Monitoring Whitefly Susceptibility to Applaud

M. Yasui, P.C. Ellsworth, J. Lublinkhof, D. Comer

Abstract

A bioassay developed by one of the authors (MY) in 1993 was used to monitor susceptibility of sweetpotato whitefly to Applaud in five different field locations. Whitefly populations were exposed to from 0 (untreated fields) to 4 (small plot trial) applications of Applaud. Susceptibilities of whiteflies, as measured by LC50s and LC95s, did not increase with exposure to Applaud (0 to 4 applications) nor since baseline measurements of susceptibility were made in 1993. Under current patterns of use (single use), risk of resistance to Applaud appears to be minimal.

Introduction

Applaud® (buprofezin) was discovered in 1977 by Nihon Nohyaku, first registered in Japan in 1983, and is now registered in over 60 countries (Yasui, 1993). Prior to 1996, Applaud had only been evaluated in the U.S. in experimental plots for efficacy (e.g., Akey & Henneberry 1994; Ellsworth et al. 1994; Natwick 1994a&b; Palumbo 1994; Watson et al. 1994). In 1996 Arizona received a Section 18 emergency exemption allowing use of Applaud against sweetpotato whitefly [Bemisia tabaci (Genn.) (Strain B) = Bemisia argentifolii (Bellows & Perring); (a.k.a. silverleaf whitefly)] in cotton. This was the first time Arizona whitefly populations had been exposed to Applaud on a relatively broad scale (ca. 70,000 A).

In 1993 while conducting research at The University of Arizona, we developed a simplified Bemisia susceptibility monitoring technique for Applaud (Yasui, unpubl. data). In 1996 in cooperation with The Univ. of Ariz. (PCE) and AgrEvo USA Company (JL & DC), Nihon Nohyaku (MY) further refined and used this technique to establish baseline data prior to wide scale use of Applaud.

Methods

Bioassay Methods (brief)

As an insect growth regulator, Applaud has no lethal effect on whitefly adults. Applaud, a chitin biosynthesis inhibitor, interferes with the normal molts of whitefly nymphs. Monitoring of nymphal mortality, therefore, is needed, instead of the more common and more convenient adult mortality bioassays (e.g., Prabhaker et al. 1992; Simmons & Dennehy 1996). Nymphal assays are difficult, especially when host material and specialized insect rearing facilities are necessary.

In 1993, Yasui developed a simplified susceptibility monitoring technique which obviated the need for “clean” host material or any specialized equipment. With further refinement this past year, we established a protocol that successfully uses foliage and whitefly eggs collected from the field of interest. We determined that the second leaf below the terminal (i.e., 1 leaf below the first folded leaf) contains the greatest concentration of similarly-aged whitefly eggs. Furthermore, we found that holding these leaves for 6–8 days at 25°C yielded nearly uniform cohorts of settled 1st instars, the ideal age for initiating the assay. With very simple tools (vials, scissors, water), a dissecting microscope, & reasonable control over near “room” temperature conditions, anyone with interest in a particular field population can conduct this bioassay, though it does require about 21 days to complete.
In brief, a 2nd main stem node leaf from terminal is collected from the field site with the petiole intact. The leaf blade is then trimmed to an approximately 3 X 4 cm rectangle. The trimmed leaf is then held at 25°C (70-80% R.H.) for 7 days in a vial with 20 ml of water. After the eggs hatch and first instar nymphs settle on the leaf, both sides of the leaf are sprayed to saturation. Serial dilutions of Applaud 70WP (40 SC in '93) were used in our bioassays in 1996 with 0.01% Triton X100 as a wetter solution (0.125% Kinetic wetter in 1993). The leaves are then held for an additional 12 days at 25°C (60-70% R.H.). Nymphs are then classified as dead or alive. LC50 & LC95 statistics can then be determined from probits.

Field Populations

Whiteflies to be assayed for susceptibility to Applaud were collected from 3 locations, Maricopa Agricultural Center (MAC), a grower's field in Peoria, and a grower's field in Eloy. Whiteflies from growers' fields in Casa Grande and Buckeye were also assayed, but these results are not reported here. The 1996 MAC was designed as a randomized complete block with 3 replicates & individual plots measuring 1600 sq. ft.. Treatments were 0 & 4 sprays of Applaud (0.35 lb a.i./A). The bi-weekly sprays started on 25 June. The 1993 data were from large, untreated areas at MAC. The Peoria & Eloy grower sites were 21 A & 30 A in size, respectively. These growers fields received one application of Applaud each. All stages of whitefly were monitored at all sites using university recommended sampling procedures (Ellsworth et al. 1995, 1996).

