EFFECT OF A NEEM BIOPESTICIDE ON REPELLENCY, MORTALITY, OVIPOSITION, AND DEVELOPMENT OF DIAPHORINA CITRI (HOMOPTERA: PSYLLIDAE)

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EFFECT OF A NEEM BIOPESTICIDE ON REPELLENCY, MORTALITY, OVIPOSITION, AND DEVELOPMENT OF *DIAPHORINA CITRI* (HOMOPTERA: PSYLLIDAE)

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ABSTRACT

The biological effects of a neem-based biopesticide, containing 4.5% azadirachtin, were assessed against the Asian citrus psyllid, *Diaphorina citri* Kuwayama, a recently introduced insect pest and potential disease vector of citrus in the United States. Over the concentration range 11-180 ppm azadirachtin, no mortality of adult psyllids was observed when exposed to treated plants. Adult psyllids demonstrated a small but significant repellent effect from treated plants in a choice experiment, but showed no preference to oviposit on treated or untreated plants. Psyllid nymphs were susceptible to azadirachtin at very low concentrations and activity perhaps was due to developmental inhibition. At a concentration of 22.5 ppm azadirachtin, ecdysis was not observed past 4 days after treatment and all nymphs were dead within 7 days. The densities of psyllid nymphs on treated plants exposed to a greenhouse population were significantly reduced by concentrations as low as 10 ppm azadirachtin. Over the range of concentrations used in these experiments, the product caused no phytotoxicity to tender foliage of either citrus or orange jasmine plants. Field trials are warranted to determine suitability of neem-based biopesticides for inclusion in citrus integrated pest management programs.

Key Words: Asian citrus psyllid, *Diaphorina citri*, citrus, neem, azadirachtin

RESUMEN

Los efectos biológicos de un bioplaguicida producido en base de neem, que contiene 4.5% de azadirachtina, fueron evaluados contra el psílido asi·tico de los cítricos, *Diaphorina citri* Kuwayama, una plaga recién introducida y vector potencial de enfermedades en cítricos en los Estados Unidos. Atravéz de un rango de concentración de 11-180 ppm de azadirachtina, ninguna mortalidad en los adultos de psílidos fue observada cuando fueron expuestos a plantas tratadas. Los adultos de psílidos demonstraron un pequeño pero significativo efecto repelente en las plantas tratadas en un experimento de seleccion, pero no mostraron ninguna preferencia para ovipositar en plantas tratadas o no tratadas. Las ninfas de los psílidos fueron susceptibles a la azadirachtina en concentraciones muy bajas y la actividad posiblemente fue debida a la inhibición para desarrollarse. En la concentración de 22.5 ppm de azadirachtina, no se observo la ecdisis después de 4 dias del tratamiento y todas las ninfas estuvieron muertas en un periodo de 7 dias. La densidad de las ninfas de psílidos sobre plantas tratadas expuestas a una población del invernadero fue reducida significativamente por concentraciones tan bajas como 10 ppm de azadirachtina. Sobre todo el rango de concentraciones usadas en estos experimentos, el producto no causo fitotoxicidad al follaje tierno de los citricos o en planta *Murraya paniculata*. Es necesario hacer mas pruebas de campo para determinar el uso de los bioplaguicidas con el base de neem para incluirlos en programas de manejo integrado de plagas en citricos.

Interest in the use of biopesticides with selectivity towards phytophagous insects has increased in recent years, particularly in cropping systems that rely on natural enemies as a major component of integrated pest management (Tengerdy & Szakács 1998; Rausell et al. 2000). Use of these natural compounds in place of conventional insecticides can reduce environmental pollution, preserve nontarget organisms, and avert insecticide-induced pest resurgence. The neem tree, *Azadirachta indica* A. Juss., produces the biodegradable and insecticidal liminoid azadirachtin (Isman 1999). The compound can be efficiently extracted from neem seeds where its concentration is greatest (Butterworth & Morgan 1968; Schroeder & Nakanishi 1987). The insecticidal activity of azadirachtin has been demonstrated against numerous insect pests (Schmutterer & Singh 1995), and its various modes of activity can include disruption of feeding, reproduction, or development (Mordue (Luntz) et al. 1998; Walter 1999). The fact that azadirachtin is selective toward phytophagous insects with minimal toxicity to beneficial insects increases its

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potential value to pest management (Lowery & Isman 1995; Naumann & Isman 1996).

