Toxicity of insecticidal soaps to the Asian citrus psyllid and two of its natural enemies

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Abstract

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), is an important pest of citrus because it transmits plant pathogens responsible for a serious disease of citrus known as huanglongbing. Conventional insecticides are frequently used to manage ACP. Insecticidal soaps (hereafter 'soaps') are an insect control option labelled for commercial use as well as for use by homeowners and organic growers. Soaps have been shown to be toxic to some insect pests and therefore might be an alternative to conventional pesticides for control of ACP, but the efficacy of soaps against ACP was largely unknown. Our objective was to test whether different concentrations of two insecticidal soaps, M-Pede and Safer Insecticidal Soap Concentrate, caused mortality of ACP adults, nymphs and eggs. In addition, we tested whether these soaps were toxic to two natural enemies of ACP, adults of the lady beetle *Cycloneda sanguinea* (L.) (Coleoptera: Coccinellidae) and the parasitoid *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae). Direct sprays of M-Pede or Safer Insecticidal Soap were acutely toxic to ACP adults (regardless of gender) and nymphs when applied in solutions of 0.8–2% in water. Insecticidal soaps were non-toxic to eggs at rates of up to 2%. Residues of soap were less toxic to adult ACP than direct sprays, even when applied at concentrations of up to 4%. M-Pede or Safer soap at high concentrations (for example, 2% v/v in water) may be an effective alternative to conventional pesticides to manage adult and nymphaal ACP, although multiple applications may be needed if a target population includes eggs. A 2% concentration of either soap applied as a direct spray was non-toxic to adult *C. sanguinea* but acutely toxic to adult *T. radiata*. Soaps therefore may be compatible with biological control of ACP by adult coccinellids but not the parasitoid *T. radiata*.

Introduction

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), is an important pest of citrus because it vectors huanglongbing (HLB). HLB is one of the world’s most serious diseases of citrus and is putatively caused by bacteria of the genus ‘*Candidatus Liberibacter*’ (McClean and Schwartz 1970; Bové 2006). ACP likely originated in Asia (Husain and Nath 1927; Capoor 1963; da Graça 1991). In North America, ACP began invading the West Indies and Caribbean during the late 1990s, subsequently invaded many North and Central American countries and is now widespread throughout Mexico and in many areas of the United States (Étienne et al. 1998; French et al. 2001; Halbert and Manjunath 2004; Halbert and Núñez 2004). Asiatic HLB caused by ‘*Ca. Liberibacter asiaticus*’ has been confirmed in a number of locations in North America and new infection sites continue to be found.

Routine applications of insecticides are made by citrus growers in Florida and Texas in an effort to mitigate
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the incidence and spread of ACP and HLB (Hall and Gottwald 2011). Conventional insecticides such as imidacloprid, fenpropatrin, chlorpyrifos, carbaryl and dimethoate are registered for citrus in Florida (Browning et al. 2011) and are effective for controlling ACP (Rogers et al. 2011). However, conventional insecticides cannot be used to control ACP in certified organic groves and are generally unacceptable for ACP control in urban settings. Other application scenarios may exist where an alternative to a conventional pesticide would be desirable, for example, regulatory purposes such as eliminating ACP from post-harvest shipments of leaves destined for culinary or medicinal markets (Hall et al. 2010a) or post-harvest shipments of fruit (Hall and McCollum 2011).

Insecticidal soaps (hereafter ‘soaps’) are labelled for use by homeowners and also can be used in citrus grown for the organic market. Soaps might be an alternative to conventional pesticides for control of ACP, but little was known regarding the efficacy of soaps against ACP. McKenzie and Puterka (2004) included an insecticidal soap treatment in a field study and reported the soap caused moderate to high mortality of ACP. Soaps are generally reported to provide significant, but short-term, control of some mortality of ACP. Soaps are generally reported to provide significant, but short-term, control of some pest usually must come into direct contact with liquid soap to be killed and there is little residual activity after insecticidal soaps dry.

