# Spray Volume and Frequency Impacts on Insecticide Efficacy Against the Citrus Mealybug (Hemiptera: Pseudococcidae) on Coleus under Greenhouse Conditions[1]

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**Abstract** The citrus mealybug *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) is a major insect pest of greenhouse-grown horticultural crops. The citrus mealybug causes direct damage by feeding on plant leaves, stems, flowers, and fruits, which can lead to substantial economic losses. Consequently, insecticides are used to suppress citrus mealybug populations in greenhouse production systems. However, application factors may influence the efficacy of insecticides in suppressing citrus mealybug populations. Therefore, experiments were conducted under greenhouse conditions to determine the effect of spray volume and application frequency on insecticide efficacy against the citrus mealybug feeding on coleus, *Solenostemon scutellarioides* (L.) Codd, plants. Four spray volumes (15, 25, 50, and 75 mL), 2 application frequencies (1 or 2 applications), and 3 insecticides (acetamiprid [TriStar®], flonicamid [Aria®], and cyfluthrin [Decathlon®]), each with a different mode of action, were tested. Based on mean percent citrus mealybug mortality, acetamiprid was more effective against citrus mealybugs than flonicamid or cyfluthrin. In the spray volume experiments, acetamiprid applied at 75 mL to each plant resulted in a mean percent citrus mealybug mortality of over 70%. In contrast, flonicamid and cyfluthrin resulted in less than 50% mean citrus mealybug mortality across all experiments. In the application frequency experiments, two spray applications did not result in a significantly higher mean citrus mealybug mortality than one spray application. Our study emphasizes the importance of spray volume and application frequency when using insecticides to suppress citrus mealybug populations.

**Key Words** application factors, frequency interval, insecticides, mortality, nymphs

The citrus mealybug *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) is an important insect pest of greenhouse-grown horticultural crops that causes direct damage by feeding on plant leaves, stems, flowers, and fruits (McKenzie 1967, Franco et al. 2009). Citrus mealybugs feed on plant fluids within the phloem by using their piercing-sucking mouthparts, and extensive infestations can lead to wilting, leaf chlorosis, leaf drop, premature fruit drop, stunted growth, and even death of host plants (Mani and Shivaraju 2016). During feeding, citrus mealybugs excrete honeydew, a clear, sticky liquid that serves as a substrate for black sooty mold. Black sooty mold can inhibit photosynthesis and reduce the overall aesthetic quality of plants (Charles 1982). Movement of infested plant material is the primary

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means of citrus mealybug dispersal because nymphs will move among plants via contacting foliage (Tanwar et al. 2007). One adult female citrus mealybug can lay more than 400 eggs during her lifetime (Copeland et al. 1985); thus, managing citrus mealybug populations by killing nymphs before they develop into adults is important.

Insecticides are primarily used against citrus mealybugs in greenhouse production systems (Parrella 1999, Franco et al. 2009). Systemic insecticides do not provide sufficient mortality of citrus mealybugs on greenhouse-grown horticultural crops, even at 8 times the label rate, which may be associated with citrus mealybugs not ingesting lethal concentrations of the active ingredient when feeding (Herrick and Cloyd 2017, Herrick et al. 2019). Therefore, greenhouse producers rely on foliar applications of contact insecticides. Nonetheless, contact insecticides have limited effectiveness against citrus mealybugs because (a) later instars and adults possess a hydrophobic waxy covering that protects them from insecticide sprays (Copeland et al. 1985, Venkatesan et al. 2016), (b) it is difficult to obtain thorough coverage with spray applications of all aboveground plant parts (leaves and stems) where citrus mealybugs feed, and (c) citrus mealybugs reside in protected or hidden areas on plants, which reduces the exposure of individuals to insecticide spray applications (Charles 1982, Franco et al. 2009, Herrick and Cloyd 2017). Insecticides are most effective against early-instar nymphs because they do not possess a hydrophobic waxy covering (Charles 1982, Ahmed and Abd-Rabou 2010, Venkatesan et al. 2016).

The efficacy of insecticide applications against target insect pests, such as the citrus mealybug, can be affected by several application factors, including spray coverage (Dibble 1962, McClure 1977, Shelton et al. 2003, Tipping et al. 2003, Shelton et al. 2006, Martini et al. 2012) and application frequency (Story and Sundstrom 1986, Ajeigbe and Singh 2006). High-volume insecticide applications that result in thorough coverage of plant parts (leaves and stems) can lead to improved suppression of citrus mealybug populations (Venkatesan et al. 2016).

No greenhouse studies have been conducted to assess the effects of spray volume (which can influence coverage of plant parts—leaves and stems) and application frequency on insecticide efficacy against citrus mealybugs. Therefore, the objectives of our study were to determine if spray volume and application frequency influence the efficacy of insecticides against citrus mealybugs (based on mortality) by conducting a series of controlled greenhouse experiments.