Pest management programs in citrus particularly rely on natural enemies to assist in maintaining destructive insect populations below economic injury levels. When chemical intervention is required to reduce pest populations, the use of selective insecticides helps to conserve natural enemies. Tang et al. (2002) demonstrated that azadirachtin was effective in controlling the brown citrus aphid, *Toxoptera citricida* (Kirkaldy), an important vector of citrus tristeza virus. Effective control of the aphid was achieved without harming its native parasitoid, *Lysiphlebus testaceipes* (Cresson). Weathersbee & Tang (2002) indicated that azadirachtin inhibited the development of *Diaprepes abbreviatus* (L.) larvae, a root weevil pest of citrus, disrupted reproduction in the adults, and protected the roots of citrus seedlings from feeding damage caused by larvae.

A more recently introduced exotic pest of citrus is the Asian citrus psyllid, *Diaphorina citri* Kuwayama, that was detected in Florida in 1998 (Hoy & Nguyen 1998). It prefers feeding on the phloem tissues of tender shoots and leaves where it induces abnormal growth and shoot dieback. Furthermore, it excretes copious honeydew that promotes the growth of sooty mold, in turn reducing photosynthesis, plant growth, and yield (Chien & Chu 1996; Tsai et al. 2002). The greatest threat associated with *D. citri* to the citrus industry is its capacity to vector *Liberobacter asiaticum* (L.) Jack, the bacterium that causes citrus greening disease. Citrus greening has been declared the most devastating disease of citrus worldwide resulting in unmarketable fruit, yield reductions, tree dieback, and eventual death of infected trees (McClean & Schwarz 1970; da Graca 1991).

Natural enemies, including the parasitoids *Tamarixia radiata* (Waterston) and *Diaphorencyrtus aligarhensis* (Shafee, Alam, and Agarwal), have been imported and released to assist in biological control of *D. citri* (McFarland & Hoy 2001). Selective biopesticides that aid in managing psyllids without harming these natural enemies may be needed. Since azadirachtin has been effective in controlling other phloem-feeding and root-feeding pests of citrus (Tang et al. 2002; Weathersbee & Tang 2002), we investigated a commercial neem seed extract containing 4.5% azadirachtin to determine its biological activity against *D. citri*, specifically its potential effects on repellency, oviposition, and mortality of adult psyllids; and development and mortality of psyllid nymphs.

Materials and Methods

Insect Source and Rearing

Laboratory colonies of *D. citri* were established with specimens collected from dooryard grapefruit trees (*Citrus paradisi* Macf.) in Fort Pierce, FL, during March 2000. Colonies were maintained in screened cages (70 × 70 × 70 cm) on ornamental orange jasmine plants, *Murraya paniculata* (L.) Jack grown in 140-cm^3 containers with potting soil (Metromix 500, Scotts, Marysville, OH). Plants were maintained in a greenhouse and regularly pruned and fertilized to promote production of new growth that is preferred for oviposition by adult females. The colony maintained a sex ratio of about 1:1 which is similar to the value of 0.5224 reported by Tsai & Liu (2000).

Biopesticide Source

Neemix 4.5® (4.5% azadirachtin) was obtained from Certis USA, Columbia, MD. The product was screened at concentrations ranging from 0 to 0.4% vol:vol (i.e., 0 to 180 ppm azadirachtin, diluted with distilled water) to evaluate a range of biological effects on psyllid adults and nymphs including potential repellency, toxicity, reproductive disruption, and developmental inhibition.

