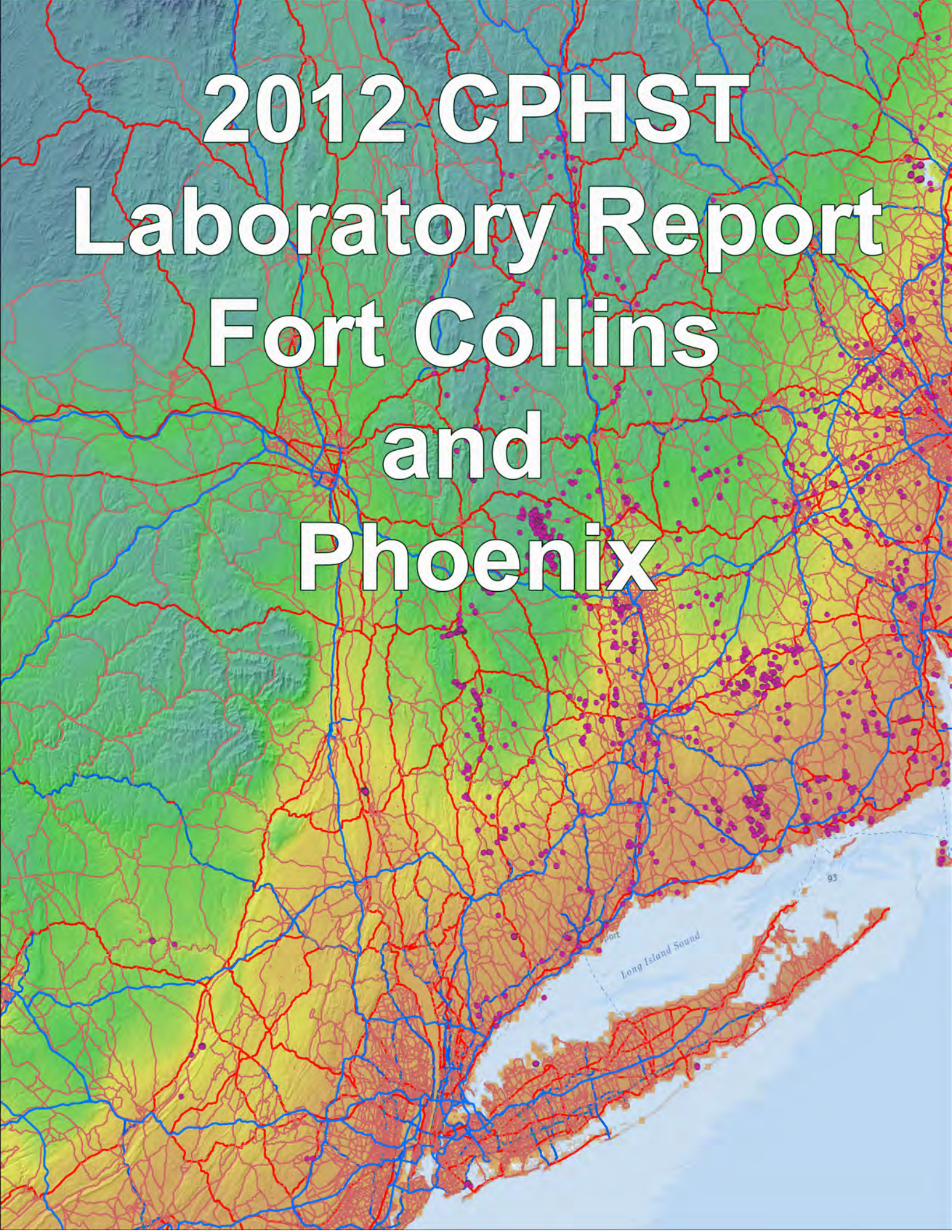
2012 CPHST Laboratory Report Fort Collins and Phoenix



United States Department of Agriculture
Animal and Plant Health Inspection Service
Plant Protection and Quarantine
Center for Plant Health Science and Technology

2012 CPHST Laboratory Report Fort Collins and Phoenix

CPHST Fort Collins Laboratory
2301 Research Boulevard, Suite 108
Fort Collins, CO 80526-1825
phone: 970-490-4464
fax: 970-482-0924

CPHST Phoenix Laboratory
3645 East Wier Avenue
Phoenix, AZ 85040-2931
phone: 602-431-3230
fax: 602-431-3258

The U.S. Department of Agriculture (USDA) prohibits discrimination against its customers, employees, and applicants for employment on the bases of race, color, national origin, age, disability, sex, gender identity, religion, reprisal, and where applicable, political beliefs, marital status, familial or parental status, sexual orientation, or all or part of an individual's income is derived from any public assistance program, or protected genetic information in employment or in any program or activity conducted or funded by the Department. (Not all prohibited bases will apply to all programs and/or employment activities.)

Mention of companies or commercial products does not imply recommendation or endorsement by the USDA over others not mentioned. USDA neither guarantees nor warrants the standard of any product mentioned. Product names are mentioned solely to report factually on available data and to provide specific information.

Table of Contents

This 2012 report highlights the diverse activities at the Fort Collins and Phoenix CPHST (Center for Plant Health Science & Technology) Laboratories that support the regulatory operations of the USDA Plant Protection and Quarantine (PPQ) programs. Our laboratories develop and transfer scientifically-based methods, innovative tools, and state-of-the-art technologies to PPQ and other federal and state agencies to reduce risk levels associated with potential, new, and established problem arthropods, molluscs, diseases, and weeds (collectively "pests"). In 2012, we made significant advancements in the following program areas: geospatial and identification technologies, pest detection and survey, waste disposal and decontamination, and weed and insect chemical and biological controls.

identification technology

Identification Technology Program: 2012 year in review 4

waste disposal and decontamination

AQI waste disposal methods development 19

Assessment of disinfectants for control of *Phytophthora ramorum* 20

Chlorine dioxide disinfectant efficacy study 22

pest detection and survey

CPHST CAPS support: bringing pests, surveys, plants, and science together 24

geospatial technology

Geospatial Technology and Predictive Modeling: 2012 year in review 30

biological weed management

Rearing and field releases of the yellow toadflax stem-mining weevil, *Mecinus janthinus* 37

Impacts of the biological control agent *Jaapiella ivannikovi* and a competitive grass on Russian knapweed plants 39

Rearing the Russian knapweed gall midge, *Jaapiella ivannikovi* 41

Field releases of the Russian knapweed gall midge, *Jaapiella ivannikovi* 43

Development of rearing systems for beneficial root feeding biological control agents 44

Survey for natural enemies of Canada thistle, *Cirsium arvense* 45

pink bollworm

Strontium Chloride as a secondary dietary marker for pink bollworm mass-reared for Sterile Insect Technique 46

Comparison of 20, 22.5 and 25 kilorad irradiations for released pink bollworm 48

grasshopper and mormon cricket

Development of a new active ingredient in bait and evaluation of a new formulation of the traditional active ingredient in bait for controlling grasshoppers and Mormon cricket on rangeland 50

Confirming an effective field dose of Chlorantraniliprole (Prevathon®) for control of rangeland grasshoppers 53

Continued operational scale evaluation of EDT Concentrate as a replacement for oil diluents in Dimilin spray mixes to control rangleand grasshoppers 56

Evaluation of ground applications of selected domestic strains of pathogenic fungi, on four hecare (10 acre) and mini polots, for control of rangeland grasshoppers (Orthoptera: Acrididae) near Dagmar, MT 2012 59

staff directories

Fort Collins Laboratory 61

Phoenix Laboratory 63

2012 laboratory report production

design, layout, and formatting Laura A. Hartmann, Amanda J. Redford, and Julia L. Scher

editors Laura A. Hartmann and Daniel Z. Mackesy

publication date June 2013

cover design Gericke Cook and Christina Southwick

print copy contact Patty Smith (patricia.m.smith@aphis.usda.gov)

electronic copy http://www.aphis.usda.gov/plant_health/cphst/nwsl.shtml

cover image. A spatial risk map generated by the Fort Collins CPHST Laboratory depicting likelihood of occurrence of *Rosa multiflora*, an invasive weed species. The likelihood was modeled on bioclimatic data and statistically determined by MaxEnt software. Also shown are point locations of known occurrence of this species (source: EDDMaps) and major roads. For more information, contact Gericke Cook (email gericke.l.cook@aphis.usda.gov).

Identification Technology Program: 2012 year in review

Terrence Walters, Julia Scher and Amanda Redford

Terrence Walters

Julia Scher

Amanda Redford

The Identification Technology Program (ITP) develops web-based products and offers services for identifying plant pest arthropods, molluscs, nematodes, weeds, and diseases (collectively, "pests"). By engaging experts and adopting advanced technologies, ITP serves USDA Plant Protection and Quarantine's (PPQ) efforts to safeguard U.S. agriculture and natural resources from the entry, establishment, and spread of pests while supporting trade and export of U.S. agricultural products. ITP maintains our clients' needs as top priority, so we continue to explore new approaches and technologies for product development, delivery, and promotion and find new ways to offer our services to better support PPQ and its collaborators and trading partners.

In 2012, ITP achieved some significant goals: for the first time, projects were funded and in development in all four program areas (*ID Tool*, *ID Resource*, *ID Image,* and *ID Mobile*), and our team expanded its offerings in the areas of client services, product-based services, and outreach. ITP was pleased to note diversity among our product users and the taxa our products cover, as well as significant international recognition and usage. This year, our team took the first steps toward fully leveraging and sharing our data through the repurposing of tool images. Finally, our team embarked on a modernization-driven initiative to help set our course for the long term.

ENGAGING WITH PPQ

ITP engaged directly with PPQ on several fronts that led to tangible results. Working with National Identification Services (NIS), part of PPQ's Policy Management core functional area, and with PPQ Field Operations managers, ITP started down the path of creating an application for identifiers to gather and share their images with one another, to be called *imageID*. Collecting the many valuable diagnostic images taken by all the identifiers in one place for easy access had proven to be quite a challenge for NIS over the years, so ITP consulted closely with PPQ's Plant Inspection Station (PIS) oversight team and conducted a thorough survey, including identifier interviews and port visits, to begin assessing the image collections. In another significant engagement, ITP delivered *AphID* to NIS in 2012, an identification tool NIS had specifically chosen ITP to oversee and develop, and for which NIS provided funding in 2010.

POSITIONING ITP FOR SUCCESS

With projects in progress in all four program areas in 2012, ITP realized the need to take stock of our situation and assess our path forward. USDA APHIS's 2012 modernization and reorganization plan also spurred us to become leaner and more efficient while being more productive and providing more services. ITP engaged a local consulting group to conduct a program-wide assessment and critically review our products and systems to recommend a path to restructure and consolidate our product software configurations, enabling ITP to streamline product development. The roadmap they will deliver in 2013 will also offer ways our team can maximize sharing, integration, and repurposing of our data and leverage recommended technologies to enhance client experiences and interactions with ITP's products, as well as expand our product line to better meet PPQ's needs and challenges.

An essential component of ITP's path forward is the promotion of our products and services. Such efforts could lead to greater awareness and more engaged usage, enabling us to more fully support PPQ's and its partners' identification needs. To help us reach this goal, our consultants started developing a separate marketing roadmap for ITP. The first steps in 2012 were to implement product usage tracking; their analysis of over four months of data will be presented in a "current state analysis" that will set the stage for iterative promotional actions and analysis in 2013.

PRODUCT HIGHLIGHTS

Optimizing tool development

ITP's identification tools are web-based products that include fact sheets, image galleries, and matrix-based keys, along with supporting pages and media, all in an easily accessible online package.

ITP made further refinements to the highly effective database-driven process for tool production we instituted in 2011. This year, a key component of this new paradigm

for tool development was rolled out: *Fact Sheet Manager* (*FSM*). *FSM* is a service-driven application that allows tool developers to easily generate fact sheets by creating and updating fact sheet content themselves, with their changes going live right away, reducing time to delivery. *FSM* is easy to use and gives developers more control, freeing up ITP project managers' time. We intend to further refine the tool developer-ITP team interaction with plans for systems that will allow developers to create and edit their own tool websites using provided templates and guidelines, offering greater efficiency, consistency, and control.

Tool highlights:

- *Flat Mites of the World* is our most popular tool to date, with over 55,000 unique visitors in 2012.
- *Citrus Resource* was the first *complete* commodity-based resource delivered.
- ITP's first plant disease (non host symptom-based) tool was initiated, focusing on type specimens of the destructive genus *Phytophthora*.
- Authors' taxonomic research for tools spawned discovery of new species and numerous revisions, which were published in peer-reviewed journals.

See Accomplishments for complete details about ITP's 2012 tools.

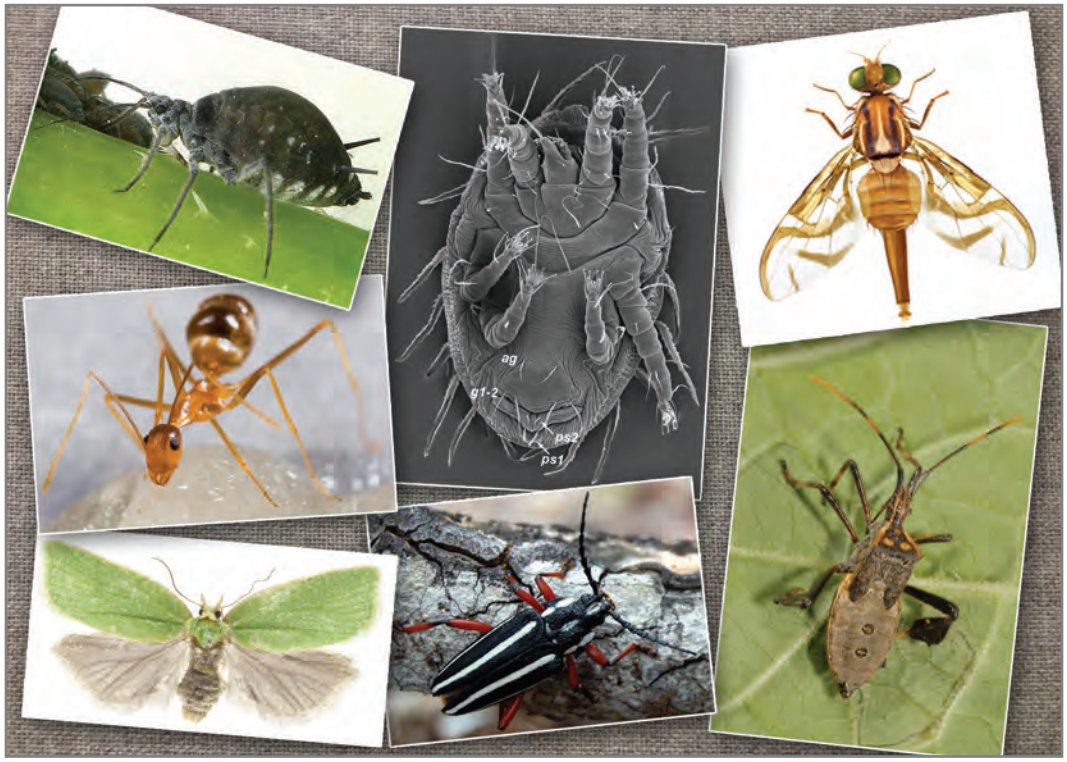
Tools go mobile

ITP is pleased to report that in 2012 several mobile application (app) projects were started that had been in the planning stages for several years, and substantial progress was made on them.

In collaboration with the University of Florida, two National Plant Diagnostic Network (NPDN)-themed citrus identification apps for Android, iPhone, and iPad were developed through a National Institute of Food and Agriculture (NIFA)-funded project. Content from two of ITP's tools, *Citrus Diseases* and *Citrus Pests*, was adapted to create these apps for national and state survey specialists and others to address and prevent threats to citrus crops. This same content will be adapted again in 2013 for a third, "Protect U.S."-themed multi-agency app specifically targeted to small farmers and homeowners. All three apps will be beta tested during hands-on workshops and delivered in mid 2013 along with presentations for educators and e-learning modules for the general public to increase awareness and encourage participation.

Another app project was initiated by Lisa Martin, a Colorado State University graduate student and ITP web developer. For her graduate thesis, Lisa will transform ITP's *Screening Aid to Palm Pests* tool content into a native iPhone app.

Thirteen Lucid matrix keys from nine tools will also be transformed to native smartphone apps suitable for field and survey work. Queensland Alliance for Agriculture and Food Innovation, Biological Information Technology (QBIT), creators of Lucid, are using ITP's keys to test conversion of keys to a mobile version of Lucid they developed in 2012. Once the conversion process is streamlined, keys in future ITP tools can be quickly made mobile-ready. Further, by combining the mobile tool content apps produced by Lisa Martin and the University of Florida with their corresponding QBIT-transformed keys, three complete tools will be available in 2013 in mobile format.



ITP tools cover a diversity of pest taxa as a result of cooperative relationships with a number of taxonomic experts at multiple academic institutions (see Accomplishments), including aphids, an important pest group we covered for the first time in 2012. These images illustrate the diversity of arthropods from tools delivered to USDA Plant Protection and Quarantine (PPQ) and its partners in 2012. Clockwise from upper left: Aphis sambuci *(elder aphid) from* AphID*, © Josef Dvořák, www.biolib.cz; ventral view of an* Australopalpus alphitoniae *female mite from* Flat Mites of the World*, by Gary Bauchan;* Anastrepha bistrigata*, a fly, from the* Anastrepha *and* Toxotrypana *tool; a leaf-footed bug nymph (*Leptoglossus *sp.) from* Citrus Pests*, by Lyle Buss;* Elateropsis rugosus*, a cerambycid beetle from* Longicorn ID*, © D. Spiller; a female* Tortrix viridana *moth from* TortAI*, by Todd Gilligan; and an* Anoplolepis gracilipies *worker ant from* Antkey*, by Eli Sarnat.*

Pursuing long-term growth for ID Source

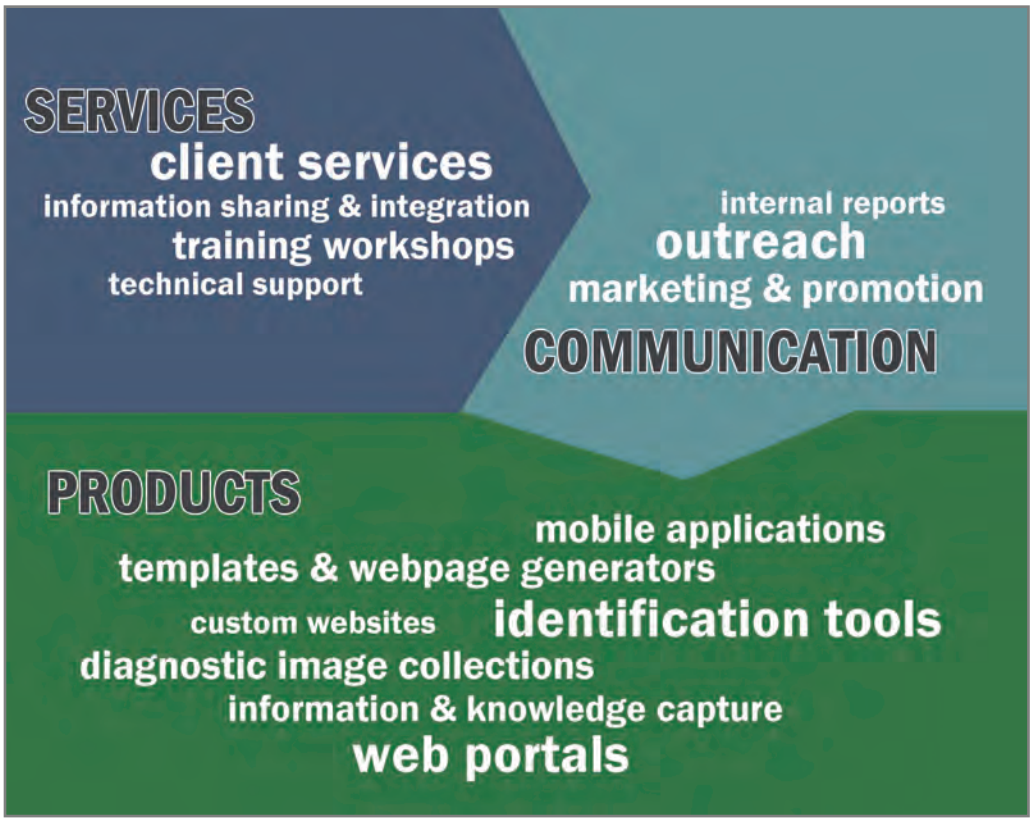
Over 500 new identification websites (called "ID Aids") were added to *ID Source,* a niche-oriented web portal, during 2012 (an increase from the previous year), and by year's end, users could browse nearly 2,000 vetted, identification-themed ID Aids for help in recognizing, identifying, and verifying plant pest organisms. *ID Source's* ID Aids are a special collection that represents many hours of gathering and screening, and then selecting and describing only those websites that are high quality, non-commercial, and relevant for protecting plants from pests of concern.

ID Source, first launched in September 2011, pursued a three-pronged approach to ongoing growth and development in 2012, all resulting from our team's engagement with local consultants. Substantial back-end changes were made that will result in vastly improved front-end functionality and new features for a better user experience. Usage tracking and analysis will indicate features that matter to users, design changes to make, and promotional actions to take so the site is more user-friendly and attracts more users. Finally, the code reviews conducted by our consultants facilitated development work on *ID Source,* and the roadmap they're completing will recommend major system changes to improve *ID Source's* architecture over the long term.

> ...a note of appreciation for the quality identification tools that you and your staff continue to "roll out" to PPQ and our stakeholders.
>
> Bill Wade, PPQ Professional Development Center

Features rolled out in 2012 include:

- A translation button on all pages so non-English users can view *ID Source* in any one of 64 languages.
- Search results shown in order of relevance for any type of search (relevance order was previously limited to just Text Searches).
- ID Aid popularity captured and displayed as number of visits, with a sort by popularity option.
- Fresh, newly added ID Aids users can see every time they visit *ID Source's* home or search results pages.
- Instant display of Text Search results for pest names.



A graphic snapshot of our program. ITP has expanded its role to provide services as well as products, supporting clients in new ways. We personally engage with clients and developers through workshops and direct support and serve the broader community through sharing of our data, which can be integrated into external datasets. ITP develops a diversity of products: those clients can use for their identification responsibilities (e.g., tools, web portals, image collections, mobile apps), as well as interfaces for contributing data and generating products (e.g., IDpic, Fact Sheet Manager (FSM), capsID*). Communication and outreach, in the form of presentations, webinars, reports, and marketing, keep ITP in touch with client needs, build awareness of our products and services, and keep us accountable to PPQ.*

Image sharing and integration

The need for high quality, scientifically valid, diagnostic images for identification purposes continues to grow within PPQ. ITP has devoted considerable energy to this area over the years, and we made more progress in 2012.

The vast trove of images that are in all ITP's tools are an incredible identification resource; our team has received many requests to access them outside the tools. *IDpic* was the first step in making them more widely available. Using the *IDpic* interface, over 4,000 images from 10 ITP tools were entered into one central database. In collaboration with the Center for Invasive Species and Ecosystem Health (CISEH) at the University of Georgia, these images were uploaded to the *IDpic Node* at CISEH and became instantly available to the public; in 2012, ITP's images were viewed 191,064 times and requested, mostly for educational and outreach purposes, 75 times.

imageID *will offer identifiers a way to upload, search, and filter the high quality, scientifically valid photographs they produce. To jump-start the* imageID *project, Terrence Walters, along with cooperators Joe LaForest (University of Georgia) and Dr. Deena Walters (Colorado State University), visited the San Francisco (SFO), Los Angeles (LAX), and Long Beach Plant Inspection Stations to discover the scope and origins of identifiers' image collections, obtain copies of valuable images and appropriate data, and find out what features identifiers would like to see in the* imageID *user interface. Phil Johnson, entomologist at SFO, discusses educational products for pests with Joe (left), and Dr. Tesfaye Tedla, plant pathologist at LAX, tells both Joe and Deena about his image collection data (right).* imageID *will be delivered in 2014.*

ITP also worked closely with NIS and the PISs to begin gathering another valuable collection of images—those taken by PIS identifiers through their Remote Pest Identification Program—as a first step toward shared image access for identifiers.