## Materials and Methods

**Citrus mealybug colony.** Citrus mealybugs were obtained from a laboratory colony in the Department of Entomology at Kansas State University (Manhattan, KS). The citrus mealybug colony is maintained on butternut squash (*Cucurbita maxima* Lamarck), purchased from a local supermarket, at 24 to 27°C, 50 to 60% relative humidity, and under constant light. The laboratory colony has been maintained for nearly 20 years and has never been exposed to insecticides. Specimens used in this research were deposited as voucher number 262 in the Kansas State University Museum of Entomological and Prairie Arthropod Research (Manhattan, KS).

**Plant material and procedures.** Coleus, *Solenostemon scutellarioides* (L.) Codd, plants were used in a series of greenhouse experiments conducted from August 2018 through November 2019. The experiments tested the effect of spray volume and application frequency on insecticide efficacy against citrus mealybugs feeding on coleus plants. Coleus plugs (young plants, either seedlings or cuttings grown as single units in modular trays) were purchased from Ball Horticultural Company (West Chicago, IL). Coleus plugs (cultivar 'Redhead') were transplanted into 15.2-cm diameter containers with a growing medium (Pro-Mix BX; Premier Tech Horticulture, Quakertown, PA, USA) composed of 75 to 85% coarse sphagnum peat moss, perlite, vermiculite, limestone, and a wetting agent. The coleus plants were arranged on a wire-mesh bench in a research greenhouse and irrigated with approximately 500 mL of tap water. In all the experiments, coleus plants were fertilized at least once with Miracle-Gro® Water Soluble All Purpose Plant Food (Scotts Miracle-Gro Products, Inc., Maryville, OH, USA) consisting of 24-3.5-13.2 (N-P-K) at a rate of 15 g/3.8 L of tap water. Each coleus plant received approximately 500 mL of fertilizer solution.

Coleus plants were positioned on a wire-mesh bench in a research greenhouse so leaves of adjacent plants were not touching to ensure no movement of nymphs among plants after they were infested with citrus mealybugs. Each plant was artificially infested with 10 to 15 second to early-third instar citrus mealybug nymphs, obtained from the laboratory colony, by using a fine-point paintbrush. Citrus mealybug nymphs were placed among several different leaves on each coleus plant. Nymphs were allowed to acclimate for 2 to 3 d before the designated treatments were applied. Greenhouse conditions for all experiments were 22 to 24°C with a relative humidity between 60 and 70% under natural daylight conditions.

All insecticide treatments were mixed in 946 mL of tap water, and spray applications were made to the designated coleus plants using a 946-mL plastic spray bottle (Spraymaster®; Delta Industries™, King of Prussia, PA). Experiments evaluated spray volume over a 4-week period with 3 weekly spray applications of the designated insecticide treatment to each coleus plant. In the spray volume experiments, 4 spray volumes were used (15, 25, 50, and 75 mL). These spray volumes were used based on the results from preliminary experiments determining spray coverage of coleus plants and associated citrus mealybug mortality. The aboveground plant parts, including the upper and lower surfaces of leaves and stems of each coleus plant, were treated with the designated spray volume. Another set of experiments tested application frequency (one or two spray applications per week using a spray volume of 50 mL per coleus plant) over a 2-week period.

For each experiment, there were four treatments, including three insecticides registered for use against citrus mealybugs in greenhouses and a water control. All experiments were set up as a completely randomized design. One coleus plant was considered an experimental unit. Coleus plant growth was measured over the course of each experiment with height (cm) and number of leaves recorded twice for each coleus plant, including once on the day the treatments were first applied and again on the day coleus plants were destructively sampled (Table 1). The insecticides and application rates used were as follows: acetamiprid (TriStar® 30 SG; Cleary Chemical Corporation, Dayton, NJ) at 0.19 g/946 mL, flonicamid (Aria®; FMC Corporation, Philadelphia, PA) at 0.15 g/946 mL, and cyfluthrin (Decathlon® 20

**Table 1. Mean plant height (cm) and number of leaves of coleus, *Solenostemon scutellarioides*, plants used as a host for citrus mealybug, *Planococcus citri*, in experiments before and after treatments were applied, and the mean difference in growth based on plant height and number of leaves (after measurement-before measurement) for each experiment.**

| Experiment Number | Experiment Type* | Number of Coleus Plants | Mean Plant Height (cm) | | | Mean Number of Leaves | | |
|---|---|---|---|---|---|---|---|---|
| | | | Before** | After*** | Difference | Before** | After*** | Difference |
| 1 | AF | 40 | 24.3 | 26.8 | 2.5 | 116.2 | 120.6 | 4.4 |
| 1 | SV | 60 | 26.5 | 32.8 | 6.3 | 120.4 | 125.3 | 4.9 |
| 2 | AF | 40 | 26.3 | 29.7 | 3.4 | 124.5 | 127.9 | 3.4 |
| 3 | SV | 48 | 23.9 | 42.3 | 18.4 | 58.2 | 122.3 | 64.1 |
| 4 | AF | 32 | 35.1 | 35.3 | 0.2 | 122.5 | 124.3 | 1.8 |
| 4 | SV | 48 | 32.3 | 32.9 | 0.6 | 124.4 | 128.4 | 4.0 |

* AF, application frequency; SV, spray volume.

** Plant height and number of leaves were measured the day treatments were applied.

*** Plant height and number of leaves were measured the day coleus plants were destructively sampled.

WP; OHP, Inc., Bluffton, SC) at 0.13 g/946 mL. All three insecticides have different modes of action (IRAC 2019).

