Toxicity of Sucrose Octanoate to Egg, Nymphal, and Adult *Bemisia tabaci* (Hemiptera: Aleyrodidae) Using a Novel Plant-Based Bioassay

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ABSTRACT The sweetpotato whitefly, *Bemisia tabaci* (Gennadius), B biotype, presents a unique problem for vegetable growers by serving as a vector of plant viruses and by inducing physiological disorders of leaves and fruit. An action threshold of a single whitefly is necessary because of the threat of disease in many areas and growers rely heavily on a single class of insecticides (neonicotinoids) for whitefly control. Additional control methods are needed to manage this pest in commercial vegetables. Extracts of wild tobacco contain natural sugar esters that have previously been shown effective in controlling many soft-bodied insects. We developed a novel tomato leaf bioassay system to assess a synthetic sugar ester derivative, sucrose octanoate, for insecticidal activity against the eggs, nymphs, and adults of *B tabaci*. The LC_{50} values for sucrose octanoate against adults, second instars, and fourth instars of the whitefly were 880, 686, and 1,571 ppm, respectively. The LC_{50} against whitefly eggs was higher (11,446 ppm) but indicated that some egg mortality occurred at the recommended application rate of 0.8–1.2% (3,200–4,800 ppm [AI]). Toxicity of sugar esters to whitefly eggs has not been reported previously. The tomato leaf bioassay produced reliable and repeatable results for whitefly toxicity studies and predicted that effective nymph and adult whitefly control can be achieved with sucrose octanoate at application rates ≤1% (4,000 ppm [AI]). Field efficacy studies are warranted to determine whether this biorational pesticide has application in commercial tomato production.

KEY WORDS biorational, sugar ester, *Bemisia argentifolii*, silverleaf whitefly

WHITEFLIES ARE IMPORTANT PLANT PESTS and disease vectors in the southern United States where the climate supports their survival year-round. The sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Sternorrhyncha: Aleyrodidae), B biotype (=silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring), was introduced into the United States in 1987 and has persisted for almost two decades, in part because of an expanded host range, increased intercrop mobility, high fecundity, and enhanced ability to develop insecticide resistance compared with the A biotype (Perring et al. 1993, Bellows et al. 1994). The B biotype is associated with the appearance of new crop disorders in Florida, including the appearance of a silverleaf disorder of squash and an irregular ripening disorder of tomato (Schuster et al. 1990, 1991). The B biotype is also a vector of Begomoviruses, and the emergence of newly recognized plant viruses associated with this biotype is alarming (Polston and Anderson 1997). Bean golden mosaic, tomato mottle, and tomato yellow leaf curl viruses have all occurred for the first time in Florida since the introduction of the B biotype (Blair et al. 1995, Kring et al. 1991). These viruses increase the economic impact of whiteflies on vegetable production by reducing the economic threshold to one whitefly per plant, resulting in a zero tolerance of infestations by this pest. Growers currently rely heavily on a single class of insecticides (neonicotinoids) for whitefly control, which is expected to lead to increased tolerance or the development of resistance, further stressing the economic inputs required to sustain profitability (Byrne et al. 2003).

The continuing search for new crop protection alternatives has led to the discovery of the insecticidal properties of sugar esters that occur naturally in plants, are benign to the environment, and can be commercially synthesized. Natural and synthetic sugar esters have been shown to be effective biorational insecticides against a range of insect species. Soft-bodied arthropods, including mites, lepidopteran larvae, aphids, whiteflies, and psyllids, are killed rapidly upon contact (Parr and Thurston 1968, Neal et al. 1994, Puterka and Severson 1995, Liu et al. 1996, McKenzie and Puterka 2004, McKenzie et al. 2004). In addition, sugar esters have demonstrated ovipositional and feeding deterrence against mites (Neal et al.

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Fig. 1. Petri dish tomato-based bioassay system.

1994), whiteflies (Liu and Stansly 1995a), and leafminers (Hawthorne et al. 1992). Although the primary mode of action is unknown, it has been suggested that possible modes of action against pear psylla, *Cacopsylla pyricola* Foerster, include desiccation brought about by alterations in the insect cuticle, or suffocation by the sugar ester suspension (Puterka et al. 2003).

