

# Comparative effects of two novel Betaproteobacteria-based insecticides on *Myzus persicae* (Hemiptera: Aphididae) and *Phenacoccus madeirensis* (Hemiptera: Pseudococcidae)

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## Abstract

We compared the lethal and sublethal effects of 2 novel Betaproteobacteria-based insecticides (*Burkholderia* spp. strain A396 as Venerate® XC; *Chromobacterium subtsugae* strain PRAA4-1 as Grandevo® WDG) for suppression of 2 polyphagous insect pests of world-wide importance: green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), and Madeira mealybug, *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae). In laboratory and screenhouse tests, the insects were exposed to residues applied by leaf dipping, or by spraying the insects and foliage. These novel products also were compared to a well-established product, spirotetramat (Movento® 240 SC). Spirotetramat was generally effective for suppression of both species of insects, and *Burkholderia* (Venerate) induced mortality levels that made it competitive with spirotetramat. *Chromobacterium subtsugae* (Grandevo) was less satisfactory, inducing only moderate levels of mortality in both species. Reproduction by aphids surviving exposure to *Burkholderia* was slightly affected, whereas *C. subtsugae* did not affect reproduction. New Betaproteobacteria-based insecticides show promise for a useful role in suppressing important insect pests such as *M. persicae* and *P. madeirensis*.

Key Words: spirotetramat; Venerate; Grandevo; Movento; green peach aphid; Madeira mealybug

## Resumen

Comparamos los efectos letales y subletales de 2 nuevos insecticidas basados en Betaproteobacteria (cepa *Burkholderia* spp. A396 como Venerate® XC; cepa PRAA4-1 de *Chromobacterium subtsugae* como Grandevo® WDG) para la supresión de 2 plagas de insectos polífagos de importancia mundial: el pulgón (áfido) verde del melocotón, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) y la cochinilla harinosa de Madeira, *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae). En pruebas de laboratorio y en una casa de malla, los insectos fueron expuestos a los residuos aplicados por la inmersión de hojas o al rociar los insectos y el follaje. Estos nuevos productos también se compararon con un producto bien establecido, spirotetramat (Movento® 240 SC). Spirotetramat fue generalmente efectivo para la supresión de ambas especies de insectos y *Burkholderia* (Venerate) indujo niveles de mortalidad que lo hicieron competitivo con spirotetramat. *Chromobacterium subtsugae* (Grandevo) fue menos satisfactorio, induciendo solo niveles moderados de mortalidad en ambas especies. La reproducción de los áfidos que sobrevivieron a la exposición de *Burkholderia* se vio ligeramente afectada, mientras que *C. subtsugae* no afectó la reproducción. Los nuevos insecticidas basados en Betaproteobacteria prometen tener un papel útil en la supresión de plagas de insectos importantes, como *M. persicae* y *P. madeirensis*.

Palabras Clave: spirotetramat; Venerar; Grandevo; Movento; pulgón verde del melocotón; cochinilla harinosa de Madeira

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Arthropod pest management relies heavily on the use of synthetic insecticides. This approach has immensely benefited agricultural production in the past. However, there is a nearly universal view that synthetic insecticides have failed to provide all the desired outcomes in the management of crop pests because they have induced many ecological and health problems (Jeyasankar & Jesudasan 2005). Awareness of the harmful effects of this approach has resulted in public concern and debate on the wisdom of the indiscriminate use of synthetic insecticides, ultimately leading to the search for environment-friendly management options. Also, control of these pests is a tremendous challenge for organic farmers, who rely heavily on alternative methods such as natural biological control, promoting natural enemies, and cultural control to prevent injury to crops.

The vast reserves of available biodiversity provide numerous opportunities to harness the ability of other organisms, and their chemical constituents, to sustainably minimize damage from pests, and to increase agricultural productivity and production. Today, the potential and usefulness of several bio-pesticides (microbes, microbial products, or products derived from plants) in sustainable agriculture have been realized and promoted globally (Chandler et al. 2008). These plant protectants are viewed as more environmentally benign than their synthetically produced chemical counterparts because they often do not persist in the environment, have few effects on vertebrates, usually have high host selectivity, and often are compatible with biological control agents (Gupta & Dikshit 2010). Currently, there are at least 200 bio-pesticide products registered for use in the U.S.A, and new prod-

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ucts are being developed as consumers demand more sustainably produced foods (Chandler et al. 2008; Thakore 2006).

