Toxicity of Selected Adjuvants Against *Nipaecoccus viridis* Instars, Adults, and Ovisacs, and Against Their Predator *Cryptolaemus montrouzieri*, 2021–2022

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*Citrus volkameriana*

Hibiscus mealybug | *Nipaecoccus viridis*

Mealybug destroyer | *Cryptolaemus montrouzieri*

*Nipaecoccus viridis* is an emerging pest in Florida citrus that has historically been difficult to manage, in part due to a layer of wax that protects it from insecticides. As a result, management programs have had to rely primarily on naturally occurring biological control. Our objective was to determine if adjuvants can penetrate this protective wax and induce mortality of different life stages of *N. viridis* on their own, and if they would be harmful to the predator *Cryptolaemus montrouzieri*.

Between 2021 and 2022, we evaluated six commercially available adjuvants to determine their impact on ovisacs, second- and third-instar nymphs, and adults of *N. viridis*, as well as larvae and adults of *C. montrouzieri*. DI water was used as a negative control, and the insecticide Delegate (Spinetoram) was used as a positive control. Adjuvants were mixed with DI water at maximum label rates, and applied with 236-ml plastic spray bottles (equate, Bentonville, AR). *Nipaecoccus viridis* were obtained from a lab colony reared on Volkamer lemon trees kept at ~27°C. *Cryptolaemus montrouzieri* were obtained from a hibiscus plant (*Hibiscus sp.*) heavily infested with mealybugs donated by a homeowner in Polk County, FL.

For trials with second to third *N. viridis* instars, fully expanded young Volkamer lemon leaves were excised from the host tree and had their petioles placed into a microcentrifuge tube (1.5-ml polypropylene microcentrifuge tubes, Millipore Sigma, Darmstadt, Germany) filled with DI water and sealed with parafilm (Prafilm, Bemis Company, Inc., Sheboygan Falls, WI). Five second to third *N. viridis* instars were placed on each leaf using a fine tipped paintbrush (size 2 camel hair, Torrington Brush Works, CT) and allowed to settle and feed for 24 h. After 24 h, leaves with instars were sprayed with adjuvants until leaves were dripping, and allowed to air dry for 1–2 h. Once dry, adults were assessed for mortality by gently probing with a fine-tipped brush. After an initial mortality check on day 0, leaves with *N. viridis* were placed into plastic petri dishes, sealed with parafilm, and kept in an incubator at 16:8 L:D cycle and 28 ± 2°C. Eleven replicates with five *N. viridis* instars each were conducted with each adjuvant and the controls. On days 3, 5, and 7 after adjuvants were applied, *N. viridis* instar mortality was assessed by gently probing instars with a fine-tipped brush. Instars that moved in response were considered alive, and those that did not were considered dead. The number of dead mealybugs did not meet assumptions of normality, and nonparametric Kruskal–Wallis tests were used to determine the effect of treatment on the number of dead instars. Dunn tests were used to determine means separation between treatments. All analyses were conducted in R.

For trials with *N. viridis* adults and ovisacs, excised Volkamer lemon leaves were created as before with their petioles in microcentrifuge tubes filled with DI water. Adult female *N. viridis* were placed onto the excised leaves and allowed to lay eggs and develop ovisacs over the course of 1–3 wk. Adults with ovisacs were then sprayed with adjuvants until leaves were dripping and allowed to air dry for 1–2 h. Once dry, adults were assessed for mortality by gently probing with a fine-tipped brush. After an initial mortality check on day 0, leaves with *N. viridis* were placed into plastic petri dishes, sealed with parafilm, and kept in an incubator at 16:8 L:D cycle and 28 ± 2°C. On days 3 and 5 after adjuvants were applied, *N. viridis* adult mortality was assessed again. Ovisac mortality was determined by holding the leaves and ovisacs for up to 3 wk and monitoring for the emergence of first instars. If instars were found, the ovisac was considered to have survived. If no first instars were found after 3 wk, the ovisac was considered dead. Fifteen replicates of one *N. viridis* adult and ovisac were conducted with each adjuvant and the controls except for Trio and 800 Plus, which only had...
10 replicates. Kruskal–Wallis tests were used to determine the effect of treatment on adult and ovisac mortality, and Dunn tests were used to determine means separation between treatments. All analyses were conducted in R.