M-Pede (Gowan Company, LLC, Yuma, AZ) and Safer Insecticidal Soap Concentrate (Woodstream Corporation, Lititz, PA) are two soaps marketed as approximately 50% concentrations of potassium salts of fatty acids. Our objective was to test whether M-Pede or Safer soap caused mortality of adults, nymphs and eggs of ACP. In addition, we tested whether these soaps were toxic to two natural enemies of ACP, adults of the lady beetle *Cycloneda sanguinea* (L.) (Coleoptera: Coccinellidae) and adults of the parasitoid *Tamarixia radiata* (Waterston) (Hymenoptera: Eulo-

**Materials and Methods**

Asian citrus psyllid adults, nymphs and eggs were obtained from a colony described by Hall et al. (2010b). Briefly, the colony was established during early 2000 at the USDA-ARS U.S. Horticultural Research Laboratory (Fort Pierce, FL). The psyllids originally were collected from citrus in the field and subsequently reared in a greenhouse in cages containing orange jasmine, *Murraya paniculata* (L.) Jack, until March 2010, when *Citrus macrophylla* Wester was substituted as the rearing plant. The colony is maintained by transferring adults to new plants every 14 days using procedures similar to those described by Skelley and Hoy (2004).

The toxicity of insecticidal soaps to ACP and two ACP natural enemies was assessed in a series of six experiments. In each replicated experiment, one to several concentrations of soap were prepared (v/v in water) by adding the appropriate amount of soap to tap water for 100 ml (except where noted) of soapy water. Each soap at the concentrations studied readily mixed with tap water leaving no film, suggesting that no softening of the water was required (Culbert 2005). In four of the six experiments, subject insects were treated with soap and then placed onto a leaf disc for subsequent observations on mortality. These leaf discs were prepared as described by Hall and Nguyen (2010). Briefly, we excised mature citrus leaves (‘Duncan’ grapefruit, *Citrus paradisi* Macf.) from young trees maintained in a greenhouse. A 2.34-cm-diameter copper pipe with sharpened edges similar to a cork borer was used to cut each leaf disc. Each leaf disc was then embedded on agar (7 g/500 ml water) in a small Petri dish (suspension culture dish, 35 × 10 mm, non-treated polystyrene, #430588; Corning Inc., Corning, NY), and then the Petri dish lid was put in place and secured with a rubber band. The water associated with the agar generally maintains relative humidity (RH) in the dishes at 98–100% whether the dishes are held at ambient conditions or in an environmental chamber at 25°C and 75% RH (D. G. Hall, unpublished data).

**Experiment 1: direct sprays of adults with 0.2%, 0.4%, 0.8%, 1.6% or 2% soap**

Three groups of five male and three groups of five female ACPs (7 days after final moult) were each treated with a soap concentration or plain water (total
n = 60 groups not including water controls). Each group of five adults was aspirated into a vial, briefly anaesthetized with CO₂, placed onto filter paper and sprayed with soap until thoroughly wet using a 180-ml Nalgene aerosol spray bottle (#2430-0200; Fisher Scientific, Pittsburgh, PA). Each group of ACP was then transferred to a leaf disc (described earlier) using a small brush moistened with tap water. Petri dishes were closed and placed into an environmental chamber (26°C, 75% RH, 14-h daily illumination). Numbers of dead psyllids were determined after 48 h by examining them under a dissecting microscope fitted with a fibre optic light. An adult was considered dead if it exhibited no leg or antennal movement during a 10–15 s period.

Experiment 2: exposure of adults to residues of 1%, 2% or 4% soap

Six leaf discs were dipped into a soap solution (or plain water) and placed in a fume hood until dry (1–2 h) before embedding them on agar in a small Petri dish. For each soap and percentage solution, 30 ACPs (7 days after final moult) were divided into six groups (five individuals per group, gender not determined) (total n = 36 groups not including water controls). Using the methods previously described, each group was transferred to a treated leaf disc and then placed into an environmental chamber (26°C, 75% RH, 14-h daily illumination). Numbers of dead psyllids were determined after 48 h using the same mortality criteria as in experiment 1, and the gender of each adult in the experiment was determined.