Recently, certain Betaproteobacteria species, such as *Burkholderia* spp. and *Chromobacterium* spp., have gained great scientific and commercial interest due to the production of various metabolites acting as potent insecticides. *Chromobacterium subtsugae* strain PRAA4-1 is a gram-negative, violet-pigmented bacterium that was isolated first from soil in Maryland, USA. Martin et al. (2007a) and Hoshino (2011) published initial assessments of the potential of *C. subtsugae* for insect control. A new fermentation product was developed in 2016 by Marrone Bio Innovations Inc., Davis, California, USA, from secondary metabolites produced from *C. subtsugae* strain PRAA4-1 and is currently labeled (Grandevo® WDG, Marrone Bio Innovations Inc.) for management of lepidopteran larvae, aphids, phytophagous mites, thrips, whiteflies, psyllids, *Lygus* (Myridae) bugs, and mealybugs on vegetable and fruit crops (30% active ingredient). It functions primarily as a stomach poison, but also reduces fecundity and oviposition, and deters feeding (Koivunen et al. 2009; Asolkar et al. 2012).

In 2014, a new biological insecticide (Venerate® XC) also was developed by Marrone Bio Innovations Inc., and is composed of killed cells and fermentation solids of *Burkholderia* spp. strain A396 as an active ingredient. It is registered for use on agricultural crops in both the field and greenhouse for many chewing pests including caterpillars and beetles, as well as sucking arthropods such as aphids, whiteflies, and mites. Several active compounds in Venerate provide multiple modes of action, resulting in enzymatic degradation of exoskeletal structures by contact and ingestion of the product (Asolkar et al. 2013).

Although extracts from the microbes *C. subtsugae* and *Burkholderia* spp. have been reported to have broad activity against various sucking insects, algae, arachnids, mites, and nematodes (Asolkar et al. 2012, 2013), their effectiveness is not entirely consistent. For example, Lee et al. (2014) and Morehead (2016) found that certified *Burkholderia* sp. (MBI-206), and *C. subtsugae* (MBI-203) insecticides were effective on nymphs and adults of the brown marmorated stink bug, *Halyomorpha halys* Stål (Hemiptera: Pentatomidae), in bioassays and field trials. However, foliar applications of *Burkholderia* sp. (Venerate) did not reduce abundance of stink bugs and other heteropterans attacking tomatoes in California (Carson et al. 2014). On the other hand, *Burkholderia* sp. caused significantly higher mortality to eggs of two-spotted spider mite than other botanical, chemical, and microbial pesticides on strawberries in California (Dara 2015).

Because there is inconsistent or inadequate information on the effects of the recently developed microbe-based control agents *C. subtsugae* and *Burkholderia*, this study was carried out to evaluate their efficacy on 2 pests of world-wide importance, the green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), and the Madeira mealybug, *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae). Their performance was assessed relative to spirotetramat (Movento®), which is an effective and widely accepted insecticide that interferes with lipid biosynthesis and produces toxicity to many groups of insects including aphids and mealybugs. Thus, in this study spirotetramat was used as a reference (standard) insecticide.

## Materials and Methods

### INSECT COLONIES AND PLANT CULTURE

A colony of *M. persicae* was established from apterous individuals originally obtained from aphid culture kept for several generations on pepper plants at the Department of Entomology and Nematology, University of Florida, Gainesville, Florida, USA. Aphids were maintained

on young pepper plants grown in potting soil-filled plastic pots (15 cm diam) in a growth room at  $25 \pm 3$  °C with a 16:8 h (L:D) photoperiod. A continuous supply of new greenhouse-grown plants was provided as needed for the colony replenishment when old plants senesced due to high feeding pressure of aphids.

Individuals of *P. madeirensis* originated from infested cotton plants in a greenhouse at the Department of Entomology and Nematology, University of Florida. They were maintained on young cotton plants, Bollgard II XtendFlex, planted in potting soil-filled plastic pots (15 cm diam) under greenhouse conditions at  $25 \pm 3$  °C with a 16:8 (L:D) photoperiod. The mealybug culture was maintained in a 60 × 60 × 100 cm cage covered with fine mesh on all sides. Host plants in the rearing cages were replaced as needed.

Seeds of pepper and cotton plants were planted in 50-cavity plastic seedling trays (50 × 30 × 0.6 cm) containing commercial potting soil. After growing for several d under the aforementioned greenhouse conditions, seedlings of both plants at the primary leaf stage were moved into larger plastic pots (15 cm diam) filled with commercial potting soil. Plants were fertilized weekly using 20-9-20 water-soluble fertilizer (N:P:K) and irrigated as needed until needed for trials.

### INSECTICIDES

Commercially available microbe-based insecticides (Grandevo WDG [30% *C. subtsugae* strain PRAA4-1 and spent fermentation media] and Venerate XC [94.46% heat-killed *Burkholderia* spp. strain A396 cells and spent fermentation media]) were obtained from Marrone Bio Innovations Inc., Davis, California, USA. Movento 240 SC (22.4% spirotetramat: cis-3-(2,5-dimethylphenyl)-8-methoxy-2-oxo-1-azaspiro [4.5] dec-3-en-4-yl-ethyl carbonate) was obtained from Bayer Crop Science LP, Research Triangle Park, North Carolina, USA. The efficacy of these products applied directly to insects and residually via the host plant was evaluated on young and old nymphs of aphids and mealybugs, and at different concentrations in the laboratory. Also, the highest labeled concentrations were evaluated in a screenhouse trial to control aphids and their progeny on pepper plants.

