
**Executive Summary**

The erythrina gall wasp, *Quadrasticus erythrinae*, (EGW) is one of the most devastating invasive species introduced into the State of Hawaii. EGW host range includes *Erythrina sandwicensis*, a native and large component of dry land forest areas, as well as *Erythrina variegata*, abundant in landscapes. Our work focused on immediate control of this pest with safe and effective insecticides. Insecticides and application methods were selected based on criteria of efficacy, treatment longevity and non target impact. Five studies were conducted on different host spp. in dryland forest areas, resorts and landscapes on both east and west sides of the Island of Hawaii. Native species were more tolerant of EGW infestation than *E variegata*. Imidacloprid applied systemically as a root drench or injected through trunks was effective against EGW. Root drenches were inconsistent and recommended only for containerized trees or those irrigated and naturally contained. Trunk injection systems were very efficacious but varied in response among injection systems. One of the most effective injection systems evaluated was the Arborjet system (arborjet.com); it performed consistently and allowed for the most volume of liquid to be injected into a trunk through the fewest locations. Imidacloprid was very persistent within the leaves and can provide season- or year-long control. Our results were shared with clientele at site visits and at formal meetings and seminars. Results were published in a refereed journal and presented at national and branch meetings of the Entomological Society of America. Adoption of treatment recommendations has occurred but has been limited by the devastating nature of the wasp, remoteness of certain areas and costs associated with treatment.
1. Conduct chemical treatment studies to evaluate the effectiveness of the following against EGW in *Erythrina* seedlings and saplings:
   a. Acephate, carbaryl and/or abamectin as foliar treatments
   b. Imidacloprid, acephate, and/or dinotefuran.

   Foliar treatment of carbaryl, (Sevin) known to be highly toxic against wasps, was conducted in July and August 2006. Five spray applications of carbaryl repeated every two weeks at the recommended label rate provided only minimum effect on heavily infested saplings of *E. sandwicenesis* and did not provide the residual activity to make it cost-effective. Therefore, foliar applications with other more expensive insecticides were not conducted. Efficacy of imidacloprid (Merit) and dinotefuran (Safari) applied as drenches at the recommended labeled rate against EGW were conducted in collaboration with a resort in West Hawaii on windbreak wiliwili (Fig 1). Imidacloprid and dinotefuran were applied at the point of irrigation in conjunction with liquid fertilizer to optimize uptake and increase plant vigor. Response to imidacloprid drench noted by new flush growth was observed 3 weeks after treatment and effectiveness continued to 4 months after treatment with no emergence of adult wasps from the few new galls that were observed on treated trees. Dinotefuran drenches were effective within 2.5 weeks of application but severe damage reoccurred in <4 months. Apparently, dinotefuran is much more water soluble than imidacloprid and explains the shorter residual activity. Systemic insecticide drenches will have a greater likelihood of success in treatment of containerized seedlings and saplings due to the confined root systems as compared with trees in the landscape with sprawling roots and groundcover.

2. Conduct chemical treatment studies to evaluate the effectiveness of the following against EGW in *E. sandwicenesis* and other *E. spp* used for landscaping and wind breaks:
   a. Imidacloprid and dinotefuran as drench treatments and soil injections
   b. Imidacloprid and abamectin with and without irrigation using the Mauget, Wedgle, and Sidewinder injection systems

   The initial study determined that imidacloprid can be effective against the EGW when trunk injected with the Mauget system; it reduced emergence from galls over a period of four months (Fig 1). Drenching with imidacloprid was not effective. Abamectin was not effective applied as an injection. The second and third trials were installed on endemic
wiliwili tree, *Erythrina sandwicensis* O. Deg., trees in a native dryland forest at Pu‘u Wa‘awa‘a and Waikoloa, Hawaii. The fourth trial was established in an irrigated resort landscape at the Hualalai Resort, Hualalai, Hawaii, on the coral tree, *Erythrina variegata*. At the irrigated resort setting, drenches of imidacloprid and also dinotefuran were included as treatments. Results from drench treatments repeatedly showed little or no results except in situations where roots were confined and concentrated because of containerization and controlled irrigation. The natural root system of erythrina which appear to be sparse and spread across a large area contributed to poor systemic uptake. Competition by neighboring plants or turf exacerbated the problem of uptake. Drenching with imidacloprid and dinotefuran was effective in one situation where tree roots were confined between a wall and sidewalk and received irrigation to a small area around them (Fig 2). Imidacloprid treatments were made using commercially available injection equipment and according to label recommendations of the formulations. Wedge Direct-Inject (Arbor Systems, Omaha, NE), Sidewinder Precision Tree Injector (Noosaville, QLD, Australia) and the Mauget Imicide (JJ Mauget Co, Arcadia, CA) were tested. Data was collected on emergence from galled leaf samples, galling severity, and imidacloprid residue within leaves using ELISA and HPLC techniques. Among the three injection systems tested, Wedge, Sidewinder and Mauget, Mauget delivered the highest concentration of imidacloprid in leaves but all systems were confounded by variability of uptake as indicated by large variability among and within the injection systems and locations (Fig 3). Despite the variability, a trend of reduced wasp emergence with increasing imidacloprid levels were observed. High correlation between concentration of imidacloprid and emergence of wasps or infestation severity rating of samples was demonstrated (Fig. 4); therefore, tissue analysis may be used to predict when re-treatment of trees is necessary. Trials have shown that imidacloprid is stable in the tissue with high residues detectable for more than 6 months. In studies at Pu‘uwa‘awa‘a, monthly fluctuations in gall wasp populations, may have been related to rainfall, new flushes and availability of food resources (ungalled leaf tissue) (Fig. 5).

The fifth and final trial was conducted in East Hawaii and included another injection system, Arborjet Tree IV (Arborjet, Woburn, MA; arborjet.com) injection system tested on tall or windbreak form of *E. variegata*. In this study, the highest concentrations of imidacloprid within tissue were measured in the Arborjet injected trees as compared with Mauget or Wedge (Tables 2 to 4). Imidacloprid levels were >300ppm using the Arborjet formulation, IMA-Jet (5% imidacloprid) and Arborjet injector. Stability of imidacloprid within the tissue was demonstrated yielding complete season long control with one trunk injection. This study determined that in addition to the concentration of imidacloprid injected, the volume of carrier injected into the tree is important to allow sufficient transport from the injection site to leaves. The Merit 200SL (17.1% imidacloprid) treatment delivered with the Arborjet system provided more active ingredient of imidacloprid, but did not result in leaves with the highest concentrations of residue, which likely due to less overall injection volume (Tables 2 and 3). The IMA-Jet (5% imidacloprid) delivered the highest volume. The IMA-Jet and Merit 200 SL treatments remained effective through complete leaf senescence, dormancy and into the second growing season of the tall erythrina trees (Table 4). This study indicates that one injection may possibly deliver two seasons of control. Further evaluation is needed to confirm the longevity of one injection.
3. Sample the plant tissues to measure both the concentration of chemical and the number of wasps produced per sample (See Fig 1,2 to 5; Table 2 to 4).

   Methodologies were effectively developed to quantify emergence of wasps. Galled leaf tissue samples were collected and galls that lack emergence holes were excised from surrounding tissue, weighed, and held in paper bowls covered in silkscreen for 2 weeks. At the end of that time emerged wasps were counted and numbers of wasps/g of gall tissue calculated. In addition to calculating emergence of wasps, a 5 point rating index of infestation severity was created to evaluate degree of galling. Concentration of imidacloprid in leaf tissue was measured by both HPLC and ELISA methods.

4. From the results of the above studies, evaluate the following:
   a. Optimal application time in relation to tree biology
   b. Optimal application for drenches and soil injection
   c. Duration of protection offered by drenches and injections
   d. Optimal number of injections per year

   The optimal time for application of imidacloprid is prior to leaf flushing and development of severe galling. This is especially important in areas where trees are growing under stressful conditions and have a limited ability to initiate new leaves or flush only seasonally. When imidacloprid is injected after severe galling and defoliation, trees have taken more than two months to begin to respond and develop new leaves in areas with abundant natural rainfall. Imidacloprid can be trunk injected prior to break of dormancy. Due to the limited success of drenches, drenches are currently recommended for small establishing trees, containerized trees, or trees with confined root systems. Although there is no perfect commercial trunk injection system, the Arborjet system has outperformed the others we have evaluated and would be recommended for most situations. One injection per year is a likely treatment regime. In certain circumstances, control in a second year following dormancy is possible through trunk injection.