ITP's ultimate goal is to bring both of these groups of amazing images together into one huge, searchable, interactive image resource where the power of shared images in supporting identification is available for PISs as well as PPQ and its partners.

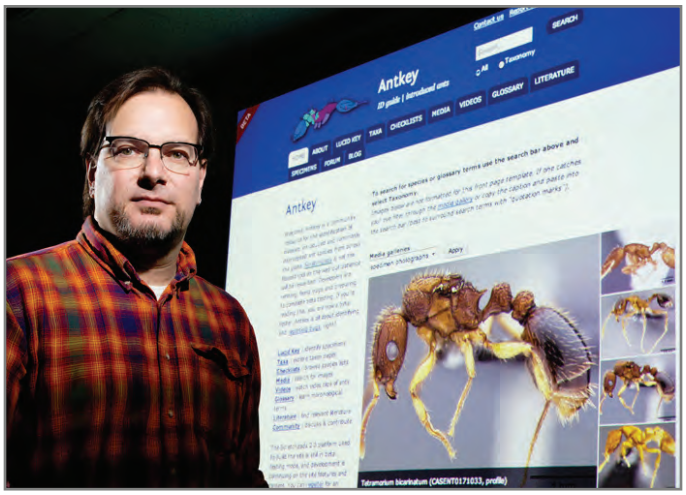
SUPPORTING DOMESTIC IDENTIFICATION AND CAPS

After two years of planning and discussions with CAPS (Cooperative Agricultural Pest Survey) representatives and the National CAPS committee (NCC) concerning their ongoing need to produce manuals and pest datasheets more efficiently, ITP delivered to the representatives and NCC a beta version of *FSM*. Originally created to enter and easily update data for tool fact sheets, we created an additional *FSM* module to capture data for CAPS pest datasheets and other survey manual content. CAPS survey manuals and pest datasheets would then be viewable by the CAPS community on a new site linked to the *FSM* database, called *capsID*, allowing CAPS representatives to easily update survey materials in a far timelier manner. CAPS and ITP worked together closely to test and improve both *FSM* and *capsID*, and ITP will present more polished versions of *FSM* and *capsID* to the NCC in 2013.

INTERNATIONAL ENGAGEMENT

ITP recognizes the importance of international collaboration in preventing cross-border spread of invasive pests, a by-product of globalization in today's world. As part of our continuing leadership role in Quad's (Quadrilateral Scientific Collaboration in Plant Biosecurity) Digital Identification Tools project area, ITP delivered to the Quad community seven tools that met key 2012 objectives, including covering three of the pest groups and two of the update priorities from the

Antkey *was a standout tool for ITP in 2012. Cooperators University of Illinois entomology professor Andy Suarez, shown below with the* Antkey *home page in the background, and his postdoc Eli Sarnat developed* Antkey *to be an interactive community resource that both experts and non-experts can use to identify invasive, introduced, and commonly intercepted ant species from across the globe. But there is more—the authors have added many features that push the boundaries of what a tool can be. A small sampling reveals external links to Antweb and Antwiki species pages, dynamic search results from Google Scholar and the Biodiversity Heritage Library, and dynamic mapping of specimens that have georeferenced location nodes. Plus, there's a blog with the latest updates and a forum for entering new species page requests, recording new introductions, and requesting an identification.*



2007 Quad Priority List of urgently needed tools. With these numbers, ITP surpassed all previous years' tool delivery to the Quad community. ITP also supported the European Phytosanitary Research Coordination (EUPHRESCO) network, hosting a delegation from Ukraine (Partner country No. 30) for a three-day workshop to jump-start Ukraine's effort to support screening and identification of their country's plant pests. Additionally, ITP funded two international post-docs, from Australia and Belarus, who lent their taxonomic expertise on mites (*Flat Mites of the World*) and beetles (*Diabrotica ID*), respectively.

A significant number of visitors to ITP's products were from outside the U.S., averaging around 30% of total visitors, with an impressive nearly 50% international users among those visiting *PIAkey*. Furthermore, a number of international plant protection organizations recognized our products in their newsletters, websites, or emails, including the North American Plant Protection Organization (NAPPO), European and Mediterranean Plant Protection Organization (EPPO), International Plant Protection Convention (IPPC), the Costa Rican Ministry of Agriculture and Livestock, and CABI.

TRAINING AND SUPPORT SERVICES

Based on positive results in previous years, ITP started incorporating training services for tool developers directly into their cooperative agreements. For the first time, we conducted workshops at the beginning of the development process, and found that these tool kickoff meetings go a long way to ensuring each project proceeds in an efficient manner, timelines are met, and tool products are high quality. (See Accomplishments for details.)

A major service ITP provides is technical and customer support for our tool developers and users. Throughout the year, ITP helped clients build keys optimally and troubleshoot software issues. In particular, during the USDA APHIS transition to a new operating system, ITP dealt with a large increase in help requests due to users' problems accessing the Lucid version 2 keys. By collaborating with PPQ-Information Technology (IT), ITP ensured users' computers could run the required software. ITP will work with PPQ-IT again in 2013 to add Lucid to employee computer configurations, which should sharply reduce problems experienced.

> Thanks so much for the quick implementation [of my suggestion]! The site looks great and the browsing is now all the easier.
>
> Florida environmental specialist, user of *Citrus Diseases* tool

NEW DIRECTIONS

The ITP team strives to deliver products and services that are relevant by keeping up with current technology and media trends while staying attuned to clients' changing needs. These goals led us to pursue several important efforts in 2012 that could significantly improve what our program has to offer in the coming years.

ITP moved closer to our goal of sharing data with other plant protection organizations and government agencies. We began sharing images, and we also set up an RSS feed of *ID Source* ID Aids to be displayed on the InsectImages website in 2013. But the maximum benefit will be realized when our projects sit within an API (application program interface) web services framework that allows electronic devices to communicate via the Internet; we could then share and integrate all the data and information we produce. Part of the plan will include a single ITP website for accessing all our products and services. In 2013, our consultants will present an optimal architecture for our program with a clear path we can take to achieve this new level of communication.

ITP supported international collaboration through EUPHRESCO, Phytosanitary European Research Area Network, by hosting a delegation from Ukraine, who came to the U.S. to learn about our program and our tool development approaches, as part of an initiative to build a program similar to ITP and to develop tools for Ukraine's pest identification needs. During the intensive, three-day workshop (left), Amanda Redford (with laptop) demonstrates features of ITP's tools during training on the key and website development process; listening are, from left to right, Terrence Walters, Mr. Vitaliy Romanchenko, First Deputy Head, Main State Phytosanitary Inspection Service of Ukraine Ministry of Agricultural Policy and Food of Ukraine, and from the National Academy of Agrarian Sciences of Ukraine, Dr. Liliya Pylypenko, Head, Scientific - Organizational Department and Dr. Oleksandr Borzykh, Director, Institute of Plant Protection. At right, Deena Walters (Colorado State University; seated) demonstrates ITP's sophisticated imaging system for Drs. Borzykh and Pylypenko.

ITP's proximity to Colorado State University (CSU) has led to an extensive and mutually beneficial partnership: in 2012, ITP employed 13 individuals through cooperative agreements with CSU, the highest number ever. ITP has built a network of four departments representing four CSU colleges, which led to constructive interactions through work on ITP's products. The cooperators pictured, most of whom are students who worked onsite at our Fort Collins Laboratory, brought a diversity of knowledge and expertise that continues to benefit and enhance our products. Through this partnership, we were able to provide funding to recent graduates (with bachelor's, master's, and doctoral degrees) as well as both undergraduate and graduate students, sometimes supporting them throughout their degree, as was the case for Todd Gilligan and Matt Trice. The value of ITP's relationship with CSU and the contributions of our many, diverse CSU cooperators cannot be overstated.

ITP became fully committed to promoting our products in 2012 to maximize their potential to serve our clients long term. The first step was engaging the consultants, who will deliver in 2013 a "current state analysis" and recommended marketing actions to improve product performance and usability, understand product users and how we can better serve them, and improve visibility and user retention. We are also eager to explore social media, especially in light of the recent USDA trend in that direction. A social media presence has the potential to heighten awareness of our products and services in new and exciting ways.

Finally, we are committed to being accountable for the value of our products. To that end, in 2013 we will begin generating regular reports on the usage of all ITP's products. These metrics will be clearly presented in a concise form and sent to managers and stakeholders, so they can see where and how our products are being used and can guide our program's direction. By offering these reports we aim to be responsive and transparent in our support of PPQ.

Accomplishments, Identification Technology Program, 2012

Funding requests approved

Source: USDA National Institute of Food and Agriculture (via University of Florida)
New technologies and educational materials to address national emergent citrus pests and diseases
Purpose: Deliver citrus pest and citrus disease smartphone mobile applications (apps) to farmers and the general public

Source: Section 10201 2008 Farm Bill
Digital identification of microlepidoptera on Solanaceae
Purpose: Produce a web-accessible digital tool to facilitate identification of microlepidopteran pests on solanaceous crops

Source: Section 10201 2008 Farm Bill
Digital imagery for pest screening, detection, identification, and report production
Purpose: Develop *imageID*, a web-based site that maximizes the usefulness and value of PPQ's existing and future image resources to support the screening, detection, and identification of plant pests

Source: Section 10201 2008 Farm Bill
Longicorn ID: a digital identification tool for diagnosing cerambycoid beetles (Cerambycidae, Disteniidae, Oxypeltidae, Vesperidae), Edition 2
Purpose: Add the potentially invasive longhorned wood boring beetle pests included within the Cerambycinae and Lamiinae subfamilies (Coleoptera) to the *Longicorn ID* tool

Source: Section 10201 2008 Farm Bill
Mobile app for plant pests
Purpose: Create a mobile application that enables the public to screen for and report on major domestic plant pests of concern to PPQ

Source: Section 10201 2008 Farm Bill
Morphological and molecular diagnostic tools for Lepidoptera larvae intercepted at U.S. ports of entry
Purpose: Deliver a comprehensive, multimedia, web-based tool for rapid identification of frequently intercepted Lepidoptera at U.S. ports

Source: Section 10201 2008 Farm Bill
Safeguarding against scale insect pests: a digital tool for training, screening, and identification
Purpose: Update website and add new species of recent concern since the release of Edition 1

ITP's U.S. cooperator institutions and organizations (by state)

AZ	Arizona State University
CA	California Department of Food and Agriculture (CDFA)
CO	Colorado State University
DC	Smithsonian National Museum of Natural History
FL	University of Florida, Florida A&M University, Florida Department of Agriculture & Consumer Services - Division of Plant Industry (FDACS DPI), Southern Plant Diagnostic Network (SPDN), National Plant Diagnostic Network (NPDN)
GA	University of Georgia
IL	University of Illinois
MD	University of Maryland, USDA Agricultural Research Service (ARS) Systematic Entomology Laboratory (SEL)
NC	North Carolina State University
NE	Chadron State College, University of Nebraska
NM	University of New Mexico
OR	Oregon State University

Presentations by or associated with ITP

USDA APHIS Eastern Regional Office Board of Directors Annual Seminar Series
January 4 in Raleigh, North Carolina
Design and delivery of digital identification resources for PPQ and its partners
Presenter: Terrence Walters

Colorado State University, Department of Bioagricultural Sciences and Pest Management Seminar Series
January 25 in Fort Collins, Colorado
Digital technology-based identification tools for plant protection
Presenter: Julia Scher

Cornell Horticulture Seminar Series
January 30 in Ithaca, New York
Images for horticulture and IPM [integrated pest management] education: introducing the Cornell Node of the Bugwood image database
Presenter: Joe LaForest, ITP cooperator (University of Georgia)

Southern Region Integrated Pest Management Working Group
February 15–16 in Tifton, Georgia
Introduction to the Center for Invasive Species and Ecosystem Health
Presenter: Joe LaForest, ITP cooperator (University of Georgia)

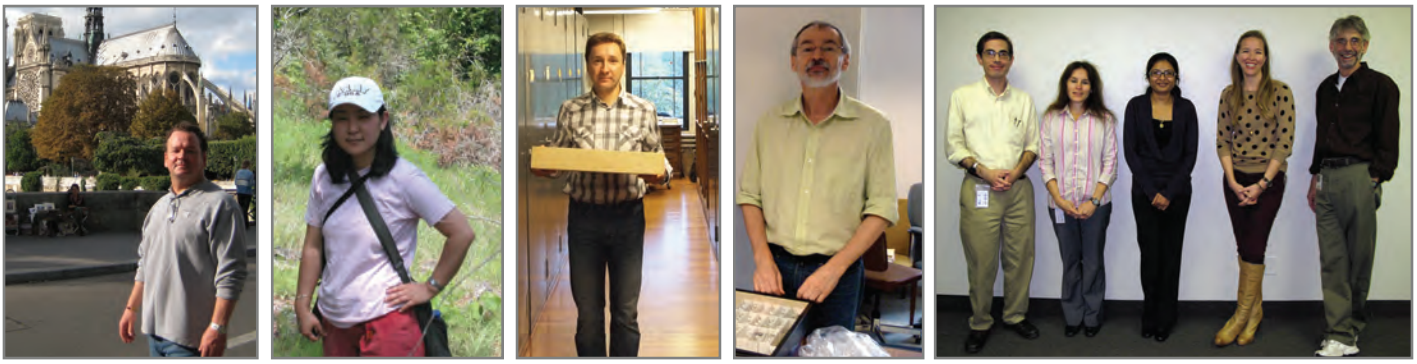
Entomological Society of America, Joint Annual Meeting of Southeastern and Southwestern Branches
March 7 in Little Rock, Arkansas
Citrus pest Lucid key: utilizing a web-based digital identification system for educating first detectors
Presenter: Sarahlynne Guerrero, ITP cooperator (University of Florida)

Center for Plant Health Science and Technology (CPHST) Fort Collins Laboratory Seminar Series
March 20 in Fort Collins, Colorado
Design and development of web-based identification tools for wood boring beetles: a case study
Presenter: Eugenio Nearns, ITP cooperator (University of New Mexico)

USDA APHIS Annual Agreements Meeting
May 24 in Riverdale, Maryland
Increasing ITP's production efficiency and product quality and value through APHIS agreements
Presenter: Terrence Walters

Southern IPM Center Advisory Council, Small Farms Working Group
June 12 and 26 in Raleigh, North Carolina and Clemson, South Carolina, respectively
Partnership to manage the Southern IPM Center: Facilitation of Innovation Through Technology (FITT)
Presenter: Joe LaForest, ITP cooperator (University of Georgia)

Ecological Society of America 97th Annual Meeting
August 7 in Portland, Oregon
What's in a name? Crowdsourcing citizens, experts, and the web to populate the ID Source *plant pest identification search tool*
Presenter: Greg Newman, ITP cooperator (Colorado State University)



ITP cooperators from left to right: Dr. Eugenio Nearns (University of New Mexico) in Paris to conduct museum research for Longicorn ID*; Dr. Sangmi Lee (Arizona State University), co-author of upcoming* Microlepidoptera on Solanaceous Plants *tool; Dr. Alexander Derunkov (Smithsonian Institution, National Museum of Natural History) holding a tray in the Smithsonian insect collection and in the next photo Dr. Alexander Konstantinov (USDA Agricultural Research Service, Systematic Entomology Laboratory [ARS SEL]) in Dr. Derunkov's laboratory, both working on* Diabrotica ID*; tool developer kickoff workshop participants with Amanda Redford and Terrence Walters, left to right: Dr. James Hayden (Florida Department of Agriculture & Consumer Services - Division of Plant Industry [FDACS DPI]), who is embarking on a tool to microlepidoptera, and Dr. Alessandra Rung and Grishma Parikh (California Department of Food and Agriculture [CDFA]) two of the authors of the forthcoming* Scale Insects Edition 2*.*

International Congress of Entomology (ICE) Agricultural Acarology Symposium
August 24 in Daegu, South Korea
Designing digital multimedia identification tools for invasive mites
Presenter: Terrence Walters

Colorado State University School of Business
August 28 in Fort Collins, Colorado
ID Source*: project for Colorado State University fall marketing capstone course*
Presenter: Julia Scher

Workshop of TECRO-AIT Cooperative Program in Agricultural Science Achievements
September 24 at the Council of Agriculture, Taipei City, Taiwan
The biogeography of ant invasions and its implications for biosecurity
Presenter: Andrew Suarez, ITP cooperator (University of Illinois)

Fall Meeting of the Southern Lepidopterists' Society and Association for Tropical Lepidoptera
September 29 in Gainesville, Florida
Microlepidoptera on Solanaceae: an on-line resource
Presenter: James Hayden, ITP cooperator (University of Florida)

Colorado State University, Department of Bioagricultural Sciences and Pest Management Seminar Series
October 10 in Fort Collins, Colorado
Moths, molecules, and money: identifying lepidopteran pests
Presenter: Todd Gilligan, ITP cooperator (Colorado State University)

American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America International Annual Meeting
October 22 in Cincinnati, Ohio
Bugwood: a plant image archive with multiple applications
Presenter: Joe LaForest, ITP cooperator (University of Georgia)

Colorado State University, Department of Bioagricultural Sciences and Pest Management, Dissertation Defense Seminar
October 23 in Fort Collins, Colorado
Advances in tortricid systematics and identification (Lepidoptera: Tortricidae)
Presenter: Todd Gilligan, ITP cooperator (Colorado State University)

University of New Mexico, Department of Biology, Museum of Southwestern Biology, Dissertation Defense Seminar
November 2 in Albuquerque, New Mexico
Systematics of longhorned beetles (Insecta: Coleoptera: Cerambycidae)
Presenter: Eugenio Nearns, ITP cooperator (University of New Mexico)

Sociedad Entomologica Peruana, Liv Convención Nacional de Entomologia
November 6 in Cusco, Perú
Anastrepha *identification system*
Presenter: Allen Norrbom, ITP cooperator (USDA ARS SEL)

ITP cooperators from left to right: Dr. Eli Sarnat (University of Illinois) in the Darlingtonia bog collecting specimens for Antkey*; Dr. Jennifer Beard (Australia Queensland Museum and University of Maryland; left) and Dr. Ron Ochoa (USDA ARS SEL; right), co-authors of* Flat Mites of the World*, with Dr. David Walter, acarologist and former ITP cooperator; Russell Scarpino (left) and Prathamesh Verlekar, along with Dr. Gregory Newman in the next photo, all members of the* ID Source *development team from Colorado State University.*

Entomological Society of America Annual Meeting
November 13–14 in Knoxville, Tennessee
Bugwood's tools as a common forum for ecological entomology
Presenter: Joe LaForest, ITP cooperator (University of Georgia)
A taxonomic and morphological overview of Tuta absoluta *(Lepidoptera: Gelechiidae)*
Presenter: Sangmi Lee, ITP cooperator (Arizona State University)

Ohio State University
December 6 and 12 online and in Wooster, Ohio
Bugwood image database administrator training (parts 1 and 2)
Presenter: Joe LaForest, ITP cooperator (University of Georgia)

Workshops given by ITP

Instructor: Amanda Redford
January 27 in College Park, Maryland
This second workshop with the *Phytophthora* team included reviewing the team's fact sheets and providing guidance on building the interactive key, as well as checking on the team's progress overall and addressing outstanding issues.

Instructors: Terrence Walters, Amanda Redford, and Deena Walters
November 13–15 in Fort Collins, Colorado
ITP provided an overview of our program, along with sessions on tool features and tool development tasks, for officials from two Ukraine agricultural plant protection organizations, to make them better prepared as they seek to create an organization similar to ITP and embark on building tools for their country's pest identification needs.

Instructors: Terrence Walters, Amanda Redford, and Deena Walters
November 27–28 in Fort Collins, Colorado
Participants: Cooperating scientists from CDFA and FDACS DPI
This was a kickoff workshop designed to start development in the right direction for two teams who were just beginning work on a microlepidoptera and a scale insect tool, respectively. Training included in-depth instruction in Lucid keys, *Fact Sheet Manager*, and tool content.

Workshops given by ITP's cooperators

Cornell Node training for the Bugwood image database
Instructor: Joe LaForest, ITP Cooperator (University of Georgia)
January 29–February 4 in Ithaca, New York
This workshop for staff and faculty of Cornell University and University of Maine introduced participants to image databases and node concepts now being developed at the University of Georgia. Demonstrations showed how individuals can contribute images to the Cornell Node and use the Node's image collection to support identification of pests and diseases.

Ant taxonomy training workshop
Instructor: Eli Sarnat, ITP cooperator (University of Illinois)
March 19–23 in Mangilao, Guam
During this intensive five-day ant taxonomy outreach workshop at the University of Guam, participants—including 20 quarantine personnel from the Micronesian islands of Palau, Saipan, Rota, Tinian, Yap, Pohnpei, Chuuk, and Kosrae—were trained in the identification of invasive Pacific region ants using ITP's *Pacific Invasive Ant Key* (*PIAkey*).

Tortricidae of agricultural importance workshop
Instructor: Marc Epstein, ITP cooperator (CDFA)
May 17 in Van Nuys, California
The primary objective of this workshop was to demonstrate the various modules, galleries, and keys contained in ITP's *TortAI: Tortricids of Agricultural Importance* to CDFA biologists and southern California county entomologists. Training was provided on how to identify an unknown tortricid using the tool's adult and larval matrix-based keys.

4th international workshop on Oomycetes: Phytophthora, Pythium, and Phytopythium
Instructors: Gloria Abad (CPHST Beltsville Laboratory) and Yilmaz Balci, ITP Cooperator (University of Maryland)
May 21–25 in College Park, Maryland
This University of Maryland workshop provided hands-on training on morphological and molecular tools (e.g., Lucid keys) used to identify species within these three genera of plant pests and included a full-day international web symposium ("*Oomycetes of Concern in International Trade*").