### DIRECT AND RESIDUAL EFFECTS OF INSECTICIDES ON APHIDS AND MEALYBUGS

Leaf disc bioassays were used to measure the direct and residual effects of the formulated insecticides on immature stages of both insect species using plastic petri dishes (5.5 cm diam) with gauze-covered ventilation holes in lids. The efficacy resulting from direct application of the insecticides to the insects was assessed using clean, untreated pepper (for aphids) or cotton (for mealybugs) foliage cut into discs (5 cm diam) with a sharp-edged plastic tube. Leaf discs in each treatment were placed with their abaxial surface facing upward on a 4 mm layer of 1% agar that was poured in plates 1 d before testing. We transferred 25 same-aged last instar nymphs for each species individually onto the surface of each leaf disc using a fine camel-hair brush. Subsequently, leaf discs accommodating insects were directly treated with spirotetramat at concentrations of 0.1, 0.2, 0.4, 0.6, and 0.8 mL per L, *Burkholderia* at 3.75, 7.5, 15, 22.5 and 30 mL per L, and *C. subtsugae* at 3.9, 7.8, 15.6, 23.4, and 31.2 mg per L of the test products using 500 mL plastic water spray bottles until saturating the individual leaf disc. The Petri dishes were inverted immediately to remove excess spray solution and maintained in an inverted position in a room at  $23 \pm 3$  °C and 16:8 h (L:D) photoperiod. Six replicates were used for each treatment.

Similar procedures were used to quantify the residual effects of spirotetramat, *Burkholderia*, and *C. subtsugae* on immature stages of both insects using the aforementioned concentrations. For this experi-

ment, 6 leaf discs (5 cm diam) were first dipped in each dilution of test products for 10 s with gentle agitation and placed on paper towels, abaxial surface facing upwards, to air dry. Thereafter, individual leaf discs were placed in a plastic petri dish (5.5 cm diam) lined in the bottom with a layer of agar, with abaxial surface upward. The leaf disc in each petri dish was infested with second instar nymphs for both insect species at a density of 25 nymphs per dish. Water control treatments for both experiments consisted of leaf discs treated with tap water. Each treatment was replicated 6 times. Mortality was monitored at 24 h intervals and continued until the time when there was no additional increase in mortality. Thus, the length of time that insects were monitored varied with the pattern of mortality.

To estimate the reproductive effects of treatment, adult aphids developing from the second instar nymphs that survived the residual effect of bio-insecticides were maintained on young, untreated leaf discs on agar at a density of 10 adults per dish for an additional 7 d, and their progeny were counted and removed at 1-d intervals. Each treatment was replicated 6 times. We could not make observations on reproduction by aphids that developed from the nymphs treated with spirotetramat due to the death of all aphids after treatment.

The efficacy of insecticides to *M. persicae* under greenhouse conditions was assessed by infesting pepper plants at the 6 true-leaf stage with 50 adult aphids of the same age. Each experimental plant was enclosed by a clear plexiglass cylinder (15 cm diam) with a gauze mesh-covered top and holes on the side for ventilation. Aphids were left to reproduce freely on the plants for 2 wk in a very lightly shaded screen house. Subsequently, experimental plants were sprayed with insecticides at the highest labeled concentration (13.6 gm per L for *C. subtsugae*, 0.4 mL per L for spirotetramat, and 10 mL per L for *Burkholderia*), whereas plants treated with water served as a control. For each treatment, 10 plants were used. Aphid mortality was recorded 72 h after treatment.

## STATISTICAL ANALYSIS

Analysis of the effects of insecticide concentration and post-treatment interval (time) were assessed with two-way ANOVA (GraphPad Software, San Diego, California, USA). Some analyses did not consider time as a variable, so for these we used one-way ANOVA. All data were evaluated with the D'Agostino and Pearson omnibus normality test

to assess need for transformation or use of nonparametric analysis. Mortality data were adjusted for water control (check) mortality using Abbott's formula (Abbott 1925). Statistical differences among mean values were assessed using Bonferroni's Multiple Comparison Test (significant if  $P = 0.05$ ).

## Results

### MORTALITY ATTRIBUTABLE TO SPIROTETRAMAT (MOVENTO)

All concentrations of spirotetramat were toxic to the second instar nymphs of aphids when they fed on leaves that had been dipped in an aqueous solution and allowed to dry (residual toxicity) (Table 1). Reduction in the survival rate of young nymphs was significantly related to increasing concentration ( $F = 11.77$ ;  $df = 4,100$ ;  $P < 0.0001$ ). Likewise, exposure time was a significant factor in aphid survival ( $F = 1055.78$ ;  $df = 4,100$ ;  $P < 0.0001$ ), and the interaction of concentration and time was significant ( $F = 3.34$ ;  $df = 12,100$ ;  $P < 0.0004$ ). Application of spirotetramat at any concentration resulted in nearly complete mortality of the aphid population at 96 h after treatment. Because all concentrations resulted in about the same levels of mortality, the dominant variable in the analysis was time, which accounted for over 94% of the total variance.