Adult Psyllid Choice Experiment

Pineapple sweet orange, *Citrus sinensis* (L.), seedlings were grown to 1.5-2.5 cm tall in Rootcubes® Growing Media (Smithers-Oasis, Inc., OH, USA). The seedlings were arranged four per container inside polycarbonate Magenta vessels (77 × 77 × 97 mm) with vented polypropylene lids (Sigma). Two each of the seedlings per container were treated with azadirachtin at concentrations of 11.3, 45, or 180 ppm and two each were untreated to facilitate choice in the experiment. The aerial portion of each seedling was dipped for 5 s in the appropriate azadirachtin suspension or distilled water and then dried for 1 h before they were randomly arranged in the vessels. The vessels were labeled according to azadirachtin concentration and seedlings within each vessel were labeled according to choice option (treated or untreated). Twenty adult psyllids were introduced into each vessel and were allowed to settle on the plants or inner surfaces of the enclosure. The vessels were maintained in a growth chamber at 25 ± 1°C, 60-80% RH, and a photoperiod of 14:10 (L:D). There were six replications for each azadirachtin concentration. The numbers of psyllids that settled on each of the seedlings per vessel were recorded after 4 and 8 h, and at 1, 2, 3, 4, and 7 d. The total numbers of eggs deposited on each seedling were recorded after 7 d.

Effect on Psyllid Nymph Development

Pineapple sweet orange seedlings were grown and treated as described above at concentrations of 0, 11.3, 22.5, 45, 90, or 180 ppm azadirachtin. Eight 2nd instar psyllid nymphs were transferred to each seedling and the seedlings were placed singly in Petri dishes (9 cm diameter × 3 cm deep) and maintained in a growth chamber at 25 ± 1°C, 60-80% RH, and a photoperiod of 14:10 (L:D). There were eight replications for each azadirachtin concentration. The numbers of live nymphs remaining and exuviae produced in each petri dish were recorded after 1, 2, 3, 4, and 7 d.

Effect on Survival of Nymphs in Greenhouse

Orange jasmine seedlings were pruned to 10 cm tall and fertilized to force new growth. The seedlings (120) were placed in a screened cage (70 × 70 × 70 cm) and maintained in a small greenhouse. Approximately 400 adult psyllids were introduced into the cage and psyllid populations were allowed to develop for 7 d after which time the seedlings had become infested with 50-150 nymphs per plant. Each seedling was randomly assigned to one of four treatments (30 replications per treatment): water containing 0, 10, 30, or 90 ppm azadirachtin. Plants were sprayed until runoff with a hand-held sprayer, dried for 1 h, and then returned to the cage. Half of the 30 replications (plants) for each treatment were examined after 5 d to determine the numbers of eggs, dead nymphs, and live nymphs per plant. Data were collected on the remaining 15 replications after 7 d.

Data Analyses and Statistics

Data collected from the adult psyllid choice experiment were subjected to PROC FREQ and differences in the frequencies of adult settling and oviposition were determined with Pearson's chi-square test (SAS Institute 1999). Data from the nymph development and greenhouse population experiments were subjected to analysis of variance by PROC GLM and treatment differences were determined by Tukey's studentized range test or the least-squares means procedure (SAS Institute 1999). Differences among means were considered significant at a probability level of five percent ($P \leq 0.05$).

Data from the adult psyllid choice experiment were analyzed to determine the effects of treatment concentration and choice (treated or untreated) on the numbers of adult psyllids and eggs observed on each citrus seedling. Data from the nymph development experiment were analyzed to determine the effects of treatments on daily nymph survival and cumulative production of exuviae in each petri dish. Data from the greenhouse population experiment were analyzed to determine the effects of treatments on the numbers of eggs, live nymphs and dead nymphs found on orange jasmine seedlings after 5- and 7 d. Survival rates for nymphs during the greenhouse population experiment were calculated for each treatment as the ratio of live nymphs to total nymphs (live plus dead) per seedling. Reduction rates for nymphs were calculated as 1 minus the population survival rate. Linear regression was used to fit a polynomial model to these data to describe the dose response (SAS Institute 1999).

Results

Adult Psyllid Choice Experiment

Adult psyllids given a choice of citrus seedlings either treated or untreated with azadirachtin demonstrated a small but highly significant preference for settling on untreated seedlings (χ^2 = 31.40; df = 1; $P < 0.0001$), indicating there was repellency due to treatments over the 7-d period of the experiment. The cumulative percentages of adult psyllids observed settling on treated and untreated seedlings were 44.03 and 55.97, respectively (Table 1). The frequency of settling within each treatment did not change over time (χ^2 = 11.20; df = 6; P = 0.0825) indicating that the repellent effect was consistent for 7 d. Adult settling was not affected by the concentration of azadirachtin (χ^2 = 0.59; df = 2; P = 0.7438), where the cumulative percentages settled on the seedlings were 32.82, 34.09, and 33.09 in the high, medium, and low treatments, respectively (data not shown but values can be calculated from Table 1). The interaction of azadirachtin concentration and

Table 1. Effect of azadirachtin on the frequency of *D. citri* adults settling on a choice of treated or untreated citrus seedlings. Counts were made after 4 and 8 h, and 1, 2, 3, 4, and 7 d.