For trials with both *C. montrouzieri* adults and larvae, single individuals were placed into a 150 mm × 15 mm polystyrene petri dish and sprayed with adjuvants until the bottom of the dish was fully coated. *Cryptolaemus montrouzieri* were then removed and placed into individual 33-ml polystyrene vials (Thornton Plastics, Salt Lake City, UT) and kept in an incubator at 16:8 L:D cycle and 28 ± 2°C. Ten replicates of one larva and one adult were tested for each adjuvant and for both controls, except for larvae tested with Delegate, where we had 20 individuals. On days 3, 5, and 7 after adjuvants were applied, *C. montrouzieri* mortality was assessed by gently probing individuals with a fine-tipped brush. Kruskal Wallis tests were used to determine the effect of treatment on the number of dead individuals. Dunn tests were used to determine means separation between treatments, and all analyses were conducted in R.

There was a significant effect of treatment for *N. viridis* instar mortality on day 3 ($\chi^2 = 34.328$, df = 7, $P = 1.495\times10^{-5}$), day 5 ($\chi^2 = 38.095$, df = 7, $P = 2.907\times10^{-6}$), and day 7 ($\chi^2 = 38.228$, df = 7, $P = 2.742\times10^{-6}$), with all adjuvants leading to increased mortality, and multiple adjuvants resulting in comparable mortality to Delegate (Table 1). There was also a significant effect of treatment on adult *N. viridis* mortality on day 0 ($\chi^2 = 38.535$, df = 7, $P = 2.397\times10^{-6}$), day 3 ($\chi^2 = 21.354$, df = 7, $P = 0.00328$), and day 5 ($\chi^2 = 31.697$, df = 7, $P = 4.623\times10^{-5}$), although only the adjuvant Wake Up led to increased mortality compared to the control across all three days (Table 2). Finally, there was a significant effect of treatment on ovisac mortality ($\chi^2 = 23.323$, df = 7, $P = 0.001497$), although no adjuvants led to significantly increased ovisac mortality compared to the control (Table 2).
There was a significant effect of treatment on *C. montrouzieri* larval mortality on day 3 ($\chi^2 = 53.178$, $df = 7$, $P = 3.419e-09$), day 5 ($\chi^2 = 47.264$, $df = 7$, $P = 4.958e-08$), and day 7 ($\chi^2 = 36.495$ $df = 7$, $P = 5.846e-06$), with 435 Oil, and Wake Up leading to increased mortality, while Delegate did not (Table 3). There was also a significant effect of treatment on adult *C. montrouzieri* mortality on day 3 ($\chi^2 = 32.529$, $df = 7$, $P = 3.239e-05$), day 5 ($\chi^2 = 39.092$, $df = 7$, $P = 1.877e-06$), and day 7 ($\chi^2 = 53.72$, $df = 7$, $P = 2.672e-09$), although only Delegate led to increased mortality on any day (Table 4).

### Table 3.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rate (V/V)</th>
<th>Percent <em>C. montrouzieri</em> larvae dead</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 3</td>
</tr>
<tr>
<td>Water (deionized)</td>
<td>NA</td>
<td>0b</td>
</tr>
<tr>
<td>Clearsurf90 (nonionic surfactant)</td>
<td>0.5%</td>
<td>10b</td>
</tr>
<tr>
<td>Trio (surfactant)</td>
<td>0.375%</td>
<td>0b</td>
</tr>
<tr>
<td>800 Plus (emulsifier and surfactant)</td>
<td>0.25%</td>
<td>0b</td>
</tr>
<tr>
<td>SuffOil-X (spray oil and insecticide)</td>
<td>2%</td>
<td>0b</td>
</tr>
<tr>
<td>435 Oil (spray oil)</td>
<td>3%</td>
<td>70a</td>
</tr>
<tr>
<td>Wake Up (surfactant and penetrant)</td>
<td>0.39%</td>
<td>80a</td>
</tr>
<tr>
<td>Delegate (spinetoram)</td>
<td>2.35%</td>
<td>5b</td>
</tr>
</tbody>
</table>

Different letters denote statistically significant differences between treatments in the same day (Dunn tests, $P < 0.05$).

### Table 4.

<table>
<thead>
<tr>
<th>Treatment</th>
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<tbody>
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<td></td>
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<td>70a</td>
</tr>
</tbody>
</table>

Different letters within columns denote statistically significant differences between treatments (Dunn tests, $P < 0.05$).