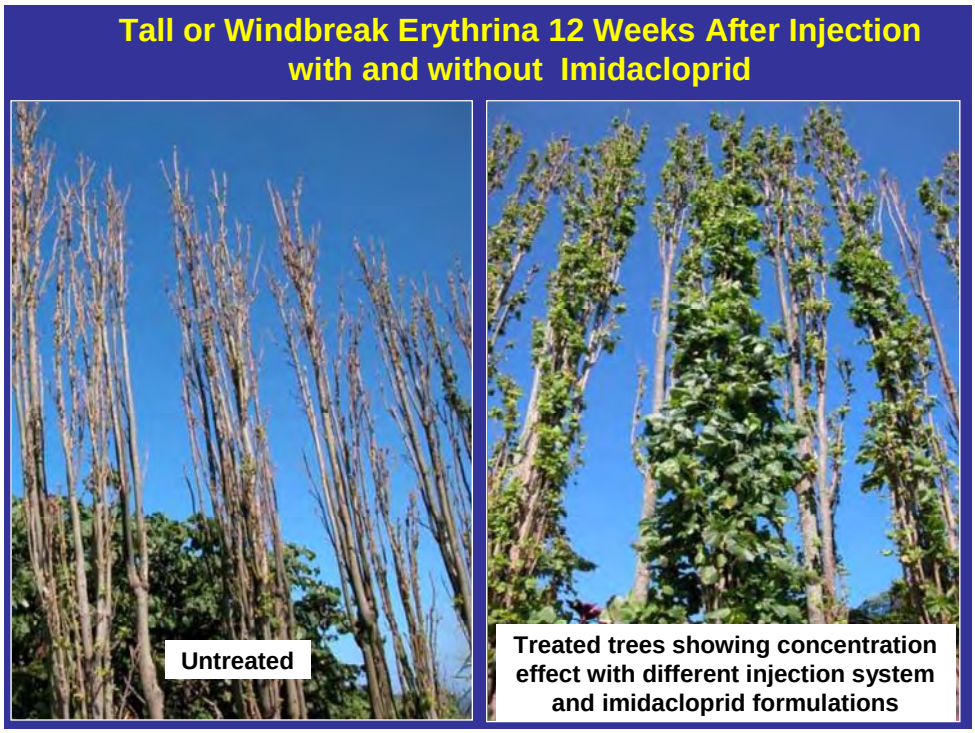
Management of the Invasive Erythrina Gall Wasp, *Quadrasticus erythrinae* Kim (Hymenoptera: Eulophidae) on Native and Landscape *Erythrina* spp.

Executive Summary

The erythrina gall wasp, *Quadrastichus 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.



FINAL REPORT: "Management of the Invasive Erythrina Gall Wasp, *Quadrasticus erythrinae* Kim (Hymenoptera: Eulophidae) on Native and Landscape *Erythrina* spp."

PRINCIPAL INVESTIGATOR: Arnold H. Hara, Entomologist
University of Hawai'i at Mānoa, College of Tropical Agriculture and Human Resources

PERIOD COVERING: July 01, 2006 to September 04, 2007

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. sandwicencsis* 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. sandwicensis* 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. Wedgle 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, Wedgle, 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 Wedgle (Tables 2 to 4). Imdacloprid 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 formaulation 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:

   Xu, T., C. Jacobsen, A. Hara and Q. Li. 2006. 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*. J. Agric. Food Chem. .54: 8444-8449.

   Presentations:

   Jacobsen, C. and A. H. Hara 2006. Chemical control of *Quadrastichus erythrinae* infesting *Erythrina* spp. in Hawaii's diverse environmental conditions. Symposium: Recent Advances in the Biological Control and Chemical Control of Arthropods in Floriculture. Pacific Branch, Entomological Society of America, 90th Annual Meeting, Wailea Resort, Maui, Hawaii.

   Jacobsen, C.M., A.H. Hara, T. Xym Q. X. Li, A.M. LaRosa and R. Hauff. 2006. Analysis of imidacloprid concentrations within Erythrina spp. leaf tissues as a monitoring and predictive tool for control of erythrina gall wasp, Quadrastichus erythrinae Kim, infestations. 54th Annual Meeting of the Entomological Society of America, Dec. 2006, Indianapolis, IN (poster).

   Hara, A.H. 2006. Chemical control of the erythrina gall wasp – Trials and tribulation. Annual Landscape Industry Council of Hawaii Conference, Honolulu, HI.

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

Jacobsen, C.M. 2007 Erythrina Gall Wasp Control Update: Chemical control recommendations, Seminar for landscapers and arborists. Kona Outdoor Circle, Feb. 2007, Kailua-Kona, HI.

Hara, A.H. 2007 and C.M. Jacobsen. Update on Control of the Erythrina Gall Wasp and Other Invasive Pests. United Agri-Products Seminar, May 17, 2007, Honolulu, HI.