Concentration (ppm azadirachtin)[a]	Frequency (%) of psyllids settling		χ^2
	Treated	Untreated	
11.3	349 (47.87)	380 (52.13)	1.32
45	305 (40.61)	446 (59.39)	26.47***
180	316 (43.71)	407 (56.29)	11.45***
Cumulative	970 (44.03)	1233 (55.97)	31.40***

[a]Each treatment was replicated 6 times and comprised 20 adult psyllids given choices of 2 treated and 2 untreated citrus seedlings.
***$P < 0.001$; chi-squared test of settling frequency on treated versus untreated citrus seedlings, df = 1 [SAS Institute 1999]).

choice was significant (χ^2 = 7.96; *df* = 2; *P* = 0.0187), and further examination of the data indicated that no significant repellency of adult psyllids occurred at the lowest rate of azadirachtin (Table 1).

Adult psyllids demonstrated no preference to oviposit on treated or untreated citrus seedlings. The cumulative percentages of eggs laid on treated (49.50) and untreated (50.50) seedlings were similar during the 7 d of the experiment (χ^2 = 0.09; *df* = 1; *P* = 0.7530) (Table 2). However, the concentration of azadirachtin affected the numbers of eggs produced by adult psyllids in each treatment (χ^2 = 136.83; *df* = 2; *P* < 0.0001). The frequency of eggs produced in each treatment increased from 200 to 500 as the concentration of azadirachtin increased from 11.3 to 180 ppm, indicating that treatments may have influenced adult psyllid reproduction (Table 2).

Effect on Psyllid Nymph Development

Both the survival (*F* = 193.89; *df* = 5, 140; *P* < 0.0001) and molting (*F* = 1316.74; *df* = 5, 140; *P* < 0.0001) of psyllid nymphs maintained on citrus seedlings were affected by azadirachtin concentration. Across 7-sampling d, the mean numbers of surviving nymphs and exuviae produced in each treatment declined with increasing treatment concentration (Fig. 1). The effect of time also was significant for both the numbers of nymphs (*F* = 863.61; *df* = 4, 140; *P* < 0.0001) and exuviae (*F* = 203.38; *df* = 4, 140; *P* < 0.0001) indicating that the numbers of nymphs and exuviae in each treatment changed during the 7 d of the experiment. The interaction of azadirachtin concentration and time also was significant for both nymphs (*F* = 18.26; *df* = 20, 140; *P* < 0.0001) and exuviae (*F* = 143.15; *df* = 20, 140; *P* < 0.0001), indicating that the responses to concentration over time differed among one or more levels of treatment.

Survival of nymphs was significantly (*P* ≤ 0.05, least-squares means) lower in all treatments than in the control for counts taken beyond 1 d after treatment (Fig. 1A). By d 7 of the experiment, no psyllid nymphs survived in any of the treatments with azadirachtin concentrations >11.3 ppm while survival in the control was similar to that reported by Tsai and Liu (2000) for psyllid nymphs developing on citrus.

The cumulative production of exuviae was significantly (*P* ≤ 0.05, least-squares means) greater in the control than in the other treatments for all sample intervals (Fig. 1B). Over the 7 d of the experiment, an average of 23 exuviae per treatment were produced by nymphs in the control while less than 5 were produced by those treated with even the lowest concentration of azadirachtin. The data indicate that azadirachtin may have acted as a developmental inhibitor.