5. Evaluate injection systems for management of large trees and trees in forested areas.

   This project developed a table that displays the relative advantages and disadvantages of the different commercial injection systems (Table 1). The Arborjet Tree IV is the most efficacious injection system because it is capable of injecting the greatest quantity of formulation into the tree using the fewest injection holes in a manner that allows assurance that the volume was successfully injected. The IMA-Jet formulation of imidacloprid for use with the Arborjet system also appears to have certain characteristics that allow for better mobility within the tree. Using the IMA-Jet formulation in the Sidewinder system may be less labor intensive than the Arborjet system and a good choice injecting in more remote locations (e.g., forest situations) where self-contained equipment is important.
6. Conduct tests to determine the long-term effect of drilling/boring into trees and the ability of plants to translocate chemicals in natural conditions with little or no rainfall.

   This study did not observe any negative effects of drilling into trees more than a year after injections. As a precaution, our treatment recommendations were developed to require drilling the fewest holes with the greatest interval between treatments. This study has determined that imidacloprid can be translocated in arid situations, including natural dryland forest areas. However, most of the injection systems did not perform as well and reliably under these dry arid conditions as compared with higher rainfall and irrigated areas.

7. Publicize research results via web pages, an outreach bulletin, press releases, and manuscripts submitted for publication in scientific, forestry, landscape trade journals, and newsletters.

   In addition to numerous personal meetings with landscape professionals at resorts throughout Hawaii, seminars were held on the Big Island, Maui and Oahu (Attachment 1). The meetings were attended on the average by 50 landscapers, arborist and other professionals. A manuscript, “Application of an enzyme-linked immunosorbent assay for the analysis of imidacloprid in wiliwili tree, *Erythrina sandwichensis* O. Deg, for control of the wasp *Quadrastichus erythrinae*.” by Ting Xu, Christopher Jacobsen, Arnold Hara and Qing Li has been published in the Journal of Agricultural and Food Chemistry (Attachment 2). A second manuscript has been prepared for Arthropod Management Tests and for another referred publication (Attachment 3). Our work has also been presented at both the Pacific Branch and national meetings of the Entomological Society of America and has been of great interest to researchers in both Florida and California since the gall wasp was recently introduced into Florida and California.

Refereed Publication:


Presentations:


Hara, A.H. 2006. Chemical control of the erythrina gall wasp. United Agri-Products Seminar, Honolulu, HI.


Website presentation:


Press Release:

Fig. 1. Number of erythrina gall wasps emerging from gall tissue after treatment with Imicide (imidacloprid) and Abacide (abamectin) delivered by the Mauget injection system, and Merit (imidacloprid) and Safari (dinotefuran) drenches.

![Graph showing mean emerged wasps per gram of gall tissue over months after treatment](image)

Fig. 2. Successful drench of imidacloprid in confined irrigated location.

![Image of person drenching plants](image)
Fig. 3. Correlation between treatments of *E. sandwicensis* trees and concentration of imidacloprid or emergence of wasps.
Fig. 4. Correlation between concentration of imidacloprid and emergence of wasps or infestation severity rating of samples.
Fig. 5.

**Fluctuation of levels of gall wasps and damage symptoms at Pu’u Wa’awa’a during the first 6 months post infestation inception.**

* Ratings were on a scale to 5. For galling severity, 0 represented no symptoms while a rating of 3 represented samples with heavy galling of leaves but minimal leaf deformity. Samples with ratings of 5 exhibited extreme galling and deformity. For emergence density, 0 represented no emergence from galls within the sample while 3 represented 30-45 emergence holes/ g gall tissue. A rating of 5 represented >60 emergence holes/ g gall tissue.
Table 1. Comparison of different tree injection systems under evaluation of efficacy studies.

<table>
<thead>
<tr>
<th>Treatment System</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Cost of System</th>
</tr>
</thead>
<tbody>
<tr>
<td>ArborSystems Wedge Direct-Inject</td>
<td>Creates least wounding of tree trunk among systems.</td>
<td>Some leaking of chemical (&lt;0.3ml) during treatment. The least quantity of AI is applied of any system. Must use ArborSystems’ chemical formulation.</td>
<td>$605 for Wedge Direct-Inject Pointer $305/ 120ml (5% AI) $28-41</td>
</tr>
<tr>
<td>Arborjet Tree IV Micro Infusion System</td>
<td>Injects the largest volume of insecticide through the fewest injection sites. Compatible with other formulations if desired. Able to see chemical uptake. Pretreatment holes need to be drilled. Occasional leaking. Must wait for treatment to finish (usually 15-20 min up to 1 hr). Remote application is impractical due to bulky equipment.</td>
<td>$699 for 2 tree IVs &amp; kit; $315 for each additional IV IMA-jet $175/ 500ml (5% AI) $56</td>
<td></td>
</tr>
<tr>
<td>Mauget Ready to use 3ml Micro injector Capsules</td>
<td>Formulation is premeasured and ready for placement. No additional equipment other than a drill is required. Able to see chemical uptake. Pretreatment holes need to be drilled. Wound remains unplugged. Passive system; tree does not always uptake product. Need to return later to collect the caps.</td>
<td>Imicide $116 for 24, 3ml capsules (10% AI) $48</td>
<td></td>
</tr>
<tr>
<td>Sidewinder Tree Injectors Backpack Tree Injector</td>
<td>Complete unit is carried on the back and includes drill and injection equipment. Somewhat heavy but practical for remote locations. No waiting for uptake Compatible with different formulations. Pretreatment holes need to be drilled. Occasional leakage. Difficult to assure the entire dose was administered. More injection sites are needed as compared with Arborjet Tree IV.</td>
<td>$1584 for Backpack Injector Insecticide is from other manufacturers following their labeled rates.</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Efficacy of various treatments applied to *Erythrina variegata* 10 weeks after treatment.

<table>
<thead>
<tr>
<th>Treatment Formulation/Equipment</th>
<th>Rate AI/ Inch Diameter</th>
<th>Galling Severity Rating* P=0.005</th>
<th>Emergence Rating* P&lt;0.0005</th>
<th>Emerged Wasps/g Tissue P&lt;0.0005</th>
<th>Imidacloprid Concentration µg µg/g P&lt;0.0005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>-----------</td>
<td>4.8a ± 0.25</td>
<td>3.8a ± 0.25</td>
<td>21.4a ± 2.04</td>
<td>0.0a ± 0.0</td>
</tr>
<tr>
<td>Imicide/ Mauget Capsules 10%AI</td>
<td>0.15 ml</td>
<td>3.3ab ± 0.48</td>
<td>1.5bc ± 0.29</td>
<td>8.9bc ± 2.88</td>
<td>2.9a ± 0.06</td>
</tr>
<tr>
<td>Pointer/ ArborSystems Wedgle 5% AI</td>
<td>0.026 ml</td>
<td>3.3ab ± 0.48</td>
<td>1.8bc ± 0.25</td>
<td>4.8c ± 0.87</td>
<td>7.3ab ± 0.12</td>
</tr>
<tr>
<td>Merit 200 SL/ Arbor Jet Tree IV 17.1%AI</td>
<td>0.77 ml</td>
<td>3.5ab ± 0.29</td>
<td>1.3bc ± 0.25</td>
<td>8.7bc ± 3.87</td>
<td>38.7b ± 1.45</td>
</tr>
<tr>
<td>IMA-jet/ Arbor Jet Tree IV 5% AI</td>
<td>0.40 ml</td>
<td>2.0b ± 0.71</td>
<td>0.8c ± 0.25</td>
<td>0.7c ± 0.51</td>
<td>320.7c ± 17.30</td>
</tr>
<tr>
<td>Merit 2/ Root Drench 21.4% Al</td>
<td>1.28 ml</td>
<td>4.5a ± 0.29</td>
<td>2.3b ± 0.25</td>
<td>15.8ab ± 2.56</td>
<td>0.2a ± 0.0</td>
</tr>
</tbody>
</table>

*Ratings were on a scale to 5. For galling severity, 0 represented no symptoms while a rating of 3 represented samples with heavy galling of leaves but minimal leaf deformity. Samples with ratings of 5 exhibited extreme galling and deformity. For emergence density, 0 represented no emergence from galls within the sample while 3 represented 30-45 existing emergence holes/ galled leaf sample. A rating of 5 represented >60 existing emergence holes/ galled leaf sample.

Means in a column with different letters are significantly different by Tukey’s multiple Comparison procedure (P<0.05).
Table 3. Efficacy of various treatments applied to *Erythrina variegata* 20 weeks after treatment.