A similar trend in residual toxicity was observed for the effects of spirotetramat on young nymphs of Madeira mealybug. Its influence on nymphal mortality was significantly related to insecticide concentration ( $F = 19.29$ ;  $df = 4,125$ ;  $P < 0.0001$ ). Exposure time also was a significant variable ( $F = 52.60$ ;  $df = 4.125$ ;  $P < 0.0001$ ), but the interaction of time and concentration was not significant ( $F = 0.52$ ;  $df = 16,125$ ;  $P = 0.93$ ), indicating consistency in the mortality response. Nevertheless, the reduction in population of *P. madeirensis* nymphs did not exceed 52% at any concentration, indicating that the young Madeira mealybugs were less susceptible to spirotetramat than the green peach aphids (Table 1).

As shown in Table 2, spirotetramat was quite effective on the late instar nymphs of the green peach aphid and Madeira mealybug when the insecticide was applied directly to insects infesting the host plant. Abundance was reduced by up to 90% for the aphids and 84% for mealybugs, relative to the control. Concentration significantly affected

**Table 1.** Effects of different concentrations of spirotetramat (Movento®) at various time intervals post treatment on the percent mortality of the second instar nymphs of *Myzus persicae* aphids and *Phenacoccus madeirensis* mealybugs when the insecticide was applied to the plant foliage on which the insects later fed. Mortality was corrected using Abbott's formula (Abbott 1925).

Concentration	<i>Myzus persicae</i>				
	24 h	48 h	72 h	96 h	
0.1 mL per L	1.67 ± 1.07bA	33.23 ± 2.76bB	75.02 ± 1.77bC	97.95 ± 1.30aD	
0.2 mL per L	12.05 ± 1.99abA	37.17 ± 3.14bB	67.48 ± 2.63abC	100.0 ± 0.00aD	
0.4 mL per L	2.32 ± 1.61abA	28.23 ± 1.51bB	65.78 ± 5.85abC	100.0 ± 0.00aD	
0.6 mL per L	6.85 ± 1.65abA	38.30 ± 2.15bB	74.50 ± 3.60abC	100.0 ± 0.00aD	
0.8 mL per L	17.07 ± 1.99aA	53.87 ± 6.64aB	77.60 ± 2.67aC	98.37 ± 0.90aD	
Concentration	<i>Phenacoccus madeirensis</i>				
	24 h	48 h	72 h	96 h	120 h
0.1 mL per L	6.58 ± 3.25aA	9.57 ± 0.77aAB	20.53 ± 1.76bBC	26.13 ± 3.92bC	40.83 ± 3.26aD
0.2 mL per L	8.38 ± 2.08aA	13.57 ± 1.90aA	20.05 ± 3.25bAB	31.17 ± 4.68bB	42.37 ± 2.73aC
0.4 mL per L	16.30 ± 1.40aA	18.05 ± 1.50aA	29.68 ± 4.21abA	32.03 ± 4.51bAB	46.45 ± 5.45aB
0.6 mL per L	19.18 ± 3.26aA	17.95 ± 2.33aA	36.40 ± 2.35abAB	42.18 ± 6.55bB	51.82 ± 6.63aB
0.8 mL per L	27.43 ± 5.18aA	27.42 ± 5.59aA	46.73 ± 4.95aAB	47.22 ± 5.02aAB	52.32 ± 5.29aB

Means ± SE within columns followed by the same lower-case letter, or within rows followed by the same upper-case letter, are not significantly different ( $P > 0.05$ ).

**Table 2.** Effects of different concentrations of spirotetramat (Movento®) at various time intervals post treatment on the percent mortality of late instar nymphs of *Myzus persicae* aphids and *Phenacoccus madeirensis* mealybugs when the insecticide was applied directly to nymphs on host foliage.

Concentration	<i>Myzus persicae</i>			
	24 h	48 h	72 h	96 h
0.1 mL per L	11.50 ± 1.06bA	34.47 ± 1.72bB	68.50 ± 2.32aC	75.30 ± 3.92bC
0.2 mL per L	14.73 ± 2.48bA	24.89 ± 2.98bA	62.62 ± 4.85abB	73.93 ± 4.85bB
0.4 mL per L	12.70 ± 1.44bA	30.85 ± 4.23bB	54.52 ± 3.94abC	85.10 ± 2.10abD
0.6 mL per L	29.70 ± 2.24aA	49.18 ± 3.01abB	68.40 ± 3.93abC	88.62 ± 1.75abD
0.8 mL per L	36.37 ± 2.11aA	54.63 ± 3.69aB	70.95 ± 3.94aBC	90.47 ± 2.75aC
Concentration	<i>Phenacoccus madeirensis</i>			
	24 h	48 h	72 h	96 h
0.1 mL per L	14.35 ± 1.29bA	24.23 ± 1.35cA	44.02 ± 3.29bB	46.37 ± 2.49bB
0.2 mL per L	10.77 ± 2.31bA	39.10 ± 1.31bcB	59.90 ± 5.36abC	84.20 ± 3.81aD
0.4 mL per L	29.00 ± 1.88bA	57.00 ± 5.23abB	75.57 ± 2.40aC	79.63 ± 4.19aC
0.6 mL per L	53.58 ± 4.69aA	66.78 ± 5.03abA	72.95 ± 4.83aAB	83.10 ± 4.65aB
0.8 mL per L	54.87 ± 3.86aA	67.55 ± 5.18aAB	76.87 ± 4.76aB	84.28 ± 3.09aB