The objective of this study was to determine the insecticidal activity and dosage-mortality relationships of sucrose octanoate (α-D-glucopyranoside, β-D-fructofuranosyl octanoate), a synthetic analog of natural sugar esters found in leaf trichomes of wild tobacco, *Nicotiana gossei* Domin, to egg, nymphal, and adult sweetpotato whitefly. Determination of the effect of any product on immature whiteflies requires prolonged observation up to adult emergence because mortality of whitefly immatures is very difficult to assess because of their sessile nature and flat morphological characteristics. Therefore, we developed a simple, cost-effective, plant-based insecticide bioassay that could accommodate all whitefly life stages.

Materials and Methods

Source of Insects, Plants, and Biorationals. Adult male and female *B. tabaci* biotype B were obtained from laboratory colonies maintained by the U.S. Horticultural Research Laboratory, Fort Pierce, FL. All stages of whitefly have been maintained on dwarf cherry tomato, *Lycopersicon esculentum* 'Florida Lanai', since 1996 by serial transfer and were housed in large screened Plexiglas cages located in air-conditioned greenhouses with ambient light and humidity. Temperatures fluctuated between day and night highs of 29.4 and 26.7°C, respectively, with an overall low of 23.9°C. Whitefly biotyping was based on random amplification of polymorphic DNA polymerase chain reaction analysis using primers developed by De Barro and Driver (1997) and confirmed by mitochondrial DNA markers developed by Frohlich et al. (1999). Sucrose octanoate, a synthetic analog of natural sugar esters, was provided by AVACHEM (AVA Chemical Ventures, L.L.C., Portsmouth, NH) as a 40% (AI) formulation and was used in all whitefly bioassays.

Whitefly Petri Dish Bioassay System. A petri dish bioassay system was developed (Fig. 1) to hold single detached tomato leaves infested with whiteflies for up to extended periods (egg to adult). The bioassay system was modified from one developed by McKenzie et al. (2002) to apply insect viruses. A ventilation hole was cut in the lid of a polystyrene petri dish (100 by 20 mm) and covered with ultrafine screen mesh. Polystyrene conical tubes (15 ml, 17 by 120 mm) were filled with 0.8% agar solution (Phytotechnology Laboratories, Mission, KS) enriched with N–K–P (20–10-20) fertilizer mixture (Scotts-Siera Horticultural Products Company, Marysville, OH) and allowed to cool. A hole was cut in the side of the petri dish to provide a tight fit for the conical tube. Whole tomato leaves were selected from intact plants and trimmed with a razor blade so that only the terminal leaflet remained. Single leaflets with petiole/rachis were

Table 1. Time-specific survivorship of whitefly stages after treatment with sucrose octanoate concentrations

Sucrose octanoate (ppm)	Mean no. of whiteflies (% survival)			
	Eggs	Third/fourth instars	Red-eye/adults	Cumulative adults
	0 DAT	14 DAT	21 DAT	28 DAT
0	56.2 ± 7.7	52.8 ± 7.7 (93)a	55.5 ± 6.5 (90)a	53.5 ± 7.4 (87)a
750	74.0 ± 14.5	64.6 ± 11.8 (90)a	61.5 ± 10.2 (84)a	57.4 ± 11.9 (86)a
1,500	49.5 ± 8.9	43.8 ± 8.7 (82)ab	45.5 ± 9.0 (81)a	37.8 ± 7.6 (77)ab
3,000	43.4 ± 7.3	33.7 ± 6.8 (74)abc	32.9 ± 6.0 (71)ab	31.2 ± 7.0 (69)ab
6,000	46.6 ± 7.5	32.8 ± 7.0 (67)bc	32.4 ± 6.6 (64)ab	31.7 ± 7.9 (60)ab
12,000	44.4 ± 6.4	26.2 ± 5.4 (59)c	22.9 ± 3.8 (50)bc	21.3 ± 3.9 (49)bc
24,000	57.7 ± 9.1	22.3 ± 6.4 (29)d	23.2 ± 5.6 (27)cd	20.8 ± 5.1 (24)cd
48,000	39.0 ± 18.2	6.0 ± 4.6 (16)d	3.5 ± 3.5 (11)d	2.0 ± 1.5 (6)d

Means within a column followed by the same letter are not significantly different ($P > 0.05$; REGWQ).