Experiment 3: direct sprays of nymphs with 0.8%, 1.6% or 2% soap

Five-fifth instar nymphs were transferred from an infested flush shoot to a filter paper disc using a small brush moistened with tap water for each soap concentration tested. Each group of nymphs on six separate discs was then sprayed until wet with a concentration of 0.8%, 1.6% or 2% of Safer Soap or M-Pede in water using the aforementioned spray bottles (total n = 36 groups). Six additional paper discs with nymphs were each sprayed with tap water as controls (0% soap). After treating the nymphs, they were transferred to a leaf disc. The discs were placed on a tray in an environmental chamber (25°C, 75% RH, 14-h daily illumination). The number of dead psyllid nymphs was counted after 48 h.

Experiment 4: direct sprays of eggs with 0.8%, 1.6% or 2% soap

Thirty-five flushing M. paniculata seedlings growing in Cone-tainers (SC-10 super cell Cone-tainers; Stuewe and Sons, Corvalis, OR) in a rack were placed together in a cage, and approximately 400 adult ACPs were released into the cage. After 48 h, the adults were removed from the plants and the number of ACP eggs on each plant was counted. There were five seedlings (replications) of each soap concentration (total n = 30 seedlings). Each concentration of soap in tap water was prepared for a finished volume of 200 ml. Five additional egg-infested plants were treated with tap water as controls (0% soap). The egg-infested flush shoots on each plant were individually dipped into water or a concentration of soap and allowed to air dry, and the plants were then placed into an environmental chamber at 25°C, 75% RH and 14-h daily illumination. Six days later, each plant was examined to count the number of eggs that did not hatch.

Experiment 5: toxicity of 2% soap to adult Cycloneda sanguinea

Adults of C. sanguinea were collected from wild thistle (Cirsium horridulum Michaux) growing at the Teague Hammock Natural Preserve area in Saint Lucie County, Florida several days prior to the experiment. Ten adults were treated as a group with a 2% solution of Safer Soap, and ten were treated as a group with a 2% solution of M-Pede. There were four groups (replications) of each treatment (total n = 8 groups). Four additional groups of ten beetles were each treated with tap water as controls (0% soap). Each group of ten adults was briefly anaesthetized with CO₂, placed onto filter paper moistened with tap water, sprayed with a treatment until thoroughly wet using the aforementioned spray bottles and then transferred to a leaf disc held in an environmental chamber (25°C, 75% RH, 14-h daily illumination). Numbers of dead C. sanguinea were determined after 48 h using the same methods described for adult ACP, and the gender of each beetle was determined.

Experiment 6: toxicity of 2% soap to adult Tamarixia radiata

Adult T. radiata were obtained from a USDA-ARS colony started during 2010 using parasitoids reared from nymphal ACP collected at an ornamental planting of M. paniculata in St. Lucie County, Florida. The colony is maintained using procedures similar to those
described by Skelley and Hoy (2004). New adults (≤ 24 h old) were separated into six groups of ten individuals (gender not determined). One group of ten adults was sprayed with each soap concentration, and there were six replications (total n = 12 groups). In addition, six groups of ten adults were sprayed with tap water as controls (0% soap). Each group of ten adults was aspirated into a vial, briefly subdued with CO₂, placed onto filter paper moistened with tap water, sprayed until thoroughly wet with a 2% soap solution using the aforementioned spray bottles and transferred to a leaf disc in a Petri dish along with a small piece of tissue paper saturated with honey for a food source. The Petri dishes were placed into an environmental chamber (26°C, 75% RH, 14-h daily illumination). Numbers of dead adults were determined after 48 h using the same methods described for adult ACP, and the gender of each parasitoid was determined.

Statistical analyses

For each experiment, percentage mortality data associated with soap treatments were corrected for mortality of control insects (treated with tap water) using Abbott’s formula (Abbott 1925). Percentage data were arcsine-transformed (Gomez and Gomez 1984) for statistical analyses. Normality of the data from each experiment was verified using the Shapiro–Wilk test (PROC UNIVARIATE; SAS Institute 2008; Shapiro and Wilk 1965). For normal data, mortality because of the type and concentration of soap was compared using analyses of variance (PROC TTEST or PROC GLM; SAS Institute 2008), the latter followed by Tukey’s test for pairwise comparisons of means. In cases where data could not be normalized, a nonparametric analysis of variance was used (PROC GLIMMIX in conjunction with PROC RANK; SAS Institute 2008). All evaluations of statistical significance were conducted at P = 0.05.