<table>
<thead>
<tr>
<th>Treatment: Formulation/Equipment</th>
<th>Rate Al/ Inch Diameter</th>
<th>Galling Severity Rating P&lt;0.0005</th>
<th>Emergence Rating P=0.001</th>
<th>Emerged Wasps/g Tissue</th>
<th>Imidacloprid Concentration µg/g P&lt;0.0005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>154 ± 0.15 ml</td>
<td>5.0a ± 0.0</td>
<td>3.5a ± 0.50</td>
<td>15.2a ± 2.66</td>
<td>0.0a ± 0.0</td>
</tr>
<tr>
<td>Imicide/ Mauget Capsules 10 % Al</td>
<td>0.15 ml</td>
<td>3.3ab ± 0.25</td>
<td>1.8ab ± 0.48</td>
<td>3.2b ± 1.46</td>
<td>5.4a ± 0.47</td>
</tr>
<tr>
<td>Pointer/ ArborSystems Wedge 5 % Al</td>
<td>0.026 ml</td>
<td>3.3ab ± 0.25</td>
<td>1.5abc ± 0.29</td>
<td>3.0b ± 1.57</td>
<td>3.0a ± 0.27</td>
</tr>
<tr>
<td>Merit 200 SL/ Arbor Jet Tree IV 17.1 % Al</td>
<td>0.77 ml</td>
<td>1.5bc ± 0.87</td>
<td>0.5bc ± 0.50</td>
<td>0.4b ± 0.24</td>
<td>36.3b ± 2.03</td>
</tr>
<tr>
<td>IMA-jet/ Arbor Jet Tree IV 5% Al</td>
<td>0.40 ml</td>
<td>0.3c ± 0.25</td>
<td>0.0c ± 0.0</td>
<td>0.07b ± 0.07</td>
<td>234.7c ± 12.4</td>
</tr>
<tr>
<td>Merit 2/ Root Drench 21.4 % Al</td>
<td>1.28 ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Ratings were on a scale to 5. For galling severity, 0 represented no symptoms while a rating of 3 represented samples with heavy galling of leaves but minimal leaf deformity. Samples with ratings of 5 exhibited extreme galling and deformity. For emergence density, 0 represented no emergence from galls within the sample while 3 represented 30-45 existing emergence holes/ galled leaf sample. A rating of 5 represented >60 existing emergence holes/ galled leaf sample.

Means in a column with different letters are significantly different by Tukey’s multiple Comparison procedure (P<0.05).
Table 4. Efficacy of various treatments applied to *Erythrina variegata* 12 months after treatment.

<table>
<thead>
<tr>
<th>Treatment: Formulation/Equipment</th>
<th>Rate Al/ Inch Diameter</th>
<th>Galling Severity Rating* P=0.206</th>
<th>Emergence Rating* P=0.001</th>
<th>Emerged Wasps/g Tissue P=0.071</th>
<th>Imidacloprid Concentration µg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>0.15 ml</td>
<td>3.0a ± 0.58</td>
<td>2.3a ± 0.33</td>
<td>6.3a ± 1.23</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Imicide/ Mauget Capsules 10 % Al</td>
<td>0.026 ml</td>
<td>3.8a ± 0.25</td>
<td>3.3a ± 0.25</td>
<td>6.2a ± 1.57</td>
<td>0.7 ± 0.15</td>
</tr>
<tr>
<td>Pointer/ ArborSystems Wedge 5 % Al</td>
<td>0.77 ml</td>
<td>1.3a ± 0.88</td>
<td>0.3b ± 0.33</td>
<td>2.5a ± 0.80</td>
<td>21.0 ± 0.58</td>
</tr>
<tr>
<td>Merit 200 SL/ Arbor Jet Tree IV 17.1 % Al</td>
<td>0.40 ml</td>
<td>2.3a ± 1.03</td>
<td>0.8b ± 0.48</td>
<td>1.5a ± 1.27</td>
<td>41.0 ± 4.0</td>
</tr>
<tr>
<td>Merit 2/ Root Drench 21.4 % Al</td>
<td>1.28 ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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SEMINAR
FOR
LANDSCAPERS & ARBORISTS

“Erythrina Gall Wasp Control”

Topics:

- Update: chemical control recommendations – Chris Jacobsen, Univ. of Hawaii
- Update: biological control research, HDOA - Chris Jacobsen, Univ. of Hawaii
- Tree injection with the Arborjet Injection System – Arborjet representatives
- Informal Discussion - Participants share their info and results treating for EGW
- Demonstration of actual tree injection – Arborjet reps, in KOC garden

Date: Thursday, February 1, 2007
Time: 9 am – 12:00 pm
Place: Kona Outdoor Circle

Sponsored by the Cooperative Extension Service-UH Manoa and Arborjet
EGW Seminar
Kona Outdoor Circle
Feb 1, 2007

Below: Russ Davis of Arborjet provides a powerpoint presentation at the seminar.
Below: Joe Doccola of Arborjet demonstrates an injection technique with a live tree.
UAP 7TH Annual Seminar
Thursday, May 17, 2007
Pearl Country Club

11:30-12:15  45 Minute Lunch Break

12:15-1:00  #1 Update on Control of the Erythrina Gall Wasp & Other Invasive Species
Dr. Arnold H. Hara, Professor & Entomologist, Dept of Plant and Environmental Sciences
University of Hawaii at Manoa
www.ctahr.hawaii.edu/haraa
DOA Credits 1/ Pvt 1, Cat. 1A, 2, 3, 4, 6, 8, 9, 10
Certified Arborists: Tree worker CEUs: 0.75 Credits
Arborist CEUs: 0.75 Credits
Board Certified Arborist: Science CEUs: 0.75 Credits
Course Code: WE-07-180

#2  Biology & Control of Soil Borne Pathogens of Turfgrass: Fairy Ring Bermuda Decline & Spring Dead Spot
Dr. Frank Wong, Cooperative Extension Specialist, University of California, Riverside
www.turfpathology.ucr.edu
Sponsored By: Randy Rider, Syngenta www.syngenta.com
DOA Credits 1/ Cat. 3

#4  Establishing Bermuda grass and Seashore Paspalum as Golf Turf
Dr. Sebastian Braum, Manager, Agronomic Services & Marketing Support
www.yara.com

1:00-1:05  5 Minute Break

1:05-1:50  #1 Vertebrate Pest Management
Scott McCalley, Western Regional Manager, LiphaTech
www.liphatech.com
DOA Credits 1/ Pvt 1, Cat. 1A, 2, 3, 7C, 8, 10

#2  Application Principles & Their Effect on Turf Disease Control
Richard F. Fletcher, Director, Product Development, Cleary Chemical Corporation
www.clearychemical.com
DOA Credits 1/ Cat. 3

#3  Landscape Fertility Cultural / Fertilizer Recommendations for Landscape Plants
To: Landscape & Golf Course Industries

From: Norman Nagata, Assistant Extension Agent

You are invited to this seminar on invasive landscape pests that will be presented by Dr. Arnold Hara, Entomologist, University of Hawaii, College of Tropical Agriculture & Human Resources.

### Update on the Control of the Erythrina Gall Wasp (EGW) and Other Invasive Pests

**Date:** June 7, 2007 (Thursday)
**Time:** 3:00 to 4:00 pm
**Place:** Maui Community College, Science Building 10A
**Registration:** You may register to attend this “free seminar” by responding to this email notice or by calling the Cooperative Extension Service at 244-3242.

Your registration will assure that you will receive any handouts that may be provided and that you have attended this program for auditing purposes: for recertification credits.

### PROGRAM

1. **History & Status of the EGW in Hawaii**

2. **Biology of the EGW**
   - A. Identification
   - B. Duration of life stages
   - C. Host list
   - D. Gall formation
   - E. Effect on host

3. **Control Strategies**
   - A. Chemical
     - 1. Foliar
     - 2. Systemic drench
   - B. Non-Chemical
     - 1. Cultural control – replacement cultivars or species
     - 2. Classical biological control
       - a. Status of parasites from Africa
       - b. Potential for long-term control of EGW

4. **Other Invasive Species Updates**
   - A. Papaya mealybug
   - B. Coqui frog
   - C. Nettle caterpillar
   - D. Little fire ant

5. **Conclusions**

If you have any needs due to your disability, please contact Norman Nagata at 244-3242 by May 21, 2007.

cc: Arnold Hara, Robert Paull, Kenneth Grace, Harold Keyser & Wayne Nishigama

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Wai`alae-Kahala Extension Service 57-2-07
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Telephone: (808) 244-3242, Facsimile: (808) 244-7090, E-Mail: kahului@clahr.hawaii.edu, Web: www2.clahr.hawaii.edu

An Equal Opportunity/Affirmative Action Institution
Application of an Enzyme-linked Immunosorbent Assay for the
Analysis of Imidacloprid in Willwill Tree, Erythrina
sandwicensis O. Deg, for Control of the Wasp Quadrastichus
erthrinae

TING XU,† CHRISTOPHER M. JACOBSEN,† II KYU CHO,† ARNOLD H. HARA,‡ and
QING X. LI††

Department of Molecular Biosciences and Bioengineering, University of Hawaii, Honolulu,
Hawaii 96822, and Benedict Agricultural Research Center, 873 Kamehameha Street, University of
Hawaii, Hilo, Hawaii 96720

A monoclonal antibody-based enzyme-linked immunosorbent assay (ELISA) for the neonicotinoid
insecticide imidacloprid was evaluated for its reproducibility, accuracy, and comparability to results
from a conventional high-performance liquid chromatography (HPLC) for the analysis of imidacloprid
in the endemic willow tree (Erythrina sandwicensis O. Deg) found in dryland forests and landscapes
in Hawaii. Imidacloprid was applied to willow trees in an attempt to control the newly introduced
erythrina gall wasp, Quadrastichus erythrinae Kam. Leaf samples were freeze-dried and extracted
with acetic aqueous methanol followed by methylene chloride partitioning. After solvent removal, the
extract residue was reconstituted in 1 mL of water/methanol (1:1 v/v) for ELISA; no significant matrix
interference was observed at 10-fold or more dilution. The average recoveries of imidacloprid from
fortified samples ranged from 78% to 100% by ELISA. The correlation between the ELISA and
HPLC results was excellent (r² = 0.98). Imidacloprid was detected with the ELISA in all treated samples
and its level varied in the samples among different treatments and in those from different parts of the
trees. The infestation severity rating of leaf samples was inversely related to the concentration of
imidacloprid. It is clear that imidacloprid effectively controls the wasps. The ELISA is a suitable method
for quantitative and reliable determination of imidacloprid in willow trees and the application provides
information to understand how to control the wasps.