Table 2. Toxicity of sucrose octanoate applied to freshly laid eggs (determined by adult emergence) or applied to adults and determined 24 HAT

Bioassay	*n*	Slope ± SE	LC_{50}[a] (95% FL)	LC_{90}[a] (95% FL)
Egg spray[b]	4,639	1.65 ± 0.05	11,446 (7,505–18,607)	68,222 (35,222–281,424)
Adult[c]	2,408	2.26 ± 0.13	880 (779–978)	3,255 (2,945–3,653)

[a] LC values expressed in ppm (AI) of commercially formulated sucrose octanoate.
[b] Newly laid whitefly eggs (20–40 h old) were sprayed with commercially formulated sucrose octanoate, and mortality was calculated from eggs that did not result in an adult 28 DAT; five replications, seven concentrations, and bioassays were repeated four times.
[c] Mixed populations of adult male and female whiteflies were caged, chilled, and sprayed with commercially formulated sucrose octanoate and mortality calculated 24 HAT; five replications, five concentrations, and bioassays were repeated five times.

placed in water until all were processed to ameliorate shock and prevent wilting. Once all leaflets were processed, the petiole/rachis from each leaflet was placed as far as possible into the conical tubes filled with solidified agar solution. The prepared leaflet bioassays were acclimated in an incubator at 25 ± 1°C and photoperiod 16:8 (L:D) h for 48 h before they were infested with whitefly adults. A small hole was cut into the top of the conical tube above the surface of the culture media to facilitate addition of water when the petri dish covers were in place. Petri dish covers were placed over the leaflets during whitefly egg deposition and adult emergence. Cages were removed during nymphal development and then replaced before adult emergence.

Spray Application Device. All treatments were applied using an ultralow-volume spray device developed by Puterka and Severson (1995). The device consisted of a spray platform that holds a pressurizable Nalgene aerosol spray bottle (Nalge Nunc International, Rochester, NY) at the proper distance and angle to provide uniform and consistent spray coverage to each petri dish. The nozzle provided with the spray bottle was used to deliver a fine aerosol spray. Measured amounts (200 μl) of each sucrose octanoate suspension were placed in individual test tubes (5 ml, 12 by 75 mm). Parafilm was wrapped around the top of the test tube to ensure a tight fit. The bottle was pressurized to ≈10 psi with 20 strokes of the pump mechanism for each application. Both the abaxial and adaxial sides of the leaf were sprayed until no product remained in the test tube.

Whitefly Petri Dish Bioassay Experiments. Whitefly adults (15–20) were caged for 24 to 48 h to deposit eggs (25–50) on the tomato leaf for egg and nymphal bioassays. Whiteflies for adult bioassays were chilled in a cold room (4°C) and directly sprayed. For assays with immature whiteflies, adults and cages were removed and eggs were either sprayed directly or allowed to hatch and develop into small (crawler, second) or large (third–fourth) instars that were directly sprayed. Eggs, nymphal, and adult whiteflies were counted in each replicate before treatment applications. Sucrose octanoate suspensions were prepared in concentrations of 750–24,000 ppm (0.19–6.0% formulated product) in deionized H_2O plus a deionized distilled H_2O control for nymphal and adult whitefly bioassays. Egg bioassays included a higher dosage of 48,000 ppm (12% formulated product). Abaxial and adaxial leaf surfaces were each sprayed with 200 μl of the appropriate sucrose octanoate suspension and controls were sprayed with deionized H_2O alone. Conical tubes with treated bioassay leaflets were placed upright in racks in an incubator at 25 ± 1°C and photoperiod 16:8 (L:D) h. Whitefly egg bioassays were examined at 7-d intervals, and counts of each life stage were made until the emergence of adults was complete. Whitefly nymphs were counted at 1, 3, 7, and 14 d after treatment (DAT), or until all adults had emerged. Whitefly adults were counted at 3, 6, and 24 h after treatment. Each concentration was replicated five times, and each bioassay was repeated a minimum of three times.

Statistics. Relationships between mortality and concentration of sucrose octanoate were evaluated by probit analysis (Sparks and Sparks 1987). Differences between LC values were determined by overlapping fiducial limits. Data were analyzed by the General Linear Models (GLM) procedure, and differences among treatment means were determined by Ryan–Einot–Gabriel–Welsch multiple range test (REGWQ) at $\alpha = 0.05$ (SAS Institute 2000).