Results

Experiment 1: direct sprays of adults with 0.2%, 0.4%, 0.8%, 1.6% or 2% soap

When adults were sprayed directly, a mean ± SEM of 3 ± 8% adults died under the water-only control treatment. Arcsine-transformed data were not normal (W = 0.97, P = 0.04), so the nonparametric analysis of variance was used. Mortality of adults was differentially influenced by the concentration of soap (F₄, ₄₉ = 32.5, P < 0.0001) but not the type of soap (F₁, ₄₉ = 2.9, P = 0.10), gender (F₁, ₄₉ = 3.3, P = 0.08) or the interaction between the type of soap and concentration (F₄, ₄₉ = 0.9, P = 0.47) (table 1).

Experiment 2: exposure of adults to residues of 1%, 2% or 4% soap

Females accounted for 53% of the ACP used in this experiment, and no difference in gender ratio was observed across treatments (F₇, ₃₅ = 1.1, P = 0.4). No mortality occurred among adults placed on leaf discs that had been treated with tap water. Percentage mortality of adults exposed to soap residues followed the normal distribution (for arcsine-transformed data, W = 0.95, P = 0.12). Mortality of adults was differentially influenced by the concentration of soap (F₂, ₃₀ = 10.7, P = 0.0003), but not the type of soap (F₁, ₃₀ = 0.3, P = 0.6) or the interaction between the type of soap and concentration (F₂, ₃₀ = 0.5, P = 0.6) (table 2).

Experiment 3: direct sprays of nymphs with 0.8%, 1.6%, or 2% soap

All nymphs sprayed with 0.8%, 1.6% or 2.0% Safer Soap or M-Pede died within 48 h, whereas all of the nymphs sprayed with tap water remained alive.

Experiment 4: direct sprays of eggs with 0.8%, 1.6% or 2% soap

A mean ± SEM of 24 ± 1.7 eggs were oviposited on the flush of each seedling plant. Arcsine-transformed

Table 1 Mortality of Asian citrus psyllid adults treated with different concentrations of M-Pede or Safer Insecticidal Soap, adjusted for mortality of adults treated with water

<table>
<thead>
<tr>
<th>Effect</th>
<th>Mean per cent control (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>72 ± 8/a</td>
</tr>
<tr>
<td>Female</td>
<td>64 ± 8/a</td>
</tr>
<tr>
<td>Soap</td>
<td></td>
</tr>
<tr>
<td>M-Pede</td>
<td>72 ± 8/a</td>
</tr>
<tr>
<td>Safer</td>
<td>65 ± 8/a</td>
</tr>
<tr>
<td>Concentration</td>
<td></td>
</tr>
<tr>
<td>0.2%</td>
<td>5 ± 4/c</td>
</tr>
<tr>
<td>0.4%</td>
<td>60 ± 11/b</td>
</tr>
<tr>
<td>0.8%</td>
<td>90 ± 4/a</td>
</tr>
<tr>
<td>1.6%</td>
<td>96 ± 3/a</td>
</tr>
<tr>
<td>2.0%</td>
<td>100 ± 0/a</td>
</tr>
</tbody>
</table>

For each effect (gender, soap or concentration), means followed by the same letter are not significantly different (P < 0.05). Three groups of adults of each gender were sprayed with each concentration of each soap (total n = 60).
percentage data were normal for eggs that hatched \( (W = 0.94, P = 0.35) \). A mean ± SEM of 91 ± 6% eggs hatched when they were treated with tap water. For both soaps and concentrations, an adjusted mean ± SEM of 91 ± 4% of eggs treated hatched normally. There was no significant difference in percentage egg hatch between soaps \( (F_{1, 24} = 0.1, P = 0.8) \) or soap concentration \( (F_{2, 24} = 1.8, P = 0.2) \) (table 3).

### Experiment 5: Toxicity of 2% soap to adult Cycloneda sanguinea

Females accounted for 32% of the beetles used in this experiment, and no difference in gender ratio was observed among the soap and water treatments \( (F_{2, 6} = 0.1, P = 0.9) \). No beetles sprayed with tap water died, and the mortality data between treatments were normal \( (W = 0.93, P = 0.52) \). A mean ± SEM of 15 ± 5% and 5 ± 5% beetles died within 48 h after being sprayed with a 2% solution of M-Pede or Safer soap, respectively; these mortality rates were not significantly different \( (t = -1.4, \text{ d.f.} = 6, P = 0.2) \).