For the spray volume experiments, treatments were applied to coleus plants once every 7 d for 3 weeks. On the fourth week, 7 d after the final spray application, whole coleus plants were destructively sampled. The number of live, dead, and total number of citrus mealybugs associated with each coleus plant were recorded. Percent mortality of citrus mealybugs was calculated by dividing the number of dead citrus mealybugs (those that did not move after prodding with a dissecting probe) by the total number of citrus mealybugs recovered on each coleus plant and multiplying by 100. Egg-laying females and male pupae were counted as alive because they survived the treatments long enough to develop beyond the second and third instars. In addition, first-instar nymphs, associated with the $F_1$ generation, were counted on each coleus plant based on increments of 50 individuals.

In the application frequency experiments, all coleus plants were treated on the same day. Seven days after the first spray application, the number of live, dead, and total number of citrus mealybugs associated with each coleus plant designated to receive one spray application were recorded, and coleus plants assigned to two spray applications per week were treated a second time. Seven days after the second spray application, the number of live, dead, and total number of citrus mealybugs affiliated with each coleus plant were recorded for the remaining coleus plants. Percent citrus mealybug mortality was calculated by dividing the number of dead citrus mealybugs by the total number of citrus mealybugs recovered on each coleus plant and multiplying by 100. Egg-laying females, male pupae, and first-instar nymphs were counted similar to the spray volume portion of the experiment.

**Experiment 1: Spray volume and application frequency.** Three spray volumes were used (25, 50, and 75 mL). Coleus plants associated with each spray volume were treated three times, and then destructively sampled 7 d after the final spray application.

For application frequency, a spray volume of 50 mL was used. Coleus plants were treated and then those coleus plants that received one spray application were destructively sampled. Coleus plants designated to receive two spray applications were treated a second time. After 7 d, the number of live, dead, and total number of citrus mealybugs on each coleus plant were recorded for the remaining coleus plants. There were 5 replications for each treatment combination for a total of 40 coleus plants for spray volume and 60 coleus plants for application frequency.

**Experiment 2: Application frequency.** A spray volume of 50 mL was used. Coleus plants received the first spray application, and 7 d later, the coleus plants designated to receive 1 spray application were destructively sampled. Coleus plants designated to receive 2 spray applications were treated a second time and then destructively sampled 7 d after the final spray application. There were 5 replications per treatment combination for a total of 40 coleus plants.

**Experiment 3: Spray volume on plants started from cuttings.** Cuttings were taken from established coleus plants. Each cutting (12.7 to 15.2 cm in length) was removed from the terminal growth of a single coleus plant. A single cutting was placed into a 15.2-cm diameter container with a standard soilless growing medium. Once the coleus plants (formerly cuttings) developed an established root system, they were fertilized in a manner similar to previous experiments.

Because the coleus plants originated from cuttings and not plugs, the plant architecture was different from coleus plants used in the other experiments. Plant architecture is the spatial arrangement of aboveground plant parts, including leaves, stems, and branches (Cloyd and Sadof, 2000). As the coleus plants, originating from cuttings, established, they developed two main branches and adopted a "V" shape. Furthermore, the coleus plants were shorter in height (cm) and had fewer leaves in the beginning of the experiment, which is why there was such variability in the before and after growth difference measurements compared to the other coleus plants (Table 1). The spray volumes used were 15, 50, and 75 mL to compensate for the differences in plant architecture. Coleus plants associated with each spray volume were treated three times, and then destructively sampled 7 d after the final spray application. There were 4 replications per treatment combination for a total of 48 coleus plants.

**Experiment 4: Spray volume and application frequency.** Coleus plants were pruned for uniformity before starting the experiment by removing approximately 10 cm of terminal growth. For spray volume, three spray volumes were used (25, 50, and 75 mL). Coleus plants associated with each spray volume were treated three times, and then each coleus plant was destructively sampled 7 d after the final spray application.

For application frequency, a spray volume of 50 mL was used. Coleus plants were treated with the first spray application, and coleus plants designated to receive one spray application were destructively sampled 7 d after the first spray application. The remaining coleus plants were treated a second time and then destructively sampled 7 d after the second spray application. There were 4 replications per treatment combination for a total of 48 coleus plants for the spray volume portion of the experiment and 32 coleus plants for the application frequency portion of the experiment.

**Statistical analysis.** There were two factors affiliated with each experiment, namely, treatment (four levels), and spray volume (three levels) or application frequency (two levels). Citrus mealybug percent mortality estimates were subject to a two-way analysis of variance with treatment and spray volume or application frequency as the main effects. Data were analyzed using PROC GLIMMIX in a SAS software program (SAS Institute 2012). Significant treatment means were separated using Tukey's honestly significant difference (HSD) test at $\alpha = 0.05$. Pairwise comparisons were conducted using Tukey's HSD adjustment for multiple comparisons to avoid type I error rate inflation.