KEYWORDS: ELISA; imidacloprid; willow trees; leaves; wasps

INTRODUCTION

Erythrina sandwicensis O. Deg, is an endemic deciduous tree
that grows in dryland forests areas of leeward portions of the
Hawaiian Islands up to elevations of about 1500 ft (1). It is
also known as the willow or Hawaiian coral tree and produces
showy claw-shaped flowers that are commonly orange but other
forms can produce red, salmon, peach, light green, yellow, or
white flowers (2). In addition to growing in natural areas, E.
sandwicensis can be found in resort landscape settings. One of
the most recent threatening invasive species to willow trees is
the erythrina gall wasp (EGW), Quadrastichus erythrinae Kam
(3–4). In addition to E. sandwicensis, EGW attacks E. artemisia
and E. crista-galli (3).

The EGW was described in 2004 as a new species by Kim
et al. (5) from specimens from Singapore, Mauritius, and

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Reunion. The adult female wasp inserts eggs into the young
leaves. Larvae develop in the leaf tissue and the trees respond
to its feeding by producing galls. After pupation, the wasp exits
through a small hole in the gall. Heavily infested trees stop
growing, lose vigor, and may die. Since its discovery on Oahu
in April 2005, EGWs have spread rapidly to all the other major
islands of Hawaii (3).

Presently, chemical and biological controls are being inves-
tigated. Chemical control is a short term measure which mainly
focuses on effective use of insecticides. For long-term control,
classical biological control, involving the importation of specific
natural enemies, is the optimal choice because it is long lasting
and friendly to the environment and biological diversity. Preliminary systemic insecticide trials suggest that imidacloprid
can help in reducing damage to erythrina caused by the gall
wasp (3). Imidacloprid, 1-(6-chloronicotinyl)-2-(methylamino)
imidazolone (Figure 1), is a neonicotinoid insecticide with high activity
against sucking insects (6). It is the most widely used systemic
insecticide in the world (around 70 crops in more than 100

20
In order to develop a guideline for managing the wasps in wildlily trees, it is clear that groundwork information on the activity profile of imidaclopid in wildlily trees would be required. This information is less apparent for a systemic insecticide such as imidaclopid than for a fumigant insecticide, in part because of the longer period required for translation throughout a plant compared with the immediate contact and exposure of a fumigant-applied insecticide. Therefore, in this study, our goal is to apply a monoclonal-based enzyme-linked immunosorbent assay (ELISA) to monitor imidaclopid residues in wildlily leaves. The assay should be a suitable tool for researchers to use to improve imidaclopid application for control of insect pests.

MATERIALS AND METHODS

Reagents. All reagents were of analytical grade unless specified otherwise. Analytical standard imidaclopid (96.5% purity) was obtained from Bayer Corp, Shawnee, KS. Goat anti-mouse IgG-peroxidase conjugates ( IgG-KPL) phosphatase-conjugate buffer capsules with sodium perborate, carbonate–bicarbonate buffer capsules, and 0-glucuronidase–

Figure 1. Structures of imidaclopid and hapten.

Table 1. Infestation Severity Ratings

<table>
<thead>
<tr>
<th>rating</th>
<th>description</th>
<th>appl. gall weight</th>
<th>20 g leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>very light infestation, only very slight galling</td>
<td>3 g</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>moderate galling</td>
<td>3–5 g</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>heavy galling of leaves but minimal leaf defoliation</td>
<td>6–14 g</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>heavy galling of leaves but heavy leaf defoliation</td>
<td>14–18 g</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>extreme galling and defoliation with 8–10 expanded leaves</td>
<td>&gt; 18 g</td>
<td></td>
</tr>
</tbody>
</table>

Magenta Co, Arcadia, CA) was applied through the underside equip-

ment at 3 mL and 2 mL per inch diameter measured at breast height
(DBH) for 16–16 mL (25–30 cm) and > 16 mL (> 30 cm) DBH trees,
respectively. The field treatment was Magenta bacillus (90% imado-
clid, 37 Magenta Co, Arcadia, CA) packaged in ready-to-use 3 mL
microprojection capsules. An 11.94 mL (4.4 mm) hole was drilled into
the trunk of the tree, and a capsule fitted with a feeder tube was placed
at a depth corresponding to the conductive system tissue. The number
of capsules used was determined by dividing the diameter by 2. Unlike
the other treatments that were applied to the trunk near the ground,
the capsules were applied to the main limbs of the tree 4–6 ft from the
ground. Treatments were applied to wildlily trees in a native dryland
forest at Pu‘u Wa‘a’a‘a and Waialohi, Hawaii, and in an irrigated
resort landscape at the Hualalai Resort, Hualalai, Hawaii, all located
on the Island of Hawai‘i. Hualalai Resort treatment was using Magna-
capsules occurred March 16, 2008. At Pu‘u Wa‘a’a‘a and Wildside
Siderow treatment occurred November 10 and December 2, 2005,
respectively. Waialohi Wildside and Siderow treatment occurred
December 7 and December 19, 2005, respectively.

Sampling. Leaf samples were obtained by cutting 1.5 cm long
growing tips from the outer edge of the canopy. The samples were
collected at the lower, mid, and upper canopy levels in at least four
different locations at each level. The samples for imidaclopid analy-
s were stored at –20 °C until analyzed. The samples were cut using a
knife to remove any damaged tissue. The samples were placed in clear
plastic bags and stored at –20 °C until analyzed.

Evaluation of infestation. Samples were evaluated for
totality of infestation by a five-point numerical rating system (Table
1). A rating of 1 signifies very light infestation levels with only very
slight galling. A rating of 5 represents samples with heavy galling of
leaves but minimal leaf defoliation. Samples with ratings of 5 exhibited
extreme galling and defoliation with no expanded leaves. Wasp
emergence was quantified by excising galls that included emergence holes
and holding them in many 45 mL mixed-paper beakers (Georgia Pacific,
Atlanta, GA) covered with adhesive to prevent escape. Galls were
weighed at the time of excision so that the number of wasps per gram
of gall tissue could be calculated. Three weeks after collection, wasps
were counted with the aid of a dissecting microscope.

Extraction Procedures. Leaf samples without peduncles were freeze-
dried and ground to powder. One gram of leaf powder was weighed in
a 100 mL bottle. Imidaclopid was extracted ultrasonically with 10 mL
of methanol/H2O:0.014 M H2SO4 (1:1, v/v) at 60 °C for 20 min. The
extract was vacuum filtered through Whatman No. 4 filter paper (ID
9 cm, pore size 3.5 μm) with 1 g of Celite 545 in it. The filtrate was
concentrated to 10–15 mL of water by evaporating with a rotary
evaporator, at 35 °C, under vacuum. The residue was centrifuged (6000 g) at
10 min, and the supernatant was transferred to a 50 mL separation
funnel.

For ELISA determination, the supernatant was extracted with
methanol (20 mL x 3). The methylene chloride layer was
collected and concentrated to 1–2 mL with a rotary evaporator.

180

The organic remainder was transferred into a tube and dried under nitrogen.

The residue was dissolved in 1 mL of water-methanol (1:1, v/v) which
was diluted at least 10-fold with water for ELISA.

For HPLC determination, the supernatant was washed with 20 mL
of hexane and the aqueous layer was collected. The hexane layer
was extracted once again with 20 mL of 0.024 M H2SO4. The aqueous phases
were combined and transferred to a 125 mL separatory funnel followed
by extraction with methylene chloride (20 mL x 3). The combined
methylene chloride extract was concentrated to 2 mL with a rotary
evaporator. The organic remainder was passed through a C18 cartridge
(Analytichem, Inc., Norwalk, DE) that was preconditioned with 5 mL of

Xu et al.