Hara, A.H. and C.M. Jacobsen 2007. Update on the Control of the Erythrina Gall Wasp (EGW) and Other Invasive Pests. Cooperative Extension Service, Maui Community College, June 2007, Kahului, HI

Website presentation:

Hara, A.H. and C.M. Jacobsen. 2007. Update on the Control of the Erythrina Gall Wasp and Other Invasive Pests.
http://www.ctahr.hawaii.edu/haraa/EGWotherinvasive0507%20(NXPowerLite)_files/frame.htm

Press Release:

"Wasp swap: A relief for wiliwili?" By Travis Kaya, Maui News, June 18, 2007.
http://www.mauinews.com/news/2007/6/18/01wasp0618.html

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

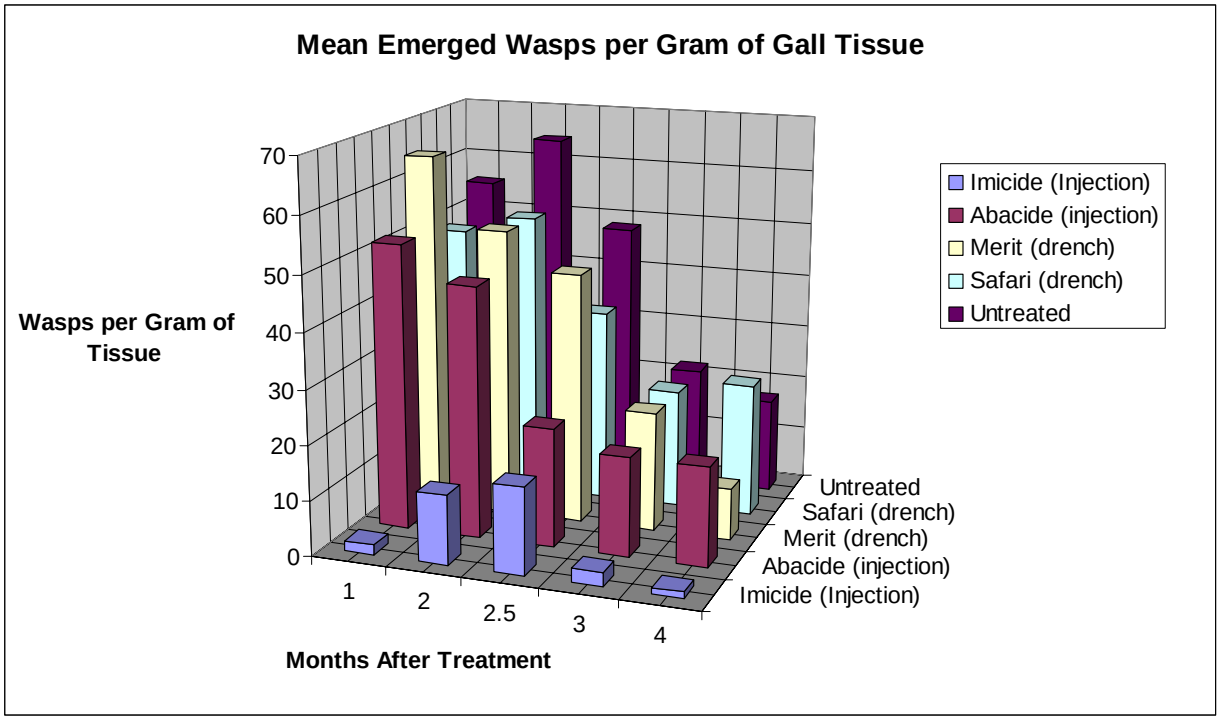


Fig. 2. Successful drench of imidacloprid in confined irrigated location.

Fig. 3. Correlation between treatments of *E. sandwicensis* trees and concentration of imidacloprid or emergence of wasps.