## Results

**Experiment 1: Spray volume and application frequency.** The two-way interaction between treatment and spray volume was significant ($F = 6.61$; df = 6, 48; $P < 0.0001$). Acetamiprid had a significantly higher mean percent citrus mealybug mortality when applied at 75 mL (80.7%; $n = 50$) than 50 mL (46.9%; $n = 58$) or 25 mL (39.9%; $n = 60$). Flonicamid applied at 75 mL had a significantly higher mean percent citrus mealybug mortality (46.3%; $n = 52$) than 25 mL (27.8%; $n = 55$) (Fig. 1). There was no significant difference in mean percent citrus mealybug mortality for cyfluthrin among the three spray volumes applied (Fig. 1).

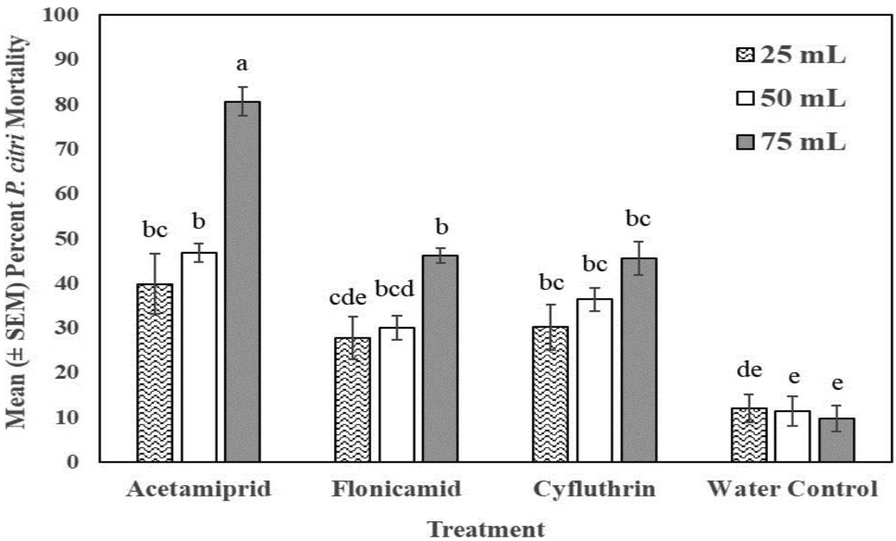


**Fig. 1. Mean (± SEM) percent citrus mealybug, *Planococcus citri*, mortality in experiment 1 using coleus, *Solenostemon scutellarioides*, plants after exposure to three weekly spray applications. The spray volumes were 25, 50, or 75 mL associated with the following treatments: (a) acetamiprid (TriStar®) at 0.19 g/946 mL, (b) flonicamid (Aria®) at 0.15 g/946 mL, (c) cyfluthrin (Decathlon®) at 0.13 g/946 mL, and (d) water control. Means with the same letter across all treatments are not significantly different ($P > 0.05$) based on a Tukey' honestly significant difference test.**

Coleus plants treated with 75 mL of acetamiprid had live male pupae, but no egg-laying females or first-instar nymphs were present. Nonetheless, first-instar nymphs and egg-laying females were present on every other coleus plant, except for those treated with acetamiprid at 75 mL. More than 100 first-instar nymphs were present on each coleus plant treated with water.

The two-way interaction between treatment and application frequency was not significant ($P > 0.05$). The effect of application frequency on mean percent citrus mealybug mortality was not significant ($P > 0.05$), but there was a significant effect of treatment on mean percent citrus mealybug mortality ($F = 51.16$; df $= 3, 32$; $P < 0.0001$). There were no significant differences in mean percent citrus mealybug mortality between coleus plants, within the insecticide treatments, that received one spray application and those that received two spray applications (Fig. 2). The mean percent citrus mealybug mortalities associated with coleus plants treated with flonicamid were not significantly different from the water control (Fig. 2).

No first-instar nymphs were present on coleus plants that were destructively sampled after 1 spray application, but at least 50 first-instar nymphs were present on each coleus plant that received 2 spray applications, regardless of treatment.

**Experiment 2: Application frequency.** The two-way interaction between treatment and application frequency was not significant ($P > 0.05$), but the main