Means ± SE within columns followed by the same lower-case letter, or within rows followed by the same upper-case letter, are not significantly different ( $P > 0.05$ ).

mortality ( $F = 29.53$ ;  $df = 4,100$ ;  $P < 0.0001$  for aphids;  $F = 63.23$ ;  $df = 4,100$ ;  $P < 0.0001$  for mealybugs), as did length of exposure time ( $F = 375.22$ ;  $df = 3,100$ ;  $P < 0.0001$  for aphids;  $F = 107.78$ ;  $df = 3,100$ ;  $P < 0.0001$  for mealybugs). The interaction of concentration and time also was significant for aphids ( $F = 3.12$ ;  $df = 12,100$ ;  $P < 0.0008$ ) and for mealybugs ( $F = 4.60$ ;  $df = 12,100$ ;  $P < 0.0001$ ), but did not account for much of the variation in response (2.7 and 7.5%, respectively).

#### MORTALITY ATTRIBUTABLE TO *BURKHOLDERIA* (VENERATE)

The residual activity of *Burkholderia* demonstrated moderate effectiveness against young aphid nymphs (Table 3). Significant levels of mortality up to 49% were recorded, depending on the concentrations ( $F = 54.07$ ;  $df = 4,60$ ;  $P < 0.0001$ ). The length of exposure time also was a significant factor affecting mortality ( $F = 18.11$ ;  $df = 2,60$ ;  $P < 0.0001$ ). A significant interaction was observed between these factors, indicating that the nature of the mortality response varied in time ( $F = 2.85$ ;  $df = 8,60$ ;  $P < 0.009$ ), but it was not particularly strong, accounting for only 10.8% of the variance.

In contrast, the residual activity of *Burkholderia* on young mealybug nymphs was higher (up to 77%) than on aphids. Insecticide concentration ( $F = 43.21$ ;  $df = 4,75$ ;  $P < 0.0001$ ) and exposure time ( $F = 22.11$ ;  $df = 2,75$ ;  $P < 0.0001$ ) significantly affected mortality. The interaction of concentration and exposure time was significant ( $F = 4.00$ ;  $df = 8,75$ ;  $P < 0.0005$ ), though the interaction accounted for only 9.8% of the variance.

Relative to direct toxicity, *Burkholderia* induced high levels of toxicity to late instars of both insects within the first 24 h, causing over 90%

mortality in each species when nymphs were directly treated (Table 4). The toxicity level varied considerably with the concentration ( $F = 37.26$ ;  $df = 4,25$ ;  $P < 0.0001$  for aphids;  $F = 228.6$ ;  $df = 4,25$ ;  $P < 0.0001$  for mealybugs).

#### MORTALITY ATTRIBUTABLE TO *CHROMOBACTERIUM SUBTSUGAE* (GRANDEVO)

The residual effects of *C. subtsugae* applied at different concentrations to the second instars of both insects tested are shown in Table 5. Exposure of young nymphs to treated leaves eventually resulted in a substantial reduction in the survival of both insect species in a significant concentration-dependent manner ( $F = 55.44$ ;  $df = 4,75$ ;  $P < 0.0001$  for aphids;  $F = 32.80$ ;  $df = 4,75$ ;  $P < 0.0001$  for mealybugs). Exposure time was a significant factor also, though less so ( $F = 7.08$ ;  $df = 2,75$ ;  $P = 0.0015$  for aphids;  $F = 16.26$ ;  $df = 2,75$ ;  $P < 0.0001$  for mealybugs). For aphids, the interaction term was significant ( $F = 4.11$ ;  $df = 8,75$ ;  $P < 0.0004$ ), so the response to concentration varied with time. In contrast, the interaction term was not significant with mealybugs, so the response to concentration on mealybugs was similar regardless of exposure time ( $F = 1.20$ ;  $df = 8,75$ ;  $P = 0.3100$ ). In general, the residual toxicities of *C. subtsugae* to both insects were less effective at suppressing the pest populations to a low level, relative to the other insecticides. Application of *C. subtsugae* at the highest labeled rate (15.6 mL per L) killed only about 25% of the insects at 72 h. Additional increase in concentration to twice the recommended field dosage did not induce a large suppressive effect; a maximum mortality of 44% was achieved at 72 h for aphids. There was no significant increase in the

**Table 3.** Effects of different concentrations of *Burkholderia* (Venerate®) at various time intervals post-treatment on the percent mortality of second instar nymphs of *Myzus persicae* aphids and *Phenacoccus madeirensis* mealybugs when the insecticide was applied to the plant foliage on which the insects later fed.