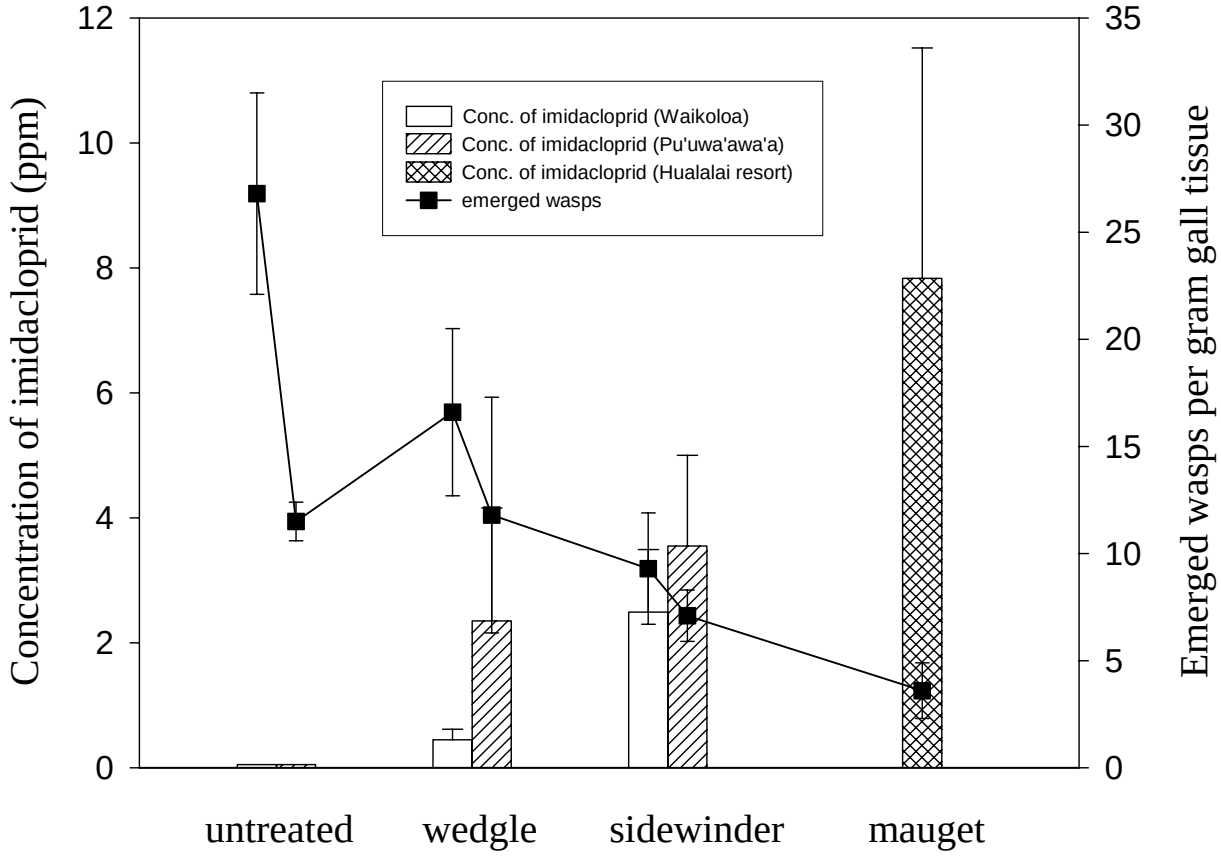


Fig. 4. Correlation between concentration of imidacloprid and emergence of wasps or infestation severity rating of samples.

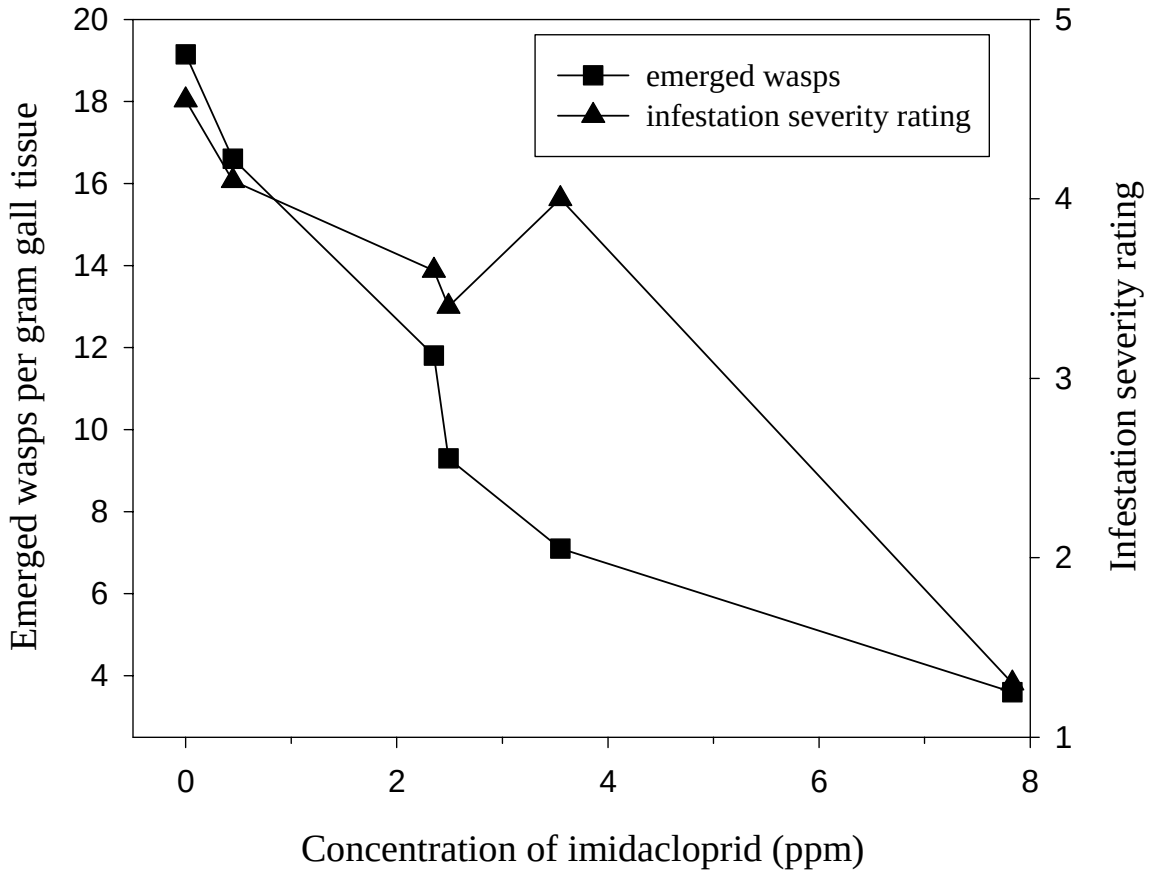
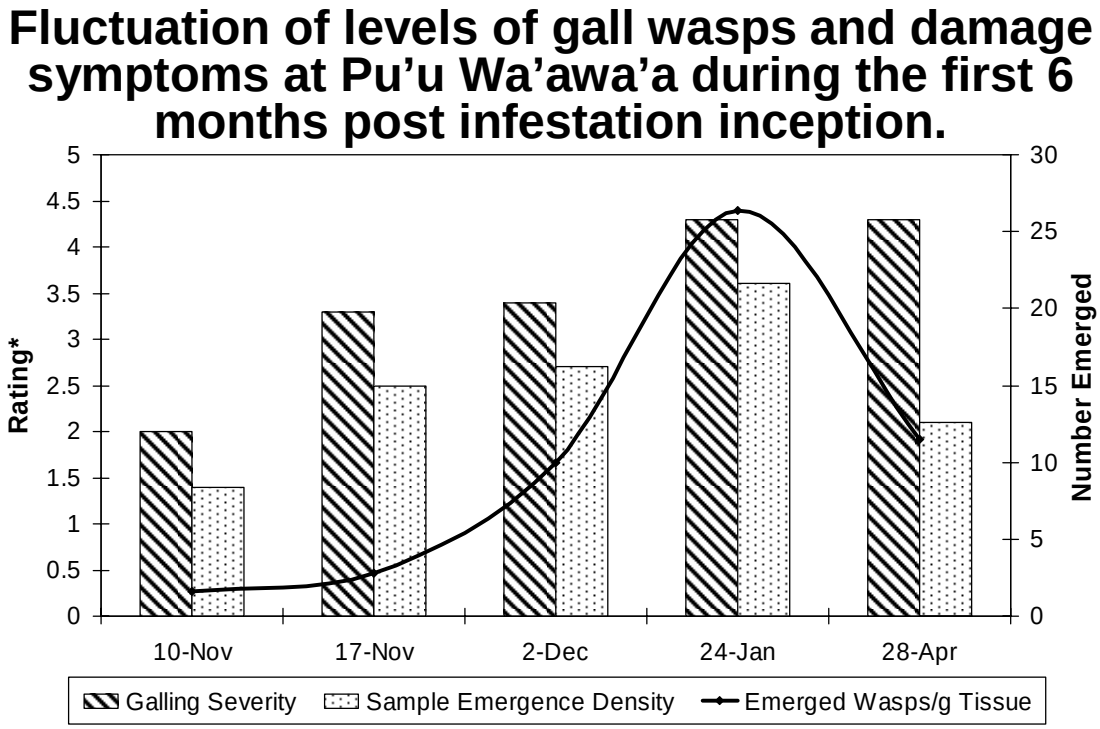