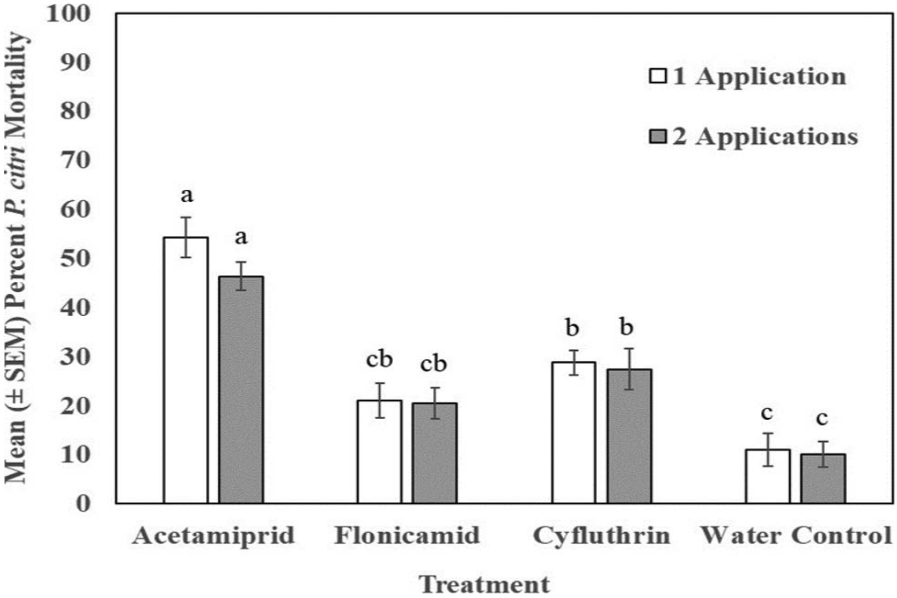


**Fig. 2. Mean (± SEM) percent citrus mealybug, *Planococcus citri*, mortality in experiment 1 using coleus, *Solenostemon scutellarioides*, plants after exposure to one or two spray applications of the following treatments at 50 mL of spray solution per plant: (a) acetamiprid (TriStar®) at 0.19 g/946 mL, (b) flonicamid (Aria®) at 0.15 g/946 mL, (c) cyfluthrin (Decathlon®) at 0.13 g/946 mL, and (d) water control. Means with the same letter across all treatments are not significantly different ($P > 0.05$) based on a Tukey's honestly significant difference test.**

effects of application frequency ($F = 5.31$; df = 1, 32; $P = 0.028$) and treatment ($F = 143.57$; df = 3, 32; $P < 0.0001$) associated with mean percent citrus mealybug mortality were significant. Within the two-way means for each treatment, there were no significant differences in mean percent citrus mealybug mortality between coleus plants treated once and those treated twice (Fig. 3). Acetamiprid applications, regardless of frequency, had a significantly higher mean percent citrus mealybug mortality than flonicamid and cyfluthrin (Fig. 3). Two spray applications of flonicamid or cyfluthrin resulted in a significantly higher mean percent citrus mealybug mortality than the water control (Fig. 3). However, mean percent citrus mealybug mortality for the water control was similar to one spray application of flonicamid or cyfluthrin (Fig. 3).

None of the coleus plants destructively sampled after one spray application had any first-instar nymphs. However, all coleus plants that received two spray applications had first-instar nymphs by the end of the experiment. Coleus plants treated with acetamiprid had less than 50 first-instar nymphs per coleus plant, whereas coleus plants treated with flonicamid, cyfluthrin, or water had more than 100 first-instar nymphs on each coleus plant.

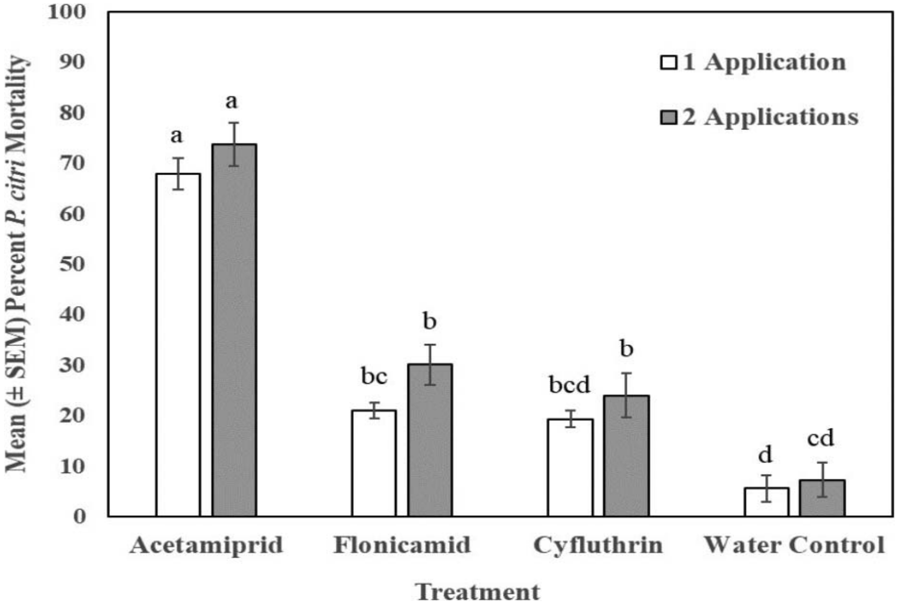


**Fig. 3. Mean (± SEM) percent citrus mealybug, *Planococcus citri*, mortality in experiment 2 using coleus, *Solenostemon scutellarioides*, plants after exposure to one or two spray applications of the following treatments at 50 mL of spray solution per plant: (a) acetamiprid (TriStar®) at 0.19 g/946 mL, (b) flonicamid (Aria®) at 0.15 g/946 mL, (c) cyfluthrin (Decathlon®) at 0.13 g/946 mL, and (d) water control. Means with the same letter across all treatments are not significantly different ($P > 0.05$) based on a Tukey's honestly significant difference test.**

**Experiment 3: Spray volume on plants started from cuttings.** The two-way interaction between spray volume and treatment was significant ($F = 4.31$; df = 6, 36; $P = 0.0023$). Acetamiprid applied at 75 mL resulted in a significantly higher mean percent citrus mealybug mortality (76.2%; $n = 28$) than 50 mL (44.7%; $n = 26$) or 15 mL (30.8%; $n = 32$) (Fig. 4). Flonicamid and cyfluthrin applied at 75 mL had a significantly higher mean percent citrus mealybug mortality than when applied at 15 mL (Fig. 4). Across all insecticide treatments, mean percent citrus mealybug mortalities affiliated with the 15-mL spray volume were not significantly different from the water control (Fig. 4).