21
metanol followed by 5 mL of water. The cartridge was eluted with 5 mL of methylene chloride/acetonitrile (55:45, v/v). The eluate was collected and dried under a gentle nitrogen stream. The residue was reconstituted in 2 mL of acetonitrile/water (1:1, v/v) and filtered through a 0.45-μm syringe filter (Gelman Sciences, Ann Arbor, MI) before HPLC analysis.

ELISA Determination. The ELISAs were carried out in 96-well poly styrene microplates (Maxisorp F96; Nalgene Nunc International, Copenhagen, Denmark) as previously described (22). Briefly, microplate wells were coated with antigen (4 μg in 100 μL well in 0.05 M carbonate–bicarbonate buffer, pH 9.6) of hapten (Figure 1) and BSA overnight at 4 °C. The following day, the plates were washed four times with PBS containing 0.05% Tween 20 (PBST) and then blocked with 1% BSA in PBS (150 μL per well) for 1 h at room temperature. The plates were washed again four times; a solution of 50 μl per well of samples or standard diluted in PBST and 50 μl per well (0.2 μg of antibody per well) of unlabelled MAb 2A8 was added and incubated at room temperature for 1 h. Peroxidase-labeled goat anti mouse IgG (1:5000 in PBST; 100 μL per well) was then added, and the plates were incubated at room temperature for 1 h. The plates were then washed four times as above, and then substrate solution (100 μL per well of 0.05 M citrate–phosphate buffer, pH 5.6, containing 0.03% o-phenylenediamine and 1.0 mg/mL of OPD) was added. After 10–15 min at room temperature, the reaction was stopped with sulfuric acid (4 N, 50 μL per well), and absorbance at 490 nm was read with a Vmax kinetic microplate reader (Molecular Devices, Sunnyvale, CA). Samples and standards were analyzed in four replicate wells. Inhibition curves were fitted with the four-parameter logistic equation using Softmax version 2.35 software (Molecular Devices).

HPLC Determinations. A Dionex BioLC system ( Dionex Corp., Sunnyvale, CA) consisted of a 100 photodiode array detector, AS50 autosampler, GP50 gradient pump, and column oven, which were controlled by Chromeleon software. The HPLC was operated at the following conditions: mobile phase, acetonitrile/methanol (55:45, v/v); injection volume, 30 μL; flow rate, 1.5 mL/min; column, Inertsil ODS-3; 5 μm, 4.6 × 250 mm; column temperature, 30 °C; wavelength, 270 nm.

RESULTS AND DISCUSSION

Matrix Interference. Several instrument methods (12–14) and immunoassays (11–16) have been reported for the analysis of imidacloprid in environmental matrices and agricultural products. As it is well-known, immunocatalytic methods for residual pesticides have many advantages. On the other hand, although these methods are susceptible to matrix interference from sample matrices, especially biological samples, they can be overcome by simple dilution with water or appropriate buffer without troublesome clean-up steps (17). The ELISA for residual imidacloprid monitoring was highly specific and sensitive (17). No significant matrix interferenc from the water and cucumber samples was observed after sample dilution of the extracts before analysis in our previous studies (17). This indicated that the ELISA method could be suitable to perform residual analysis for imidacloprid in the environment and biological matrices. In this study, we applied the ELISA method to analyze imidacloprid in walnut leaf extracts.

An ultrasonic extraction with a mixture of methanol and 0.04% H2SO4 (4:1, v/v) was applied to walnut leaf samples (13, 15). The extracts may contain numerous constituents such as chlorophylls, carotenoids, and wax, and therefore, it is essential to assess the influence of interference on the ELISA performance. The optimal dilution factor with water was investigated for the extract (Figure 2). Although the IC50 value slightly shifts slightly, the curve of the 5-fold dilution sample is apart from the standard curve, which is apparently due to the matrix interference. Little position shift of the curves of 10-fold or more dilutions relative to the standard curve indicates no significant matrix interference on the assay. Therefore, it is necessary to dilute the extracts at least 10-fold for ELISA to minimize the matrix effects.

The extracts of the plant leaves were too complicated for direct analysis by HPLC. Thus, a further cleanup procedure was necessary after extraction. Several methods such as liquid–liquid partition (LLP) (18), supercritical fluid extraction (SFE) (19), and solid-phase extraction (SPE) (20) have been successfully applied to clean up the extract of imidacloprid residues from environmental samples. In this study, LLP and SFE were used to clean up the extracts. Methane chloride was used to eliminate polar compounds followed by a C18 column cleanup to remove nonpolar interference such as lipids from the matrixes. Elution of imidacloprid was carried out with a different solvent and its proportions to establish the best elution procedure.

Elution with 100% of methanol or acetonitrile provided good recoveries of imidacloprid, but the eluates obtained were dairy because of water and pigments. In contrast, elution with 100% of methylene chloride gave low recoveries of imidacloprid and required more solvent. In this study, different ratios of methylene chloride and acetonitrile were tested. Elution with a mixture of methylene chloride/acetonitrile at 65:15 (v/v) had a minimal amount of co-extractives and gave satisfactory recoveries. The eluates did not interfere with the accurate determination of imidacloprid by HPLC (Figure 3).

HPLC Separation. The chromatographic separation of imidacloprid using different mobile phases was investigated in detail according to the method of Lin et al. (21). The imidacloprid peak was relatively wide and tailed using aqueous acetonitrile (20%) as a mobile phase. This problem was overcome by adding ammonium acetate to the mobile phase. Further investigations showed that a reasonable retention time for imidacloprid could be obtained at about 11.2 min by adjusting the ratio of acetonitrile/5 mM ammonium acetate solution at 20:80 (v/v). With this mobile phase, imidacloprid could be completely separated from the matrix interferences (Figure 3). The concentrations of imidacloprid were calculated by calibration with the peak areas of external imidacloprid standard.

Comparison of Recoveries Determined on HPLC and ELISA. Recovery experiments were performed in control samples at four fortification levels (Table 2). The average recoveries of imidacloprid from the leaf samples were in a range of 78–100% for ELISA and 76–114% for HPLC, respectively.
Table 2. Recovery of imidacloprid from fortified samples determined by ELISA and HPLC

<table>
<thead>
<tr>
<th>fortified concentration (μg/g)</th>
<th>ELISA (μg/g)</th>
<th>HPLC (μg/g)</th>
<th>recovery (%)</th>
<th>n = 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>0.1</td>
<td>0.06 ± 0.04</td>
<td>0.04 ± 0.02</td>
<td>91</td>
<td>84</td>
</tr>
<tr>
<td>0.5</td>
<td>0.36 ± 0.02</td>
<td>0.36 ± 0.04</td>
<td>78</td>
<td>76</td>
</tr>
<tr>
<td>2</td>
<td>1.77 ± 0.1</td>
<td>1.70 ± 0.22</td>
<td>114</td>
<td>114</td>
</tr>
<tr>
<td>10</td>
<td>9.91 ± 0.05</td>
<td>10.54 ± 0.19</td>
<td>109</td>
<td>105</td>
</tr>
</tbody>
</table>

ND, not detected.

Both the ELISA and HPLC procedures are sensitive enough to detect 0.1 ppm of imidacloprid in the leaf samples.

To validate the ELISA, correlation studies were performed. Figure 4 shows an excellent correlation (r² = 0.98) between the results obtained by ELISA and those by HPLC analysis of samples which contained different levels of imidacloprid.

The satisfactory recovery and correlation suggested that both ELISA and HPLC methods were suitable for the analysis of imidacloprid in the leaves. However, there are some differences of pretreatment between these two methods. Compared with ELISA, sample cleanup procedures are required for HPLC analysis. In addition, HPLC requires more organic solvents and generates solvent wastes, which need proper disposal. Since ELISA has far higher sample throughput than HPLC analysis and can fulfill the requirements for monitoring imidacloprid in the leaves, it was used to analyze the real samples.

Application to Real Samples. There had been very limited experience with imidacloprid against wasps in widespread (27). Expectations were highly based on knowledge of the superior performance of imidacloprid against sucking insects in various crop settings (27–29). Decision making in pest management has traditionally relied upon field efficacy data related to a particular activity profile for any given insecticide. Thus, measuring insecticide concentrations within a plant may provide information on effective doses and help us improve wasp management. In the present study, imidacloprid was injected into trees in three different ways including Wedge, Sidewinder, and Mauger. Imidacloprid was detected in all the samples collected from treated trees and low emergence of the wasps was observed for treated trees compared with untreated trees (Figure 5). Actually, no imidacloprid was detected in untreated trees. More wasps emerged from untreated trees in Waikoloa than those at Pu‘u Wa‘awa‘a as shown in Figure 5. It is clear that the infestation of wasps is different at two locations. Maybe that is a reason why a more significant decrease of wasps was observed at Waikoloa than at Pu‘u Wa‘awa‘a under the same
Figure 6. Correlation between concentration of imidacloprid and emergence of the wasps and infestation severity rating of samples. The error bars are standard deviations.