### Experiment 6: toxicity of 2% soap to adult Tamarixia radiata

Female \( T. \ radiata \) accounted for 78% of the parasitoids used in this experiment, and no difference in gender ratio was observed among the soap and water treatments \( (F_{2, 10} = 1.6, P = 0.3) \). Mortality of \( T. \ radiata \) adults sprayed with water averaged 12 ± 5%, and arcsine-transformed percentage control data were normal \( (W = 0.96, P = 0.74) \). After correcting for mortality of water controls, mean ± SEM mortality of \( T. \ radiata \) averaged 86 ± 9% or 83 ± 17% when sprayed with 2% Safer or M-Pede soap, respectively, and these percentages did not differ between the soaps \( (t = -0.02, \text{ d.f.} = 10, P = 0.98) \).

### Discussion

Potassium salts of fatty acids (PSFA) are commonly referred to as soap salts and are used as insecticides, herbicides, fungicides and algacides (National Pesticide Information Center 2001). Fatty acids are extracted from animal fats or plant oils and treated with potassium hydroxide to produce PSFA (National Pesticide Information Center 2001). As such, there is no single active ingredient in an insecticidal soap. PSFA work by penetrating an insect’s cuticle and disrupting cell membranes, causing dehydration and death (Osborne and Henley 1982). Although there may be different recipes for producing PSFA as well as differences in how they may be formulated for use, we found no published information suggesting that some insecticidal soaps are more effective than others for insect control.

This laboratory evaluation provided insight into the toxicity of commercially formulated insecticidal soaps to ACP and two of its natural enemies, the results of which serve as guidelines for future laboratory studies and more importantly for application scenarios to be tested under field conditions. Our laboratory procedures for exposing adult ACP and adult \( T. \ radiata \) to direct sprays resulted in some mortality among insects treated with tap water, which was probably a consequence of trauma associated with the insects being saturated with water on wet filter paper and subsequently being transferred by brush to a leaf disc. We could not attribute control mortality to the use of \( \text{CO}_2 \) to subdue ACP adults or subsequently holding them.
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on leaf discs, as both of these steps were followed in experiment 2 where no mortality of adult ACP occurred in the control treatment. Using similar procedures to assess the toxicity of chemicals to \( T. \ radiata \), Hall and Nguyen (2010) reported that 12% of parasitoids sprayed with water died – this was the same level of mortality observed in the current study. However, only 1.5% of the parasitoids died when they were placed onto leaf discs without being sprayed with water (Hall and Nguyen 2010).

Direct sprays of M-Pede or Safer Insecticidal Soap (50% concentrates of PSFA) were acutely toxic to adults and nymphs of ACP when applied in solutions of 0.8% or higher. Each of the two soaps was equally toxic to adult males and females. Dry residues of these soaps were only moderately toxic to adult ACP, suggesting that residual control by either soap would be incomplete even at soap concentrations of up to 4% in water. Although not apparent to the eye, it remained possible in experiment 2 that the dry residues of soap could have been partially rehydrated by the presence of moisture associated with agar, that is, rehydrated residues might be higher in toxicity than residues that are completely dried. Neither of the two insecticidal soaps was effective against eggs at concentrations of up to 2%. A 2% solution of M-Pede was previously reported to be ineffective against eggs in field evaluations (McKenzie and Puterka 2004).