Results

Table 1 shows the calculated LC50s and LC95s for all populations tested. There is no clear pattern of decreasing susceptibility (as would be indicated by increasing LC measurements) for any of the populations post-Applaud application. Baseline LC50s are around 3-12 ppm. Figure 1 shows decreasing slopes for LC50s & LC50s when regressed on number of Applaud sprays. Applaud susceptibility does not apparently decrease within season with as many as 4 sprays (in a small plot context) nor after the recommended practice of 1 spray in the commercial fields.

Maricopa Populations

Figures 2 and 3 show very similar probits for whiteflies from untreated cotton in 1993 and 1996, indicating, as expected, no substantial changes in our populations' susceptibilities to Applaud since 1993. Figure 4 shows the probit lines for whiteflies before and after successive Applaud sprays. No significant differences existed among these 4 probits. This indicates a pattern of sustained susceptibility with multiple uses of Applaud. Because Applaud treatments were made in small plots, populations potentially could have been subjected to the diluting influences of localized migration.

Large Commercial Field Populations

In Eloy, 2 pretreatment and 2 post-treatment bioassays were run with the second one conducted on F1 progeny of collected adults. All 4 probits are similar with LC50s of ca. 3-12 ppm (figure 2). The Peoria location was evaluated twice before treatment & once after treatment with Applaud. There were no significant differences among the 3 probits. LC50s were ca. 4-9 ppm (figure 6).

Conclusions

From the 5 monitoring sites, LC50 values ranged from ca. 3-12 ppm. This range was not related to any pattern of time (1993 or 1996 sampling dates) or Applaud use (once, 4 times, or not at all). Thus there is no appreciable difference in susceptibility among populations studied to date.

In spite of the refractory nature of whitefly susceptibilities to Applaud in these studies, recommendations will con-
tinue to reinforce the prudent use of this valuable active ingredient within an IPM and resistance management pro-
grains. Only one use of Applaud will be allowed for cotton in 1997. These data would indicate that under this pattern of use, risk of resistance to Applaud is minimal.

We have developed and presented a simplified, field-based, susceptibility monitoring technique which minimizes the time and effort associated with an otherwise difficult bioassay. These data serve as a baseline for future susceptibility monitoring.

References Cited


Yasui, M. 1993. Biological action of an IGR, buprofezin. 5th International ICIPE Mobile Seminar, Tokyo. (Abstract)

Acknowledgments

The authors wish to recognize the significant role that Dr. Leon Moore (Emeritus Professor, Univ. Ariz.) had in organizing, monitoring, and sampling the commercial field trials in 1996. We wish to thank Philip Odim who assisted in all aspects of research and development in 1993 and set the stage for a successful year in 1996. We also thank Donna Meade and Jon Diehl and the rest of the technical staff at MAC and the grower cooperators for their assistance in this work. Thanks to Dr. Jon P. Chernicky and his staff of Arid Ag Research who set up and implemented the 1996 small plot field design. Finally, we wish to thank Fred Strachan (AgR Evo) and Yosuke “Hank” Tomoi (Nihon Nohyaku) who assisted in all phases of design and conception of these studies and most importantly sponsored the senior author’s visits to the University of Arizona – Maricopa Agricultural Center in 1993 & 1996.

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Table 1. Monitoring sites, dates, exposure history, and bioassay results of field populations from Arizona. Populations designated as ‘F1’ are the progeny of field collected nymphal populations bioassayed on beans.