The concentrations of imidacloprid in the leaf samples correlated inversely with the emergence of the wasps and infestation severity rating (Figure 6). Trees were treated with the Maugat Incisive microinjection capsules at 0.15 mL a.i./inch diameter were sampled approximately 3 weeks after treatment and contained the highest concentration of imidacloprid in the leaves (Figure 5) and consequently had the lowest control efficacy among the three treatment methods (Figure 6). Wedge (applied at 0.025 mL a.i./inch diameter) and SideWinder (applied at 0.15–0.7 mL a.i./inch diameter) treatments were applied during approximately the same period and were sampled between 4 and 5 months after treatment. The Wedge system is purported by the manufacturer to provide greater efficiency of imidacloprid utilization due to the targeted nature of the injection method. The results of this study may indicate greater utilization despite lower concentration values for the Wedge treatment. The Wedge treatment had 1.5 and 6.5 times less imidacloprid than those treated by SideWinder which applied 5.8–7.7 times more imidacloprid. This study focused on imidacloprid extraction and measurement for the E. aceris. The results indicate that the analytical method could be used to determine efficiency differences among injection equipment and method, efficacy thresholds, and control periods. Imidacloprid distribution in willow trees was obtained to relate to injection techniques and control efficacy. The tests were carried out by analyzing the leaves collected from lower, middle, and upper canopies of the trees treated via the SideWinder technique and the gall or non-galling leaves collected from the middle canopy. It is interesting that the concentration of imidacloprid in the leaves decreased gradually from the low canopy to the top canopy. The imidacloprid levels in the non-galling leaves from two of the three trees were much higher than those in the galling leaves (Table 3). The imidacloprid level in the non-galling leaves from tree-2 was slightly lower than that in the galling leaves. The data suggest field control variations. After imidacloprid was injected into trunks or main limbs, it was slowly taken up into different parts of the tree.

ELISA is an effective method to quantify and monitor imidacloprid in willow trees. We will continue to use this assay in our further work on gathering more basic knowledge of imidacloprid in willow trees such as the nature of its exposure to wasps, its spatial and temporal dynamics, and the intrinsic susceptibility of the wasps to imidacloprid.

Table 3. Spatial Distribution of Imidacloprid in Trees with SideWinder Treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tree 1</th>
<th>Tree 2</th>
<th>Tree 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>upper canopy</td>
<td>1.15 ± 0.05</td>
<td>0.78 ± 0.05</td>
<td>0.93 ± 0.02</td>
</tr>
<tr>
<td>middle canopy</td>
<td>1.32 ± 0.13</td>
<td>1.34 ± 0.16</td>
<td>1.12 ± 0.14</td>
</tr>
<tr>
<td>lower canopy</td>
<td>2.18 ± 0.26</td>
<td>2.15 ± 0.25</td>
<td>1.31 ± 0.05</td>
</tr>
<tr>
<td>galling absent</td>
<td>1.85 ± 0.05</td>
<td>0.72 ± 0.01</td>
<td>1.05 ± 0.03</td>
</tr>
<tr>
<td>galling present</td>
<td>0.73 ± 0.03</td>
<td>1.00 ± 0.03</td>
<td>0.85 ± 0.03</td>
</tr>
</tbody>
</table>

Conclusion. A monoclonal antibody-based ELISA was used to measure concentrations of imidacloprid in willow leaf samples for control of the gall wasp, Quadristicta aceris. The satisfactory recovery of imidacloprid by ELISA and the good correlation between ELISA and HI-PLC results suggest that ELISA is a highly sensitive and relatively simple method to quantify imidacloprid in willow tree leaves. Imidacloprid was distributed into different parts of the trees after treatment. The inverse relationship between the imidacloprid concentration and the infestation severity rating suggests imidacloprid works effectively against the wasps. The ELISA is a useful tool to measure imidacloprid for management and control of the wasps.

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25
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Erythrina gall wasp: Quadrastichus erythrinae Kim

This experiment was conducted using upright or windbreak Erythrina variegata
trees approx 25-35 ft tall growing near Hilo, Hawaii. The study was arranged along a
row of the trees in a 4 replicate CRD with a replicate consisting of a single tree. Trees
were heavily infested and nearly defoliated at the time of treatment on 23 June 2006.
Five treatments (4 trunk injections and 1 soil drench) and the untreated control were
applied according to labeled rates and were: 1 untreated control; 2 Imicide via Mauget
ready to use 3ml capsules (diameter/2 = number of capsules); 3 Pointer via ArborSystems
Wedgle (1ml injection every 6 inches around trunk circumference); 4 Merit 200 SL via
Arbor Jet Tree IV (4.7ml/ inch diameter); 5 IMA-jet via Arbor Jet Tree IV (8ml/ inch
diameter); 6 Merit 2F soil drench (6ml/ inch diameter). Diameters were measured at breast height and multiple trunks were measured individually and then summed to get a total diameter for dose calculation. Efficacy data consisted of collecting leaf samples mid canopy in at least 4 different locations. Samples were then returned to the lab and evaluated for severity of galling and rating of wasp emergence holes within the samples. Both were evaluated on a scale to five. For galling severity, 0 represented no symptoms while 3 represented samples with heavy galling but minimal leaf deformity. Samples with 5 exhibited extreme galling and deformity or stunting. For emergence density 0 represented no emergence from galls while 3 represented 30-45 emergence holes/galled leaf sample. A rating of 5 represented > 60 emergence holes/ galled leaf sample. In addition to ratings, actual wasp emergence/ g of galled tissue was determined by excising galls from leaf samples, holding them in paper bowls covered with silkscreen for 3 weeks and with the aid of a dissecting microscope counting the number of wasps emerged from the excised galls. Following ratings and excising of galls for wasp emergence data, samples were frozen and shipped to Honolulu, HI for analyses of imidacloprid concentrations within leaf tissue. Concentrations were determined by both HPLC and ELISA methodologies.

Prior to study initiation all trees were severely infested and exhibited heavily deformed/stunted leaves and petioles; trees were largely defoliated. The first evaluation, 3WAT, revealed detectable levels of imidacloprid in all treatments (Table 1). IMA-jet had the greatest concentration of imidacloprid (179ppm) and was the only treatment in which trees physically displayed a response to treatment; leaves showed slightly less incidence of galling. At 5 WAT reduction in emergence from galls was displayed by all treatments except the soil drench of Merit 2F, which was not efficacious in this study. The sparse spread out root system of these windbreak trees appears to have prevented
sufficient uptake of the drenches. By 10 WAT trees had begun to regain and retain leaves within the canopies. Wasps/g gall tissue was approx 21 wasps for untreated trees and ranged from <1 - 9 among injection treatments (Table 3). IMA-jet with the greatest concentration of imidacloprid showed the least wasp emergence. Merit 200SL had very high levels of imidacloprid (38.7 ppm) but displayed greater than expected wasp emergence from tissue. It is possible that the low injection volume of this treatment slowed dispersal throughout the canopy and created areas of different concentrations within leaves and galls formed in those areas of lower concentration. By 15 WAT wasp emergence from Merit 200SL showed a reduction to 2.7 wasps/g which correlates much better with the imidacloprid concentration levels found (Table 4.). All injections showed reduced emergence of wasps and trees treated with Merit 200SL and IMA-jet had greatly reduced galling of leaves. At 20 WAT all trunk injection treatments were still efficacious. IMA-jet was superior among treatments and displayed practically no galling of leaves (0.3 galling severity rating) (Table 5). After 20 WAT treatment, trees began to naturally drop their leaves for the winter months and evaluations were discontinued until the spring flush (10 months after treatment). Ten months after treatment with a whole new canopy of leaves imidacloprid was still measurable in all trunk injections. Untreated trees were almost completely dead and were no longer rated or quantified for wasp emergence. Imicide, Pointer and IMA-jet treatments had moderate infestation severity ratings (2-3.3) while Merit 200SL still showed low infestation (<1) (Table 6.). One year following treatment, Merit 200SL and IMA-jet treatments had concentrations of 28.6 and 46.8 ppm, respectively, which are levels great enough to control gall wasp; emergence ratings were lower than efficacious levels the remaining treatments (<1) (Table 7.).