Low percentages of the adult lady beetle \( C. \ sanguinea \) died within 48 h whether they were sprayed with a 2% solution of M-Pede or Safer soap. M-Pede can cause significant levels of mortality of some species of lady beetles, but this may depend on the species (Smith and Krischik 2000), life stage (Kraiss and Cullen 2008) and concentration of soap. M-Pede (2% v/v) was consistently toxic to adult \( C. \ maculata \) (DeGreer) as compared to adults treated with tap water, although percentages of adults killed by the soap were variable (42–90%); in contrast, M-Pede killed lower percentages of adult \( H. \ axyridis \) (Pallas) (7–33%) (Smith and Krischik 2000). Kraiss and Cullen (2008) reported that M-Pede (2% v/v) was completely non-toxic to adult and pupae of \( H. \ axyridis \) but moderately toxic to first and third instars (39–40% mortality). Toxicity of insecticidal soaps to immature stages of \( C. \ sanguinea \) remains to be determined and warrants further research. A full understanding of the compatibility of insecticidal soaps with biological control of ACP would take into consideration soap toxicity to the entire complex of ACP predators, including other species of Coccinellidae as well as syrphid flies (Diptera: Syrphidae), lacewings (Neuroptera: Chrysopidae, Hemerobiidae) and spiders (Aranae) (Hall 2008). With respect to the ACP parasitoid \( T. \ radiata \), 2% solutions of the soaps were acutely toxic to adults, which supports previous evidence that insecticidal soaps can be incompatible with biological control of invertebrate pests by parasitoids (Weinzierl 2000; Tremblay et al. 2008). Other non-conventional pesticides such as petroleum oil and sulphur have been also shown to be toxic to adult \( T. \ radiata \) (Hall and Nguyen 2010).

Application rates of from 1% to 2% M-Pede or Safer Insecticidal Soap are labelled for control of insect pests, whereas rates higher than 2% are excluded from the labels for these soaps, perhaps as a safeguard against phytotoxicity. Culbert (2005) presents a discussion on the toxicity of insecticidal soaps to plants and notes that higher concentrations of soap may not only disrupt the cuticle of an insect but also the waxy surface of plant leaves and fruit, resulting in yellow or brown spots, scorching or death of plant tissue. The two soaps differ with respect to inert ingredients. Safer Insecticidal Soap contains <35% ethyl and methyl alcohol (the presence of alcohols imparts a slight flammability) and 15% undisclosed inert ingredients. Propylene glycol and potassium hydroxide constitute 51% of M-Pede. In spite of these differences in inert ingredients, no differences between the soaps were found with respect to their toxicity to ACP adults, eggs or nymphs or the two natural enemies. How well these laboratory findings may relate to field efficacy of insecticidal soaps should be investigated. It is possible that the toxicity of soap might be influenced by environmental conditions (for example, temperature and humidity), and there may be times during the day that are better than others to apply a soap (for example, a good time might be during early morning before the dew evaporates).

The current market prices for M-Pede and Safer soap concentrate are US$10 and $23 per litre ($36 and $87 per gallon), respectively. Growers often use airblast sprayers to apply insecticides and other chemicals to citrus in a wide array of spray volumes ranging from 238 to 4732 l/ha (25–500 gallons per acre) depending on the size of trees, the specific design of the sprayer and the reason for applying the chemical (Salyani 2012). The cost of a 2% solution of M-Pede or Safer Insecticidal Soap at 946 spray litres per ha (100 spray gallons per acre) would be approximately US$180 or $438 per ha ($72 or $175 per acre), respectively (not including application cost). A smaller spray volume of 238 l/ha might suffice for control of ACP because they aggregate primarily on flush (predominately located near the outside of the tree canopy), which would reduce cost by 75% compared to the
cost at a spray volume of 946 l/ha. Using concentrations lower than 2% also would reduce cost, but may not provide complete control. As an alternative to M-Pede, Safer or other commercially formulated soaps for insect control, insecticidal soaps can be made from scratch (Culbert 2005) and may be less expensive. However, a downside of home-made soaps is that these lack use instructions and care must be taken to assure they are not phytotoxic if applied to plants. Regardless of the type of insecticidal soap, if soap is applied to control a population of ACP that includes eggs, two or more applications timed several days apart may be required because soaps were basically not-toxic to eggs.

Insecticidal soap at a high concentration (for example, 2% v/v in water) may be an effective alternative to conventional pesticides to manage ACP adults and nymphs in commercial citrus and other settings, although multiple applications may be needed if a target population includes eggs. A 2% concentration of either Safer Soap or M-Pede applied as a direct spray was relatively non-toxic to adult *C. sanguinea* but acutely toxic to adult *T. radiata*. Soaps therefore may be compatible with biological control of ACP by some adult coccinellids such as *C. sanguinea* but not by parasitoids, at least *T. radiata*.

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