Publications from ITP's cooperators

Revisiting the ants of Melanesia and the taxon cycle: historical and human-mediated invasions of a tropical archipelago
The American Naturalist, Volume 180, Number 1, Pages E1–E16
Cooperating author: Eli Sarnat (University of Illinois)

A new species of Anastrepha *(Diptera: Tephritidae) from Colombia, Costa Rica, and Panama*
Canadian Journal of Entomology, Volume 144, Pages 158–168
Cooperating authors: Cheslavo Korytowski (Universidad de Panamá) and Allen Norrbom (USDA ARS SEL)

Raoiella indica *(Acari: Tenuipalpidae): an exploding mite pest in the neotropics*
Experimental and Applied Acarology, Volume 57, Issue 3–4, Pages 215–225
Cooperating authors: Jennifer Beard (University of Maryland) and Ronald Ochoa (USDA ARS SEL)

External mouthpart morphology in the Tenuipalpidae (Tetranychoidea): Raoiella *a case study*
Experimental and Applied Acarology, Volume 57, Issue 3–4, Pages 227–255
Cooperating authors: Jennifer Beard (University of Maryland) and Ronald Ochoa (USDA ARS SEL)

New taxa and combinations in Onciderini Thomson, 1860 (Coleoptera: Cerambycidae: Lamiinae) from Central and South America, with notes on additional taxa
Insecta Mundi, Volume 0231, Pages 1–24
Cooperating author: Eugenio Nearns (University of New Mexico)

A new genus and five new species of Onciderini Thomson, 1860 (Coleoptera: Cerambycidae: Lamiinae) from South America, with notes on additional taxa
Insecta Mundi, Volume 0266, Pages 1–23
Cooperating author: Eugenio Nearns (University of New Mexico)

A Lucid key to the common species of Phytophthora
Plant Disease, Volume 96, Number 6, Pages 897–903
Cooperating author: Jean Ristaino (North Carolina State University)

Identification and detection of Phytophthora*: reviewing our progress, identifying our needs*
Plant Disease, Volume 96, Number 8, Pages 1080–1103
Cooperating author: Yilmaz Balci (University of Maryland)

New species of Anastrepha *(Diptera: Tephritidae), with a key for the species of the* megacantha *clade*
Zootaxa, Volume 3478, Pages 510–552
Cooperating authors: Cheslavo Korytowski (Universidad de Panamá) and Allen Norrbom (USDA ARS SEL)

The role of behavioural variation in the invasion of new areas
Behavioural Responses to a Changing World, Eds. U. Canolin and B. Wong, Oxford University Press, Chapter 14, Pages 190–200
Cooperating author: Andrew Suarez (University of Illinois)

Elton's insights into the ecology of ant invasions: lessons learned and lessons still to be learned
Fifty Years of Invasion Ecology: The Legacy of Charles Elton, Ed. D. Richardson, Wiley-Blackwell, Chapter 18, Pages 239–251
Cooperating author: Andrew Suarez (University of Illinois)

The ants of Fiji
University of California Publications in Entomology, University of California Press, Volume 132, 400 pages
Cooperating author: Eli Sarnat (University of Illinois)

Product promotion highlights

Boletín ProNAP
The April issue of this bulletin from the Costa Rican Ministry of Agriculture and Livestock featured an article announcing ITP's *Flat Mites of the World* tool for the Costa Rican regional agricultural community.

Bugwood Blog
The Center for Invasive Species and Ecosystem Health announced the release of *Antkey* through a blog post describing the tool's features. The post also noted the use (ITP's first) of Scratchpads, the taxonomically-focused social networking platform upon which *Antkey* is built.

Colorado Weed Management Association Newsletter
The spring issue of this newsletter featured *ID Source* in its "Tools and Technology" section, where it described *ID Source's* ID Aids, its design and search capabilities, and ongoing development.

NAPPO Newsletter
This organization's March newsletter featured *ID Source* in a detailed article titled "*ID Source*, a new search tool for plant identification on the web," which described the function, highlights, and recent upgrades of the resource. The newsletter is available in both English and Spanish.

NPDN First Detector Network News
Six articles appeared in this online newsletter from January through December of 2012, most of which were announcements of new tools released by ITP. The articles described *Flat Mites of the World, TortAI: Tortricids of Agricultural Importance, Longicorn ID,* Anastrepha *and* Toxotrypana*,* and *Hispines of the World*. Also noted was *Citrus Pests*, which completed the *Citrus Resource.*

University of Illinois *News Bureau*
This November article titled "Name that ant! New online tool helps identify alien ant invaders" described the recently released *Antkey* tool for a general university audience. The article mentions how interactive websites like *Antkey* can help prevent costly invasions of new pests into the United States, and it also highlights how citizens can participate in the protection of agriculture through the use of *Antkey*, which was designed with non-specialists in mind.

Identification products ITP delivered to PPQ and cooperators

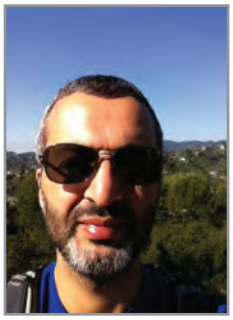
Anastrepha *and* Toxotrypana*: Descriptions, Illustrations, and Interactive Keys*
Authors: Allen Norrbom, Cheslavo Korytkowski, Roberto Zucchi, Keiko Uramoto, George Venable, Jerrett McCormick, and Michael Dallwitz
Cooperators: USDA ARS SEL, Universidad de Panamá
Collaborator:* Commonwealth Scientific and Industrial Research Organization (CSIRO) Australia
<http://delta-intkey.com/anatox/intro.htm>

Antkey
Authors: Eli Sarnat and Andrew Suarez
Cooperator: University of Illinois
<http://www.antkey.org>

AphID: Identification Guide for Cosmopolitan and Polyphagous Aphid Species
Authors: Colin Favret and Gary Miller
Cooperators: University of Maryland, Université de Montréal, USDA ARS SEL
<http://aphid.aphidnet.org>

Citrus Pests
Authors: Sarahlynne Guerrero, Jennifer Weeks, Amanda Hodges, Kirk Martin, and Norman Leppla
Cooperators: University of Florida, SPDN
<http://idtools.org/id/citrus/resource>

* ITP's collaborators contribute their expertise to help guide ITP during critical steps of product development.



ITP cooperators from left to right: Dr. Marc Epstein (CDFA; left), co-author of TortAI*, instructing two participants during his Tortricidae of Agricultural Importance workshop; the* Citrus Pests *team, from left to right: Dr. Jennifer Weeks, Sarahlynne Guerrero, and Dr. Amanda Hodges (University of Florida); Nathan Lord (University of New Mexico) examining longicorn beetles for the forthcoming second edition of the* Longicorn ID *tool; Dr. Yilmaz Balci, co-author with USDA Center for Plant Health Science and Technology's (CPHST) Gloria Abad, of identification tools for* Phytophthora*.*

Flat Mites of the World
Authors: Jennifer Beard, Ronald Ochoa, Gary Bauchan, Matthew Trice, Amanda Redford, Terrence Walters, and Charlie Mitter
Cooperators: University of Maryland, USDA ARS SEL
Collaborator: Australia's Queensland Museum
<http://idtools.org/id/mites/flatmites>

Hispines of the World
Author: Charles Staines
Collaborator: Smithsonian National Museum of Natural History
<http://idtools.org/id/beetles/hispines>

IDpic
Authors: Deena Walters and Joe LaForest
Cooperators: Colorado State University, University of Georgia

IDpic Node
Authors: Deena Walters and Joe LaForest
Cooperators: Colorado State University, University of Georgia
<http://www.ipmimages.org/browse/NodeThumb.cfm?Node=5>

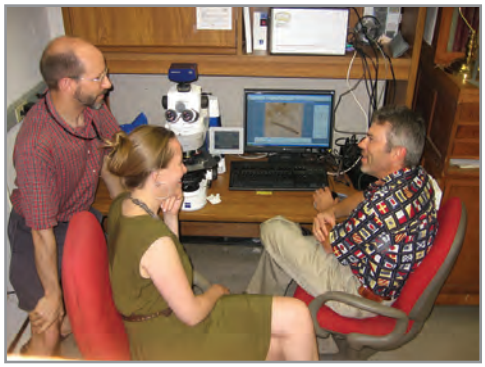
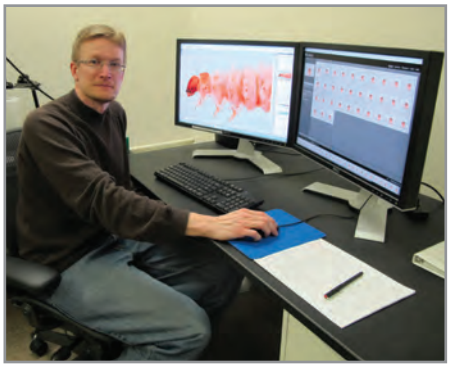
Longicorn ID: Tool for Diagnosing Cerambycoid Families, Subfamilies, and Tribes
Authors: Eugenio Nearns, Nathan Lord, Steven Lingafelter, Antonio Santos-Silva, and Kelly Miller
Cooperator: University of New Mexico
Collaborators: USDA ARS SEL, CSIRO Australia
<http://cerambycids.com/longicornid>

Table Grape Spider ID
Collaborator: University of California Riverside
<http://idtools.org/id/table_grape/spider/login.php>

TortAI: Tortricids of Agricultural Importance
Authors: Todd Gilligan and Marc Epstein
Cooperators: Colorado State University, CDFA
<http://idtools.org/id/leps/tortai>

Ongoing identification products

ID Source
Project lead: Julia Scher
Cooperator: Colorado State University
<http://idsource.colostate.edu>

ITP cooperators from left to right: Joe LaForest (University of Georgia) and Dr. Deena Walters (Colorado State University), co-authors of IDpic *and* IDpic Node*, discussing* IDpic *during Joe's visit to the CPHST Fort Collins Laboratory; the* AphID *team: Dr. Colin Favret (Université de Montréal and University of Maryland; left) and Dr. Gary Miller (USDA ARS SEL; right), with Amanda Redford during a training session at Dr. Miller's ARS laboratory in Beltsville, MD; Dr. Todd Gilligan (Colorado State University) photographing moth larvae with ITP's imaging system for the upcoming tool for Lepidoptera larvae intercepted at ports of entry.*

ID Tools
Project lead: Amanda Redford
Cooperator: Colorado State University
<http://idtools.org/>

A Resource for Wood Boring Beetles of the World
Project lead: Eugenio Nearns
Cooperator: University of New Mexico
<http://wbbresource.org>

Beta versions of identification products completed and under review

Diabrotica *ID: Identification of* Diabrotica *species (Coleoptera: Chrysomelidae) from North and Central America*
Cooperators: University of Maryland, USDA ARS SEL
Expected release: 2013
<http://idtools.org/id/beetles/diabrotica>

Fact Sheet Manager (FSM)
Cooperator: Colorado State University
Expected release: 2013

Identification products initiated or in development

capsID
Cooperators: Colorado State University
Expected release: 2013

Digital Identification of Microlepidoptera on Solanaceous Plants
Cooperator: FDACS DPI
Collaborators: Arizona State University, Florida Museum of Natural History, Dresden Museum of Zoology
Expected release: 2013

Grasshoppers of the Western United States, Edition 4
Cooperators: Chadron State College, University of Nebraska
Expected release: 2013

imageID
Cooperators: Colorado State University, University of Georgia
Expected release: 2014

Longicorn ID: Tool for Diagnosing Cerambycoid Families, Subfamilies, and Tribes, Edition 2
Cooperator: University of New Mexico
Collaborators: USDA ARS SEL, CSIRO Australia
Expected release: 2013

Morphological and Molecular Diagnostic Tools for Lepidoptera Larvae Intercepted at U.S. Ports of Entry
Cooperator: Colorado State University
Collaborator: The Ohio State University, Smithsonian National Museum of Natural History
Expected release: 2013

Online Identification Tools for Phytophthora
Collaborators: University of Maryland, University of California Riverside, USDA ARS
Expected release: 2014

Safeguarding Against Scale Insect Pests: A Digital Tool for Training, Screening, and Identification, Edition 2
Cooperator: CDFA
Collaborator: USDA ARS SEL
Expected release: 2013

Notable uses of ITP's products in publications and for training

Product: *Citrus ID*
This tool was recommended to Leland Cseke (University of Alabama in Huntsville), who needed to know whether various genera were considered to be citrus or other members of the Rutaceae for his sequence analysis project.

Product: *IDpic Node*
A sampling of image usage requests received in 2012 reveal a variety of ways in which images requested were to be used:
- "Grasses of the NSW Tablelands," an Australian identification booklet for landholders
- Women's lunch club presentation
- University of Georgia Natural Resources Conservation Service Curriculum
- Student presentation for college dendrology class
- For teaching weed ID in undergraduate weed management course
- USDA newsletter concerning pest detection for Customs & Border Protection
- Training videos
- Lab training

Product: *ID Source*
Business students in an upper level marketing class at Colorado State University studied this resource as part of a capstone project, analyzing the website, its usage and its goals, conducting a user survey, and presenting ITP with detailed marketing plans. The students benefitted from exposure to a real government client with plant pest concerns, and ITP benefitted by gaining some promising recommendations which may be implemented when roadmap marketing actions are undertaken in 2013.

Product: *LBAM ID*
CDFA specifically requested 24 CD copies of this tool for use in the field during tomato pest surveys, with the hope that this tool would reduce the number of lepidopteran specimens forwarded to laboratories for identification.

Product: Lucid key from *Online Identification Tools for* Phytophthora
Participants of the international web symposium "Oomycetes of Concern in International Trade" had the opportunity to beta test a draft version of this key during University of Maryland's "4th International Workshop on Oomycetes: *Phytophthora*, *Pythium*, and *Phytopythium*."

Product: *PIAkey*
ITP cooperator Eli Sarnat used *PIAkey* as the primary training resource during an outreach workshop on ant taxonomy held at the University of Guam.

Product: *A Resource for Pests and Diseases of Cultivated Palms: Screening Aid to Pests*
This tool was provided to USDA APHIS when they requested identification resources from ITP for use by individuals in Texas needing to detect South American palm weevil.

Product: *TortAI: Tortricids of Agricultural Importance*
This past year, the newly released *TortAI* was used during a California state and county workshop for identifying tortricid adults and larvae both in the laboratory and during Cooperative Agricultural Pest Survey (CAPS) surveys.

Thank you to the institutions and organizations that provided beta reviewers for ITP's identification tools

Alberta Museum, Canada
Australian Quarantine and Inspection Service
Brigham Young University
California Department of Food and Agriculture
Canadian National Collection of Insects, Arachnids & Nematodes
Colorado State University
Department of Homeland Security
Florida Department of Agriculture & Consumer Services - Division of Plant Industry
Louisiana State University
Museu Nacional-Universidade Federal do Rio de Janeiro, Brazil
Queensland Museum, Australia
Smithsonian National Museum of Natural History
Texas A&M University
USDA APHIS Plant Protection and Quarantine
USDA APHIS PPQ National Identification Services
USDA Agricultural Research Service, Systematic Entomology Laboratory
University of Illinois
University of Maryland
Washington State Department of Agriculture

Award won by cooperator

2011 Deputy Administrator's Safeguarding Award: Offshore Pest Information System (OPIS) Pest List Project
Sponsoring organization: USDA APHIS PPQ
Recipient: ITP cooperator Todd Gilligan

Todd was a member of the Lepidoptera group (one of 16 groups) within the OPIS Pest List Team. This team compiled and ranked a web-based list of the most significant plant health threats from foreign countries. The resultant OPIS list enables more than 1,200 stakeholders to use various strategies to focus their pest exclusion efforts.

AQI waste disposal methods development

Craig Ramsey and Debra Newman

In 2012, two waste disposal studies, funded by the AQI program, were conducted at the Fort Collins CPHST lab in collaboration with Biosafe Life Sciences and MicroChem laboratory. The goal of the studies was to develop and evaluate alternative methods to devitalize and dispose of mixed agricultural waste (plant and animal materials) that is seized at U.S. ports of entry. There are three USDA approved methods for waste disposal: 1) incineration to ash; 2) sterilization (cooking regulated garbage to an internal temperature of 212 °F for 30 minutes); and 3) grinding followed by discharge into an approved sewage system (USDA Manual for Agricultural Clearance). These methods can incur high costs and high carbon footprints, may not devitalize all threats (namely seeds and certain microbes) and may not be practical for all border stations.

Two disposal systems were evaluated for their sterilization efficacy: a steamer and a small electric incinerator. The steamer system (STI) is manufactured by BioSAFE Life Science, and the electric incinerator (Incinolet) was manufactured by Research Products. *Bacillus subtilis*, a non-pathogenic, spore-forming bacteria, and Gymnocladus dioicus, a hard-coated tree seed, were chosen for the efficacy tests to represent microbial pathogens and plant seeds because they are particularly difficult to devitalize. *B. subtilis* spore preparation and treatment assays were conducted by MicroChem Laboratory.

The objective of the electric incinerator study was to determine the efficacy of the Incinolate unit on *B. subtilis* spores, over six burn times ranging from 100 to 240 minutes. This study had only one replicate test, consisting of five spiked mangoes, for each of the six burn times. The mangoes were injected with 2ml of a *B. subtilis* spore suspension and then incinerated using the Incinolate. The ashes were collected in vials with sterile methods and sent to MicroChem for analysis.

Analysis of the incinerator data showed that the length of burn time had no effect on *B. subtilis* efficacy (Figure1). There was an average of 16 viable Colony Forming Unit (CFU)/vial ash sample for burn times of 160, 180, and 200 minutes. The *B. subtilis* spore count for the untreated samples was 8.0 x 107 CFU/ml. The viable spores were reduced by an average of 7.145 log10 CFU/vial ash sample (1.4 x 107), across the six burn times. There was an average of 16 CFU/ml viable spores for the 160, 180, and 200 minute burn time tests. The 220 and 240 minute tests had 100% efficacy of the spores. Also, the pure ash and the partially burned samples had the same viable CFU counts. In summary, the small electric incinerator was able to sanitize spiked mangoes across four burn times, and had 100 % efficacy for burn times of 220 and 240 minutes. The capacity of the incinerator is limited to about five large mangoes. This incinerator could be used to sanitize mixed animal and plant material that is seized at small border stations.

There were two objectives in the STI steamer study: 1) to determine the efficacy of the STI system on *B. subtilis* spores embedded onto wool samples; and 2) to measure the temperature of wood chips inside an auger using remote data loggers. The study was replicated over three test runs with 80 *B. subtilis* fabric samples treated per run and each test run consisted of five wool treatments (Table 1). One run was with hay as the waste carrier and two tests were run with wood chips purchased from a local tree service company. The *B. subtilis* samples and the temperature sensors were added into the wood chips for each of the runs. The five *B. subtilis* treatments included untreated wool fabric, uncovered wool fabric, cut and uncovered wool fabric, fabric covered by poster board and wool fabric covered by leather. MicroChem laboratory prepared and assayed the *B. subtilis* samples.

The initial *B. subtilis* spore counts on the control samples was 1.9 x 10^8 CFU/ml. The log10 reduction and percent reduction of viable *B. subtilis* spores (CFU/wool sample) for each of the six treatments, averaged across all three runs, is listed in Table 1. The results show that two out of four STI treatments and the autoclave test had 100% efficacy. The two other treated samples had an average log10 reduction of 6.42. Sporicidal efficacy base on log10 reduction is calculated as log10 initial count (CFU/carrier) – log10 test results (CFU/carrier). The samples that were covered with either the poster board or the leather strips, which simulated material that was not shredded, had 100% efficacy. These

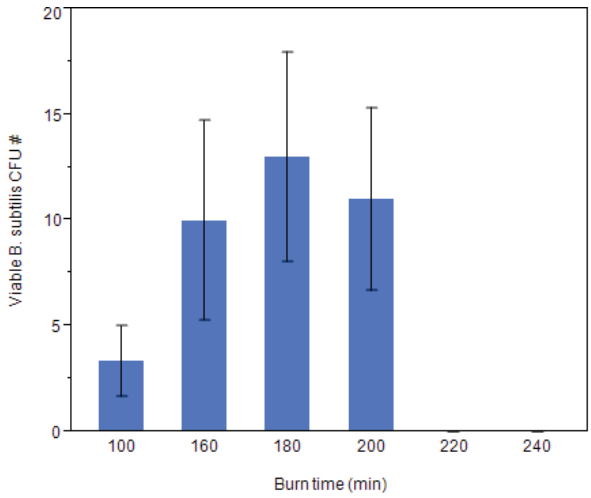


Figure 1. Graph of the B. subtilis viable spore counts for burn times of 100, 160, 180, 200, 220, and 240 minutes. Regression analysis of viable CFU counts over the six burn times revealed that burn time had no effect on incinerator efficacy of the spores (p-value = 0.5891).

results indicate that the temperature and time of exposure was sufficient to kill the spores that were protected within the poster board or the leather "sandwiches".

The wood chip temperature was recorded over time to delineate how long the wood chips were heated to a minimum of 100 C, while in the auger (Figure 2). There was a pressure release problem in run 1, thus the temperature dropped below 100 C for both sensors. STI runs 2 and 3 both had temperatures averaging 103 to 105 C for longer than 30 minutes. Therefore, the modified STI steam system met the USDA waste disposal regulations that require 100 C for 30 min for runs 2 and 3. Another set of STI tests are planned in 2013 to confirm the wood chip temperature inside the auger.

In summary, both waste disposal systems had high sporicidal efficacy results, and the STI system also met the USDA time and temperature requirements for waste disposal heat treatments. The time of exposure was different between the Incinolet and the STI steam systems. The average time of exposure for the STI system, for temperatures equal to or above 100 C, was 71 minutes. The time of exposure for the Incinolet ranged from 100 to 240 minutes.

Table 1. B. subtilis sample description, viable spore log10 reduction, and viable spore percent reduction after treatment.

Sample description	Viable spores - Log10 reduction	Viable spores - Percent reduction
Untreated fabric preloaded with B. subtilis	0	0
Wool fabric alone	6.37	99.99998
Wool fabric inside poster board sandwich sample (plant waste)	Complete control	100
Wool fabric inside leather sandwich sample (plant waste)	Complete control	100
Cut samples (shredded) but treated along with sandwich samples	6.47	99.999967
Autoclave	Complete control	100

The five mangoes in the Incinolate test were not completely burned to ash until the time of exposure reached 220 minutes. This was 3.1 times longer than the STI average time of exposure. Sporicidal efficacy comparisons between these two disposal systems should include any differences in time of exposure, because it is a critical factor in heat treatments and incineration tests. Time of exposure not only affects deactivation rates for bacteria spores, it also affects the number of batch cycles allowed per day. Time of exposure also affects operational costs, as energy costs increase with longer heating times.

Acknowledgements: The authors would like to acknowledge Mike Hennessey, Russ Bullock, Lori Miller, and Jose Ceballos for project support.

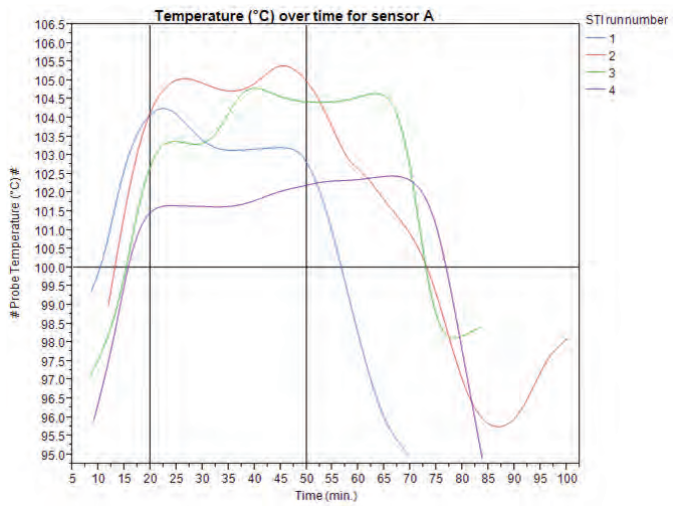


Figure 2. The graph shows the auger temperature (y-axis labeled as C) over time (x-axis labeled as min.) for one sensor (upper x-axis) for the four test runs (upper right legend). The black vertical reference lines indicate a time interval of 30 minutes, and the horizontal reference line indicates the temperature at 100 C.

Assessment of disinfectants for control of *Phytophthora ramorum*

Craig Ramsey and Debra Newman

This project is a cooperative agreement between USDA-APHIS and Colorado State University. The project started in 2011 with the overall goal of assessing disinfectants for controlling *P. ramorum* in nurseries. A master's degree student, Heather Hammack, was assigned to this project in the Horticultural Department. She completed three studies in 2012 which were: 1) a ClO2 disinfectant and rhododendron foliar injury study; 2) a circadian rhythm study with kidney beans; and 3) a foliar disease resistance study with kidney beans. The primary objective of this project was to determine if two separate oxidant disinfectants are safe to apply to nursery plants for controlling *P. ramorum* spores. The second objective is to determine the effects of oxidant applications on nursery plant natural defense activities.