Concentration	<i>Myzus persicae</i>			<i>Phenacoccus madeirensis</i>		
	24 h	48 h	72 h	24 h	48 h	72 h
3.75 mL per L	5.60 ± 1.8.2bA	7.40 ± 2.22cA	4.62 ± 2.20cA	3.75 ± 1.41bA	2.35 ± 1.62cA	6.70 ± 1.27dA
7.5 mL per L	15.60 ± 2.84abA	15.08 ± 1.37cA	20.30 ± 3.46bA	13.98 ± 3.55abA	14.05 ± 1.92cA	23.05 ± 3.75cdA
15 mL per L	12.02 ± 2.13abA	19.36 ± 1.35cAB	24.82 ± 2.28abB	15.85 ± 2.13abA	20.02 ± 2.29bcA	33.70 ± 5.07bcB
22.5 mL per L	23.72 ± 2.25abA	33.72 ± 4.09abAB	39.88 ± 4.85aB	37.07 ± 6.33aA	35.33 ± 7.09abA	53.92 ± 6.73abA
30 mL per L	25.50 ± 3.18aA	37.04 ± 3.26aAB	49.48 ± 4.53aB	24.82 ± 3.48abA	48.50 ± 10.99aA	77.80 ± 5.31aB

Means ± SE within columns followed by the same lower-case letter, or within rows followed by the same upper-case letter, are not significantly different ( $P > 0.05$ ).

**Table 4.** Effects of different concentrations of *Burkholderia* (Venerate®) on the percent mortality of late instar nymphs of *Myzus persicae* aphids and *Phenacoccus madeirensis* mealybugs when the insecticide was applied directly to nymphs on infested host foliage. Mortality was corrected using Abbott's formula (Abbott 1925). Only 24 h results are presented because there were no increases in mortality thereafter.

Concentration	<i>Myzus persicae</i>	<i>Phenacoccus madeirensis</i>
	24 h	24 h
3.75 mL per L	35.78 ± 5.21c	5.42 ± 1.35d
7.5 mL per L	68.28 ± 6.35b	54.00 ± 3.33c
15 mL per L	89.22 ± 2.44a	76.97 ± 3.51b
22.5 mL per L	91.28 ± 2.23a	94.53 ± 2.28a
30 mL per L	94.73 ± 1.83a	97.70 ± 1.03a

Means ± SE within columns followed by the same lower-case letter are not significantly different ( $P > 0.05$ ).

mortality of either insect as a function of exposure time, except application of *C. subtsugae* at a concentration of 31.2 g per mL for aphids and 7.8 g per mL for the mealybug after 72 h.

The late nymphal instars of aphids responded to direct spray with *C. subtsugae* in a manner similar to that observed for residual activity. *Chromobacterium subtsugae* displayed the ability to reduce the survival of older nymphs by up to 47% at 72 h (Table 6). Mortality was significantly affected by insecticide concentration ( $F = 94.00$ ;  $df = 4,75$ ;  $P < 0.0001$ ), and exposure time affected mortality to a small degree ( $F = 3.65$ ;  $df = 2,75$ ;  $P < 0.03$ ). There was no significant interaction between concentration and exposure time ( $F = 0.64$ ;  $df = 8,75$ ;  $P = 0.74$ ).

The acute contact toxicity of *C. subtsugae* to older nymphs of *P. madeirensis* occurred only at 1 d after direct treatment, and in a dose-dependent manner ( $F = 56.57$ ;  $df = 4,25$ ;  $P < 0.0001$ ). The effect of *C. subtsugae* on the mortality of old mealybug nymphs at 24 h was equivalent to that seen with this product on late instar nymphs of aphids, but 3 d were required for the aphid mortality to accrue. Even with application of *C. subtsugae* at double the labeled rate, the mortality of late instars of both species was only about 50% (Table 6).

#### SUBLETHAL EFFECTS OF *BURKHOLDERIA* AND *CHROMOBACTERIUM*

The sublethal effects of different concentrations of *C. subtsugae* (Grandevo) and *Burkholderia* (Venerate) on the reproduction of adult aphids developing from second instars that survived exposure to residual insecticide treatment are illustrated in Table 7. *Chromobacterium subtsugae* did not diminish the reproductive rate of aphids at any concentration tested ( $F = 0.42$ ;  $df = 5,30$ ;  $P = 0.9300$ ). In contrast, we observed a statistically significant reduction in reproduction by aphids after exposure to the residues of *Burkholderia* on pepper foliage ( $F = 4.91$ ;  $df = 4,20$ ;  $P = 0.0064$ ).