In summary all treatments except the soil applied drench were effective against the erythrina gall wasp throughout the growing season and remained detectable the
following year. It may be possible to refrain from treating the following year with IMA-jet and Merit 200SL treatments but Imicide and Pointer applied at study dosages require yearly reapplication. IMA-jet treatment resulted in the greatest concentration of imidaclorpid within the leaves and the greatest reduction in galling. Merit 200SL was applied at the highest rate AI but did not result in the greatest concentration within leaf tissue. It may be that the limited volume of carrier contributed to reduced movement into the leaf tissue. Imicide and Pointer treatments were applied at much lower rates AI as compared with Merit 200 SL and IMA-jet. It is likely greater efficacy with those treatments would result from increases in dose.
Materials Tested

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Product Name: Imicide
Manufacturer’s Name: J.J. Mauget Company
Address: Arcadia, CA 91006
Active Ingredient: 10% Imidacloprid

Product Name: Pointer
Manufacturer’s Name: ArborSystems
Address: Omaha, NE 68134
Active Ingredient: 5% Imidacloprid

Product Name: Merit 200 SL
Manufacturer’s Name: Bayer Environmental Science
Address: Research Triangle Park, NC 27709
Active Ingredient: 17.1% Imidacloprid

Product Name: IMA-jet
Manufacturer’s Name: Arborjet Inc.
Address: Winchester, MA 01890
Active Ingredient: 10% Imidacloprid
Product Name: Merit 2F

Manufacturer’s Name: Bayer Environmental Science

Address: Research Triangle Park, NC 27709

Active Ingredient: 21.4% Imidacloprid
### Table 1. 3 WAT

<table>
<thead>
<tr>
<th>Treatment Formulation/Equipment</th>
<th>Rate AI/ Inch Diameter</th>
<th>Galling Severity Rating* P=0.003</th>
<th>Emergence Rating* P=0.006</th>
<th>Emerged Wasps/g Tissue P=0.337</th>
<th>Imidacloprid Concentration µg/g P&lt;0.0005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>---</td>
<td>4.5a ± 0.29</td>
<td>3.0a ± 0.0</td>
<td>7.2a ± 1.31</td>
<td>0.0a ± 0.0</td>
</tr>
<tr>
<td>Imicide/ Mauget Capsules 10%AI</td>
<td>0.15 ml</td>
<td>4.3ab ± 0.25</td>
<td>2.8a ± 0.25</td>
<td>6.1a ± 2.05</td>
<td>2.9a ± 0.33</td>
</tr>
<tr>
<td>Pointer/ ArborSystems Wedgle 5% AI</td>
<td>0.026 ml</td>
<td>4.0ab ± 0.41</td>
<td>2.8a ± 0.25</td>
<td>4.1a ± 0.50</td>
<td>3.8a ± 0.04</td>
</tr>
<tr>
<td>Merit 200 SL/ Arbor Jet Tree IV 17.1% AI</td>
<td>0.77 ml</td>
<td>4.0ab ± 0.0</td>
<td>1.8ab ± 0.48</td>
<td>4.9a ± 0.60</td>
<td>70.0b ± 1.73</td>
</tr>
<tr>
<td>IMA-jet/ Arbor Jet Tree IV 5% AI</td>
<td>0.40 ml</td>
<td>3.3b ± 0.25</td>
<td>1.3b ± 0.25</td>
<td>2.4a ± 1.80</td>
<td>178.6c ± 2.72</td>
</tr>
<tr>
<td>Merit 2/ Root Drench 21.4% AI</td>
<td>1.28 ml</td>
<td>5.0a ± 0.0</td>
<td>1.8ab ± 0.48</td>
<td>5.2a ± 1.97</td>
<td>0.4a ± 0.02</td>
</tr>
</tbody>
</table>

*Ratings were on a scale to 5. For galling severity, 0 represented no symptoms while a rating of 3 represented samples with heavy galling of leaves but minimal leaf deformity. Samples with ratings of 5 exhibited extreme galling and deformity. For emergence density, 0 represented no emergence from galls within the sample while 3 represented 30-45 existing emergence holes/ galled leaf sample. A rating of 5 represented >60 existing emergence holes/ galled leaf sample.
Table 2. 5 WAT

<table>
<thead>
<tr>
<th>Treatment Formulation/Equipment</th>
<th>Rate AI/ Inch Diameter</th>
<th>Galling Severity Rating* P=0.06</th>
<th>Emergence Rating* P=0.02</th>
<th>Emerged Wasps/g Tissue P=0.004</th>
<th>Imidacloprid Concentration µg/g P&lt;0.0005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>--</td>
<td>4.8 ± 0.25</td>
<td>3.5 ± 0.29</td>
<td>15.9 ± 2.64</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Imicide/ Mauget Capsules 10%AI</td>
<td>0.15 ml</td>
<td>3.5 ± 0.29</td>
<td>1.8 ± 0.48</td>
<td>8.1 ± 2.50</td>
<td>6.4 ± 0.09</td>
</tr>
<tr>
<td>Pointer/ ArborSystems Wedgle 5% AI</td>
<td>0.026 ml</td>
<td>4.0 ± 0.4</td>
<td>2.3 ± 0.63</td>
<td>7.4 ± 2.34</td>
<td>2.4 ± 0.03</td>
</tr>
<tr>
<td>Merit 200 SL/ Arbor Jet Tree IV 17.1%AI</td>
<td>0.77 ml</td>
<td>3.8 ± 0.48</td>
<td>1.5 ± 0.29</td>
<td>9.5 ± 2.66</td>
<td>28.3 ± 1.20</td>
</tr>
<tr>
<td>IMA-jet/ Arbor Jet Tree IV 5% AI</td>
<td>0.40 ml</td>
<td>3.5 ± 0.65</td>
<td>1.8 ± 0.48</td>
<td>3.0 ± 0.84</td>
<td>98.8 ± 4.34</td>
</tr>
<tr>
<td>Merit 2/ Root Drench 21.4% AI</td>
<td>1.28 ml</td>
<td>5.0 ± 0.0</td>
<td>3.3 ± 0.48</td>
<td>16.1 ± 1.88</td>
<td>0.1 ± 0.0</td>
</tr>
</tbody>
</table>

*Ratings were on a scale to 5. For galling severity, 0 represented no symptoms while a rating of 3 represented samples with heavy galling of leaves but minimal leaf deformity. Samples with ratings of 5 exhibited extreme galling and deformity. For emergence density, 0 represented no emergence from galls within the sample while 3 represented 30-45 existing emergence holes/ galled leaf sample. A rating of 5 represented >60 existing emergence holes/ galled leaf sample.
Table 3. 10 WAT

<table>
<thead>
<tr>
<th>Treatment Formulation/Equipment</th>
<th>Rate AI/ Inch Diameter</th>
<th>Galling Severity Rating* P=0.005</th>
<th>Emergence Rating* P&lt;0.0005</th>
<th>Emerged Wasps/g Tissue P&lt;0.0005</th>
<th>Imidacloprid Concentration µg µg/g P&lt;0.0005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>-----</td>
<td>4.8a ± 0.25</td>
<td>3.8a ± 0.25</td>
<td>21.4a ± 2.04</td>
<td>0.0a ± 0.0</td>
</tr>
<tr>
<td>Imicide/ Mauget Capsules 10%AI</td>
<td>0.15 ml</td>
<td>3.3ab ± 0.48</td>
<td>1.5bc ± 0.29</td>
<td>8.9bc ± 2.88</td>
<td>2.9a ± 0.06</td>
</tr>
<tr>
<td>Pointer/ ArborSystems Wedgle 5%AI</td>
<td>0.026 ml</td>
<td>3.3ab ± 0.48</td>
<td>1.8bc ± 0.25</td>
<td>4.8c ± 0.87</td>
<td>7.3ab ± 0.12</td>
</tr>
<tr>
<td>Merit 200 SL/ Arbor Jet Tree IV 17.1%AI</td>
<td>0.77 ml</td>
<td>3.5ab ± 0.29</td>
<td>1.3bc ± 0.25</td>
<td>8.7bc ± 3.87</td>
<td>38.7b ± 1.45</td>
</tr>
<tr>
<td>IMA-jet/ Arbor Jet Tree IV 5% AI</td>
<td>0.40 ml</td>
<td>2.0b ± 0.71</td>
<td>0.8c ± 0.25</td>
<td>0.7c ± 0.51</td>
<td>320.7c ± 17.30</td>
</tr>
<tr>
<td>Merit 2/ Root Drench 21.4% AI</td>
<td>1.28 ml</td>
<td>4.5a ± 0.29</td>
<td>2.3b ± 0.25</td>
<td>15.8ab ± 2.56</td>
<td>0.2a ± 0.0</td>
</tr>
</tbody>
</table>

*Ratings were on a scale to 5. For galling severity, 0 represented no symptoms while a rating of 3 represented samples with heavy galling of leaves but minimal leaf deformity. Samples with ratings of 5 exhibited extreme galling and deformity. For emergence density, 0 represented no emergence from galls within the sample while 3 represented 30-45 existing emergence holes/ galled leaf sample. A rating of 5 represented >60 existing emergence holes/ galled leaf sample.
Table 4. 15 WAT