The objective of the first greenhouse study was to determine the effects of several chlorine dioxide formulations and three sprayer types on rhododendron foliar injury. Rhododendrons were selected for this study because they are primary host plants for *P. ramorum*. Electro-Biocide (EB) was the commercial chlorine dioxide disinfectant used for this study. Different formulations of EB were generated locally containing mixtures of the following additives: surfactants, pH buffers, and anticorrosion inhibitors. Fluorescence was measured with a LICOR 6400 instrument to evaluate foliar stress levels for the two study factors. Plant stress may be detected by changes in foliar fluorescent parameters such as the maximum quantum efficiency (Fv/Fm), which measures the efficiency at which light is absorbed by the PS II

center inside of chlorophyll. Plants with a higher relative efficiency have a higher rate of photons converted into photochemistry. This indicates that the plant is more vigorous and under less biotic or abiotic stress.

The results from the first study show there were significant interactions between factors, i.e. plant fluorescence was dependent on the interaction of EB rates with surfactants and sprayer types. The negatively charged, electrostatic sprayer and EB + surfactant had the highest Fv/Fm across four EB rates (0, 200, 400, 800 ppm). The uncharged, hand bottle sprayer and EB + surfactant had the highest Fv/Fm value when applied at 1,600 ppm. Fv/Fm measurements ranged from 71.5 to 80.5% maximum efficiency for photo conversion, across all treatments. Treatment differences could be detected at the1% maximum quantum efficiency scale. The rhododendrons showed no visible signs of foliar injury from the EB applications up to a month after the treatments. This was a non-replicated study conducted so that the best combination of treatments could be selected for a more detailed study in the future.

Figure 1. Heather Hammack, a master's degree student in the Horticultural Department at Colorado State University.

The objective of the second greenhouse study was to determine if there was a daily circadian rhythm of plant physiology functions that may affect dark adapted foliar fluorescence measurements. This study estimated the time-of-day by measuring effects on fluorescence taken from kidney beans and rhododendron plants. Any circadian rhythm patterns in plants during their nighttime cycle would be a confounding factor when measuring dark adapted fluorescence over 6 to 8 hour time periods. The study results show that there were no circadian rhythm patterns in fluorescence, as compared to the diurnal patterns in photosynthetic rates. Instead, there was a linear decrease in Fv/Fm over time that was affected by plant species and leaf temperature. Fv/Fm decreased from 79.8 to 79.0% and 81.9 to 79.2% maximum quantum efficiency for rhododendron and kidney beans, respectively, over a 12 hour measurement period. These results indicate that time of fluorescent measurement could be used as a covariate in future studies to minimize the confounding effects of time. A second option would be to shorten the measurement window for each day, to avoid a significant decrease in Fv/Fm over time. Although Fv/Fm estimates are often used to detect PS II chlorophyll injury in water stressed plants, this study could not find a significant linear relationship between Fv/Fm and a wide range of soil moisture levels in the kidney bean test. Despite these findings, the data showed a strong, direct relationship between Fv/Fm and soil temperature. This study validates one of the claims that plant fluorescence can be used to measure the degree of plant stress to an abiotic stressor such as soil temperature.

The objective of the third greenhouse study was to determine the effects of two oxidant disinfectants applied to young kidney bean plants that were inoculated with a bacterial wilt, *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (Cff or bacterial wilt of common bean). Six plant responses were measured in this study to estimate the effects of the oxidants on suppression of the wilt pathogen, overall plant growth, and indirect evidence for increased natural plant defense activity against the foliar pathogen. Plant researchers have found that plants may receive chemical signals or be stimulated by biotic stresses by developing a natural plant defense known as Systemic Acquired Resistance (SAR). This defense activity partially protects plants and increases their resistance to pathogens and insect herbivory. Oxidants such as chlorine dioxide and hydrogen peroxide may be SAR signals/stimulants that initiate a cascade of cellular activities which cause the production of numerous compounds that help protect photosynthesis and other functions from biotic and abiotic stressors. The study factors for the kidney bean growth and morphology responses were: 1) oxidants applied either four days before plants were inoculated with the wilt, or four days after plants were inoculated; 2) non-inoculated and inoculated plants with Cff,; and 3) four oxidant treatments. The EB formulations were applied at 200 ppm and Oxidate was applied at 100 ppm, with each oxidant mixed with two different surfactants (ethylated alcohol and sarcosinate). The kidney bean plant growth and morphology responses were total leaf area, specific leaf area, total fresh weight, total oven dry weight, and relative growth rate, when averaged across the six plants. The plants were harvested 48 to 51 days after planting in order to take the growth and leaf morphology data.

Analysis of the data shows that the three study factors interacted together to affect plant growth and defense activities. In three out of six plant growth tests, the study factors included two, 2-way interactions. As an example, the Relative Growth Rate (RGR) was a function of the three study factors interacting together (Figure 2). The total above-ground, dry biomass response increased by 35 and 24% for the non-inoculated and inoculated plants, respectively, when plants were sprayed with EB plus sarcosinate surfactant at nine days after planting. These results indicate that EB plus sarcosinate surfactant should be applied very early, during the primary leaf stage, in order to achieve higher growth rates.

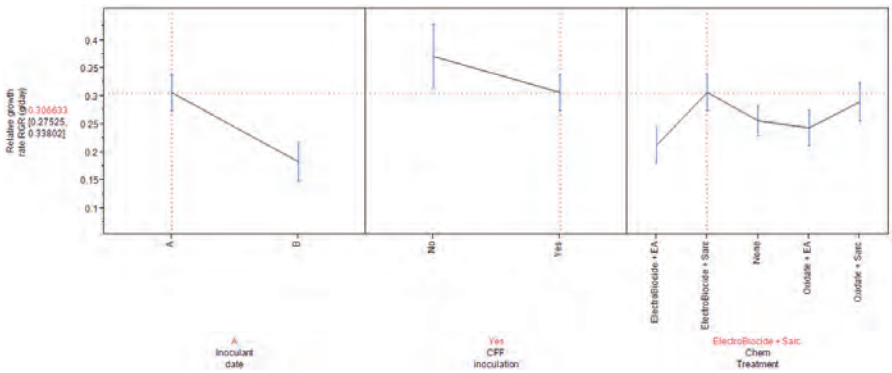


Figure 2. The graph shows the Relative Growth Rate (RGR) for aboveground, oven dry biomass per day for kidney bean seedlings sprayed nine days after planting (red dotted line on A in left plot), for seedling inoculated with the Cff bacterial wilt (red dotted line on yes in middle plot) and for seedlings treated with EB plus sarcosinate (red dotted line on EB+ sarc in right plot). The vertical red lines indicate the level within a study factor which in turn represents the statistical adjustment for the interactions among the three study factors mentioned above. The RGR ranged from 0.068 to 0.31oven dry g/day among all the study factors, with the highest RGR (0.31 g/day) shown on the left y-axis in this graph.

The highest RGR treatment for inoculated plants was EB plus sarcosinate applied four days before the CFF wilt inoculation which had an RGR of 0.31 g/day. The highest RGR (0.31 g/day) was 19.2% higher than the inoculated, control plants (RGR = 0.26 g/day). Inoculated kidney beans sprayed once with EB at 200 ppm had a 20% increase in RGR, when compared to the inoculated control treatment. These results show that oxidant disinfectants sprayed before the plants were inoculated had increased plant growth.

Also, the oxidant disinfectants show promise for signaling natural plant defenses against foliar injury from bacterial wilt. Future studies will focus on the most effective treatments and increase the number of plant replications in order to reduce variation between treatments.

In summary, EB could be applied to woody nursery plants without foliar injury that would prevent the plants from being sold either wholesale or from local nurseries. A secondary, but very significant benefit from applying disinfectants may be that the oxidants serve as signals to activate the plant's defenses. Further studies are needed to verify that plant defense activities have been primed by the signals, and whether the signals create a biochemical "imprint or memory" so that a plant's defenses can be readily re-activated against future pathogen attacks. This is a continuing project with Colorado State University. A second graduate student will continue to study the effects of oxidant disinfectants on nursery plant foliar injury, activating plant defenses, and sanitizing nursery supplies and soil.

Acknowledgements: The authors would like to acknowledge Russ Bullock, Mike Hennessey, Charla Hollingsworth, Prakash Hebbar, and Stacy Scott for project support.

Chlorine dioxide disinfectant efficacy study

Craig Ramsey and Debra Newman

I have been collaborating with Intelagard and Strategic Resource Optimization (SRO) since 2009 with their hydrogen peroxide (H2O2) and chlorine dioxide (ClO2) disinfectants. SRO has recently patented a series of chlorine dioxide formulations, which were registered with EPA as a general use disinfectant in 2010. Their ClO2 disinfectant is marketed as ElectroBiocide (EB), which can include formulations with different concentrations, surfactant mixtures, pH buffering mixtures, and anti-corrosion additives. The benefits of SRO's formulations are that it can be generated on demand, and pH buffered to prevent corrosion. In September 2012, the Fort Collins CPHST lab collaborated with Colorado State University and ATS microbiology laboratory to conduct a bacterial efficacy study with EB. The ATS lab is a nationally renowned lab that specializes in disinfectant studies.

The objective of the study was to determine the effects of several chlorine dioxide formulations on the efficacy of two bacteria, *Staphylococcus aureus* and *Bacillus subtilis* (Figure 1). This study was conducted to test the majority of

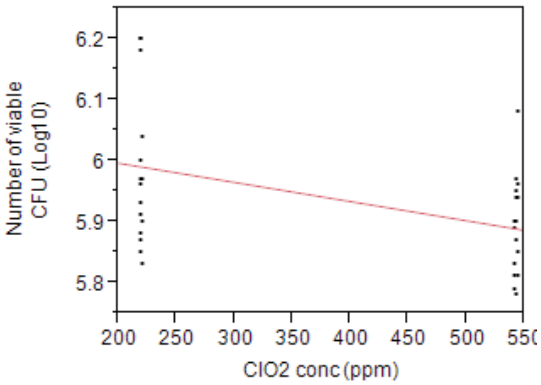


Figure 1. Graph shows viable, spore counts (CFU/carrier) for ElectroBiocide (pH = 6, conc. = 0 and 500 ppm, sarcosinate surfactant) applied to B. subtilis spores with 0% calf serum. The average CFU count was 5.89 and 6.14 log10 CFU/carrier for calf serum at 0 and 5%, respectively. Thus, there was a 4% relative loss (log 10 CFU/carrier basis) or a 77% relative loss (integer CFU/carrier basis) in EB sporicidal efficacy when calf serum level was increased up to 5% (p-value = 0.0069)..

the EB formulations in a factorial study. The formulations were fully crossed and included all combinations of chlorine dioxide applied at 200 or 500 ppm, pH levels of 6 or 7, three surfactants (EA, ME, and sarcosinate), and applied with two organic challenges (0 and 5% calf serum). Calf serum reduces EB activity and mimics spray conditions on slightly dirty surfaces. ElectroBiocide was applied with a hand bottle (4 trigger pulls) on bacteria or spores that were dried on a hard glass slide. The initial *S. aureus* density was 5.07 and 5.87 log10/ml, with and without calf serum, respectively. The initial *B. subtilis* density was 6.58 and 6.28 log10/ml, with and without calf serum, respectively. EB was exposed on the slide for 10 minutes and then was neutralized.

S. aureus is a vegetative bacterium that is relatively easy to kill with disinfectants. *B. subtilis* is a spore forming bacteria that is commonly used in disinfectant tests and is much harder to kill when induced in the spore stage. The results from the second study show that all of the chlorine dioxide treatments had a 99.9 and 99.99% efficacy, when applied to *S. aureus* with and without the 5% organic challenge. In other words, EB at 200 ppm resulted in virtually complete control of *S. aureus* and increasing EB to 500 ppm did not improve the efficacy. Due to the complete control of *S. aureus* across all ClO2 treatments it was not possible to detect any efficacy effects for the four study factors.

Analysis of the *B. subtilis* data reveals three 2-way interactions among the four study factors (EB rates, calf serum rates, pH, and surfactant types). Thus, the results are reported based on these three interaction terms: 1)EB rates x calf serum; 2) EB rates x surfactants; and 3) and EB rates x pH. As the EB concentration increased from 200 to 500 ppm with no calf serum (0%), the Colony Forming Unit (CFU) counts decreased and efficacy improved (p-value = 0.0018) (Figure 2). The addition of calf serum (5%) reduced efficacy by 77% (CFU/carrier). The sarcosinate surfactant also improved efficacy (p-value = 0.0404) (Figure 3). The average viable CFU count was 6.08, 6.06 and 6.01log10 for the EA, ME, and sarcosinate surfactants respectively, across all treatments. Lowering the pH from 6.5 to 5 may increase efficacy by 22%, with a borderline p-value of 0.0569

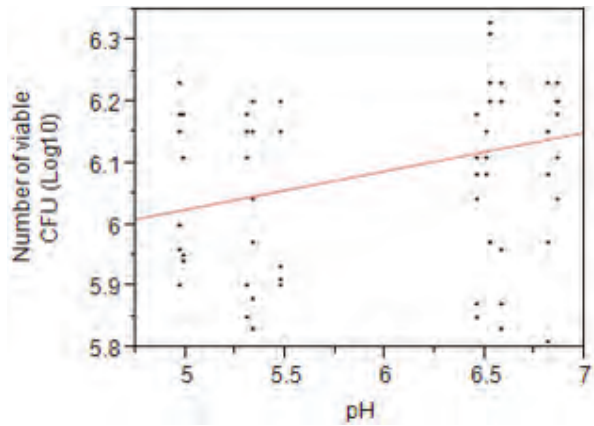


Figure 3. Graph shows the regression analysis between pH and viable CFU counts (p-value = 0.0569) for EB at 200 ppm, calf serum = 0%, and sarcosinate surfactant. For this EB formulation the B. subtilis efficacy was 5.97 and 5.86 log10 CFU/carrier (933,254 and 724,436 CFU/carrier), for pH levels of 6.5 and 5, respectively. This is a 22% increase (integer CFU count basis) for a 1.5 decrease in pH levels.

(Figure 4). The highest EB efficacy was only 67.6% (0.49 log reduction) for ClO2 at 500 ppm, EA surfactant, and pH 5.

This disinfectant study reveals that chlorine dioxide at 200 pm had 100% efficacy for *S. aureus*, independent of pH, surfactants, or organic challenge. The *B. subtilis* spores were more resistant to control with EB than the *S. aureus* vegetative cells. Consequently, with *B. subtilis* we could use statistical analysis to discriminate the effects of the EB concentration, surfactant type, and calf serum organic challenge on the spore efficacy. In general, spore efficacy was enhanced by the sarconsinate surfactant, and the efficacy increased as the EB concentration increased from 200 to 500 ppm. Also, calf serum reduced spore efficacy and pH level had no statistical effect on efficacy, but may increase efficacy with decreasing pH levels.

Acknowledgements: The authors would like to acknowledge Russ Bullock, Mike Hennessey, Charla Hollingsworth, Prakash Hebbar, and Stacy Scott for project support.

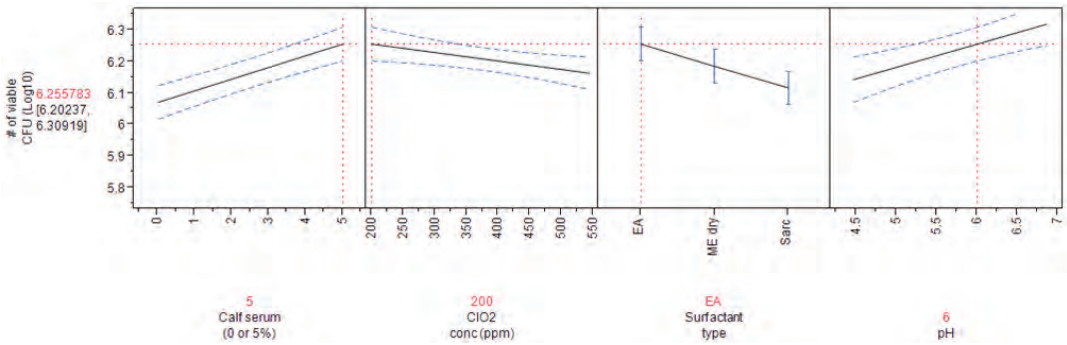


Figure 2. Graph shows viable B. subtilis spore counts (6.12, 6.19, and 6.25 log10 CFU/carrier) for the sarcosinate, ME, and EA surfactants, when EB = 200 ppm, calf serum = 5%, and pH = 6. Thus, the sarcosinate surfactant increased B. subtilis efficacy by 26% (integer CFU/carrier basis) over the EA surfactant. The interaction between ClO2 and surfactant had a p-value =0.0404.

CPHST CAPS Support: Bringing Pests, Surveys, Plants, and Science Together

Melinda Sullivan, Lisa Jackson, Talitha Molet and Christina Southwick

Cooperators: Esther Daniells and Ashanti Robinson

The Cooperative Agricultural Pest Survey (CAPS) is a combined effort by Federal and State agricultural organizations to conduct surveillance, detection, and monitoring of agricultural plant pests and biological control agents. Survey targets include insects and mites, nematodes, weeds, plant pathogens, and mollusks. The goals of the CAPS program include protecting American agriculture and facilitating the export of U.S. agricultural products. To protect American agriculture, one of the primary functions of the CAPS program is to detect exotic pests before they can become well established. The economic costs associated with eradication of a pest that is not well established within a particular area are much less than when the pest is established and reproducing.

The CPHST CAPS Support team provides the scientific foundation for the CAPS program and develops products/tools for the CAPS program at the request of Plant Protection and Quarantine (PPQ) and state cooperators. The CPHST CAPS Support team also provides day-to-day support functions for the CAPS program. Lisa Jackson leads the support effort for arthropods and mollusks. Melinda Sullivan leads the support effort for pathogens, nematodes, and weeds. Talitha Molet, Christina Southwick, Esther Daniells, and Ashanti Robinson provide technical support as needed.

Lymantria mathura

Commodity-Based and Taxon-Based Survey Manuals

In the past, the CAPS surveys have focused on surveying for one to a few organisms at a time. In 2005/2006, the program shifted to a commodity-based or bundled survey approach. The purpose of these commodity-based (pests with the same host plant) and taxon-based (similar pest taxa; e.g., cyst nematodes, bark beetles) surveys was to increase efficiency of these surveys by surveying for a suite of exotic pests at the same time, including those that may only be considered minor pests. By increasing survey efficiency, the odds of detecting a pest before it becomes established will be greatly enhanced.

A series of survey references and survey guidelines have been developed for CAPS cooperators by the CPHST CAPS Support team to assist with commodity-based surveys. The Commodity-Based Survey References are comprised of a series of pest datasheets, which include images of the pest and information gathered from Pest Risk Assessments (PRAs) and scientific literature. Each pest section contains detailed information on the biology, host-range, distribution, survey, and identification of the pest in appropriate detail for CAPS surveyors. The second document, the Commodity-Based Survey Guidelines, provides guidelines for survey and identification for a smaller number of pests, determined by a subcommittee of the National CAPS Committee. The methods are intended to increase homogeneity of the national data set and increase the statistical confidence in negative data (e.g., demonstration of "free from" status). Upon completion, each document goes through a CPHST peer review. This is followed by a one month review that is open to the entire CAPS community. Final edits are made and the document is posted on the CAPS Resource and Collaboration website (http://caps.ceris.purdue.edu) for use by the CAPS community. After this initial review process, documents are open for comment on a yearly basis.

In past years, commodity-based manuals for citrus, corn, grape, oak, pine, small grains, soybean, and stone fruit have been developed; as well as taxon-based survey manuals for exotic wood borers and bark beetles. In 2012, the Cotton Commodity-based Survey Reference and Guideline and the Asian Defoliator Pathway-based National Survey Reference were completed. The Asian defoliator manual is the first manual developed to date that uses a pathway-based approach for survey.

In 2012, the CPHST CAPS Support team also updated and made available a new version of the Exotic Wood Borer/Bark Beetle (EWB/BB) National Survey Guidelines and the Pine Survey Reference and Guidelines. Updates to the EWB/BB manual included: 1) the development of full datasheets for PPQ Program Pests (Asian longhorned beetle, emerald ash borer, and pine shoot beetle); 2) the addition of Biology and Ecology sections to datasheets; 3) the addition of new approved survey methods for *Agrilus auroguttatus*, *Agrilus biguttatus*, and *Trichoferus campestris*; and 4) revisions to the visual survey protocol for *Agrilus biguttatus.* Updates to the Pine Survey Reference and Guidelines included: 1) addition of datasheets for *Dendrolimus punctatus* and *Panolis flammea*; 2) updated NAPPFAST maps; 3) trap and lure information that is synchronized with the IPHIS Ordering Da-

tabase; and 4) the removal of *Dendrolimus superans* and *Diprion pini* as survey targets until attractants are identified.

New Manual Format

How CAPS manuals will be offered in the future is changing dramatically. In 2012, we began to transition away from the large commodity-based survey reference and guideline documents. The manuals were previously delivered as very large PDF files, which were time-consuming to download and cumbersome to navigate. We are moving toward having only one manual per commodity or taxon survey. In this new format, the guideline document information has been shortened and is included as an introduction to the survey manual instead of as a separate manual. The Asian defoliator manual was developed in this new format in 2012. Instead of the manual consisting of one large PDF file, the Introduction and individual pest datasheets were created as separate documents for easier navigation by the end-user and easier updating by the CPHST CAPS Support team. All manuals developed in the future will have this new format and older manuals will gradually be converted into this new format.

Tomato torradovirus symptoms

Pest Datasheet Development

Several pests on the CAPS Prioritized Pest List are not currently included in a commodity-based or taxon-based survey. The CPHST CAPS Support team worked in 2012 to develop standalone datasheets, similar to those provided in the commodity-based reference documents, for these pests. Several new pests were added to the CAPS pest lists during 2012. To support the new pest lists, the CPHST CAPS Support team created datasheets for the new pests and updated several existing datasheets. Each pest datasheet contains detailed information for CAPS surveyors on the biology, host-range, distribution, survey, and identification of the pest. Additionally, pictures and the symptoms and signs of infection/infestation are provided for each pest (when available).

Leek yellow stripe virus

For 2012, new datasheets were prepared for *Acrolepiopsis assectella* (leek moth), *Bursaphelenchus cocophilus* (red ring nematode), *Candidatus* Phytoplasma palmae and related strains (lethal yellowing of palm), Coconut cadang-cadang viroid, *Cylindrocladium pseudonaviculatum* (boxwood blight), *Heterodera cajani* (pigeonpea cyst nematode), *Heterodera ciceri* (chickpea cyst nematode), *Lymantria dispar asiatica* (Asian gypsy moth), *Leek yellow stripe virus*, *Lymantria mathura* (rosy moth), *Meloidogyne fallax* (false Columbia root knot nematode), *Neoleucinodes elegantalis* (tomato fruit borer), *Pseudomonas syringae* pv. *actinidiae* (kiwi canker), *Oryctes rhinoceros* (coconut rhinoceros beetle), *Otiorhynchus dieckmanni* (wingless weevil), *Ralstonia solanacearum* race 3 biovar 2 (bacterial wilt, Southern wilt), *Tecia solanivora* (Guatemalan potato tuber moth), torradoviruses (specifically focusing on *Tomato torrado virus*), *Xanthomonas oryzae* pv. *oryzae* (bacterial blight), *Xanthomonas oryzae* pv. *oryzicola* (bacterial leaf streak), and *Yponomeuta malinellus* (apple ermine moth).