Effect on Survival of Nymphs in Greenhouse

After 5 d, the numbers of eggs found on orange jasmine seedlings treated with azadirachtin at concentrations of 0, 10, 30, and 90 ppm were not significantly different (*F* = 2.01; *df* = 3, 56; *P* = 0.1233). The average number of eggs ranged from 9.2 to 24.9 among treatments; and although there appeared to be a trend for reduced egg eclosion, this could not be determined due to the variability within treatments (Table 3). The numbers of dead nymphs differed significantly (*F* = 4.63; *df* = 3, 56; *P* = 0.0058) among treatments while the numbers of live nymphs were only marginally different (*F* = 2.74; *df* = 3, 56; *P* = 0.0521). Regression analysis of the data indicated a significant relationship between azadirachtin concentration and population reduction of nymphs 5 d after treatment (*F* = 118.27; *df* = 3, 57; *P* < 0.0001). The relationship between nymph mortality and azadirachtin concentration was described by the cubic equation $y = 3.096x\ (\pm 0.514) - 0.100x^2\ (\pm 0.024) + 0.001x^3\ (\pm 0.000)$; $r^2 = 0.86$ (Fig. 2A). The intercept of the regression model was not significant; therefore, the data were fitted to a model that assumed a zero intercept. The predicted values given by the model for percent population reductions in each treatment corresponded well with observed values. The standard errors of the predicted means did not exceed ±3.0%. Though significant, the regression model indicated that azadirachtin did

TABLE 2. EFFECT OF AZADIRACHTIN ON THE FREQUENCY OF EGGS OVIPOSITED BY *D. CITRI* S ON A CHOICE OF TREATED OR UNTREATED CITRUS SEEDLINGS. COUNTS OF EGGS WERE MADE AFTER 7 D.

Concentration (ppm azadirachtin)[a]	Frequency (%) of eggs oviposited		
	Treated	Untreated	χ^2
11.3	100 (50.00)	100 (50.00)	0.00
45	170 (54.84)	140 (45.16)	2.90
180	230 (46.00)	270 (54.00)	3.20
Cumulative	500 (49.50)	510 (50.50)	0.09

[a]Each treatment was replicated 6 times and comprised 20 adult psyllids given choices of 2 treated and 2 untreated citrus seedlings.
****P* < 0.001; chi-square test of oviposition frequency on treated versus untreated citrus seedlings, *df* = 1 [SAS Institute 1999]).

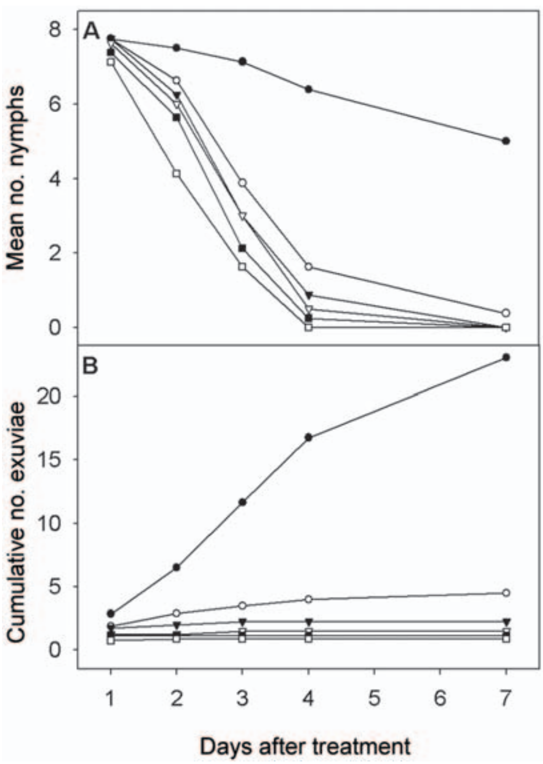

Fig. 1. Survival (A) and development (B) of *D. citri* nymphs on citrus seedlings treated with azadirachtin. The number of nymphs and exuviae were counted on 1, 2, 3, 4, and 7 days after treatment. 0 ppm = ●; 11.3 ppm = ○; 22.5 ppm = ▼; 45 ppm = ▽; 90 ppm = ■; and 180 ppm = □. Least-squares means for the number of nymphs and cumulative exuviae in each treatment are presented by day; SEs of least-squares means for nymphs and cumulative exuviae were ± 0.24 and ± 0.27, respectively.

not effectively control psyllid populations in 5 d since the highest concentration produced only a 50% reduction in the treated population.