Fig. 5.



*** 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.

Treatment System	**Advantages**	**Disadvantages**	**Cost of System Cost per Tree (20")**
ArborSystems Wedgle Direct-Inject	Creates least wounding of tree trunk among systems. Efficient placement and uptake of chemical.Treatment is relatively quick; no waiting for chemical uptake.	Some leaking of chemical (<0.3ml) during treatment. The least quantity of AI is applied of any system.Must use ArborSystems' chemical formulation.	$605 for Wedgle Direct-Inject Pointer $305/ 120ml (5% AI) $28-41
Arborjet Tree IV Micro Infusion System	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.	$699 for 2 tree IVs & kit; $315 for each additional IV IMA-jet $175/ 500ml (5% AI) $56
Mauget Ready to use 3ml Micro injector Capsules	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.	Imicide $116 for 24, 3ml capsules (10% AI) $48
Sidewinder Tree Injectors Backpack Tree Injector	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.	$1584 for Backpack Injector Insecticide is from other manufacturers following their labeled rates.

Table 2. Efficacy of various treatments applied to *Erythrina variegata* 10 weeks after treatment.

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

*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.

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

*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.

Treatment: Formulation/Equipment	Rate AI/ Inch Diameter	Galling Severity Rating* P=0.206	Emergence Rating* P=0.001	Emerged Wasps/g Tissue P=0.071	Imidacloprid Concentration µg/g
Untreated	-------				0.0 ± 0.0
Imicide/ Mauget Capsules 10 % AI	0.15 ml	3.0a ± 0.58	2.3a ± 0.33	6.3a ± 1.23	1.7 ± 0.25
Pointer/ ArborSystems Wedgle 5 % AI	0.026 ml	3.8a ± 0.25	3.3a ± 0.25	6.2a ± 1.57	0.7 ± 0.15
Merit 200 SL/ Arbor Jet Tree IV 17.1 % AI	0.77 ml	1.3a ± 0.88	0.3b ± 0.33	2.5a ± 0.80	21.0 ± 0.58
IMA-jet/ Arbor Jet Tree IV 5% AI	0.40 ml	2.3a ± 1.03	0.8b ± 0.48	1.5a ± 1.27	41.0 ± 4.0
Merit 2/ Root Drench 21.4 % AI	1.28 ml				