The following datasheets were revised: *Agrilus plannipennis* (emerald ash borer), *Anoplophora glabripennis* (Asian longhorned beetle), *Ceroplastes destructor* (soft wax scale), *Ceroplastes japonicus* (Japanese wax scale), *Dendrolimus punctatus* (masson pine moth), *Lissachatina fulica* (giant African snail), *Tomicus piniperda* (pine shoot beetle), and *Unaspis yanonensis (*arrowhead scale).

This effort will continue into 2013 as updates are made to the CAPS Prioritized Pest List or updates to existing datasheets are needed.

New Manual and Datasheet Delivery Options

Flip PDF:

Flip PDF is a new delivery option that is being used during our transition period until all manuals can be converted into the new manual format discussed previously where the introduction and datasheets are offered as individual files. Flip PDF enables the large PDF manuals that we are currently using to be viewed similar to a book. The content is the same as traditional PDF files, but the files download more quickly and the user does not have to scroll with a mouse. The pages can be manually flipped or can be flipped using arrows. All manuals are also searchable by keyword. In 2012, we offered the Stone Fruit Commodity-based Survey Reference as a Flip PDF side by side with the older PDF version and asked for comment from the CAPS community. Based on positive comments about the Stone Fruit Reference being available as a Flip PDF, we are working on delivering other manuals in this manner. Recently, the 2013 Pine Commodity-based Survey Reference and the 2012 EWB/BB National Guideline were made available as Flip PDFs.

capsID/FSM:

In 2012, the CPHST CAPS Support team and CPHST's Identification Technology Program (ITP) joined forces to access ITP's technology and expertise for 1) increasing efficiency for the production of CAPS manuals and 2) delivering user-friendly, dynamically-generated, web-based manuals to the CAPS community. Two applications to meet the above objectives were designed: Fact Sheet Manager (FSM) and capsID. Application development and extensive testing for these two applications began in the fall. FSM allows the CAPS manual and pest data sheet developers to collectively input datasheet content and images as well as introductory manual content into easy-to-use web templates. FSM automatically feeds all of this information into capsID, a new website for the CAPS community where the end-user has access to manuals and associated web pages are dynamically generated using a pre-designed template.

Symptoms associated with kiwi canker

All updates and changes to a manual in FSM automatically become live and accessible to end-users in capsID. The manuals will be available as an Introduction document and individual pest datasheets. FSM is now being populated with past and current manuals. The beta version of capsID will be released for testing in 2013.

CAPS-Approved Survey and Diagnostic Methods

Standardizing survey methods across all surveys is important in order to homogenize the data collected and to streamline the trap and lure ordering process. To accomplish this goal, the CPHST CAPS Support team developed a suite of tables for the CAPS program that provides the CAPS-approved survey and identification/diagnostic method for each of the CAPS target pests in a commodity–based survey, taxon–based survey, or on the CAPS Prioritized Pest List in 2010. In order to enter survey data, the CAPS community must use the CAPS-approved methods for survey and identification. Since that time, CPHST continues to develop approved methods for new CAPS target pests. In 2012, four new pests were added to the 2013 Approved Methods page based on New Pest Advisory Group recommendations. These pests will be evaluated for the 2014 Prioritized Pest List. The approved methods information is accessible in a web-based format on the CAPS Resource and Collaboration website (http://caps.ceris.purdue.edu/approved_methods).

To develop the approved methods, the CPHST CAPS Support team conducted scientific literature reviews for each target pest. They then communicated with subject matter experts, including scientists in countries where the pests are known to occur. Additional CPHST and PPQ scientists, including domestic and national identifiers, then reviewed the recommendations. The final product is a combination of scientific recommendations, practicality at the field level, identifier needs, and cost. The CPHST CAPS Support team then worked closely with Purdue University to make the approved methods information available in web-based format.

The CPHST CAPS Support team continues to work closely with Purdue University to develop new functionality for the approved methods page. In 2012, the CPHST CAPS Support team and Purdue worked on creating a search function for the change log, which documents changes made to a pest's information page. This will allow end-users to search by pest name or date to find out what changes have been made and when.

The CAPS community continues to be very collaborative in the approved methods process and contacts the CPHST CAPS Support team directly with feedback and concerns. They have suggested additional traps and lures for evaluation, informed CPHST of novel approaches to survey, offered valuable critiques when methods were not practical or affordable, and alerted the CPHST CAPS Support team to any problems that occur in the field.

This website also provides additional resources for each pest in a pest information sheet. Each sheet provided for the CAPS community includes: symptoms, signs, vector relationships, trap and lure recommendations, links to identification aids and references, links to the pest datasheets, and other available relevant information. This information is being used by the CAPS National Survey Coordinator and Program Managers to identify gaps in survey and diagnostic tools. This effort will continue into 2013 as updates are made to the commodity-based surveys, taxon-based surveys, and/or to the CAPS Prioritized Pest List.

***Tuta absoluta* Trap Efficacy Project**

During 2011-2012 the CPHST CAPS Support team co-led a project with scientists in the CPHST Miami Lab and PPQ Florida to evaluate traps for *Tuta absoluta*, the tomato leaf miner. The previous *Tuta absoluta* trap and lure combination captured many non-target moths, which increased the difficulty and amount of time needed to identify the specimens. The goals of the project were 1) to determine if trapping

of non-target species could be reduced by using different types of lures, and 2) if specimens would be easier to identify using other trap types or trap inserts.

The scientists conducted trap and lure trials in Panama and Florida to determine if the pheromone type, trap type, or trap insert type could improve the identification process. The scientists also worked with a PPQ Domestic Identifier to assess whether the new traps or insert types improved the identification process.

In 2012, the CPHST scientists prepared the final summary of the field and lab trials. As a result of these studies, the CAPS program will now recommend using the new trap inserts in its *Tuta absoluta* surveys. This was a great success story of how the CPHST CAPS Support team identified a need, quickly conducted appropriate studies, and supplied a practical recommendation to the CAPS leadership.

Survey Protocols

***Cerceris* Wasp Survey Protocol:** In 2012, the CPHST CAPS Support team co-wrote the *Cerceris* Wasp Survey Protocol, which was delivered to the CAPS community via the CAPS website. This protocol was a collaborative effort by the CPHST CAPS Support team and a PPQ Pest Survey Specialist. *Cerceris* wasps are ground-nesting wasps that capture beetles in the family Buprestidae. This family of insects includes several species known to cause severe damage and mortality to trees, including the emerald ash borer. *Cerceris* wasps are an effective survey tool that can be used to detect new populations of exotic buprestids, including several CAPS targets. The protocol describes how to plan a survey, locate potential *Cerceris* nesting sites, record data, and process specimens. This project was a good example of a CAPS community member recommending a novel survey method to CPHST, and the CPHST CAPS Support team following through to make the method available to the entire CAPS community.

Cerceris wasp with Buprestid beetle.

Kiwi Canker Visual Survey Protocol:

In the United States, approximately 99% of kiwifruit is grown in California. At the recommendation of the New Pest Advisory Group (NPAG), California is planning an early detection survey for kiwi canker, caused by the bacterial plant pathogen *Pseudomonas syringae* pv. *actinidiae*. The CPHST CAPS Support team worked with California (APHIS and CDFA) to develop a visual survey protocol for kiwi canker. In 2012, the CPHST CAPS Support team provided a draft of this document to California for comment and for use in its planned early detection surveys.

Pest List Review

The Pest List Review Group was initiated in June 2011. The purpose of the group is to review the process behind the Analytical Hierarchy Process (AHP), which is used to develop the Prioritized Pest List for the CAPS program. The group is made up of the CPHST CAPS Support team and national CAPS leadership representatives.

The CPHST CAPS Support team developed two new assessments to review the AHP process. One is a Pre-assessment questionnaire that is used to assess new CAPS pest suggestions before the pests are run through the AHP model. The second is a Post-assessment questionnaire that will evaluate the survey and identification/diagnostics methods of pests that make it through the Pre-assessment and the AHP model. The group has also developed a two-year timeline that would incorporate these two new steps into the larger AHP Process.

In 2012, the Pest List Review Group began to implement this new process. The group received 29 new pest suggestions from the CAPS community, and these pests were run through the Pre-assessment questionnaire. Nineteen of the pests passed the Pre-assessment process and were run through the model. In the coming weeks, the group will run any new pests through the Post-assessment questionnaire to determine if there are sufficient survey methods, identification/diagnostic methods, and identification capacity to offer these new pests as survey targets for the 2014 survey season. Pests that do not pass the Post-assessment will be placed on a research list for method development after consideration of the resources required for new method development and likelihood of success by the CAPS leadership team. In April 2013, the 2014 AHP list will be included in the 2014 CAPS Survey Guidelines for use in the 2014 survey season.

In June 2012, the group learned that the CAPS leadership would also like to modify the AHP model. PERAL economists, Alison Neeley and Trang Vo, are leading the AHP Criteria Revision Project, and the CPHST CAPS Support team is assisting in the effort. To date, the group has developed a set of draft questions. Forty trial pests that are established in the United States will be evaluated using these questions and on their economic impacts. These trial pest evaluations will help the team develop new questions for the model, by

comparing the statistical importance of the questions based on the known economic outcome of these established pests. These questions will better predict the impact of new exotic pests based on the pest's biology, pest status in its current distribution, host presence, and the climatic suitability of the United States for the pest. These new questions will be used to develop the 2016 Prioritized Pest List.

Outreach

Pest Profiles:

In 2012, PPQ Emergency and Domestic Programs (EDP) requested that the CPHST CAPS Support team develop an outreach document for *Tuta absoluta*, since the CPHST CAPS Support team had developed the datasheet for CAPS. The CPHST CAPS Support team worked with PPQ Legislative and Public Affairs (LPA) and EDP to develop a new outreach product for imminent pest threats, called the Pest Profile. Pest Profiles, unlike most CAPS products, are targeted for public and private industry; therefore, the survey methods had to be altered. Processing insect specimens from the Tuta trap and lure requires a Lepidoptera expert; therefore, trapping was not an appropriate survey method for industry. The CPHST CAPS Support team contacted several tomato pest experts to determine what visual signs could signal a Tuta outbreak and how those would differ from damage caused by native pests. This work resulted in an outreach product that is appropriate for a new audience. The new Tuta Pest Profile can be found online at http://caps.ceris.purdue.edu/webfm_send/1563.

In addition, the CPHST CAPS Support team was approached to write a Pest Profile for another high impact pest, *Neoluecinodes elegantalis*, the tomato fruit borer. The scientist delivered the draft of the Pest Profile to EDP and is awaiting final publication of the product.

Pest Cards:

In 2012, PPQ Legislative and Public Affairs (LPA) and PPQ Emergency and Domestic Programs (EDP) requested that the CPHST CAPS Support team develop Pest Cards for the 2013 Southeast Regional Fruit and Vegetable Conference. The Pest Cards are outreach tools intended for the general public and the agriculture industry. For the conference, the CPHST CAPS Support team identified CAPS pests that could become pests on the fruit and vegetable crops of interest and that also could establish in the Southeast. The CPHST CAPS Support team on the project then worked within a tight timeframe to develop the content of the pest cards (pest biology, signs of damage, and pathways) and locate high resolution images. The content and images for three pest cards (*Adoxophyes orana* (summer fruit tortrix), *Eupoecilia ambiguella* (European grape berry moth), and *Spodoptera littoralis* (Egyptian cottonworm)) were delivered within a two-week timeframe. In addition, the CPHST CAPS Support team reviewed the content of the *Tuta absoluta* (tomato leafminer) and *Duponchelia fovealis* (European pepper moth) pest cards. All of the Pest Cards are or will be

Pest Profile

Cooperative Agricultural Pest Survey
January 2012

Tomato Leafminer *(Tuta absoluta)*

The tomato leafminer moth is a pest of tomatoes and other vegetable crops, including eggplants, peppers, and potatoes. It is considered an economically damaging pest in many areas where it is present, including South America.

Tomato leafminer larvae can feed and develop on tomato fruit, leaves, or stems. As the larvae feed, they create tunnels—called "mines"—under the plant tissue surface (see fig. 2). This feeding injures the affected tissue and weakens the plant's ability to sustain itself through photosynthesis. The larvae reduce crop yield and fruit quality in two ways: directly, through their feeding; and indirectly, by providing a pathway for disease-causing micro-organisms to enter the plant. Even a small amount of damage can make fruit unmarketable as fresh produce.

This pest is distributed throughout Europe, the Middle East, and South America. In Spain, adult moths have been found more than 6 miles (10 kilometers) from tomato fields, suggesting they can move long distances by flying or drifting with the wind. Because the larvae develop within the plant, this species is difficult to control with pesticides. The U.S. Department of Agriculture (USDA) is working closely with stakeholders to survey for the tomato leafminer as part of an early detection survey program.

Description

The tomato leafminer has four life stages: egg, larva, pupa, and adult. Eggs are cylindrical and creamy white to yellow in color. Their length, 0.015 inches (0.36 millimeters [mm]), is about one-third of a dime's thickness, and their width, 0.009 inches (0.22 mm), is about one-fifth of a dime's thickness. Initially, larvae are cream in color with a dark head. As they grow, they become greenish to light pink, and in their last growth stage, they have a black line behind the head. Adult tomato leafminers are small, brown or silver moths with black spots on their wings (see fig. 1). The adult's body length, from the head to the tip of its folded wings, is about one-fourth of an inch (7 mm).

Life Cycle

The adult female tomato leafminer can lay eggs on any host-plant part that is above

Pest profile example

available to download and print for outreach events online at http://www.hungrypests.com/partner-tools/ listed by pest name under the heading "Pest Cards."

Stronger Relationship with the New Pest Advisory Group (NPAG)

In 2012, the CPHST CAPS Support team developed a stronger working relationship with the New Pest Advisory Group (NPAG). NPAG evaluates new pests that have been detected in the United States or pests of imminent concern and provides recommendations. NPAG asked for assistance from the CPHST CAPS Support team several times over the course of the year. These requests included inquiries into the CAPS status of pests, review of NPAG draft reports, participation in NPAG meetings, and assistance with the development of NPAG recommendations and presentations to the PPQ Leadership Team. For the pest *Neoleucinodes elegantalis*, the CPHST CAPS Support team participated in a video teleconference with the PPQ Leadership Team and was able to answer questions about the trap, lure, and survey of this pest. It was decided that *Neoleucinodes elegantalis* should be offered as a CAPS survey target. The CPHST CAPS Support team also suggested a revision to a frequently used NPAG recommendation "to add the pest to the CAPS prioritization list" to more generic language that allows the pest to be evaluated according to CAPS procedures for evaluating new pests. In the future, NPAG will likely continue to interact with the CPHST CAPS Support team, and the CPHST CAPS Support team will act as informal liaisons between NPAG and CAPS.

New Pathogen Diagnostic Methodologies for Phytoplasmas and Viroids

Two farm-bill funded projects were initiated to support CAPS sample pathogen sample processing and new methods development/validation. The first project deals with diagnostics of plant pathogenic phytoplasmas and is being completed by the CPHST CAPS Support team in conjunction with the University of Florida (Nigel Harrison) and USDA Agricultural Research Service (ARS) (Robert Davis). This project will analyze phytoplasma positive samples and characterize the organisms to the strain/species level to distinguish exotic phytoplasmas from those that are known to occur in the United States. This project will also develop and improve phytoplasma diagnostic methods. The second project deals with the diagnostics of palm viroids and is being completed by the CPHST CAPS Support team and the University of Florida (Nigel Harrison) and USDA ARS (Rosemarie Hammond). The palm viroid project will provide sample processing for suspect Coconut cadang-cadang (CCCVd) and Coconut tinangaja viroid samples, examine the specificity of primers, and provide validation for a commercially available RT-PCR kit for CCCVd to assess its value for CAPS.

Support for CAPS Field Staff

The CPHST CAPS Support team serves as an informal "help desk" for the CAPS field staff. In 2012, CPHST FCL scientists received 47 insect information requests from CAPS Pest Survey Specialists and State Survey Coordinators. This was an increase from 28 requests in 2011. The requests took on average 1 to 3 hours to address. Eight requests took between 3 to 12 hours to address. Requests included: 1) clarification on which traps and lures to use for which targets; 2) inquiries into new approved methods; 3) survey methods for pests of state concern; and 4) guidance on how to use specific traps and lures.

The CPHST CAPS Support team also provided support to CAPS field staff on a range of pathogen issues including clarifying survey and diagnostic methods for phytoplasmas, late wilt of corn, Phytophthora species, Coconut cadang-cadang viroid, and kiwi canker.

Palm lethal yellowing phytoplasma symptoms. Photo courtesy of Nigel Harrison, University of Florida.

Apple ermine moth. Photo courtesy of Eric LaGasa

Geospatial Technology & Predictive Modeling: 2012 year in review

Tom Kalaris, Lisa Kennaway and Gericke Cook

Tom Kalaris
Modeling Coordinator

Lisa Kennaway
Geographer

Gericke Cook
Geographer

The CPHST Fort Collins Lab provides leadership in geospatial technology and predictive modeling to the greater CPHST community, as well as participates with other APHIS and USDA groups in supporting the use of geospatial data to inform management decisions. This team supports domestic, international, and emergency programs within APHIS's Plant Protection and Quarantine (PPQ) division and strives to provide new and innovative ways to analyze data, identify patterns of pest behavior, and inform risk. For the year 2012, the team has made the following accomplishments.

Project Specific

Asian Gypsy Moth (AGM) Trapping Model for Texas

(Lisa Kennaway)

Asian gypsy moth (*Lymantria dispar* ssp., AGM) is an exotic pest that has been detected, but not established in the United States. The threat to American agriculture is significant due to AGMs broad range of host plants, including 500 species of trees and shrubs (APHIS, 2003). The AGM is similar to the European gypsy moth. However, AGM has a much broader host range and the females are capable of flying up to 25 miles, unlike the flightless female European gypsy moth (USDA, 2006). This makes early identification of new AGM introductions very important.

AGM is native to Asia. A primary pathway of introduction into America is via ship and cargo traffic from the Far East. These trade patterns place the coastal United States in high risk of AGM introduction. Coastal states have comprehensive surveillance systems in place to identify (and eradicate if necessary) any moths prior to establishment. The trapping system is organized and managed with expert local knowledge and places higher trap densities in and near major shipping ports and population areas. To enhance the placement of traps in these areas, a geospatial model has been developed to predict areas with highest AGM introduction risk based on a variety of variables. In 2012, a model was developed for the state of Texas, which builds upon earlier models created for Washington, Oregon, and California. The goal of the model is to improve and/or validate existing trapping locations.

The analysis method uses an additive, raster based model, where data layers are normalized, weighted, and then combined to identify areas of highest AGM introduction risk. Stakeholders representing USDA APHIS PPQ, Texas Department of Agriculture, and US Department of Homeland Security participated in this process. Final model inputs included: 1) shipping ports; 2) Mexico border crossings; 3) major highways; 4) railways; 5) census population; 6) military household shipments from Japan and Korea; 7) firework distributor locations (tied to 2006 AGM interception); and 8) vegetation. Final results of the model are shown in Figure 1. A comparison of AGM risk to existing trap placement is shown in Table 1.

This effort assists 2012 gypsy moth trap allocation for the state of Texas and provides new information of where AGM could be introduced and may establish. AGM is most likely introduced through human mediated pathways that involve cargo and commodity flow. The model is based on a combination of these pathways and includes a detailed delineation of vegetation communities within the state. The model results are further enhanced by a mask of generalized GM moth establishment probability that is based on climatic parameters and was developed within the USDA APHIS PPQ Western Region.

A full understanding of how climatic constraints differ between European and Asian gypsy moth, as well as within the Asian species group itself, is not known. For this reason, the climatic portion is included as a separate mask and can be used to guide trap allocation, but should not be used as an absolute determination of where AGM can establish. Work continues on defining the climatic constraints of AGM, as well as what factors are most important in determining its introduction. This model is an interactive process and will be updated once further recommendations are made on factors that influence its introduction and establishment.

In 2013, an effort to model AGM risk at a national level will begin. The information provided by this model will have two primary goals: 1) to assist program funding allocations across the country; and 2) to support state level surveillance activities. The model will be divided into two parts: introduc-

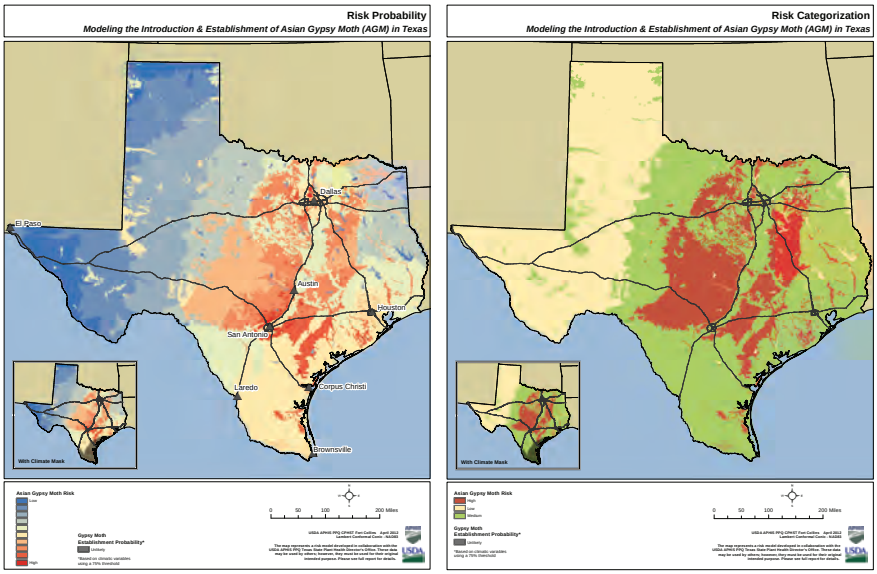


Figure 1. Final Asian gypsy moth introduction and establishment risk model, and high, medium, and low risk categorization.

tion and establishment. Introduction will focus on pathways that may bring AGM into the country such as transportation corridors, population centers, and shipping ports. Establishment will include host data and climatic parameters. As mentioned previously, it is not clear as to how certain climatic parameters influence this Asian species group. As with previous AGM efforts, the model is an iterative process and will be updated as new knowledge is available.

Also, the nationwide risk model may be further customized for six states (WA, OR, CA, MN, UT, AK) to support the Asian defoliator survey. The survey, led by the CAPS (Cooperative Agricultural Pest Survey) program, seeks to detect new infestations of a target species (i.e. AGM and related subspecies) while they are still at low population levels. The state level models will allow for customization and can add risk pathway variables that are unique for that state.

Table 1. Comparison of AGM risk to existing trap placement.

State	Risk Code	Risk Text	No. of Traps	% of Total Traps
TX	1	Low	0	0%
TX	2	Low	17	0%
TX	3	Low	5	0%
TX	4	Low	22	1%
TX	5	Medium	8	0%
TX	6	Medium	462	13%
TX	7	Medium	1570	44%
TX	8	Medium	617	17%
TX	9	High	171	5%
TX	10	High	490	14%
TX	11	High	180	5%
TX	12	High	52	1%

References

United States. Department of Agriculture. Animal and Plant Health Inspection Service. (2003). Asian Gypsy Moth (APHIS Factsheet). Retrieved January 16, 2009, from the U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) web site: http://www.aphis.usda.gov/publications/plant_health/content/printable_version/fs_phasiangm.pdf

United States. Department of Agriculture. Animal and Plant Health Inspection Service. (2006). Asian Gypsy Moth Cooperative Eradication Program, Orange County, CA (Environmental Assessment).