Two coleus plants treated with 75 mL of acetamiprid had only 1 male pupa and no egg-laying females. There were no first-instar nymphs on coleus plants treated with 50 or 75 mL of acetamiprid. Coleus plants treated with 75 mL of flonicamid had less than 50 first-instar nymphs per coleus plant. As spray volume increased, the number of first-instar nymphs on coleus plants treated with cyfluthrin decreased, especially on coleus plants treated with 75 mL of spray solution. All coleus plants treated with water had more than 100 first-instar nymphs per coleus plant at the end of the experiment.

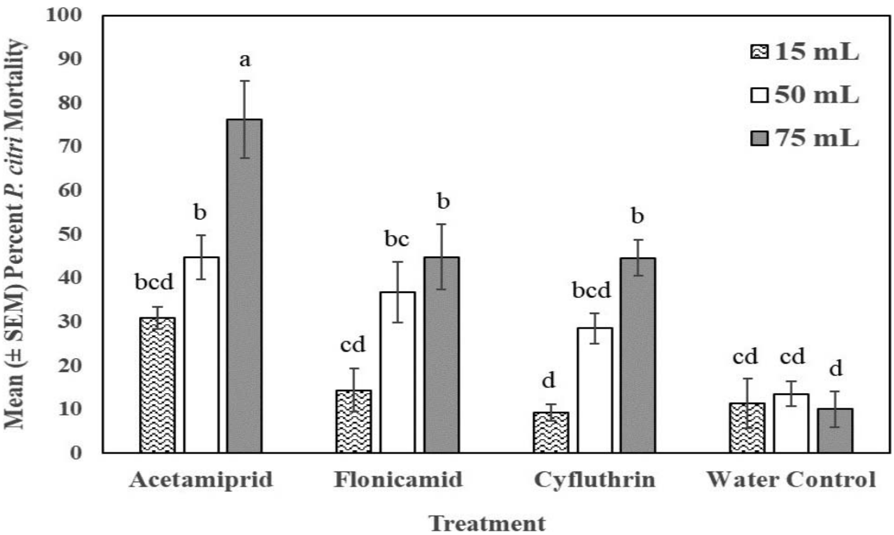


**Fig. 4. Mean (± SEM) percent citrus mealybug, *Planococcus citri*, mortality in experiment 3 using coleus, *Solenostemon scutellarioides*, plants after exposure to three weekly spray applications. The spray volumes were 15, 50, or 75 mL associated with the following treatments: (a) acetamiprid (TriStar®) at 0.19 g/946 mL, (b) flonicamid (Aria®) at 0.15 g/946 mL, (c) cyfluthrin (Decathlon®) at 0.13 g/946 mL, and (d) water control. Means with the same letter across all treatments are not significantly different ($P > 0.05$) based on a Tukey's honestly significant difference test.**

**Experiment 4: Spray volume and application frequency.** For spray volume, the two-way interaction between spray volume and treatment was significant ($F = 3.1$; df = 6, 36; $P = 0.015$). For all 3 insecticides, mean percent citrus mealybug mortality associated with the 75-mL spray volume was significantly higher than that with the 25-mL spray volume (Fig. 5). Mean percent citrus mealybug mortality was significantly higher for acetamiprid than flonicamid and cyfluthrin when applied at 75 mL (Fig. 5). Mean percent citrus mealybug mortality affiliated with flonicamid applied at 25 mL was not significantly different from the water control (Fig. 5).

Egg-laying female citrus mealybugs and male pupae were present on coleus plants across every treatment. However, less than 50 live first-instar nymphs were present on each coleus plant treated with 25 mL of acetamiprid. Coleus plants treated with 25 or 50 mL of flonicamid had more than 100 first-instar nymphs per coleus plant, and coleus plants treated with 75 mL of flonicamid had less than 50 first-instar nymphs per coleus plant. All coleus plants treated with cyfluthrin or water had more than 100 first-instar nymphs per coleus plant.