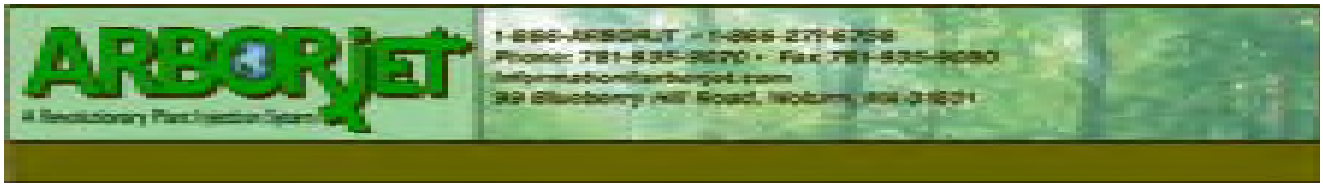
*Ratings were on a scale to 5. For galling severity, 0 represented no symptoms while a rating of 3 represented samples with heavy galling of leaves but minimal leaf deformity. Samples with ratings of 5 exhibited extreme galling and deformity. For emergence density, 0 represented no emergence from galls 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 ($P<0.05$).

Attachment 1. Flyer and photos for seminar conducted at the Kona Outdoor Circle.

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, Lipahtech
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**

C O O P E R A T I V E E X T E N S I O N S E R V I C E

College of Tropical Agriculture and Human Resources
University of Hawai'i at Mānoa
United States Department of Agriculture Cooperating

May 12, 2007

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
Recertification Credits: Pesticide (HDOA categories 1A, 2, 3, 4, 6, 8, 9 & 10), Golf Course Superintendent (GCSAA) & UH/CES Maui Landscape IPM
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 insure that you will receive any handouts that may be provided and that you have attended this program for auditing purposes for recertification credits.

PROGRAM

I. History & Status of the EGW in Hawaii

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

III. Control Strategies
- A. Chemical
  1. Foliar
  2. Systemic drench
  3. Systemic injection
  4. Field trials
- B. Non-Chemical
  1. Cultural control – replacement cultivars or species
  2. Classical biological control
     a) Status of parasitoids from Africa
     b) Potential for long-term control of EGW

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

V. 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 Nishijima,

Maui CES Office, 310 Kaahumanu Avenue, Building 214, Kahului, Hawaii 96732
Telephone: (808) 244-3242, Facsimile: (808) 244-7089, E-Mail: kahului@ctahr.hawaii.edu, Web: www2.ctahr.hawaii.edu

Attachment 2. Manuscript published in the Journal of Agricultural and Food Chemistry.

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Application of an Enzyme-linked Immunosorbent Assay for the Analysis of Imidacloprid in Wiliwili Tree, *Erythrina sandwicensis* O. Deg, for Control of the Wasp *Quadrastichus erythrinae*

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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 wiliwili tree (*Erythrina sandwicensis* O. Deg) found in dryland forests and landscapes in Hawaii. Imidacloprid was applied to these wiliwili trees in an attempt to control the newly introduced erythrina gall wasp, *Quadrastichus erythrinae* Kim. Leaf samples were freeze-dried and extracted with acidic 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 ($r^2 = 0.98$). 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 wiliwili trees and the application provides information to understand how to control the wasps.

KEYWORDS: ELISA; imidacloprid; wiliwili 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 1950 ft (*1*). It is also known as the wiliwili 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 wiliwili trees is the erythrina gall wasp (EGW), *Quadrastichus erythrinae* Kim (*3*−*4*). In addition to *E. sandwicensis*, EGW attacks *E. variegata* 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 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 investigated. 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 may help in reducing damage to erythrina caused by the gall wasp (*3*).

Imidacloprid, 1-(6 chloronicotinyl)-2-(nitroimino) imidazolidine (**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

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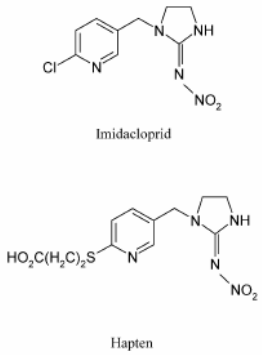

Figure 1. Structures of imidacloprid and hapten.

countries). Imidacloprid works by interfering with the transmission of stimuli in the insect's nervous system. It causes a blockage in at least one type of nicotinergic neuronal pathways that is more abundant in insects than in warm-blooded animals. This makes imidacloprid much more toxic to insects than to other animals. Furthermore, imidacloprid has a highly specific affinity to insect nicotinic acetylcholine receptors (nAChR) (*7*−*9*). Its binding leads to the accumulation of acetylcholine, resulting in the paralysis and death of insects (*10*).

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

MATERIALS AND METHODS

Reagents. All reagents were of analytical grade unless specified otherwise. Analytical standard imidacloprid (96.9% purity) was obtained from Bayer Corp, Stillwell, KS. Goat anti-mouse IgG-horseradish peroxidase (IgG-HRP), phosphate−citrate buffer capsules with sodium perborate, carbonate−bicarbonate buffer capsules, and *o*-phenylenediamine (OPD) were purchased from Sigma (St. Louis, MO). Monoclonal antibodies against imidacloprid were previously prepared in our laboratory (*11*). The purified Mab IgG in phosphate-buffered saline (PBS, 5 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 136 mM NaCl, and 2.7 mM KCl, pH 7.5) was stored at −20 °C until use.