After 7 d, the numbers of eggs found on the seedlings were not different ($F = 0.69$; $df = 3, 56$; $P = 0.5643$) among treatments. The average number of eggs per seedling ranged from 3.5 to 8.3 among treatments (Table 3). Both the numbers of dead nymphs ($F = 19.12$; $df = 3, 56$; $P < 0.0001$) and live nymphs ($F = 9.07$; $df = 3, 56$; $P < 0.0001$) were significantly influenced by treatments. The numbers of live nymphs in all azadirachtin treatments were significantly ($P \leq 0.05$) less than in the control 7 d after treatment. After 7 d, the regression of azadirachtin concentration on reduction of nymph populations was significant ($F = 149.76$; $df = 3, 56$; $P < 0.0001$). Both the F-statistic and regression coefficient were greater than those obtained for data collected after 5 d. The relationship between percent reduction of nymphs and azadirachtin concentration was described by the cubic equation $y = 6.811 (\pm 3.128) + 9.609x (\pm 0.699) - 0.311 x^2 (\pm 0.029) + 0.002x^3 (\pm 0.000)$; $r^2 = 0.89$ (Fig. 2B). The intercept was significant and representative of the population reduction in the control. The model effectively described reductions in nymph populations over the range of tested concentrations. The standard errors of the predicted means did not exceed ±3.1%. Azadirachtin concentrations from 10 to 90 ppm provided 74 to 92% reductions of psyllid nymph populations after 7 d, indicating that the biopesticide was effective at low concentrations. The delayed mortality response may have been due to inhibition of nymph development by azadirachtin.

Discussion

Our laboratory and greenhouse experiments indicated that azadirachtin was effective in controlling *D. citri* nymphs at concentrations as low 10 ppm. The compound apparently acted as a developmental inhibitor of psyllid nymphs, since ecdysis was completely arrested within 4 days after treatment. A similar pattern of activity was observed against brown citrus aphid nymphs (Tang et al. 2002). While azadirachtin has been observed to act as an antifeedent against some phloem-feeding insects (Koul 1999; Koul et al. 1997), such effects were not observed by Lowery & Isman (1994) against aphids, or here against *D. citri*. We observed that psyllid nymphs remained attached to treated plants by their mouthparts after death, perhaps indicating that they attempted to continue feeding until death. It is possible that the psyllid nymphs in our experiments ingested azadirachtin while feeding on the phloem tissues of the treated plants. The systemic activity of azadirachtin to other insects such as leafminers and thrips has been demonstrated previously (Weintraub & Horowitz 1997; Thoeming et al. 2003). *Diaphorina citri* is a serious pest to the citrus industry, not only for the damage it causes citrus due to feeding, but also because it is the most efficient vector of the bacterium that causes citrus greening disease (Tsai et al. 2002). If this disease is introduced to North America, its transmission by the psyllid throughout citrus producing regions potentially could devastate the industry. Novel integrated pest management strategies might prevent the psyllid from achieving this end. The use of biopesticides that are compatible with biological controls currently in place are desirable in crops such as citrus that often cover large acreages. Neem-based products, known for their selectivity, have been shown to effectively control other phloem-feeding insects including several species of Aphididae (Lowery et al. 1993; Lowery & Isman 1994; Schmutterer & Singh 1995; Koul et al. 1997; Koul 1998, 1999; Tang et al. 2002) and at least one species of Psyllidae, the pistachio psyllid, *Agonoscena targionii* (Lisht.) (Lababidi 2002).

TABLE 3. EFFECT OF AZADIRACHTIN ON A GREENHOUSE POPULATION OF *D. CITRI* ON ORANGE JASMINE SEEDLINGS. EGGS AND NYMPHS WERE ENUMERATED ON PLANTS AT 5 AND 7 D AFTER TREATMENT.