**Table 5.** Effects of different concentrations of *Chromobacterium subtsugae* (Grandevo®) at various time intervals post-treatment on the percent mortality of second instar nymphs of *Myzus persicae* aphids and *Phenacoccus madeirensis* mealybugs when the insecticide was applied to the plant foliage on which the insects later fed.

Concentration	<i>Myzus persicae</i>			<i>Phenacoccus madeirensis</i>		
	24 h	48 h	72 h	24 h	48 h	72 h
3.9 gm per L	11.82 ± 1.93bA	8.20 ± 0.96bA	11.45 ± 1.16cA	1.18 ± 1.18bA	2.95 ± 1.35bA	7.95 ± 3.29bA
7.8 gm per L	13.28 ± 1.88bA	11.03 ± 2.52bA	12.87 ± 1.82cA	3.05 ± 1.38bA	6.52 ± 1.62bA	21.98 ± 3.78aB
15.6 gm per L	16.23 ± 2.06abA	16.07 ± 2.04abA	19.65 ± 2.15cA	14.05 ± 1.97abA	21.20 ± 4.95aA	25.47 ± 5.18aA
23.4 gm per L	26.45 ± 2.67aA	25.97 ± 2.26aA	26.77 ± 2.32bA	22.18 ± 2.11aA	29.15 ± 1.35aA	30.48 ± 3.08aA
31.2 gm per L	23.65 ± 1.67aA	31.12 ± 4.16aA	44.00 ± 1.94aB	19.83 ± 1.99aA	26.50 ± 4.06aA	27.98 ± 2.87aA

Means ± SE within columns followed by the same lower-case letter, or within rows followed by the same upper-case letter, are not significantly different ( $P > 0.05$ ).

#### SCREENHOUSE ASSESSMENT OF APHID MORTALITY

When pepper plants that were infested initially with 50 adult aphids for 2 wk under greenhouse conditions were treated with the insecticides at the highest labeled rates, the insecticides displayed levels of efficacy very similar to that observed in the laboratory bioassay tests (Table 8). All treatments significantly reduced aphid abundance relative to the control, but provided different levels of suppression ( $F = 273.9$ ;  $df = 3,36$ ;  $P < 0.0001$ ). Spirotetramat was most effective, reducing aphid populations by about 97% after 3 d. *Burkholderia* ranked second, with a mortality rate of 76%, whereas *C. subtsugae* was least toxic, reducing the aphid population by 54%.

#### Discussion

In this study, the efficacy of 2 novel Betaproteobacteria-based insecticides on the survival rate of young and old nymphs of *M. persicae* and *P. madeirensis* was assessed, and compared to a 'standard.' Our results indicated that both species of insects were susceptible to the Betaproteobacteria-based insecticides tested, but at different magnitudes, depending on the product, concentration, and target insect. A reduction in survival of juveniles was associated with increasing concentration, and generally a significant interaction was observed between concentration and length of exposure time, indicating that the nature of the mortality response varied in time.

Spirotetramat (Movento), which represented a commonly used 'standard,' was more effective on young nymphs of *M. persicae* as compared with the Betaproteobacteria-based products. Although the residue of this product required about 3 d to provide satisfactory suppression, it eventually led to nearly complete mortality even when applied at concentrations less than recommended. However, young nymphs of Madeira mealybug were not as susceptible to spirotetramat residue as were green peach aphids, experiencing less than 52% mortality when spirotetramat was applied at the highest concentration tested. In contrast, the 2 insect species did not differ much in their susceptibility to spirotetramat when tested by direct application of the insecticide to the insects.

In leaf-dip bioassays, spirotetramat (Movento) has been shown to provide excellent efficacy against 3- to 4-d-old nymphs of several species of aphids. Aphids ingesting insecticide through feeding were completely killed (Nauen et al. 2008). However, in those studies less than 50% of aphids died when dipped into a high concentration of insecticide (contact activity). This agrees to some extent with our observations. The effectiveness of this product is attributable to systemic and translaminar efficacy, which allows the plant to acquire high and residual dosages that are effective against sucking insects, whereas its contact efficacy is rather limited (Brück et al. 2009).

The *Burkholderia* product (Venerate) exhibited a moderate residual toxicity when coming into contact with early instars of both species via

**Table 6.** Effects of different concentrations of *Chromobacterium subtsugae* (Grandevo®) at various time intervals post-treatment on the percent mortality of late instar nymphs of *Myzus persicae* aphids and *Phenacoccus madeirensis* mealybugs when the insecticide was applied directly to nymphs on infested host foliage.