For application frequency, the two-way interaction between treatment and application frequency was not significant ($P > 0.05$). The effect of application frequency was not significant ($P > 0.05$), but the effect of treatment on mean percent citrus mealybug mortality was significant ($F = 33.77$; df = 3, 24; $P <$

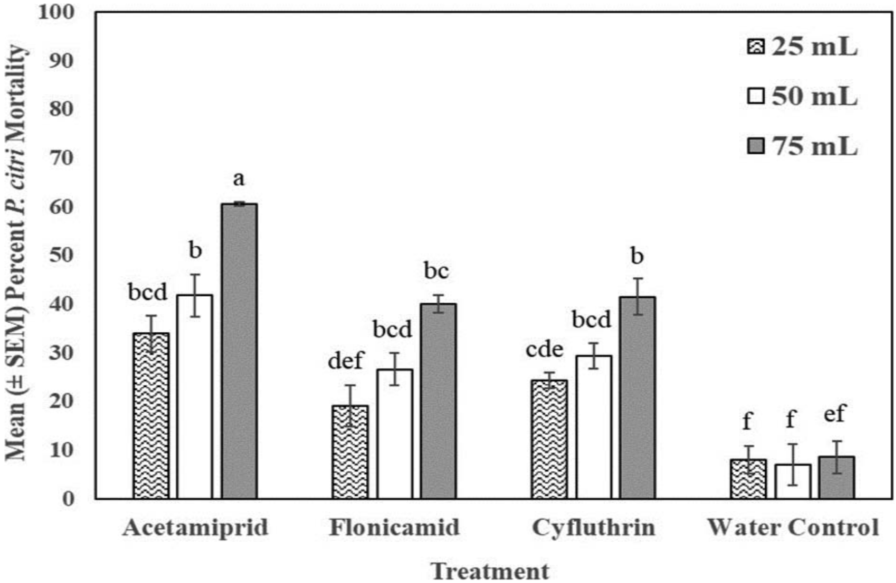


**Fig. 5. Mean (± SEM) percent citrus mealybug, *Planococcus citri*, mortality in experiment 4 using coleus, *Solenostemon scutellarioides*, plants after exposure to three weekly spray applications. The spray volumes were 25, 50, or 75 mL associated with the following treatments: (a) acetamiprid (TriStar®) at 0.19 g/946 mL, (b) flonicamid (Aria®) at 0.15 g/946 mL, (c) cyfluthrin (Decathlon®) at 0.13 g/946 mL, and (d) water control. Means with the same letter across all treatments are not significantly different ($P > 0.05$) based on a Tukey's honestly significant difference test.**

0.0001). Regardless of application frequency, mean percent citrus mealybug mortality associated with acetamiprid was similar to 2 spray applications of flonicamid (26.6%; $n = 61$) or cyfluthrin (28.1%; $n = 78$) (Fig. 6). Mean percent citrus mealybug mortality for one spray application of flonicamid or cyfluthrin was not significantly different from that for two spray applications of water (Fig. 6).

No first-instar nymphs were present on coleus plants after one spray application, but egg-laying females and male pupae were present. Nonetheless, all coleus plants that received 2 spray applications of flonicamid, cyfluthrin, and water had more than 100 first-instar nymphs per plant. After receiving 2 spray applications of acetamiprid, 2 coleus plants had no first-instar nymphs, whereas the other 2 coleus plants had less than 50 first-instar nymphs.

## Discussion

This study is the first to evaluate the effect of spray volume and application frequency on insecticide efficacy against the citrus mealybug under greenhouse conditions. Our study emphasizes the importance of understanding the application

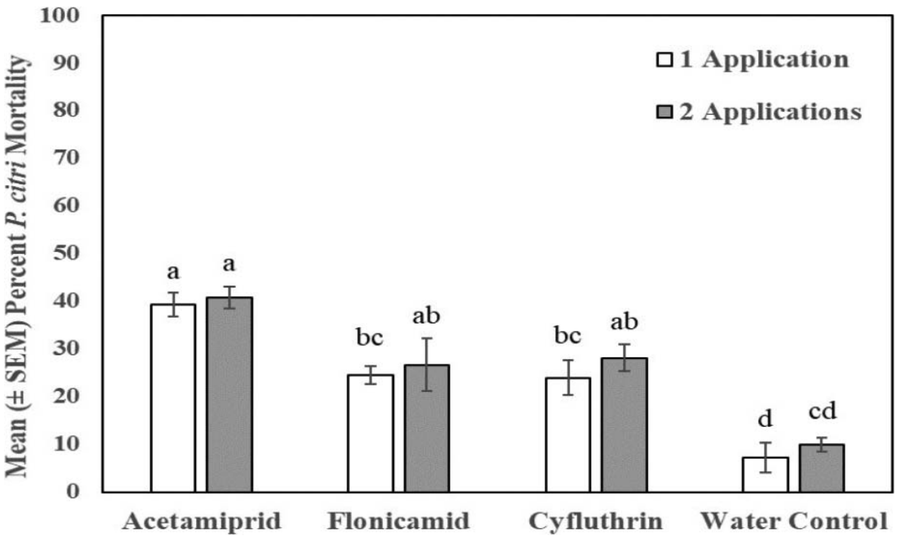


**Fig. 6. Mean (± SEM) percent citrus mealybug, *Planococcus citri*, mortality in experiment 4 using coleus, *Solenostemon scutellarioides*, plants after exposure to one or two spray applications of the following treatments at 50 mL of spray solution per plant: (a) acetamiprid (TriStar®) at 0.19 g/946 mL, (b) flonicamid (Aria®) at 0.15 g/946 mL, (c) cyfluthrin (Decathlon®) at 0.13 g/946 mL, and (d) water control. Means with the same letter across all treatments are not significantly different ($P > 0.05$) based on a Tukey's honestly significant difference test.**

factors associated with spray volume and application frequency when managing citrus mealybug populations in greenhouse production systems. Our study also compared the efficacy of three insecticides with different modes of action against the citrus mealybug. It is important to note that the laboratory colony of citrus mealybugs used in the study has never been exposed to insecticides, so the possibility of insecticide resistance is not likely.