Treatment Methods. Imidacloprid treatments were made using commercially available injection equipment and according to label recommendations of the formulations. Wedgle Direct-Inject (Arbor Systems, Omaha, NE) utilizes a 0.75 in. (19 mm) long needle-like tip which is inserted through a rubber "WedgeCheck" and is placed into the trunk acting as a stopper to prevent the chemical from escaping from the tree. One milliliter of 5% active ingredient (a.i.) imidacloprid (Pointer, Arbor Systems) was injected every 6 in. (15 cm) around the circumference of the trunk near the ground. Sidewinder precision tree injector (Noosaville, QLD, Australia) treatment was conducted by drilling a 6 mm hole into the trunk of the tree; an application nozzle was then screwed into the hole and 5 mL of imidacloprid was applied under pressure (<40 bar). Following the treatment application, a plug was screwed into the hole to seal the wound and prevent the chemical from bleeding out. Imicide HP formulation (10% imidacloprid; JJ Mauget Co, Arcadia, CA) was applied through the sidewinder equipment at 1.5 mL and 2 mL per inch diameter measured at breast height (DBH) for 10−36 in. (25−91 cm) and >36 in. (>91 cm) DBH trees, respectively. The third treatment was Mauget Imicide (10% imidacloprid; JJ Mauget Co, Arcadia, CA) packaged in ready-to-use 3-mL microinjection capsules. An 11/64 in. (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 xylem 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 ftfrom the ground. Treatments were applied to wiliwili trees in a native dryland forest at Pu'u Wa'awa'a and Waikoloa, Hawaii, and in an irrigated resort landscape at the Hualalai Resort, Hualalai, Hawaii, all located on the Island of Hawaii. Hualalai Resort treatment using Mauget capsules occurred March 13, 2006. At Pu'u Wa'awa'a, Wedgle and Sidewinder treatment occurred November 10 and December 2, 2005, respectively. Waikoloa Wedgle and Sidewinder treatment occurred December 7 and December 19, 2005, respectively.

Table 1. Infestation Severity Ratings

rating	description	approx gall weight/ 20 g leaf
1	very light infestation, only very slight galling	<3 g
2	moderate galling	3–8 g
3	heavy galling of leaves but minimal leaf deformity	8–14 g
4	heavy galling moderate leaf deformity	14–18 g
5	extreme galling and deformity with no expanded leaves	>18 g

Sampling. Leaf samples were obtained by cutting 15 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 imidacloprid analysis were stored at −20 °C until analyzed.

Evaluation of Severity of Infestation. Samples were evaluated for severity of infestation by a five-point numerical rating system (**Table 1**). A rating of 1 signified very light infestation levels with only very slight galling. 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 with no expanded leaves. Wasp emergence was quantified by excising galls that lacked emergence holes and holding them in many 473 mL waxed paper bowls (Georgia Pacific, Atlanta, GA) covered with silkscreen 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 peduncle were freeze-dried and ground to powder. One gram of leaf powder was weighed in a 100 mL beaker. Imidacloprid was extracted ultrasonically with 50 mL of methanol/H_2SO_4 0.04% (4:1, v/v) at 60 °C for 20 min. The mixture was vacuum filtered through Whatman No. 4 filter paper (ID 9.0 cm, pore size 2.5 μm) with 1 g of Celite 545 on it. The filtrate was concentrated to 10−15 mL of water by evaporating with a rotary evaporator, at 55 ± 2 °C. The residue was centrifuged (6000 rpm) for 10 min, and the aqueous supernatant was transferred to a 60 mL separation funnel.

For ELISA determination, the supernatant was extracted with methylene chloride (20 mL × 3). The methylene chloride layer was collected and concentrated to 1−2 mL with a rotary evaporator. The organic remainder was transferred to 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 0.04% H_2SO_4. The aqueous phases were combined and transferred to a 125 mL separate funnel followed by extraction with methylene chloride (30 mL × ?3). The combined methylene chloride extract was concentrated to 2 mL with a rotary evaporator. The organic remainder was passed through a C18 cartridge (Analtech, Inc., Newark, DE) that was preactivated with 5 mL of

methanol followed by 5 mL of water. The cartridge was eluted with 5 mL of methylene chloride/acetonitrile (85:15, 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 polystyrene microplates (MaxiSorp F96; Nalge Nunc International, Copenhagen, Denmark) as previously described (*11*). Briefly, microplate wells were coated with conjugates (4 ng in 100 μL per 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) by incubation for 1 h at room temperature. The plates were washed again 4 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 imidacloprid MAb 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 again washed 4 times as above, and then substrate solution (100 μL per well of 0.05 M citrate−phosphate buffer, pH 5.0, containing 0.03% sodium perborate 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/5 mM ammonium acetate (20:80, v/v); injection volume, 30 μL; flow rate, 1.5 mL/min; column, Inertsil ODS-3V, 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 (*15*−*16*) have been reported for the analysis of imidacloprid in environmental matrices and agriculture products. As it is well-known, immunochemical methods for residual pesticides have many advantages. On the other hand, although these methods are susceptible to matrix interference from samples, especially biological samples, they can be overcome by simple dilution with water or appropriate buffer without troublesome cleanup steps (*17*). The ELISA for residual imidacloprid monitoring was highly specific and sensitive (*11*). No significant matrix interference from the water and cucumber samples was observed after simple dilution of the extracts before analysis in our previous studies (*11*). The results indicated that the ELISA method could be suitable to perform residual analysis for imidacloprid in the environment and biological matrixes. So, in this study, we applied the ELISA method to analyze imidacloprid in wiliwili tree leaves.