Concentration (ppm azadirachtin)	Mean no. per seedling (n = 15)[a]		
	No. eggs	No. live nymphs	No. dead nymphs
5 d after treatment			
0	9.2 ± 5.4 a	77.1 ± 13.5 a	2.6 ± 0.5 b
10	10.6 ± 4.0 a	57.7 ± 13.5 ab	16.5 ± 4.8 ab
30	13.1 ± 6.0 a	44.0 ± 9.2 ab	13.1 ± 2.7 ab
90	24.9 ± 4.6 a	32.5 ± 9.5 b	23.8 ± 6.0 a
7 d after treatment			
0	3.5 ± 1.6 a	74.3 ± 14.4 a	5.9 ± 1.4 c
10	8.3 ± 3.4 a	13.3 ± 2.1 b	48.7 ± 9.9 ab
30	8.1 ± 3.0 a	9.5 ± 2.5 b	36.2 ± 7.8 bc
90	7.5 ± 2.7 a	6.3 ± 1.3 b	78.3 ± 15.3 a

[a]For a particular sample day, means within a column sharing the same letter were not significantly different (P > 0.05, Tukey's studentized range test [SAS Institute 1999]).
[b]Survival rate = no. live nymphs/(no. live nymphs + no. dead nymphs).

Azadirachtin did not kill adult psyllids within the range of concentrations (maximum 180 ppm) or period of evaluation (maximum 7 d) used in these experiments. Although some repellency of adults was demonstrated statistically, the observed amount was not particularly meaningful. Reduced or no activity has been reported for neem-based products against the adult stage of other insect species (Lowery & Isman 1994; Koul 1999; Tang et al. 2002; Weathersbee & Tang 2002). Also, there was no preference for adult psyllids to oviposit on treated or untreated plants; interestingly however, more eggs were oviposited as the concentration of azadirachtin increased (Table 2). Viability of the eggs was not determined in this experiment but reproductive effects such as egg sterility have been reported for neem-based products with other insect species (Ascher 1993; Weathersbee & Tang 2002).

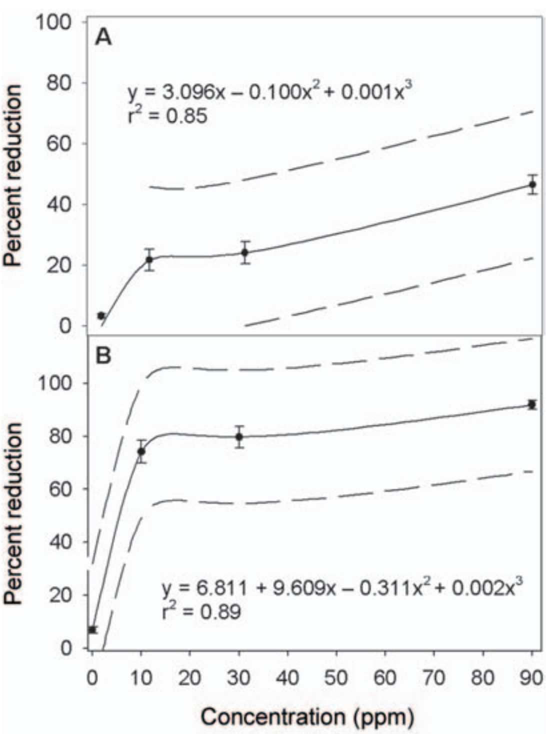

Fig 2. Relationship between percent reduction of populations of *D. citri* nymphs after 5 d (A) and 7 d (B), and concentration of azadirachtin sprayed on orange jasmine seedlings. A polynomial model was fitted to the data to develop the prediction equation. Vertical lines denote standard errors of the means and dashed lines denote 95% confidence limits for the prediction equation.

Our results indicate that azadirachtin can be applied at very low concentrations to effectively manage developing *D. citri* populations. This botanical insecticide has been found to be relatively nontoxic to beneficial insects (Lowery & Isman 1995; Naumann & Isman 1996; Walter 1999; Tang et al. 2002) and should be safe to apply in the presence of natural enemies found in citrus, particularly at concentrations required to control psyllid nymphs. Furthermore, no phytotoxicity was observed to any of the plant tissues used in these experiments. Neem biopesticides may therefore be well-suited for inclusion in citrus integrated pest management programs. Since low concentrations of neem-based products have been demonstrated to be effective against *D. citri* and other important citrus pests (Villanueva-Jiménez et al. 2000; Tang et al. 2002; Weathersbee & Tang 2002), field trials are warranted to determine appropriate methods of application and efficacy in commercial settings.

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