Table 3. Toxicity of sucrose octanoate to whitefly crawler/second instars over time by using a novel plant-based tomato bioassay

Bioassay time (DAT)	*n*	Slope ± SE	LC_{50}[a] (95% FL)	LC_{90}[a] (95% FL)
1	3,469	1.21 ± 0.06	4,807 (3,082–9,805)	54,827 (20,162–774,817)
3	3,450	1.19 ± 0.06	1,843 (1,231–2,509)	22,076 (12,210–67,793)
7	2,532	2.35 ± 0.18	605 (67–945)	2,120 (1,456–8,700)
14	2,495	2.51 ± 0.18	686 (96–1,035)	2,225 (1,517–11,255)

Whitefly nymphs were sprayed at the crawler/second instar stage, and mortality was assessed at 1, 3, 7, and 14 DAT or until adult emergence; five replications, five concentrations, and bioassays were repeated four times.
[a] LC values expressed in ppm (AI) of a commercial formulation of sucrose octanoate.

Table 4. Toxicity of sucrose octanoate to fourth instars or red-eye pupal whitefly stages over time by using a novel plant-based tomato bioassay

Bioassay time (DAT)	*n*	Slope ± SE	LC_{50}[a] (95% FL)	LC_{90}[a] (95% FL)
1	4,553	1.91 ± 0.06	12,283 (8,687–20,794)	57,505 (30,233–223,478)
3	4,553	1.77 ± 0.05	3,133 (2,403–3,929)	16,544 (12,075–26,016)
7	4,553	1.90 ± 0.06	2,012 (1,713–2,313)	9,533 (8,001–11,832)
14	3,972	2.45 ± 0.06	1,571 (1,118–2,040)	5,253 (4,087–7,181)

Whitefly nymphs were sprayed at the redeye stage, and mortality was assessed at 1, 3, 7, and 14 DAT or until adult emergence; five replications, six concentrations, and bioassays were repeated three times.

[a] LC values expressed in ppm (AI) of a commercial formulation of sucrose octanoate.

Results and Discussion

Egg Mortality. At 14 DAT, significant reductions in percentage of survival of whitefly eggs sprayed with sucrose octanoate were detected at rates of 6,000 ppm and higher (Table 1). Toxicity to eggs did not increase significantly over time, and the LC_{50} and LC_{90} values (egg to adult) were 11,446 and 68,222 ppm, respectively (Table 2). These results indicate that although the egg is the least susceptible stage to sucrose octanoate, significant mortality can be expected if eggs are treated at the recommended rate. Phytotoxicity was observed on tomato leaves treated at 48,000 ppm, but this rate is not economically feasible and was only included to aid in determining lethal concentration values. No phytotoxicity was observed at the lower rates of application. Liu and Stansly (1995b) found that *N. gossei* extracts applied at a rate of 10.0 g ([AI])/liter did not kill whitefly eggs but did cause phytotoxicity to treated tomato plants. Compared with natural sugar esters and earlier versions of synthetic sugar esters, sucrose octanoate is a superior formulation (Puterka et al. 2003), and this could explain the toxicity we observed. At the current recommended field rate of 0.8 to 1.2% (3,200–4,800 ppm [AI]) (AVA Chemical Ventures, L.C.C.) a grower might expect a 30 to 40% reduction in whitefly survival to adult stage among treated eggs, assuming efficient deposition of material was obtained. This is the first report of sucrose octanoate toxicity to whitefly eggs.

Adult Mortality. Sucrose octanoate was toxic to whitefly adults sprayed directly in the plant-based bioassay (Table 2). Adult whiteflies were killed almost immediately upon contact so mortality did not increase significantly over time (data not shown for 3, 6 h after treatment [HAT]). The LC_{50} and LC_{90} values for adult whiteflies at 24 HAT were 880 and 3,255 ppm, respectively, and they indicate that the adult stage should be easily controlled with recommended field rates.

Nymphal Mortality. Sucrose octanoate was toxic to second instars (Table 3) and slightly less toxic to fourth instars (Table 4). Mortality for both second and fourth instars increased over time (Figs. 2 and 3), unlike the stable mortality trajectories observed for egg and adult stages. The LC_{50} and LC_{90} values at 14 DAT for second instars were 686 and 2,225 ppm, respectively, whereas those for fourth instars were 1,571 and 5,253 ppm. The data indicate that a recommended field application rate of 1.2% sucrose octanoate should provide >90% control of whitefly nymphs.