<table>
<thead>
<tr>
<th>Treatment: Formulation/Equipment</th>
<th>Rate AI/ Inch Diameter</th>
<th>Galling Severity Rating P&lt;0.0005</th>
<th>Emergence Rating P&lt;0.0005</th>
<th>Emerged Wasps/g Tissue P=0.243</th>
<th>Imidacloprid Concentration μg/g P&lt;0.0005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>--</td>
<td>4.8a ± 0.25</td>
<td>4.0a ± 0.29</td>
<td>8.9a ± 3.23</td>
<td>0.0a ± 0.0</td>
</tr>
<tr>
<td>Imicide/ Mauget Capsules 10 % AI</td>
<td>0.15 ml</td>
<td>4.0a ± 0.29</td>
<td>2.3b ± 0.48</td>
<td>5.0a ± 1.97</td>
<td>6.2a ± 0.21</td>
</tr>
<tr>
<td>Pointer/ ArborSystems Wedgle 5 % AI</td>
<td>0.026 ml</td>
<td>3.8a ± 0.4</td>
<td>2.0bc ± 0.63</td>
<td>9.6a ± 4.57</td>
<td>5.8a ± 0.20</td>
</tr>
<tr>
<td>Merit 200 SL/ Arbor Jet Tree IV 17.1 % AI</td>
<td>0.77 ml</td>
<td>1.8b ± 0.48</td>
<td>0.8bc ± 0.29</td>
<td>2.7a ± 2.40</td>
<td>60.0b ± 1.73</td>
</tr>
<tr>
<td>IMA-jet/ Arbor Jet Tree IV 5% AI</td>
<td>0.40 ml</td>
<td>1.5b ± 0.65</td>
<td>0.5c ± 0.48</td>
<td>0.48a ± 0.48</td>
<td>357.3c ± 25.6</td>
</tr>
<tr>
<td>Merit 2/ Root Drench 21.4 % AI</td>
<td>1.28 ml</td>
<td>4.5a ± 0.0</td>
<td>3.0a ± 0.48</td>
<td>5.5a ± 2.74</td>
<td>2.0a ± 0.12</td>
</tr>
</tbody>
</table>

*Ratings were on a scale to 5. For galling severity, 0 represented no symptoms while a rating of 3 represented samples with heavy galling of leaves but minimal leaf deformity. Samples with ratings of 5 exhibited extreme galling and deformity. For emergence density, 0 represented no emergence from galls within the sample while 3 represented 30-45 existing emergence holes/ galled leaf sample. A rating of 5 represented >60 existing emergence holes/ galled leaf sample.*
Table 5. 20 WAT

<table>
<thead>
<tr>
<th>Treatment: Formulation/Equipment</th>
<th>Rate AI/ Inch Diameter</th>
<th>Galling Severity Rating P&lt;0.0005</th>
<th>Emergence Rating P=0.001</th>
<th>Emerged Wasps/g Tissue P&lt;0.0005</th>
<th>Imidacloprid Concentration µg/g P&lt;0.0005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>------</td>
<td>5.0a ± 0.0</td>
<td>3.5a ± 0.50</td>
<td>15.2a ± 2.66</td>
<td>0.0a ± 0.0</td>
</tr>
<tr>
<td>Imicide/ Mauget Capsules 10 % AI</td>
<td>0.15 ml</td>
<td>3.3ab ± 0.25</td>
<td>1.8ab ± 0.48</td>
<td>3.2b ± 1.46</td>
<td>5.4a ± 0.47</td>
</tr>
<tr>
<td>Pointer/ ArborSystems Wedgle 5 % AI</td>
<td>0.026 ml</td>
<td>3.3ab ± 0.25</td>
<td>1.5abc ± 0.29</td>
<td>3.0b ± 1.57</td>
<td>3.0a ± 0.27</td>
</tr>
<tr>
<td>Merit 200 SL/ Arbor Jet Tree IV 17.1 % AI</td>
<td>0.77 ml</td>
<td>1.5bc ± 0.87</td>
<td>0.5bc ± 0.50</td>
<td>0.4b ± 0.24</td>
<td>36.3b ± 2.03</td>
</tr>
<tr>
<td>IMA-jet/ Arbor Jet Tree IV 5% AI</td>
<td>0.40 ml</td>
<td>0.3c ± 0.25</td>
<td>0.0c ± 0.0</td>
<td>0.07b ± 0.07</td>
<td>234.7c ± 12.4</td>
</tr>
<tr>
<td>Merit 2/ Root Drench 21.4 % AI</td>
<td>1.28 ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Ratings were on a scale to 5. For galling severity, 0 represented no symptoms while a rating of 3 represented samples with heavy galling of leaves but minimal leaf deformity. Samples with ratings of 5 exhibited extreme galling and deformity. For emergence density, 0 represented no emergence from galls within the sample while 3 represented 30-45 existing emergence holes/galled leaf sample. A rating of 5 represented >60 existing emergence holes/galled leaf sample.
Table 6. 10 Months After Treatment

<table>
<thead>
<tr>
<th>Treatment: Formulation/Equipment</th>
<th>Rate AI/ Inch Diameter</th>
<th>Galling Severity Rating* P&lt;0.0005</th>
<th>Emergence Rating* P=0.001</th>
<th>Emerged Wasps/g Tissue P&lt;0.0005</th>
<th>Imidacloprid Concentration µg/g P&lt;0.0005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>0.15 ml</td>
<td>3.3a ± 0.25</td>
<td>0.8a ± 0.25</td>
<td>8.2a ± 1.95</td>
<td>2.2 a ± 0.07</td>
</tr>
<tr>
<td>Imicide/ Mauget Capsules 10 % AI</td>
<td>0.026 ml</td>
<td>3.3a ± 0.48</td>
<td>0.3ab ± 0.25</td>
<td>4.3ab ± 1.70</td>
<td>1.1a ± 0.06</td>
</tr>
<tr>
<td>Pointer/ ArborSystems Wedge 5 % AI</td>
<td>0.77 ml</td>
<td>0.8b ± 0.48</td>
<td>0.0b ± 0.0</td>
<td>0.3b ± 0.28</td>
<td>28.6b ± 0.91</td>
</tr>
<tr>
<td>Merit 200 SL/ Arbor Jet Tree IV 17.1 % AI</td>
<td>0.40 ml</td>
<td>2.0ab ± 0.91</td>
<td>0.0b ± 0.0</td>
<td>0.6b ± 0.36</td>
<td>46.8c ± 2.31</td>
</tr>
<tr>
<td>Merit 2/ Root Drench 21.4 % AI</td>
<td>1.28 ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Ratings were on a scale to 5. For galling severity, 0 represented no symptoms while a rating of 3 represented samples with heavy galling of leaves but minimal leaf deformity. Samples with ratings of 5 exhibited extreme galling and deformity. For emergence density, 0 represented no emergence from galls within the sample while 3 represented 30-45 existing emergence holes/ galled leaf sample. A rating of 5 represented >60 existing emergence holes/ galled leaf sample.
Table 7. 12 Months After Treatment

<table>
<thead>
<tr>
<th>Treatment: Formulation/Equipment</th>
<th>Rate Al/ Inch Diameter</th>
<th>Galling Severity Rating*</th>
<th>Emergence Rating*</th>
<th>Emerged Wasps/g Tissue</th>
<th>Imidacloprid Concentration µg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0 + 0.0</td>
</tr>
<tr>
<td>Imicide/ Mauget Capsules 10 % Al</td>
<td>0.15 ml</td>
<td>3.0a ± 0.58</td>
<td>2.3a ± 0.33</td>
<td>6.3a ± 1.23</td>
<td>1.7 ± 0.25</td>
</tr>
<tr>
<td>Pointer/ ArborSystems Wedgle 5 % Al</td>
<td>0.026 ml</td>
<td>3.8a ± 0.25</td>
<td>3.3a ± 0.25</td>
<td>6.2a ± 1.57</td>
<td>0.7 ± 0.15</td>
</tr>
<tr>
<td>Merit 200 SL/ Arbor Jet Tree IV 17.1 % Al</td>
<td>0.77 ml</td>
<td>1.3a ± 0.88</td>
<td>0.3b ± 0.33</td>
<td>2.5a ± 0.80</td>
<td>21.0 ± 0.58</td>
</tr>
<tr>
<td>IMA-jet/ Arbor Jet Tree IV 5% Al</td>
<td>0.40 ml</td>
<td>2.3a ± 1.03</td>
<td>0.8b ± 0.48</td>
<td>1.5a ± 1.27</td>
<td>41.0 ± 4.0</td>
</tr>
<tr>
<td>Merit 2/ Root Drench 21.4 % Al</td>
<td>1.28 ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Ratings were on a scale to 5. For galling severity, 0 represented no symptoms while a rating of 3 represented samples with heavy galling of leaves but minimal leaf deformity. Samples with ratings of 5 exhibited extreme galling and deformity. For emergence density, 0 represented no emergence from galls within the sample while 3 represented 30-45 existing emergence holes/ galled leaf sample. A rating of 5 represented >60 existing emergence holes/ galled leaf sample.