An ultrasonic extraction with a mixture of methanol and 0.04% H_2SO_4 (4:1, v/v) was applied to wiliwili leaf samples (*13*, *18*). The extracts may contain numerous constituents such as chlorophyll, 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 IC_{50} value 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

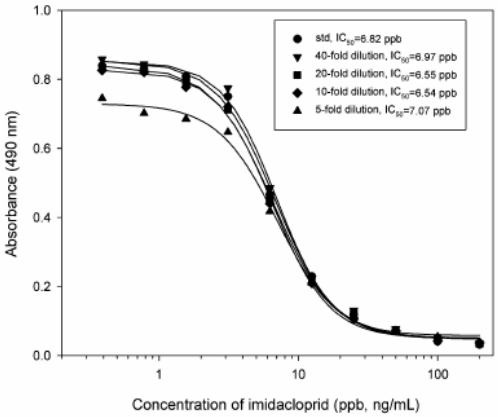

Figure 2. Inhibition curves of imidacloprid in wiliwili leaf extracts that were diluted in different folds. The data are an average of three replicates.

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 SPE were used to clean up the extracts. Methylene chloride was used to eliminate polar compounds followed by a C18 column cleanup to remove nonpolar interference such as lipids from the matrices. 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 imidachloprid, but the eluates obtained were dirty because of waxes 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 85:15 (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 Liu et al. (*18*). 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 investigation 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.

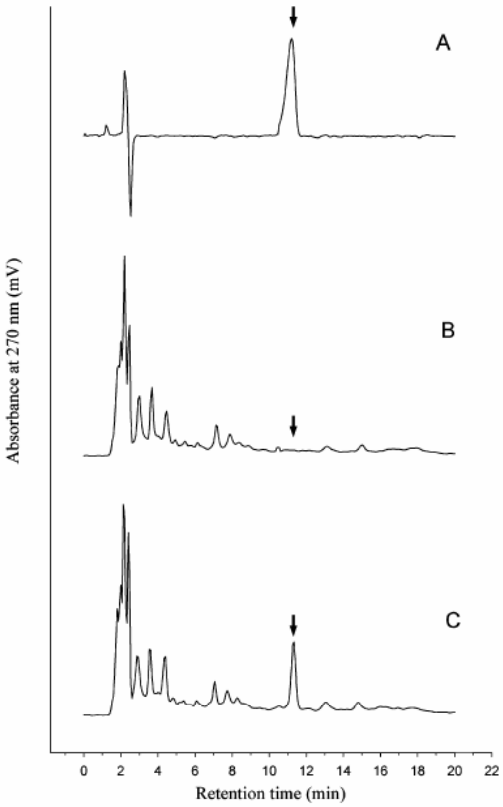

Figure 3. HPLC chromatograms of imidacloprid standard (2 μg/mL) in acetonitrile (**A**), extract of imidacloprid-free plant leaves (**B**), and extract of plant leaves fortified with imidaclopride standard (**C**).

Table 2. Recovery of Imidacloprid from Fortified Samples Determined by ELISA and HPLC

fortified concentration (g/g)	concentration ± standard deviation (μg/g)		recovery (%, $n = 3$)	
	ELISA	HPLC	ELISA	HPLC
0	ND[a]	ND		
0.1	0.09 ± 0.01	0.08 ± 0.02	91	84
0.5	0.39 ± 0.02	0.38 ± 0.04	78	76
2	1.77 ± 0.1	2.28 ± 0.22	89	114
10	9.97 ± 0.06	10.54 ± 0.19	100	105

[a] 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 ($r^2 = 0.98$) between the results obtained by ELISA and those by HPLC analyses 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

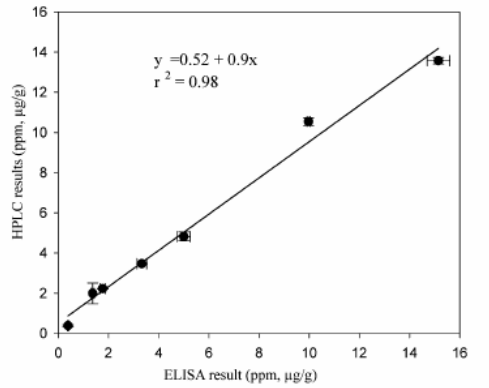

Figure 4. Correlation between ELISA and HPLC results of imidacloprid concentrations in leave samples. The error bars are standard deviations.