Evaluation of Methodology. We developed a quick, inexpensive, and repeatable plant-based bioassay that could be used to test a wide spectrum of control agents against whiteflies on tomato, including entomopathogenic viruses (McKenzie et al. 2002). The development of whiteflies on tomato leaflets in our plant-based bioassay compared well to whitefly life table

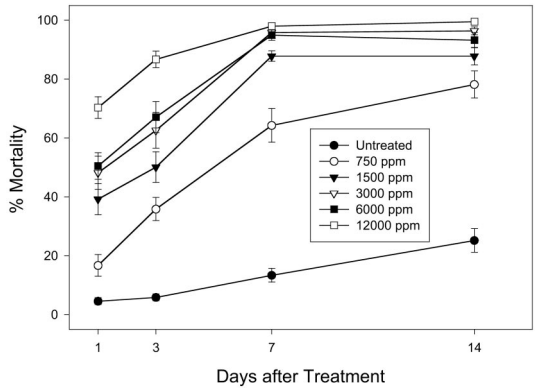

Fig. 2. Temporal mean percentage of mortality (±SE) of sucrose octanoate applied to whitefly crawler/second instars using a petri dish tomato-based bioassay system.

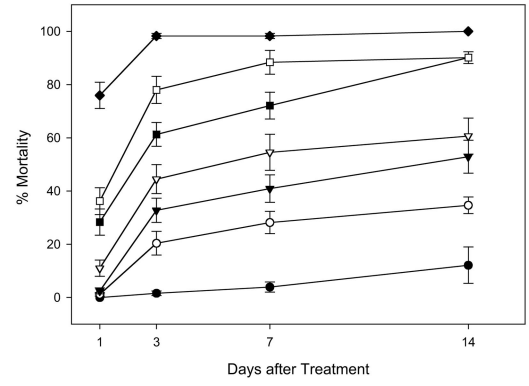

Fig. 3. Temporal mean percentage of mortality (±SE) of sucrose octanoate applied to fourth instars or red-eye pupal whitefly stages by using a petri dish tomato-based bioassay system. Untreated, ●; 750 ppm, ○; 1,500 ppm, ▼; 3,000 ppm, ▽; 6,000 ppm, ■; 12,000 ppm, □; 24,000 ppm, ◆.

studies conducted on a variety of other plant hosts. Costa et al. (1991) reported mean proportions of *B. tabaci* surviving from egg to adult stage that ranged from 0.17 on tomato to 0.83 on zucchini. Depending on the stage evaluated, natural mortality in our bioassays ranged from 12 to 22% (Table 1; Figs. 2 and 3), which is comparable with the highest rates of survival previously reported, regardless of host plant.

The plant-based bioassay was relatively quick and inexpensive compared with clip cages that require greater maintenance and greenhouse space for plant rearing. The time required to conduct the bioassay, including propagation of tomatoes, preparation of plant media and cages, manipulation and counting of whitefly populations, and application of treatments averaged 17.5 h per bioassay for five replicates of each treatment. After one-time costs for cage materials (petri dishes, screen, and glue), agar, and 10.16-cm pots, recurring costs for conical tubes, soil, and fertilizer are minimal. Bioassays can be placed upright in a metal tube rack and kept in a small incubator for the duration of the experiment. Tomato leaves occasionally wilted initially, but acclimated within 24–48 h, developed roots within 7–14 d, and remained viable for 28 d or longer. This bioassay is also conducive to leaf dip techniques in lieu of the spray device application method. In addition, whitefly populations were readily manipulated under the microscope and easily counted using this bioassay.

Sugar esters are naturally occurring botanical compounds that can be synthesized by reacting sucrose with salts of fatty acids (Puterka et al. 2003). They exhibit low toxicity to beneficial insects (Stansly and Liu 1997, McKenzie et al. 2004, Michaud and McKenzie 2004), are nontoxic to vertebrates, and are biodegradable (Ferrer et al. 1999). Liu et al. (1996) showed that sugar ester isolates of *Nicotiana* spp., and several synthetic precursors to sucrose octanoate, provided whitefly control comparable with that provided by commercial pyrethroid, organophosphate, and insect growth regulator insecticides. We have shown that synthetic sucrose octanoate is effective in controlling all life stages of *B. tabaci*, including eggs contacted at the time of application. This synthetic sugar ester shows promise in controlling homopteran pests of vegetables and also may reduce the physiological crop disorders and the viral diseases transmitted by whiteflies.

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