<table>
<thead>
<tr>
<th>Site &amp; Sampling Date</th>
<th>Exposure</th>
<th>LC$<em>{50}$ (CI$</em>{95%}$)</th>
<th>LC$_{95}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maricopa ’93</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sep-03</td>
<td>untreated</td>
<td>7.1 (3.9–11.3)</td>
<td>150</td>
</tr>
<tr>
<td>Jun-24</td>
<td>untreated</td>
<td>8.4 (5.5–12.5)</td>
<td>46.1</td>
</tr>
<tr>
<td>Jul-22</td>
<td>untreated</td>
<td>7.0 (5.7–8.4)</td>
<td>56.6</td>
</tr>
<tr>
<td>Aug-05</td>
<td>untreated</td>
<td>11.5 (3.9–26.0)</td>
<td>69.6</td>
</tr>
<tr>
<td>Aug-19</td>
<td>untreated</td>
<td>2.9 (2.2–3.8)</td>
<td>28.1</td>
</tr>
<tr>
<td>Jul-08</td>
<td>13 d after 1 spray</td>
<td>4.1 (2.6–6.0)</td>
<td>55.9</td>
</tr>
<tr>
<td>Jul-22</td>
<td>13 d after 2 sprays</td>
<td>4.7 (3.4–6.4)</td>
<td>46.8</td>
</tr>
<tr>
<td>Aug-05</td>
<td>13 d after 3 sprays</td>
<td>6.3 (2.1–9.6)</td>
<td>49.8</td>
</tr>
<tr>
<td>Aug-19</td>
<td>13 d after 4 sprays</td>
<td>4.0 (2.6–5.9)</td>
<td>33.1</td>
</tr>
<tr>
<td>Jul-02</td>
<td>untreated</td>
<td>4.5 (2.3–7.7)</td>
<td>48.9</td>
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<td><strong>Eloy ’96</strong></td>
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<td></td>
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<td>Jul-19</td>
<td>untreated</td>
<td>11.8 (6.3–20.2)</td>
<td>80.4</td>
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<tr>
<td>Aug-15</td>
<td>22 d after 1 spray</td>
<td>7.3 (4.9–11.3)</td>
<td>92.5</td>
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<td>Aug-15 (F1)</td>
<td>22 d after 1 spray</td>
<td>2.9 (2.0–3.9)</td>
<td>52.0</td>
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<td><strong>Peoria ’96</strong></td>
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<tr>
<td>Jun-27</td>
<td>untreated</td>
<td>8.9 (7.6–10.4)</td>
<td>58.8</td>
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<tr>
<td>Jul-04</td>
<td>untreated</td>
<td>3.8 (2.7–5.1)</td>
<td>69.8</td>
</tr>
<tr>
<td>Aug-08 (F1)</td>
<td>35 d after 1 spray</td>
<td>3.6 (0.5–7.2)</td>
<td>39.2</td>
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</table>
Figure 1. The LC$_{50}$ and LC$_{95}$ values for all field populations tested as a function of number of exposures (sprays in the field) with Applaud (from commercial fields and small plot tests). Susceptibility to Applaud actually increased with number of sprays (for the LC$_{50}$s). There is no clear pattern of decreasing susceptibility within season with as many as 4 sprays (in a small plot context) nor after the recommended practice of 1 spray in the commercial fields. Baseline LC$_{50}$ values for whiteflies using this bioassay method are around 3–12 ppm.

\[ LC_{50} = -0.77x + 6.55 \]
\[ R^2 = 0.177 \]
\[ P = 0.046 \]

\[ LC_{95} = -5.72x + 63.33 \]
\[ R^2 = 0.09 \]
\[ P = 0.164 \]
Figure 2: Susceptibility of whiteflies to Applaud*. Baselines from untreated cotton from 2 years; Maricopa, AZ. 1993 & 1996. *Control mortalities were 14.7 & 26.0% for 1993 & 1996.

[Graph showing mortality against Applaud concentration for 9/2/93 and 6/24/96.

Figure 3: Susceptibility of whiteflies to Applaud*. Small plots left untreated; sampling on 4 dates; Maricopa, AZ. 1996. *Control mortalities were 18.3, 17.3, 26.7, 14.4% for 6/24–8/19.

[Graph showing mortality against Applaud concentration for 6/24/96, 7/22/96, 8/5/96, and 8/19/96.]
Figure 4: Susceptibility of whiteflies to Applaud*. Small plots treated 4 times with Applaud (28 June, 6 July, 23 July, 6 August); sampling after each application; Maricopa, AZ. 1996. *Control mortalities were 25.5, 16.8, 30.5, 16.7% for 7/8–8/19.

Figure 5: Susceptibility of whiteflies to Applaud*. Commercial field treated once with Applaud (24 July); sampling pre- & post-treatment; Eloy, AZ. 1996. *Control mortalities were 28.9, 36.8, 8.8, 18.1% for 7/2–8/15.
Figure 6: Susceptibility of whiteflies to Applaud*. Commercial field treated once with Applaud (6 July); sampling pre- & post-treatment; Peoria, AZ. 1996. *Control mortalities were 34.7, 13.7, 21.4% for 6/27–8/8.

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