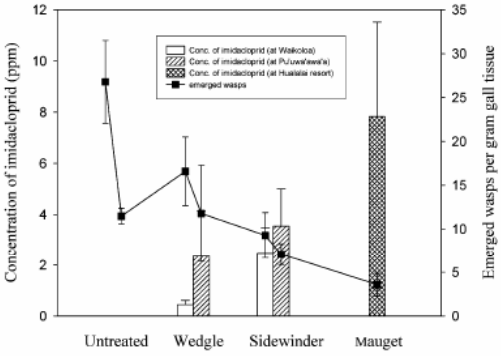

Figure 5. Correlation between treatments of wiliwili trees at different locations and concentrations of imidacloprid or emergence of the wasps. All leaf samples were collected from the middle canopy of trees. The error bars are standard deviations.

generates solvent wastes, which need proper disposal. Since ELISA has by 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 wiliwili trees (*21*). Expectations were highly based on knowledge of the superior performance of imidacloprid against sucking insects in various crop settings (*22*−*25*). 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 Wedgle, Sidewinder, and Mauget. 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 at Waikoloa than those at Pu'u Wa'awa'a as shown in **Figure 5**. It is clear the infestation of wasps was 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

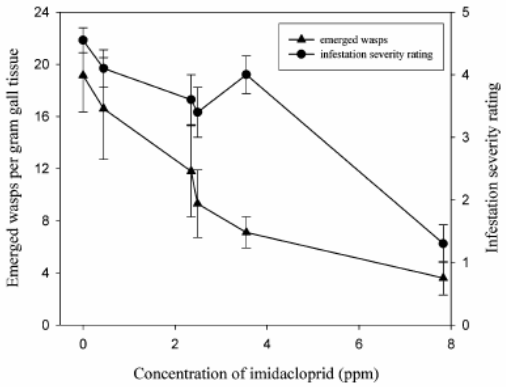

Figure 6. Correlation between concentration of imidacloprid and emergence of the wasps and infestation severity rating of samples. The error bars are standard deviations.

treatment. The concentrations of imidacloprid in the leaf samples correlated inversely with the emergence of the wasps and infestation severity rating (**Figure 6**). Trees that were treated with the Mauget Imicide 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 best control efficacy among the three treatment methods (**Figure 6**). Wedge (applied at 0.026 mL a.i./inch diameter) and Sidewinder (applied at 0.15−0.2 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 5.6 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 *Erythrina*. The analysis 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 wiliwili 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 galling 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 trees.

ELISA is an effective method to quantify and monitor imidacloprid in wiliwili trees. We will continue to use this assay in our further work on gathering more basic knowledge of imidacloprid in wiliwili trees such as the nature of the 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

source of leave samples	average concentration of imidacloprid ± standard deviation determined by ELISA (μg/g, $n = 3$)		
	Tree 1	Tree 2	Tree 3
upper canopy	1.15 ± 0.05	0.78 ± 0.05	0.59 ± 0.02
middle canopy	1.32 ± 0.13	1.34 ± 0.06	1.1 ± 0.14
lower canopy	2.18 ± 0.29	2.15 ± 0.05	1.31 ± 0.05
galling absent	1.50 ± 0.06	0.72 ± 0.01	1.03 ± 0.03
galling present	0.73 ± 0.03	1.03 ± 0.03	0.59 ± 0.03

Conclusion. A monoclonal antibody-based ELISA was used to measure concentrations of imidacloprid in wiliwili leaf samples for control of the gall wasp, *Quadrastichus erythrinae*. The satisfactory recovery of imidacloprid by ELISA and the good correlation between ELISA and HPLC results suggest that ELISA is a highly sensitive and relatively simple method to quantify imidacloprid in wiliwili 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 work effectively against the wasps. The ELISA is a useful tool to measure imidacloprid for management and control of the wasps.

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Wiliwili: *Erythrina variegata*

EFFICACY OF IMIDACLOPRID APPLIED AS TRUNK INJECTIONS AND SOIL DRENCHES FOR CONTROL OF ERYTHRINA GALL WASP 2006-2007.

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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 imidacloprid 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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Efficacy of imidacloprid applied as trunk injections and soil drenches for control of erythrina gall wasp 2006-2007.

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% Imidaclopri

Table 1. 3 WAT

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

*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

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

*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

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

*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

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

*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

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

*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

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

*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

Treatment: Formulation/Equipment	Rate AI/ Inch Diameter	Galling Severity Rating* P=0.206	Emergence Rating* P=0.001	Emerged Wasps/g Tissue P=0.071	Imidacloprid Concentration µg/g
Untreated	-------				0.0 ± 0.0
Imicide/ Mauget Capsules 10 % AI	0.15 ml	3.0a ± 0.58	2.3a ± 0.33	6.3a ± 1.23	1.7 ± 0.25
Pointer/ ArborSystems Wedgle 5 % AI	0.026 ml	3.8a ± 0.25	3.3a ± 0.25	6.2a ± 1.57	0.7 ± 0.15
Merit 200 SL/ Arbor Jet Tree IV 17.1 % AI	0.77 ml	1.3a ± 0.88	0.3b ± 0.33	2.5a ± 0.80	21.0 ± 0.58
IMA-jet/ Arbor Jet Tree IV 5% AI	0.40 ml	2.3a ± 1.03	0.8b ± 0.48	1.5a ± 1.27	41.0 ± 4.0
Merit 2/ Root Drench 21.4 % AI	1.28 ml				

*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.