Concentration	<i>Myzus persicae</i>			<i>Phenacoccus madeirensis</i>
	24 h	48 h	72 h	24 h
3.9 gm per L	6.47 ± 1.13dA	6.60 ± 1.81dA	6.68 ± 1.23dA	11.32 ± 1.64c
7.8 gm per L	8.65 ± 1.02cdA	13.35 ± 1.49cdAB	14.17 ± 1.58cdB	16.83 ± 2.06c
15.6 gm per L	20.12 ± 3.69bcA	22.17 ± 3.40cbA	27.27 ± 2.00bcA	26.73 ± 1.66b
23.4 gm per L	24.48 ± 1.28bA	26.78 ± 1.64bAB	33.95 ± 2.91bB	35.68 ± 2.00b
31.2 gm per L	45.57 ± 5.07aA	45.97 ± 4.59aA	46.62 ± 3.62aA	51.58 ± 2.97a

Means ± SE within columns followed by the same lower-case letter, or within rows followed by the same upper-case letter, are not significantly different ( $P > 0.05$ ).

Means ± SE within columns followed by the same lower-case letter are not significantly different ( $P > 0.05$ ).

**Table 7.** Reproductive rate of adult aphids developing from the second instar nymphs that survived residual exposure to different concentrations of *Burkholderia* (Venerate®) and *Chromobacterium subtsugae* (Grandevo®).

Venerate		Grandevo	
Concentration	No. of offspring per adult	Concentration	No. of offspring per adult
0	16.22 ± 1.90a	0	12.10 ± 0.72a
3.75 mL per L	15.16 ± 0.64ab	3.9 g per L	12.53 ± 0.69a
7.5 mL per L	15.82 ± 0.35ab	7.8 g per L	12.10 ± 0.46a
15 mL per L	12.86 ± 1.42ab	15.6 g per L	11.42 ± 0.56a
22.5 mL per L	11.62 ± 0.87ab	23.4 g per L	11.68 ± 0.76a
30 mL per L	9.96 ± 1.65b	31.2 g per L	12.15 ± 0.44a

dipped leaves. Over a 3-d period of exposure, the mortality increased in a concentration-dependent manner. However, the mortality of *Burkholderia* on late instars induced by direct contact occurred within 24 h of treatment, and attained > 90% mortality in each species.

The contact and residual toxicities of *C. subtsugae* (Grandevo) on both green peach aphid and Madeira mealybug were relatively low. This product does not seem to compare too favorably with the insecticidal effects observed with spirotetramat and *Burkholderia*, at least with these insects. In general, the mortality induced by *C. subtsugae* did not exceed 50% despite its use at high concentrations.

These Betaproteobacteria-based products reportedly affect insects in the orders Lepidoptera, Hemiptera, Thysanoptera, and Coleoptera, as well as mites (Martin et al. 2007a, b, c; Asolkar et al. 2012, 2013). In addition to mortality, significant sublethal effects such as feeding inhibition, fecundity, and oviposition, were observed in many cases. However, we did not observe significant sublethal effects of *C. subtsugae* on aphid reproduction.

In our screenhouse trial, the toxicity of these insecticides on *M. persicae* on pepper plants was similar to that observed in laboratory bioassays. Nearly complete mortality of the aphid population was achieved 3 d after application of spirotetramat on infested plants, whereas *Burkholderia* and *C. subtsugae* applied at the highest labeled

rate significantly reduced the aphid population, but not as effectively as spirotetramat. *Burkholderia* was more effective at population reduction than was *Chromobacterium*.

Overall, spirotetramat generally was efficacious to both species, though the mealybugs were not highly susceptible through residual contact. The *Burkholderia* product induced mortality levels that made it competitive with spirotetramat, although aphids were less susceptible than mealybugs to residual contact. *Chromobacterium subtsugae* was less satisfactory, inducing only moderate levels of mortality in both species. Reproduction by aphids surviving exposure to *Burkholderia* was slightly affected, whereas *C. subtsugae* did not affect reproduction. Consistent with the laboratory observations, direct treatment of aphid populations at the highest labeled rate on pepper plants under screenhouse conditions resulted in a reduction of aphid abundance, relative to the control, by 97% for spirotetramat, 76% for *Burkholderia*, and 54% for *C. subtsugae*, after 2 wk. Overall, *Burkholderia* seems more promising than *C. subtsugae*, though other insect species might respond differently. These new products provide opportunities to advance use of bio-based insecticides, and provide the potential to enhance insecticide resistance management. To obtain a complete picture of these novel bio-based pesticides, further investigations assessing their efficacy on their promising natural enemies are worthwhile.

**Table 8.** Effect of insecticides applied at the highest labeled rates on the population density of aphids on pepper plants under screenhouse conditions. All plants were originally infested with 50 adult aphids of the same age, aphids were allowed to reproduce freely for 2 wk, then plants were sprayed and aphid numbers were tabulated at 72 h post treatment.

Treatment	Number of aphids per plant
Control	3712 ± 153.0a
<i>Chromobacterium</i> (13.6 mg per L)	1718 ± 10.2b
<i>Burkholderia</i> (10 mL per L)	895.2 ± 58.69c
Spirotetramat (0.4 mL per L)	91.6 ± 10.64d

Means ± SE followed by the same lower-case letter are not significantly different ( $P > 0.05$ ).

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