposure to Grandevo after 72 h, at 25 °C, 45-55% RH and a 16:8 h L:D photoperiod. Significant differences compared with JMP (SAS Institute 2010); mean fecundity with the same letter are not significantly different by Tukey-Kramer test ($\alpha < 0.05$).

reducing pest populations. The results obtained in this study indicate that, although Grandevo is not harmful to either of the natural enemies tested, Grandevo did not suppress 2 of the 3 plant pests tested (Fig. 1). Only the *Phalaenopsis* mite exhibited a significant mortality after exposure to Grandevo, and mortality increased over the 72-h period. According to Marrone Bio Innovations (2012), other studies have found that Grandevo does, in fact, cause statistically significant reductions of *T. urticae* populations on strawberry, but the results of this study do not show a significant difference using Grandevo diluted with either tap or distilled water. The reasons for the difference in our results is unknown, but could be due to the population of *T. urticae* tested or the effects of the different leaf types used. Also, it is unclear how Grandevo was applied to *T. urticae* on strawberries. In a study by Martin et al. (2007), Grandevo was found to be lethal to the Southern green stink bug, sweet potato whitefly, diamond-back moth larvae, small hive beetle larvae, Western and Southern corn rootworm, and Colorado potato beetle larvae, but not to the mosquito *Culex pipens,* tobacco hornworm larvae, gypsy moth larvae, or Colorado potato beetle adults. Grandevo has also been shown to cause significant mortality to the black pecan aphid and pecan weevil when compared to a control (Shapiro-Ilan et al. 2013).

Exposure to 0.05% Silwet and 2% horticultural oil caused a significant reduction in survival of all pest and natural enemy species tested ($P < 0.0001$) (Fig. 2). All mortality occurred within the first 24 h. Previous studies using Silwet L-77 have also shown a high mortality of pests. Mortality was greater than 93% for the grape mealybug, omnivorous leafroller, Pacific spider mite, cotton aphid, and western flower thrips, though the Silwet was applied at higher concentrations (0.1, 0.25, 0.5, and 1.0%) (Tipping et al. 2003). Silwet L-77 with horticultural oil has also been shown to cause mortality of over 85% of Boisduval scales and over 98% of phalaenopsis mites (Cating et al. 2010).

Because these results were obtained under laboratory conditions, field experiments would be the next step to test these products. Results of toxicity studies under laboratory conditions may not accurately depict what might happen when products are used in the field or in a greenhouse. Coverage of the plant with the pesticide could be less thorough in the field, and natural conditions that could protect the predator or pest (e.g. webbing that some species produce) could reduce toxicity, and abiotic factors could reduce pesticide quality (e.g. rain or UV light).

In order for a pesticide to be beneficial to a pest-management program, it would need to cause a reduction in the targeted plant pest, while causing less mortality to any natural enemies present. The surviving predators could feed on any remaining pests, keeping the pest population density low. Because Grandevo caused significant mortality to the *Phalaenopsis* mite without affecting predator mortality, it could potentially be a candidate for management of the *Phalaenopsis* mite. If sprayed repeatedly at a sufficient interval, it is possible that Grandevo could also reduce fecundity of pests like *T. urticae* while not affecting the natural enemies tested here.

The 0.05% Silwet with 2% horticultural oil treatment resulted in 100% mortality of *T. urticae* and *T. pacificus,* and 82% mortality of *P. longispinus*, while leaving 18% of *M. occidentalis* females and 10% of *H. wellsina* females surviving (Fig. 2). The mortality of *T. urticae* and *T. pacificus* was significantly greater than the mortality of the predator *M. occidentalis* when exposed to 0.05% Silwet with 2% horticultural oil. When the concentration of horticultural oil was reduced to 1% with 0.05% Silwet, the survival of *M. occidentalis* increased to 64%, while survival of *T. urticae* only increased to 28% (Fig. 2). Silwet L-77 and horticultural oil thus could be an effective pest management tool, but the need for complete coverage and its short residual activity may restrict its use to plant hobbyists looking for a less-toxic way to control pests. Depending on the pest problem and natural enemies present, both of these reduced-risk pesticides could be beneficial for orchid hobbyists looking to manage pest populations in greenhouses or landscapes.

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