Nationwide Risk Model for European Gypsy Moth (EGM)

(Gericke Cook)

Since its introduction in Medford, MA in 1869, European Gypsy Moth (*Lymantria dispar dispar*) has expanded its range as an established forest pest throughout the northeastern United States and continues to spread by natural dispersion and anthropogenic introduction. This forest pest can feed on more than 300 woody species, and has the greatest impact on oaks and other hardwood species. Millions of acres of hardwood forest are at risk from EGM each year. Historic rates of natural spread were estimated to be 20 km/year before implementation of the Slow The Spread program, whose goal is to reduce the spread rate to a target that is 60% or less than the historic average (STS 2011). However, isolated populations of EGM may be artificially introduced outside the leading edge of the spread zone by the movement of outdoor household articles (APHIS 2010). The goal of APHIS-PPQ is to define the extent of the gypsy moth infestation and limit its artificial spread beyond the infested area through the use of an active trapping and monitoring program, quarantine implementation, and regulatory programs that limit anthropogenically-driven dispersal mechanisms.

The National EGM risk model is a combination of a scientific life-stage phenology model with GIS risk overlays to define and quantify known risk factors for EGM introduction and establishment on a national scale. It began as a pilot in early 2012 in the Western Region, and focused on the probability of gypsy moth establishment and climatic suitability using the BioSim software developed by Natural Resources Canada (http://cfs.nrcan.gc.ca/projects/133). The pilot testing of this software included sensitivity analysis of a model assumption (initial oviposition), stochastic weather simula-

tion trials, field data validation, and model comparison from state to national levels. Model testing during the 2012 pilot program also revealed that there is an elevation threshold to gypsy moth establishment (2500 meters), and that the program could realize cost savings by limiting trap deployment to altitudes under the 2500 meter threshold.

At the end of the 2012 gypsy moth survey season, program management requested that the BioSim modeling be expanded to a full national risk model and incorporate other factors to help target EGM risk more effectively. The risk model was partitioned into two major risk factors: introduction and establishment. Introduction risk was specifically defined as either: 1) movement from the quarantine area to campgrounds via firewood transport; or 2) the movement of at-risk outdoor household items (OHAs) from quarantine areas by using USPS address forwarding data (commonly known as "move-in" data). Establishment risk was defined as 1) climatic suitability from the BioSim model and 2) suitable host defined by Andrew Liebhold (1995) and biomass data from the Woods Hole Research Center. Data sources (Table 2) were formatted and normalized into separate GIS layers (Figure 2), and combined in an additive weighted risk model to estimate overall EGM risk (Figure 3).

The result of the weighted, additive risk model is a continuous probability risk map of European gypsy moth potential introduction and establishment. The final risk map was validated against independent field data including Forest Service defoliation areas, PPQ historical treatment and eradication zones, and PPQ survey densities.

Following model validation, the national risk map was reclassified into 10% probability classes, and area summaries for each risk class were computed for each state (minus the quarantine and Slow the Spread areas). This summary report was provided at the end of 2012 to give policy and program managers an overview of estimated risk area for each state. Ongoing modeling work in cooperation with USFS will improve on this draft product.

Following completion of the National European Gypsy Moth (EGM) risk model, state PPQ staff and cooperators will be provided GIS data layers of the individual and total risk products from the national model to use at their discretion for the planning and deployment of EGM surveys for their state. Supplemental GIS layers such as population, affluence, and special sites will also be provided to help stakeholders categorize risk areas according to the EGM program manual. National webinars will be held with state cooperators to present and discuss the risk model in an open forum. Feedback from these forums will provide opportunity to improve on model assumptions and data inputs to enhance risk modeling at the state level.

Table 1. Data sources and implementation as risk components into the national EGM risk model.

Data Source	Description	Risk Element	Map Figure
Natural Resource Canada	BioSim	Establishment- climate	2.a
NatureServe	Ecological Systems of the U.S.	Establishment - host	2.b
Kellndorder et al 2011	National Biomass and Carbon Dataset	Establishment - host	2.b
Liebhold, et al 1995	Tree species susceptibility rankings	Establishment - host	2.b
U.S. Postal Service	Cumulative address forwards from quarantine area	Introduction - OHAs	2.c
ReserveAmerica	Cumulative reservation data, visitors originating from quarantine areas	Introduction - firewood	2.d
USCampgrounds.info	Campground site info (site capacity)	Introduction - firewood	2.d

Overall Risk Model = **Establishment** (35% climate risk + 35% host biomass risk) **+**
Introduction (25% move-in risk + 5% campground risk)

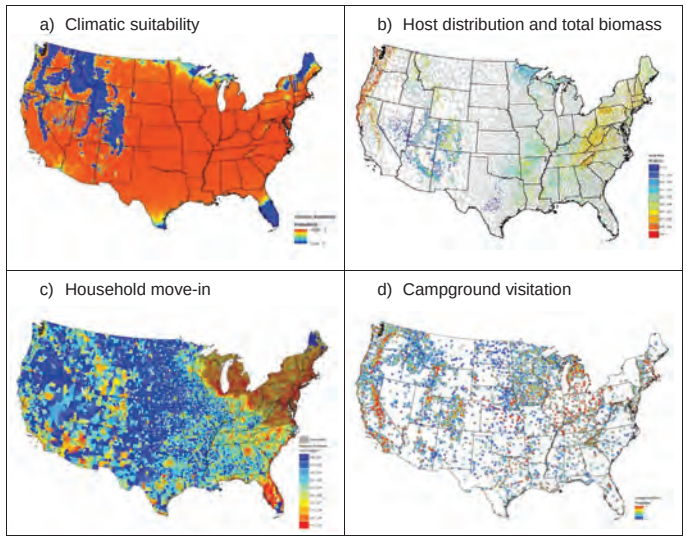


Figure 2. Mapped risk components of the national European Gypsy Moth risk model, each of which is represented by a 0-1 probability scale: a) climatic suitability, b) total woody biomass where host is present, c) risk of introduction by movement of households originating from the shaded quarantine area, and d) risk of introduction to campgrounds by visitors (assuming they bring firewood) from the quarantine area.

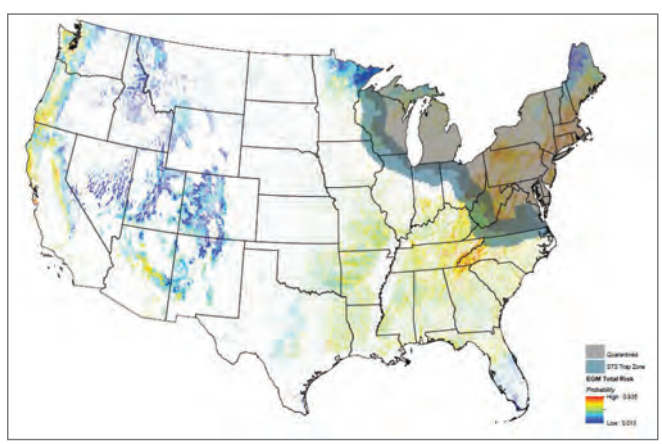


Figure 3. A draft of the European Gypsy Moth national risk model, also showing the quarantine and Slow the Spread exclusion zones.

Developing and Serving a Database of Historical Barberry Survey Data in the Pacific Northwest

(Lisa Kennaway, in cooperation with Washington State University, Dr. Tim Murray)

Black stem rust (BSR) caused by *Puccinia graminis* is a highly destructive fungal pathogen of several cereal crops such as wheat, oat, barley and rye. The fungus is able to survive over winter months and can also thrive and complete its life cycle on alternate hosts such as the common barberry (*Berberis vulgaris*), and possibly on other susceptible barberry (*Berberis*, *Mahonia* or *Mahoberberis*) species. The presence of alternate hosts near wheat fields has led to several epidemics around the world including North America over the past several centuries. In the past, eradication of the common barberry species has played an important role in reducing the impact of the pathogen, especially in the last century. Scientific research has confirmed that the benefits of the eradication program in the temperate regions of the world is directly related to, a) breaking the life cycle of the fungus on barberry species, which reduces genetic recombination and prevents the development of more virulent strains of the pathogen, and b) delaying the onset of stem rust epidemics from barberries located near wheat and barley fields. The BSR program fits well with the mission area work related to improvement and safeguarding wheat production in the Pacific Northwest (PNW). The program allows Washington State University to organize a regional (ID, MT, WA, and OR) workshop and develop a survey manual to identify common barberry that represent a threat to production of wheat, barley, and oats in the United States of America (USA), specifically in the Pacific Northwest.

Common barberry was recently found to be the alternate host for yellow rust (YR) caused by *Puccinia striiformis*, which is another destructive disease of wheat and barley especially in areas with cool climates like the USA Pacific Northwest. Little is known about the importance of barberry in the life cycle of this stripe rust pathogen and surveys are needed to answer this question.

Not all barberry species are susceptible to the stem and stripe rust pathogens and there are several thriving nursery and landscaping businesses in the USA involved in propagation, commercial planting and export of ornamental barberry (e.g. *Berberis thunbergii*) varieties. In view of the role alternate hosts play in disease epidemics, USDA-APHIS regulates the nursery industry to prevent the multiplication, commercialization and movement of susceptible barberry species, especially to the large wheat producing states (Code of Federal Regulations, Sub part: Black Stem Rust 301-38.8).

Recently, new races of black stem rust (BSR) pathogen have been discovered in Washington State and Idaho and this has been directly linked to the re-emergence of common barberry in these states.

To support the BSR program, a geospatial database of Barberry Eradication Program records (from ID, MT, WA, and OR) is being developed with three primary goals: 1) to create a permanent electronic archive of historical survey data for visualization and query; 2) to use the archive to develop tools for early warning of any new races of the rust pathogen appearing in wheat growing regions; and 3) to assist in current barberry survey efforts by directing staff to where the plant existed previously.

In 2012, a database was developed of approximately 7000 records from the Barberry Eradication Program. In 2013, database development continues and is being expanded to other states as eradication forms are made available.

Observation Sampling Analysis to Support an Area-Wide Monitoring Study for Asian Citrus Psyllid in the Lower Rio Grande Valley, Texas

(Lisa Kennaway, in cooperation with David Bartels, PPQ CPHST Mission Lab)

*This report updates the on-going geospatial contribution to the CPHST Mission Lab's Area Wide Monitoring Study. Collaborative work began in 2009.

The Asian citrus psyllid (*Diaphorina citri Kuwayama*, ACP) is a pest that acts as a vector of *Candidatus Liberibacter asiaticus*, one of the causal agents of citrus greening disease or Huanglongbing (HLB). It is an increasingly important pest for USDA APHIS as citrus greening is the most devastating disease of citrus in the world (CDFA 2009).

Within the Lower Rio Grande Valley (LRGV) of southern Texas, there is approximately 28,000 acres of citrus spanning three counties, with an industry that supports ~$160 million in economic activity for the region. ACP was first detected in Texas in 2001, and has spread to more than fifty counties throughout the LRGV. This led the CPHST Mission Laboratory to develop an Area-Wide Monitoring Study focusing on methods development to determine best control practices for ACP. The study is a joint effort between several groups including APHIS PPQ CPHST, Texas A&M University, Texas Citrus Mutual (TCM), AgriLife Cooperative Extension (ACE) and Texas Department of Agriculture (TDA).

The Area-Wide Monitoring Study incorporates a comprehensive sticky trap grid and direct sampling observations. Together, the CPHST Mission and Fort Collins Labs are using geospatial technology to manage and analyze the data, with a primary goal being to identify a trapping grid that provides an accurate view of ACP population, while also using fiscal resources wisely.

In 2009, the first phase of the geospatial effort was completed. This involved the creation of a database that houses over 9,000 ACP observation records spanning over one year. Several ACP attributes were captured in this data including number of adults, eggs, and nymphs, as well as overall percent infested observed at the trap location.

During 2010 and 2011, the second phase of the effort was completed. This involved the evaluation of trap grid spacing in an effort to understand how that spacing may impact ACP population observation. Three grid subsets were identified, including the original trapping grid, a half mile grid, and a trap tree only grid. The complete evaluation included geospatial interpolation , zonal statistical analysis, and time series data visualization.

In 2012, continued validation and review took place. Additional testing of interpolation methodology occurred, and small changes were made. The goal of determining the best spacing for ACP trapping observations will be extremely useful for achieving optimal balance between available fiscal resources and appropriate information required to protect orchards from ACP infestation and HLB disease. In addition, the overall interpolation analysis and zonal review will be expanded to on-going observation data. The CPHST Fort Collins Lab is excited about this continuing effort and is committed to assisting the Mission Lab with the geospatial component of their study.

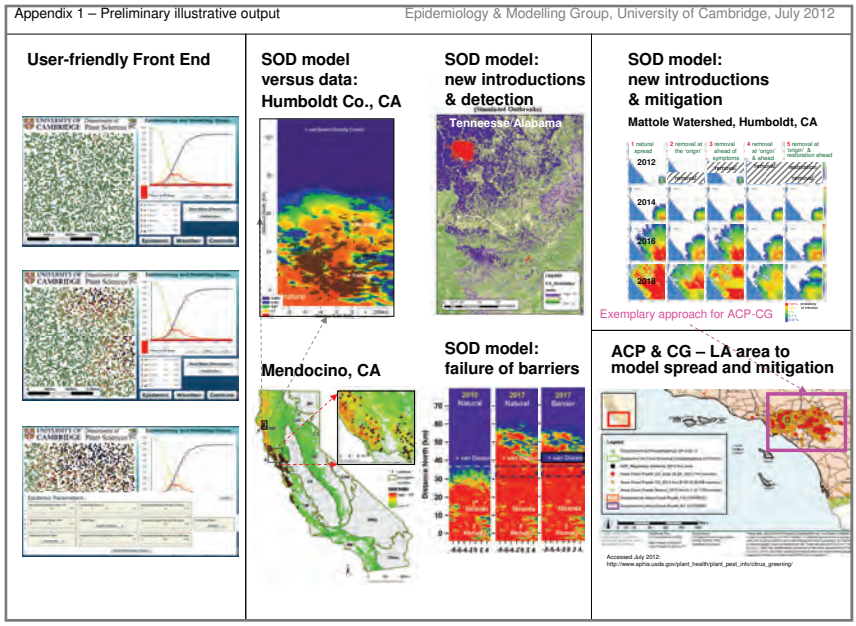


Figure 4. Cambridge spread model

References

California Department of Food & Agriculture. (2009). Asian Citrus Psyllid (ACP) Fact Sheet. Retrieved January 15, 2010, from the California Department of Food & Agriculture website: http://www.cdfa.org.

Rosson, P. 2007. Economic Impacts of Greening on the Texas Citrus Industry. Center for North American Studies, CNAS Issue Brief 2007-01 February 12, 2007.

Cooperative Work with Department of Plant Sciences, University of Cambridge, England

(Tom Kalaris)

For several years, CPHST has been working with Dr. Chris Gilligan and his staff to develop models to predict the spread of a pathogen across a landscape. Dr. Gilligan's models differ from many that CPHST uses in that they are more intricately involved with the pest biology and attempt to predict pest population dynamics and pest spread. During the past year, Dr. Gilligan has developed models that predict the spread of Sudden Oak Death (SOD) in California, Oregon, and the East Coast (Figure 4). He has also begun working on the spread of Asian citrus psyllid (ACP), a vector for Huanglongbing (HLB), in southern California and the Central Valley.

One significant contribution of the Cambridge SOD modeling came when the SOD program evaluated options for containing of the disease in California. The program managers debated whether or not establishing a barrier north of the current SOD location could effectively stop the spread of the disease to the north. The Cambridge models predicted such an effort would fail and pointed out why, hence the program's plan of a barrier to contain the disease was discarded.

Assessment, Review, and Collaboration

In addition to project specific work, the CPHST Fort Collins team contributes to the geospatial community by identifying and evaluating new tools and methods for analysis. This work involves collaboration among CPHST staff, as well as other groups including: the USDA APHIS Plant Protection and Quarantine (PPQ) Field Operations and Policy Management offices; USDA APHIS Veterinary Services; USDA Forest Service (FS); and the United States Geological Survey (USGS). Some examples of this work are detailed below.

Weather Models Comparison Analysis

(Gericke Cook)

From early to mid-2012, a CPHST review panel was formed by request of Dr. Phil Berger to identify and evaluate other providers and resources for weather data and degree day (DD) phenology models. A two-part review was conducted by this interdisciplinary panel (including representatives from Field Ops GIS, CPHST, and Eastern Hub Field Ops Program Management) to statistically validate the accuracy of these weather models and to compare the features, cost, reliability, and user friendliness of the different platforms. The platforms being evaluated were NAPPFAST, USPest.org, and BioSim. All platforms performed similarly in cal-

culating accumulated degree days against an independent weather station dataset. The results indicate a robust selection of alternative platforms for weather modeling that can be employed with reasonable user confidence in accuracy, as well as the potential for finding model agreement against other software platforms with different methods and data sources. A full analytical report detailing the statistics and feature comparisons was submitted to CPHST management.

Traffic Count Data Analysis in Partnership with USFS and Natural Resources Canada

(Gericke Cook)

The human-assisted movement of invasive species has widely been recognized as a key contributor in the spread and expansion of invasive pest populations (e.g. Emerald Ash Borer, Gypsy Moth, and Asian Longhorned Beetle). In North America, vehicle travel has been recognized as a key vector contributing to the long-distance spread of invasive forest pests beyond the organism's native biological range.

In the summer of 2012, a PPQ/CPHST geographer completed a 60-day detail with the U.S. Forest Service to develop programming that would interpolate traffic point data (Figure 5) to a road network dataset that could be used to derive risk products for different applications of invasive species spread. In cooperation with the U.S. Forest Service, USDA APHIS PPQ/CPHST, and Natural Resources Canada, they strategized a solution to develop an interpolation methodology for producing a high resolution, spatial continuous, cross-border dataset that depicts the potential spread of invasive species with the movement of commercial and passenger traffic. Once the outline of the methodology had been determined, Python code was developed for processing and analyzing traffic count data in a GIS environment, so that the traffic volume information could be interpolated continuously, and with proper directionality, onto a road network database. The ESRI Streetmap dataset (ArcGIS version 10) was used as the base street data, and the Python code determined traffic volume for both directions of each road segment found in the ESRI streets database. Part of the code development included methodologies for optimizing code efficiency and maximizing (but not overloading) available computer resources. Code optimization is still ongoing, but once the North American traffic volume data has been interpolated, an output table of traffic volume by direction for each ESRI road segment will be available in 2013. This table may be joined to the ESRI streets database by a unique identifier, allowing easy distribution to collaborators and partner agencies who have access to the same ESRI datasets.

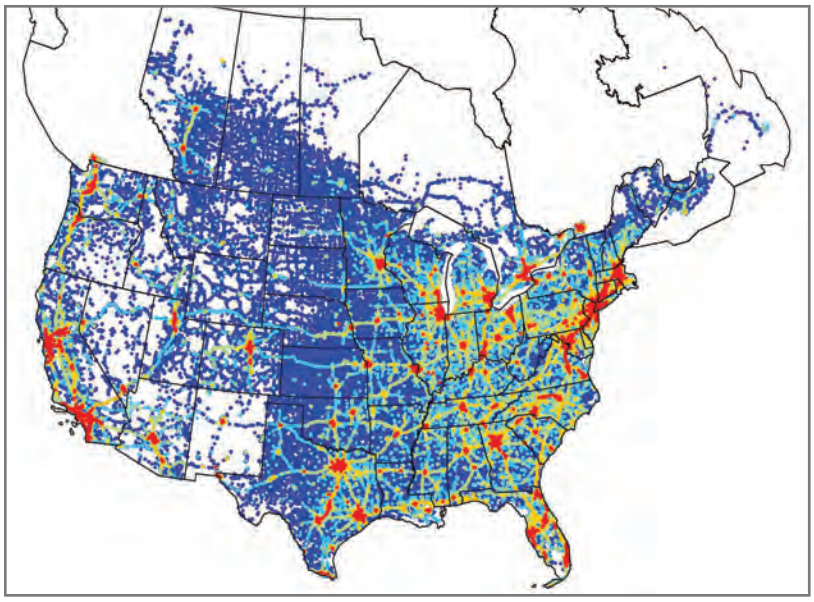


Figure 5. Traffic point locations for the United States and Canada, symbolized by traffic volume, before interpolation to a road network.

Invasive Weed Distribution Risk Model in Cooperation with CPHST PERAL Lab

(Gericke Cook)

In 2012, CPHST Fort Collins and PERAL scientists collaborated in a model comparison and evaluation project of bioclimatic and species distribution models, using two major and two minor well-known, invasive weed species as the focal subjects. The purpose of the project was to develop standards for the use of four bioclimatic modeling tools: NAPPFAST (BAMM), CLIMEX, Proto3, and MaxEnt. In early December, MaxEnt modeling software testing began in the Fort Collins lab. During the project, a cooperative relationship was formed with the USGS Fort Collins Science Center to employ a customized version of VisTrails software for automating workflows of species distribution models called Software for Habitat Modeling (SAHM). Using SAHM to run the MaxEnt model with guidance from USGS cooperators, the Fort Collins Lab has developed guidelines for data preparation, modeling templates, predictor selection, generation of pseudo-absence data, and model evaluation using minimum training presence. Given the breadth of literature debate on pseudo-absence data, the scope of the analysis was broadened to include a comparison of 4 different methods for generating pseudo-absence data so that the collaborators could fully understand the impacts of how model building can impact final performance measures. Work on model development and evaluation continues into 2013, with an anticipated goal of standard operating procedures for modeling techniques and recommendations on which model platforms are most appropriate for various management questions or applications.

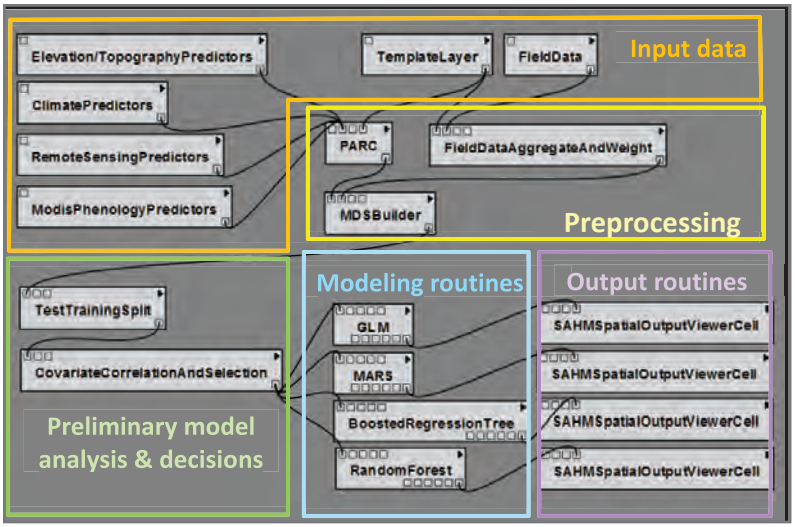


Figure 6. Sample workflow in VisTrails for presence/absence data models illustrating the full workflow: data input, pre-processing, data training, covariate selection, and modeling routines. Note that VisTrails/SAHM has the ability to execute up to four different modeling routines at the same time (or MaxEnt presence-only model by itself), allowing for consistent model comparison .