In our study, we found that additional insecticide applications do not always result in better suppression of citrus mealybug populations. There are a number of application factors that can influence the efficacy of insecticides, including spray volume, spray nozzle type, spray application method, time spent spraying, and type of spray equipment (Dibble 1962, Martini et al. 2012). In fact, studies have demonstrated that more frequent insecticide applications can substantially reduce insect damage to crops (Story and Sundstrom 1986, Ajeigbe and Singh 2006). In addition, more frequent insecticide applications can lead to higher citrus mealybug mortality (Herrick and Cloyd; unpublished data). However, increasing the frequency of insecticide applications may induce the development of resistance in citrus mealybug populations, especially if insecticides with the same mode of action are used in succession (Flaherty et al. 1982, Venkatesan et al. 2016). Consequently, greenhouse producers must develop rotation programs that do not involve using insecticides with the same mode of action (Loughner et al. 2005, Bielza 2008, Venkatesan et al. 2016).

The proper implementation of application factors can increase insecticide efficacy (Dibble 1962, McClure 1977, Shelton et al. 2003, Tipping et al. 2003, Shelton et al. 2006, Martini et al. 2012). For instance, our study demonstrated that a higher spray volume results in improved suppression of citrus mealybug populations based on mean percent mortality. Across all experiments, the 75-mL spray volume consistently resulted in a higher mean percent citrus mealybug mortality than the 15- or 25-mL spray volumes. Mean percent citrus mealybug mortality must be at least 80% to minimize plant damage and reduce the number of individuals in subsequent generations (Herrick and Cloyd, 2017). The insecticides flonicamid and cyfluthrin, which were less effective against citrus mealybugs (based on mortality), may need to be applied at higher volumes to provide suppression levels of citrus mealybug populations that are acceptable to greenhouse producers. In addition, the 15- and 25-mL spray volumes resulted in citrus mealybug mortalities that were often similar to those of the water control. For example, in experiment 3, cyfluthrin, a contact insecticide, applied at 15 mL had a lower mean percent citrus mealybug mortality than the water control. Therefore, using a higher spray volume is important for managing citrus mealybug populations with contact insecticides.

There was an effect of insecticide type on the abundance of first-instar citrus mealybug nymphs. Citrus mealybug populations can be effectively managed if insecticides are applied when early-instar nymphs are present because they do not have the protective, waxy covering that later-instar nymphs and adults possess (Charles 1982). In general, coleus plants treated with acetamiprid and flonicamid had fewer first-instar nymphs than coleus plants treated with cyfluthrin, which may be associated with the translaminar activity of acetamiprid and flonicamid (Buchholz and Nauen 2002, Morita et al. 2014). Translaminar activity refers to the ability of an active ingredient to penetrate leaf surfaces after a foliar application and form a reservoir within plant tissues. Insects are killed after ingesting the active ingredient when feeding on leaf undersides, even if the insecticide was applied to the upper leaf surface (Buchholz and Nauen 2002).

Acetamiprid has been shown to suppress adult and first-instar nymphal populations of the sweetpotato whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) for up to 10 d after a foliar application (Horowitz et al. 1998). If first-instar citrus mealybug nymphs were not killed by direct contact with the insecticide sprays, they may have ingested lethal concentrations of the active ingredient when feeding on coleus leaves. Therefore, insecticides with translaminar activity may help alleviate problems with citrus mealybugs by killing nymphs, which reduces the number of potential egg-laying females. Acetamiprid consistently resulted in a higher mean percent citrus mealybug mortality than flonicamid and cyfluthrin. Hogendorp and Cloyd (2013) reported that acetamiprid applications can result in 84% mortality of citrus mealybugs feeding on coleus plants. Flonicamid and acetamiprid possess translaminar activity, but the two active ingredients may have different rates of uptake in coleus leaves (Wang and Liu 2007). The higher efficacy of acetamiprid may be related to the mode of action. For example, flonicamid acts as a selective feeding blocker causing starvation by inhibiting the ingestion of phloem, as observed with the green peach aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) (Cho et al. 2011, Morita et al. 2014). However, the mode of action of flonicamid may delay the time required to kill insect pests. In contrast, acetamiprid acts on the nicotinic acetylcholine receptors in the central nervous system (Zhang et al. 2000), which may lead to killing citrus mealybugs more quickly.

Application factors can influence the success of pest management programs against insect pests. Our study indicates that greenhouse producers must consider spray volume and application frequency when using insecticides to manage citrus mealybugs. Further studies need to be conducted to determine how plant architecture affects spray coverage by using water-sensitive paper and subsequent citrus mealybug mortality in relation to spray volume and application frequency. Regardless, because insecticides are primarily used against citrus mealybugs, greenhouse producers will benefit from improvements in insecticide applications, which will lead to a higher mortality of citrus mealybugs and lower insecticide inputs and labor costs.

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