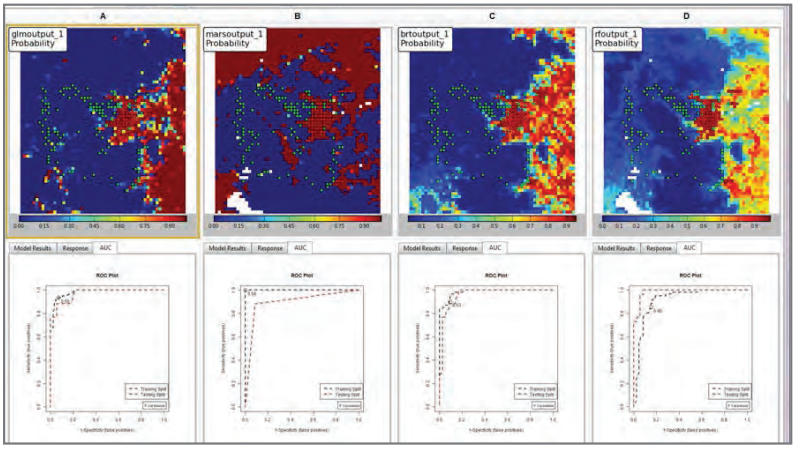


Figure 7. Model prediction surfaces and receiver operating curves (ROC) for the tutorial sample of the VisTrails/SAHM software, illustrating side-by-side comparisons of four different modeling routines: GLM, MARS, boosted regression trees, and Random Forest.

VisTrails/SAHM Modeling Tool Workshop

(Gericke Cook and Lisa Kennaway)

Members of the CPHST Fort Collins team traveled to Raleigh, NC to present a 1 ½ day training workshop in early December on the VisTrails/SAHM modeling software. VisTrails is a software platform for conducting and annotating analytical workflows that can be easily shared among cooperators. The USGS Fort Collins Science Center adapted the VisTrails software for species distribution modeling, such that modeling workflows could be easily tracked, replicated, and shared (Figure 6). This adaptation is called the Software for Assisted Habitat Modeling (SAHM), and it provides transparent tools for GIS data preparation, in-depth data exploration tools, and consistent reporting formats. The software additionally includes a built-in spatial data viewer that operates much like a stand-alone GIS, and can show side-by-side comparisons of the model outputs (Figure 7). The Fort Collins team presented an overview of the VisTrails/SAHM platform and workflow benefits, as well as an in-depth demonstration and walk-through of presence only data modeling using maximum entropy software (MaxEnt). Evaluation of this software continues within the Fort Collins lab, as well as outreach to partner agencies who are also engaging this new software.

Rearing and field releases of the yellow toadflax stem-mining weevil, *Mecinus janthinus*

Rich Hansen, Tara Costanzo and Lynn Morales

Populations of a yellow toadflax-adapted 'strain' of the stem-mining weevil, *Mecinus janthinus* (Coleoptera: Curculionidae) (Figure 1), occur at several western Montana locations (CPHST Fort Collins Laboratory [FCL] 2009 Annual Report). Weevils collected from these sites have been released in seven western states from 2009 to 2012 for yellow toadflax (*Linaria vulgaris*) biocontrol. However, we believe that field collections of *M. janthinus* are not sustainable, since host plant populations have been largely suppressed by the weevil. We began a greenhouse-based rearing program for *M. janthinus* in 2011, based on techniques developed by Colorado State University cooperators in 2009 and 2010 (CPHST FCL 2011 Annual Report). This report summarizes *M. janthinus* rearing activities in 2012. The purpose of the project is to provide adult *M. janthinus* for field release in the US, especially in midwestern and eastern states

Figure 1. Mecinus janthinus adult (L) and larvae (R).

Mecinus janthinus adult eclosion data. Weevil rearing protocols are described in our 2011 report (CPHST FCL 2011 Annual Report). Yellow toadflax stems containing dormant *M. janthinus* adults were harvested in September 2011 and divided among three large plastic containers (hereafter referred to as 'cohorts' 1, 2, and 3). To simulate overwintering, stems were placed in a cold chamber under a 10:14 hr photoperiod and temperatures of -1°C (day) and -7°C (night) from December 7, 2011 through May 1, 2012 (146 d). Stems were then held at 4°C, with a 14:10 hr (light: dark) photoperiod from May 1, 2012 until used in eclosion studies later in spring and summer 2012.

Cohorts 1, 2, and 3 were placed in clear acrylic cages in growth chambers under a 16:8 hr (light:dark) photoperiod with day and night temperatures of 25°C and 15°C, respectively. Cages were examined regularly and all eclosing *M. janthinus* adults were removed and counted. Weevils from cohorts 2 and 3 were also sexed before counting. Toadflax stems were misted with plain water at one- or two-day intervals; based on earlier research, periodic wetting of toadflax stems facilitates adult eclosion. Cages were examined until about 14 days passed with no new adult emergence (i.e. ca. 45-50 days). Stems were then dissected and all dead *M. janthinus* adults remaining in the stems sexed and counted.

Adult eclosion data are summarized in Table 1, while daily cumulative adult eclosion data are shown in Figure 2. A total of 826 live adults were collected from the three cohorts; this compares with 920 adults collected from stems reared the same way in 2011. However, 566 *M. janthinus* adults (39.9%) failed to successfully eclose from toadflax stems in 2012; we did not collect these data in 2011. For all three cohorts, adults began to emerge as soon as stems were removed from low temperatures. In general, adult eclosion was completed in about three weeks, with males eclosing somewhat earlier than females. The percentage of adults that successfully eclosed were 79.5%, 60.7%, and 51.3% for cohorts 1, 2, and 3, respectively; thus, it appears that longer periods of cold storage may have increased weevil mortality. Apparently, more female than male weevils were unable to successfully eclose.

				# eclosed *M. janthinus* adults			**# non-enclosed (dead) *M. janthinus* adults**			
Cohort	**Eclosion start date**	**Cold/cool storage (d)**	**Eclosion end date**	**Total**	**Males**	**Females**	**Total**	**Males**	**Females**	**Unk.**
1	05/17/2012	168	07/13/2012	**213**			55	17	22	16
2	06/18/2012	194	08/06/2012	**235**	103	132	152	41	92	19
3	07/16/2012	222	09/05/2012	**378**	189	189	359	103	178	78

Table 1. Total adult eclosion and residual dead adults from three cohorts of Mecinus janthinus in 2012 (progeny of parental adults that oviposited on yellow toadflax stems in summer 2011)

Live adults found in containers at the start (day 0) (Figure 2) are those which had emerged at some point during cold storage or while stems were held at 4°C (see above). The number and proportion of adults experiencing such 'early' eclosion increased with the duration of low-temperature storage (Table 2), and it appears that this phenomenon was slightly more frequent among male than female weevils.

***Mecinus janthinus* greenhouse rearing.** As in 2011, *M. janthinus* was reared within 61 x 61 x 122 cm (24 x 24 x 48 in) cages, covered with fine-mesh green polyester netting, in 2012. Each cage contained four mature yellow toadflax plants in 1-gal pots, which were watered about every other day.

We established seven rearing cages, from late May through late August 2012. We used all *M. janthinus* adults eclosed from cohorts 1, 2, and 3 (n = 826), as well as 575 weevils field-collected in Montana in early June 2012, for a total of 1401 parental adults (164 to 283 adults per cage, depending on availability). Adults were sprinkled on toadflax foliage; we attempted to provide roughly equal numbers of males and females in each cage. Caged plants were then held in the greenhouse under ambient lighting and temperature conditions (25°–27°C during the day and 15°–20°C at night) until late September or mid-October 2012. At that time, toadflax plants were moved outside, under ambient lighting and temperature conditions (25°–27°C during the day and 15°–20°C at night) until late September or mid-October 2012. At that time, toadflax plants were moved outside, under ambient conditions, to experience cooler, and ultimately subfreezing, temperatures. All toadflax stems were then harvested on December 18, 2012, placed in plastic containers, and moved to a cold chamber under a 10:14 hr photoperiod and temperatures of -1°C (day) and -7°C (night), as described above. Progeny *M. janthinus* adults will then be collected from these stems in 2013.

***Mecinus janthinus* field releases.** No FCL-reared weevils were released in 2012. However, Montana PPQ personnel and their project partners collected *M. janthinus* from Montana populations for limited distribution and field release in several western states.

***Mecinus janthinus* parasitoids.** Several hundred parasitoid and/or hyperparasitoid wasps were collected in 2011 from *Mecinus*-infested yellow toadflax stems, which we obtained from Colorado State University (CPHST FCL 2011 Annual Report). In summer 2010, yellow toadflax plants had been reared outside at CSU after colonization by *M. janthinus*. Most or all of these wasps appeared to belong to two distinct 'morphospecies', which have been submitted for identification. We believe that these insects were, in fact, attacking *M. janthinus* in the stems, but the life stage(s) attacked are unknown. In any event, no wasps were recovered during the 2012 *M. janthinus* rearing efforts described above. All 2011 and 2012 rearing was conducted in the greenhouse, with the exception of pre-overwintering out-

Cohort	No. (% total) of eclosed adults		
	All	Males	Females
1	23 (10.8%)	--	--
2	29 (12.3%)	15 (14.6%)	14 (10.6%)
3	172 (45.5%)	96 (50.8%)	76 (40.2%)

Table 2. Mecinus janthinus adult eclosion prior to removal from cold storage, 2012

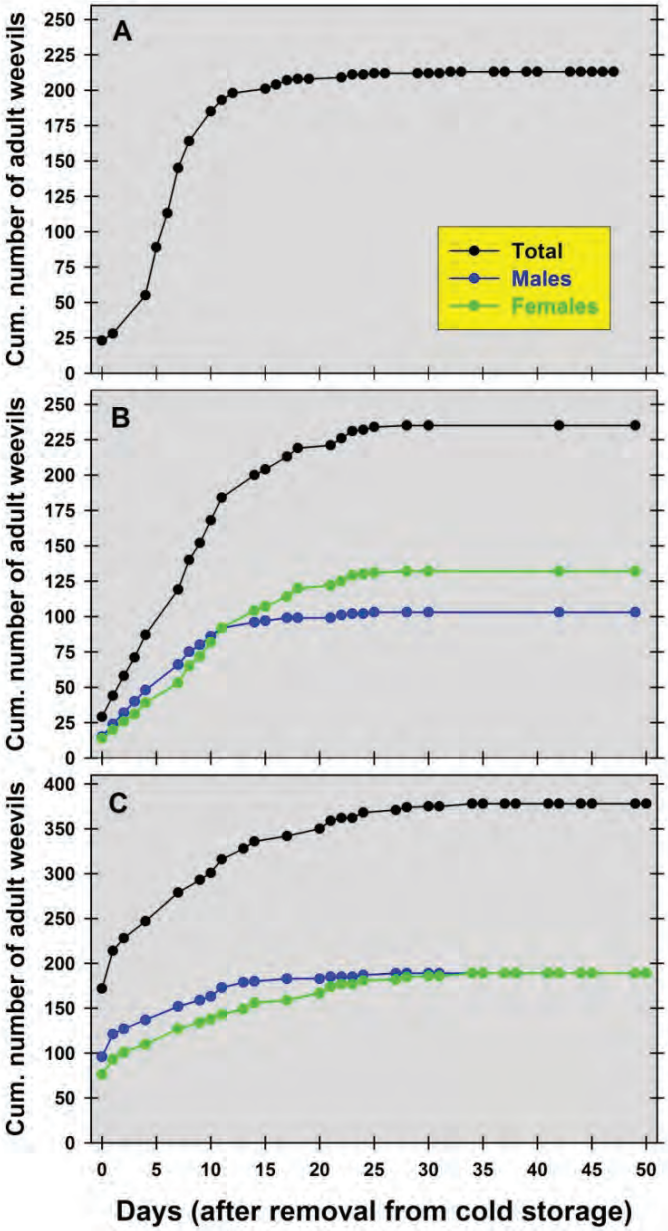


Figure 2. Cumulative adult Mecinus janthinus eclosion patterns from yellow toadflax stems, 2012: cohorts 1 (A), 2 (B), and 3 (C).

door treatments in late autumn, so it appears that *M. janthinus* were not exposed to field populations of these parasitoids. We consider that the FCL *M. janthinus* colony is now completely parasitoid-free.

Summary. In 2012, we successfully continued a greenhouse-based rearing program for the yellow toadflax stem-mining weevil that was begun in 2011. Much of the 2012 effort concentrated on develop-ing a better understanding of adult eclosion biology, which can be used to optimize rearing efforts. It appears that adult weevils can be produced from early spring through late summer by adjusting the dura-tion of cold storage, although longer cold treatment may be accompanied by increased adult mortality. We did not make field releases with FCL-reared weevils in 2012, but are planning to do so in 2013. However, it is presently uncertain if we will continue the *M. janthinus* rearing program in 2013.

[**NOTE.** Previously, the stem-mining weevils attacking Dalmatian toadflax (*Linaria dalmatica*) and yellow toadflax (*L. vulgaris*), and imported in to the US and Canada as biological control agents, were believed to belong to a single species, *Mecinus janthinus*. However, a recent taxonomic revision of this weevil group has revealed that there are two separate species on these hosts: *M. janthinus* utilizes yellow toadflax, while the newly-described *M. janthiniformis* attacks Dalmatian toadflax.

Reference: Tosevski, I., Caldara, R., Jovi, J., Hernandez-Vera, G., Baviera, C., Gassmann, A. and Emerson, B.C. 2011. Morphological, molecular and biological evidence reveal two cryptic species in *Mecinus janthinus* Germar (Coleoptera, Curculionidae), a successful biological control agent of Dalmatian toadflax, *Linaria dalmatica* (Lamiales, Plantaginaceae). Systematic Entomology 36(4): 751-763.]

Impacts of the biological control agent *Jaapiella ivannikovi* and a competitive grass on Russian knapweed plants

Rich Hansen and Tara Costanzo

Russian knapweed, *Rhaponticum (=Acroptilon) repens* (Asteraceae), is a widespread exotic weed in the western USA. The gall midge *Jaapiella ivannikovi* (Diptera: Cecidomyiidae) has been released as a classical biocontrol agent of Russian knapweed in at least eight states. Studies with Russian knapweed in its native Asian range have shown that *J. ivannikovi* attack can reduce flowering and seed production, stem height, and aboveground biomass under field conditions. However, little information is yet available on the impacts of *J. ivannikovi* under USA conditions, and also the possible integrated impacts of this biocontrol agent and other weed management techniques, such as chemical or cultural tools. This preliminary greenhouse study examines individual and combined impacts of *J. ivannikovi* and cultural control with a competitive perennial grass, on Russian knapweed plants.

This study was conducted in fall 2012. Russian knapweed plants were grown from seeds and eventually planted in 2-gallon plastic pots with standard potting soil and about 2 tbsp of slow-release fertilizer. In half the pots, three young plants of the native perennial grass sideoats grama, *Bouteloua curtipendula* (Poacaeae), were planted with a Russian knapweed plant. Pots were held in the greenhouse for several months until knapweed and grass root systems were well-established. Nine pots were placed in four fine-mesh screen cages, two containing pots with Russian knapweed plants alone and two with combined grass and knapweed pots. In one cage of each type, 40 *J. ivannikovi* galls from the FCL greenhouse colony (family 2, generation 20) were added. Thus, there were four experimental treatments: biocontrol agents alone, competitive grass alone, biocontrol agents and competitive grass, and no treatments (controls); each treatment was replicated nine times, for a total of 36 pots.

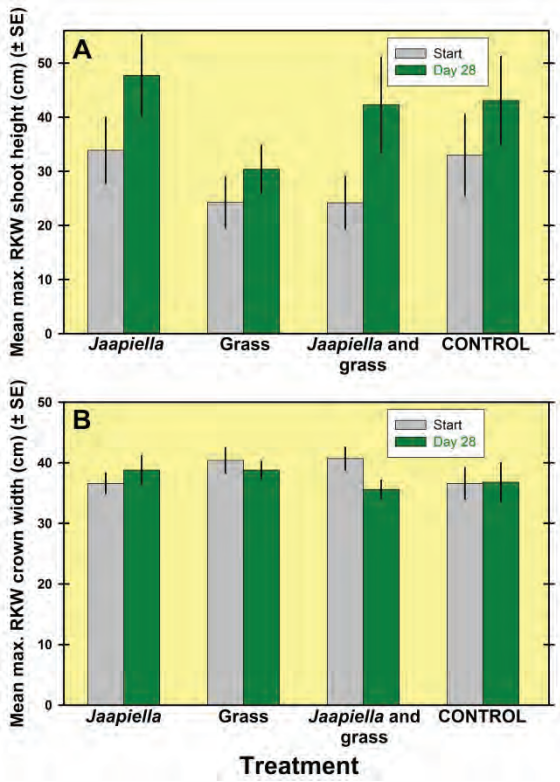


Figure 1. Mean maximum shoot height (A) and crown width (B) for Russian knapweed plants (n=9 for each treatment).

At the start of the experiment, the maximum height and crown width of each Russian knapweed plant were recorded. In addition, the number of knapweed shoots, lateral branches, and rosettes (not yet bolted) were recorded. Caged plants were held in the greenhouse under an approximately 16:8 hr light:dark photoperiod. Ambient temperatures ranged from about 25-27°C during the day and 15-20°C at night. Pots were watered at least every other day. After 28 days, Russian knapweed growth data were recorded again; in the two cages with *J. ivannikovi*, the number of progeny galls on knapweed plants was also recorded. Data collected at day 0 and day 28 were analyzed using a two-factor analysis of variance, with *Jaapiella* (yes or no) and competitive grass (yes or no) as the experimental factors.

Jaapiella galling had no significant impact on any Russian knapweed growth parameter measured (Figures 1 and 2). Grass competition significantly reduced the total number of shoots produced per plant at the start and at day 28 (Figure 2A; $F=4.94$, $p<0.05$ and $F=4.27$, $p<0.05$, respectively). Not surprisingly, the number of knapweed lateral branches was also reduced by grass competition, at least at the start (Figure 2B; $F=6.71$, $p<0.02$).

The number of *J. ivannikovi* galls produced on Russian knapweed plants with competitive grass (mean = 6.1 galls/plant; SE = 2.5) were significantly less than on knapweed plants without grass (mean = 13.6 galls/plant; SE = 2.3) ($t=2.163$; $p<0.05$). As mentioned above, the presence of *B. curtipendula* significantly decreased the number of knapweed shoots and lateral branches, thus reducing the number of terminal and lateral buds that could be utilized by *J. ivannikovi* for oviposition and subsequent gall formation.

Though this study was short-lived, it appears that a competitive grass may be able to significantly reduce Russian knapweed growth parameters by successfully competing with the weed for water, nutrients, and other resources. Reductions in Russian knapweed aboveground growth could also have negative effects on the population dynamics and host impacts of *J. ivannikovi*. However, the quantification of gall midge impacts may be underestimated, since this study only lasted 28 days, about the length of one generation in the greenhouse. Under field conditions, knapweed plants would be subjected to at least two or three *J. ivannikovi* generations annually.

Summary. We conducted a preliminary greenhouse study quantifying individual and combined impacts of a biocontrol agent (the gall midge *J. ivannikovi*) and a competitive perennial grass (sideoats grama) on Russian knapweed plants. Grass competition reduced some aboveground growth parameters while the gall midge did not; treatment interactions were not significant. We plan to expand this study in summer 2013, utilizing field cages and increased replication.

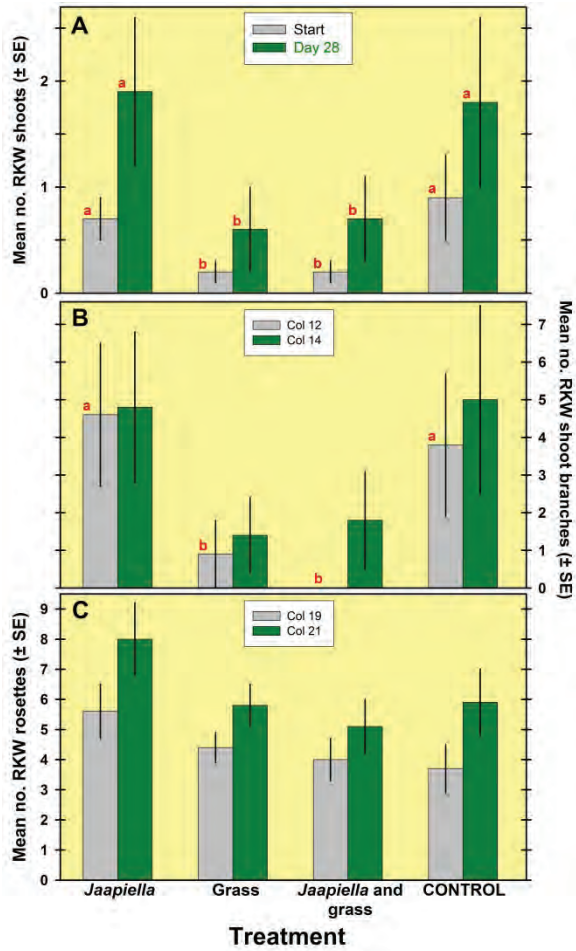


Figure 2. Mean number of shoots (A), lateral branches (B) and rosettes (C) per Russian knapweed plant. Means marked by different lowercase letters are significantly different (p<0.05) (n=9 for each treatment).

Figure 3. Sideoats grama, Bouteloua curtipendula, plant (photo by H.F. Schwartz,Colorado State Univ., bugwood.org)

Rearing the Russian knapweed gall midge (*Jaapiella ivannikovi*)

Rich Hansen, Lynn Morales and Tara Costanzo

Russian knapweed, *Rhaponticum* (=*Acroptilon*) *repens* (Asteraceae), is a perennial plant native to Asia that has become a widespread weed in western North America. It is currently listed as a noxious weed in at least 18 USA states. The 1996 canvassing effort conducted by PPQ's Western Region identified Russian knapweed as a weed target for development of a biological control program. In 2009, the gall midge *Jaapiella ivannikovi* (Diptera: Cecidomyiidae) was the second insect biocontrol agent permitted for US field release. This insect induces the formation of galls on terminal and lateral vegetative buds of Russian knapweed, stunting growth of stems and reducing flower and seed production. Initial releases were made at several sites in Colorado, Montana, and Wyoming in 2009 and 2010, utilizing *J. ivannikovi* populations originally collected in Uzbekistan and then processed in quarantine and reared at Montana State University; most appear to have become established. To expand the field release program, a greenhouse-based *J. ivannikovi* rearing program was initiated in February 2011 at the CPHST Fort Collins Laboratory. In 2011, releases using FCL-produced galls were made at 18 sites in seven states: California, Colorado, Idaho, New Mexico, Oregon, Washington, and Wyoming (2011 CPHST Fort Collins and Phoenix Laboratory Report). We continued the rearing effort in 2012; rearing production data are summarized here, while 2012 field release information is contained in a separate report.

Description of *Jaapiella ivannikovi* rearing protocols. A 'production colony' of Russian knapweed (RKW) plants was initiated in January 2011 and maintained throughout 2011 and 2012. Plants were propagated from seeds or from dormant rootstocks collected at a site near Fort Collins. Plants were grown in 1-gal plastic pots with standard potting soil and were periodically treated with slow-release and/or water-soluble fertilizers. Pest outbreaks, primarily of western flower thrips and two-spotted spider mite, were treated with sticky traps, insecticidal soap, spinosad, and predatory mites. All plants were grown in a greenhouse at ca. 25-27°C during the day and ca. 20°C at night. Ambient light was used for most of the year, though supplemental artificial lighting was provided from November through February (to deliver a minimum of about 14 hrs of light per day). We attempted to maintain a colony of 100–200 Russian knapweed plants throughout the year.

Detailed rearing procedures were described in the 2011 project report (2011 CPHST Fort Collins and Phoenix Laboratory Report) and are summarized in Figure 1. *J. ivannikovi* was reared on Russian knapweed plants in fine-mesh screen cages. We generally completed one generation in about 28 days; progeny galls were then harvested for field release or continued laboratory propagation. In 2012, we reared two *J. ivannikovi* 'families' originating with parental galls obtained from Montana State University (insects were originally collected in Uzbekistan). Family 1 was initiated on 23 February 2011 while Family 2 was cultured on 6 April 2011. As in 2011, families were maintained separately through temporal (appearance of adults was separated by at least two weeks) and spatial isolation (the use of rearing cages).

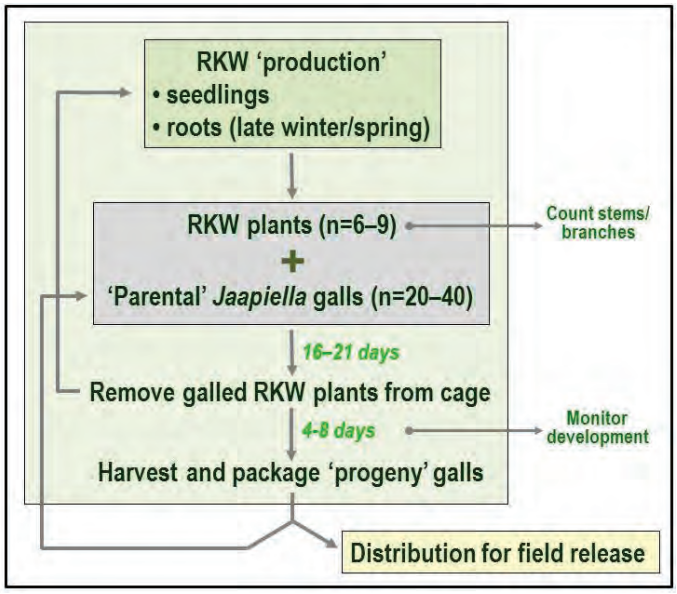


Figure 1. Summary of Jaapiella ivannikovi greenhouse rear-ing process (2011-2012)

***Jaapiella ivannikovi* rearing production data.** In 2012, we reared 27 cohorts of *Jaapiella ivannikovi*, from a total of 66 rearing cages (2-4 cages per cohort) and 554 Russian knapweed plants. We completed 14 and 13 generations, respectively, with Family 1 and Family 2 in 2012. A total of 9,031 *J. ivannikovi* galls were produced in 2012, a 264% increase from total production in 2011 (Table 1). About 42% of galls produced were used for maintaining the colony and monitoring fly development. Nearly 30% of harvested galls were not used; most of these were very small galls that would likely produce few, if any, adult flies. Other discarded galls included those that resulted from 'superoviposition', when adult *J. ivannikovi* females lay too many eggs on or near a given Russian knapweed bud, resulting in an excessive number of progeny larvae for available meristematic tissue. This induces misshapen, dessicated, abnormal galls that also, typically, produce few or no viable adults.

Production data for the 27 *J. ivannikovi* cohorts reared in 2012 are summarized in Figure 2. Average 2012 gall production ranged from 35 to 321 *Jaapiella* galls per cage, 4 to 36 galls per caged RKW plant, and 1 to 21 galls per RKW stem. In general, production data in 2012 were higher than comparable 2011 values, perhaps indicating an overall re-

finement of rearing techniques and/or increasing adaptation of *J. ivannikovi* to greenhouse rearing with time. All 2012 production measures (Figure 2) showed a decrease from May through early August, a trend that was also somewhat apparent in 2011. This phenomenon is further explained by examining minimum and maximum gall production data (Figure 3). Some plants in each cohort always produced few or no galls, but plants yielding many galls became less common during the summer. In summer 2012, we experienced a major outbreak of two-spotted spider mites in the FCL greenhouse, which led to discoloration and loss of leaves. In addition, we have observed that potted RKW plants in the greenhouse experience a significant increase in flowering in early to mid-summer, probably in response to increasing daylengths associated with the summer solstice. Flower buds are a poor gall substrate for *J. ivannikovi* when compared to vegetative buds. Thus, pest infestations and increased flowering apparently reduce the relative quality of Russian knapweed plants as gall midge hosts in the summer. By late summer into early fall 2012, spider mite populations were largely controlled and RKW flowering rates had decreased; gall production also increased, mirroring a comparable 2011 pattern.

Generally, gall production was similar for *Jaapiella* families 1 and 2 in 2011 and 2012 (Figure 4), especially when considering that there is about a two-week offset between families for a given generation. For both families, a general reduction in 'performance' for generations 15-20 in 2012 corresponds to the summer reductions in host plant quality, discussed above. In general, there has been an increase in gall production for *J. ivannikovi* families 1 and 2 through 2011 and 2012; this may be an indication of increased adaptation to the rearing environment over time.

In 2011 and 2012, we periodically assessed *J. ivannikovi* adult eclosion from reared galls, representing both Families 1 and 2. Gall length and maximum diameter were recorded (in mm). Galled stems were placed in water-filled vials, placed individually in plastic cups, and reared in a growth chamber at 25°C during the day and 15° at night, with a 16:8-hour photoperiod (light:dark). Cups were examined daily, and any adult flies observed were collected and sexed. We employed 20 galls for each cohort, which were arbitrarily selected to represent a range of sizes. Up to 128 Jaapiella adults eclosed from reared galls, with averages ranging from 8 to 30 adults per gall (Table 2). Sex ratios were strongly female-biased for all four cohorts.

Table 1. Jaapiella ivannikovi gall production and utilization in FCL green-house colony, 2011-2012

Category	2012		2011	
	No. galls	% total	No. galls	% total
Total	9031		3419	
Colony maintenance	2514	28%	1198	35%
Colony monitoring	1310	14%	576	17%
Field release	1597	18%	820	24%
Misc. experiments, photography, etc.	1000	11%	400	12%
Usable/unused	2610	29%	425	12%

Summary. We have successfully developed a greenhouse-based rearing program for the gall midge, *Jaapiella ivannikovi*, a classical biological control agent of Russian knapweed. In 2011 and 2012, this program has produced galls for field release in the western USA, and insects for use in various research projects conducted by state and federal project partners. Rearing data have been collected that document the productivity of the rear-ing program and suggest ways to improve its efficacy. The colony will be maintained for a final year in 2013, to provide *J. ivannikovi* for additional releases throughout the USA.

Table 2. Jaapiella ivannikovi adult eclosion from selected gall cohorts, 2011-2012 (n=20 galls)

		All adult eclosion			Adult Males		Adult females		
Start date	Generation	Range	Total	Mean(SE)	Total	Mean(SE)	Total	Mean(SE)	M:F ratio
***J. ivannikovi* Family 1**									
11/17/2011	G-8	0-27	157	7.9 (1.8)	61	3.1(0.9)	96	4.8 (1.0)	0.63
12/14/2011	G-9	3-60	344	17.2 (3.0)	143	7.2 (1.4)	201	10.1 (1.7)	0.71
9/25/2012	G-19	2-46	356	17.8 (3.0)	137	6.9 (1.2)	219	11.0 (1.9)	0.62
***J. ivannikovi* Family 2**									
10/21/2011	G-6	0-77	476	23.8 (5.2)	174	8.7 (2.1)	302	15.1 (3.3)	0.57
11/28/2011	G-7	0-31	204	10.2 (1.9)	81	4.1 (0.8)	123	6.2 (1.0)	0.66
04/06/2012	G-11	1-128	607	30.4 (7.6)	216	10.8 (2.9)	391	19.6 (4.8)	0.55
12/17/2012	G-21	1-79	486	24.3 (5.3)	191	9.6 (2.1)	295	14.8 (3.3)	0.65

Field releases of the Russian knapweed gall midge (*Jaapiella ivannikovi*)

Rich Hansen, Lynn Morales and Tara Costanzo

A previous document in this Laboratory Report (Rearing the Russian knapweed gall midge) describes a FCL greenhouse-based rearing project that produced *Jaapiella ivannikovi* galls for various purposes in 2011 and 2012. The 2011 report on this project (2011 CPHST Fort Collins and Phoenix Laboratory Report) details methods used to harvest, process, and ship galls to project cooperators, and recommended procedures for field release.

In 2011, we provided galls for release at 19 field sites in seven states (California, Colorado, Idaho, New Mexico, Oregon, Washington, and Wyoming) (Figure 1). About 32 galls were released, on average, at each field site in 2011. Mean eclosion data from our greenhouse colony show that approximately 20 *J. ivannikovi* adults eclose from each gall. Thus, we estimate that about 640 adults were ultimately released at each 2011 field site, with a slightly female-biased sex ratio.

We expanded *J. ivannikovi* rearing production in 2012, by increasing the number of cohorts reared during the spring-summer 2012 field season and the number of host plants used for each cohort. From May through September 2012, we provided nearly 1600 *J. ivannikovi* galls for field releases at 38 sites in eight states, which included the seven states where 2011 releases were made and Utah, in which initial releases were made in 2012 (Figure 1). Only a single 2012 release was made at a 2011 release site; 37 of the 38 releases occurred at new field locations, though often in close proximity to 2011 sites. Three Utah releases were made in field cages (Figure 2), while all other sites involved open-field releases. On average, about 40 galls were released at 2012 field sites; thus, approximately 800 *J. ivannikovi* adults should have eclosed at each site, based on the eclosion data described above.

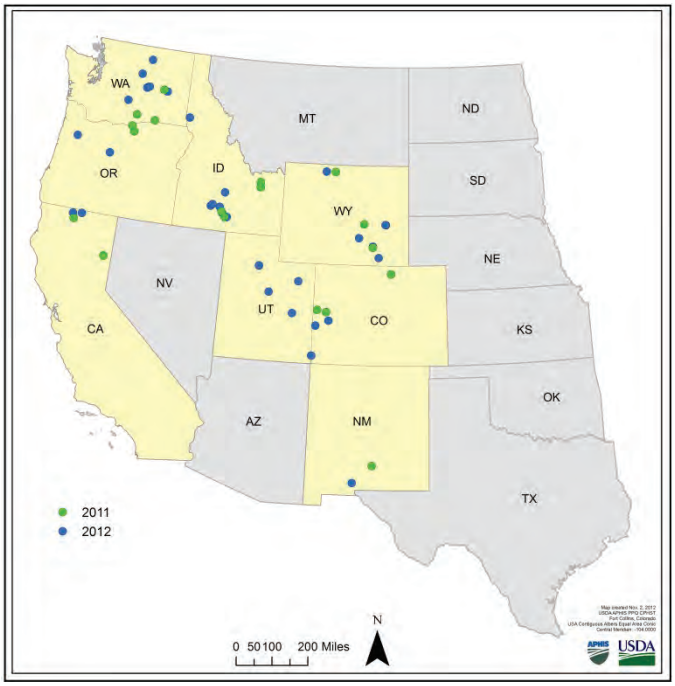


Figure 1. Jaapiella ivannikovi release sites initiated in 2011 (n=19) or 2012 (n=38) (map by T. Costanzo, FCL).

Though post-release galling was observed at some 2011 release sites later the same year, only a few supported obvious galling in 2012 (i.e. establishment). However, we are not willing to conclude that *J. ivannikovi* failed to establish at most 2011 sites, for a variety of reasons: (1) some sites were not monitored at all in 2012; (2) some sites that were monitored were visited at the wrong time of the year (typically too early in the spring, before *J. ivannikovi* eclosion); (3) project partners conducting post-release monitoring may have been unfamiliar with gall location and appearance; (4) at low densities, galls may be extremely difficult to find, especially in large, dense Russian knapweed stands; and (5) *J. ivannikovi* adults are very small and subject to wind dispersal, so post-release galling may have occurred at some distance from the initial point of release. Similarly, 2012 post-release monitoring at 2012 release sites in Colorado, Oregon, and Washington documented galling, but observations in 2013 and beyond will be needed to clarify establishment status. We are developing standardized monitoring protocols that will be distributed to all project cooperators in spring 2013. Hopefully, partners will be able to accurately assess *J. ivannikovi* establishment at all 2011 and 2012 release sites in spring

Figure 2. Caged Jaapiella ivannikovi field release in Utah, initiated in summer 2012 (photo by A. Mendenhall, PPQ).

and summer 2013.

FCL-reared *J. ivannikovi* galls have also been provided to project partners for initiation of laboratory- or greenhouse-based rearing in several states. These include Colorado (Colorado Department of Agriculture), Idaho (Nez Perce Tribe Biocontrol Center), New Mexico (New Mexico State University), and Oregon (Oregon Department of Agriculture). In 2012, these rearing projects provided galls for field release at 55 additional sites in these states.

In summary, the FCL rearing program has provided Russian knapweed gall midge, *Jaapiella ivannikovi*, galls for field release at 57 sites in eight western states. Establishment status of this biocontrol agent at most sites remains uncertain. In 2013, we plan to provide greenhouse-reared galls to project cooperators, as requested; we hope to initiate releases in at least several new states. This will likely be the final year of the FCL rearing project, since adequate numbers of field insectary sites will be established in the various states and several other rearing efforts will be able to provide *J. ivannikovi*, if needed.

Development of rearing systems for beneficial root feeding biological control agents

Nada Carruthers

Cyphocleonus achetes is a weevil introduced into North America for the biological control of diffuse and spotted knapweed. Dispersal of *C. achetes* throughout the western United States has been very slow due to low numbers of insects available for distribution. With a goal to facilitate the weevil's redistribution, a rearing system based on artificial diet was developed. A colony of *C. achetes* was successfully reared in the CPHST Albany laboratory for 16 generations on artificial diet before being terminated in 2012. Our work in 2012 focused on finishing this project and obtaining results from final field tests.

Figure 1. Pink Bollworm diet with ridges

In 2011 we produced around 530 *C. achetes* adults. Most were used in experiments; however, a group of 100 individuals was raised for field release in Idaho. This was the second release of diet-reared *C. achetes* adults within Idaho. A similar batch of adult weevils was released during the summer of 2010. Our cooperators from Nez Perce Bio-control center confirmed that insects from our releases in 2010 and 2011 successfully established in the field. These experiments showed the ability of laboratory-reared *C. achetes* to effectively establish in nature and serve as documentation for planning future biological control releases of laboratory-reared natural enemies.

Development of rearing system for *Cyphocleonus achetes* was completed in 2012.

Bradyrrhoa gilveola (pyralid moth), is an agent for biological control of rush skeleton weed, Chondrilla juncea (Asteraceae). *B. gilveola* was first introduced to Idaho in 2002 from northern Greece and Bulgaria. Its establishment here is still very limited, and numbers are not sufficient for redistribution. The larva is the damaging stage for the host plant; feeding damage decreases plant vigor and forms wounds that expose plants to pathogens. *B. gilveola* can have 2-3 generations per year, and females can lay up to 300 eggs in rosette crowns or on the soil. Newly-hatched larvae penetrate the soil and begin to feed externally on the plant roots. Older larvae spin elongated tubes that are attached to the roots, where they live and feed internally and externally. Experience from Australia (where the moth was introduced in 1973) has shown that field establishment was significantly facilitated by laboratory rearing (Cullen 1980).

The Nez Perce Bio-Control Center in Idaho requested the development of an artificial diet for rearing *B. giveola* and cooperated on the project by supplying eggs for our diet studies. Four different diets were tested: rice stem borer diet (Singh and Moore 1985), pink bollworm diet obtained from Phoenix rearing facility, Hylobius diet and cactus moth diet. Our experiments showed that diet presentation plays a crucial role in feeding induction. The highest establishment on the diet was obtained when the diet was dispensed with ridges (Figure 1). The highest and the longest survival rates of *B. gilveola* were obtained on the Hylobius diet; larvae established, molted and fed for 2 months. Establishment on other tested diets was poor and we did not observe any significant feeding.

Acknowledgements. The author would like to acknowledge project champions John Simons and Paul Brusven and would like to thank Dr. Jim Carpenter for providing cactus moth diet.

The author would like to express thanks to Nez Perce Tribe Bio-Control Center for dedicated work on our bio-control projects.

Survey for natural enemies of Canada thistle (*Cirsium arvense*)

Melinda Sullivan and Christina Southwick

Canada thistle (*Cirsium arvense*)(Figure 1), is an aggressive perennial weed that competes with other plants for water, nutrients and space, thus reducing the grazing capacity and diversity of rangeland and natural areas. It is an introduced pest in North America, Australia, New Zealand, and South Africa. *C. arvense* is the most frequently-listed weed in the United States and is considered a noxious weed in at least 28 states. A variety of herbicides are currently labeled for use against *C. arvense* but are not effective for non-agricultural use due to logistical concerns, environmental concerns, and the perennial growth habit of Canada thistle.

Figure 1. Canada thistle flower

Biological control is currently being investigated as part of an integrated pest management program for Canada thistle. The search for natural enemies of Canada thistle has been focused on Europe, its reported center of origin. However, recent evidence suggests that the range of *C. arvense* may be broader than originally thought, reaching into Northern Africa and Western Asia. Indeed, recent surveys in China have revealed the occurrence of a wealth of natural enemies to this weed (both arthropods and pathogens). Some of these natural enemies have yet to be considered for classical biological control of *C. arvense*. *C. arvense* is considered to be an occasional problem weed in China and northern India, but it is certainly not a major weed. This indicates that these eastern regions may be an import center of diversity for the species, and that it could prove valuable to investigate the potential of natural enemies that occur there for biological control of the weed. Several arthropod agents have been introduced into the United States but management of Canada thistle with these agents has been extremely variable from year to year and largely unsuccessful. No plant pathogen agents have been deliberately introduced although a number of pathogens have been recorded from its native and introduced range.

Figure 2. White rust pustules of Pustula spinulosa on Canada thistle (CABI Europe)

In 2009, CPHST Fort Collins initiated efforts to survey for and assess the efficacy of plant pathogens attacking Canada thistle in collaboration with Centre for Agricultural Bioscience International (CABI) Europe-Switzerland. CABI and its partners proposed pathogen surveys in the Xinjiang Autonomous Region of northwestern China in the summer of 2009; this region supports a diverse *Cirsium* flora and is climatically similar to much of the continental United States of America (USA). Political unrest in Xinjiang, however, precluded access. Twenty-two sites in western Mongolia were instead surveyed and a variety of pathogens were collected. It appears that the Mongolian hosts were actually the closely-related *Cirsium setosum* rather than *C. arvense*. Leaf samples were collected to confirm identity using molecular analyses.

In 2010, a survey for fungal pathogens on *C. arvense* was conducted in northwestern China by CABI Europ-Switzerland and their cooperators in China. All material is being identified and assessed for its biocontrol potential. Thus far, a number of leaf-attacking fungal pathogens have been identified: powdery mildew, *Septoria* spp., *Phoma* spp., *Alternaria* spp., and *Puccinia* cf. *punctiformis*. Despite reports that up to four rust species occur on *C. arvense* in Xinjiang Province, initial findings suggest that only one species, the systemic rust *Puccinia* cf. *punctiformis*, is actually present. The other rust species may be synonyms or on closely related but misidentified thistle hosts. Since this species is already present in North America (accidentally introduced), its potential as a classical biological agent is limited unless more aggressive strains can be identified.

The most promising pathogen collected from China to date is a white rust pathogen, *Pustula spinulosa* (formerly *Albugo tragopogonis*)(Figure 2). This pathogen has been reported as occurring in the USA on sunflower 'varieties,' and physiological 'races' of this fungus have been reported in the scientific literature. Further studies are needed to ascertain if the Chinese *Pustula spinulosa* is a different variety or race than the one known to occur in the USA. Field observations suggest that the Chinese *Pustula spinulosa* could be highly specific and damaging to *C. arvense* in the USA

Chinese scientist Huanhuan Wan visited CABI-Europe United Kingdom (UK) in early 2012 to undertake further work on *P. spinulosa* and learn specialist techniques in plant pathology, to help in his work on *C. arvense* in China. His laboratory-based inoculations of *C. arvense*, using freshly collected field material of *P. spinulosa* were successful.Inoculation tests on *C. arvense* and *Cirsium* spp. accessions from the USA were started and will continue in 2013, both in China and the UK.

Strontium Chloride as a secondary dietary marker for pink bollworm mass-reared for Sterile Insect Technique

Michelle Walters[1], John Claus[1], Guolei Tang[1], Anna Lowe[1], Nathan J. Moses[2], R. Nelson Foster[1], Barry U.Barnes[1], Joahna Solis[9], Nada Carruthers[1], Richard Zink[3], Robert Staten[4], F. Rob Anderson[5], Daryl Hill[6], Dominique Ramirez[7], David Klein[7], Doug Marsh[8], Leighton Liesner[9] and Eoin Davis[10], [1]CPHST Phoenix Lab, USDA-APHIS-PPQ, Phoenix, AZ, [2]Department of Entomology, University of Arizona, Tucson, AZ, [3]CPHST Fort Collins, USDA-APHIS-PPQ, Fort Collins, CO, [4]CPHST Phoenix, USDA-APHIS-PPQ, Retired, Gilbert, AZ, [5]Pink Bollworm Rearing Facility, USDA APHIS-PPQ, Phoenix, AZ, [6]Aircraft and Equipment Operations, USDA-APHIS-PPQ, Edinburg, TX, [7]Department of Agriculture, Chemistry Laboratory, State of Arizona, Phoenix, AZ, [8]Department of Agriculture, Agricultural Laboratory, State of Arizona, Phoenix, AZ, [9]Arizona Cotton Research and Protection Council, Phoenix, AZ, [10]Pink Bollworm Rearing Facility, USDA-APHIS-PPQ, Phoenix, AZ .

The United States Department of Agriculture, Animal Plant Health Inspection Service, Plant Protection and Quarantine Pink Bollworm Rearing Facility mass-rears and sterilizes Pectinophora gossypiella (Saunders) (pink bollworm or PBW) moths for release as part of the international Pink Bollworm Eradication Program. These mass-reared pink bollworm moths historically have been fed red dye to mark them, but program personnel have long felt that a few mass-reared moths appear unmarked (their scales get knocked off from handling during release). In addition, these finds are not related to “hot spots” of wild moths or boll infestations, as would be the case if the finds were real wild moths. The dye-depletion has not been reproduced in the lab. We have aged PBW in the lab but they do not show dye depletion. It seems to be caused by conditions in the cotton field. Dye depletion was confirmed in the field this year when we found moths high in strontium with no discernible red dye. This is a concern because a single un-dyed, wild-appearing sterilized moth caught in a monitoring trap may trigger unnecessary and expensive eradication responses. This was not such a problem when wild, un-marked moths were abundant; one more wild appearing, but sterile, moth was of little consequence as management and eradication responses were necessary regardless. As the program nears completion and wild moths are seldom seen, a mistaken identity is costly.

Numerous naturally occurring elements have been investigated to mark released pink bollworm. Of the few that looked promising (relatively non-toxic), strontium is much less expensive than rubidium, as it is more commonly found in the Earth’s surface. Strontium chloride hexahydrate ($SrCl*6H_2O$) is also used as a human dietary supplement and is available in bulk quantities at food grade. However, strontium’s abundance in the environment tends to lead to higher background levels in the soil and thus in cotton bolls (Burns Murphy et al. 1983; Burns 1984; Akey and Burns 1991). Strontium had also never been tested as a diet additive on multiple generations in a mass rearing facility. The CPHST Phoenix lab worked with the Arizona State Agricultural Chemistry Lab to develop analytical methods using inductively coupled plasma atomic emission spectrometry (ICP-AES). Together we studied the effectiveness of feeding strontium chloride to pink bollworm as a marker. The Chemistry Lab refined the ICP-AES method to measure strontium presence and quantity in a single, male, pink bollworm moth.

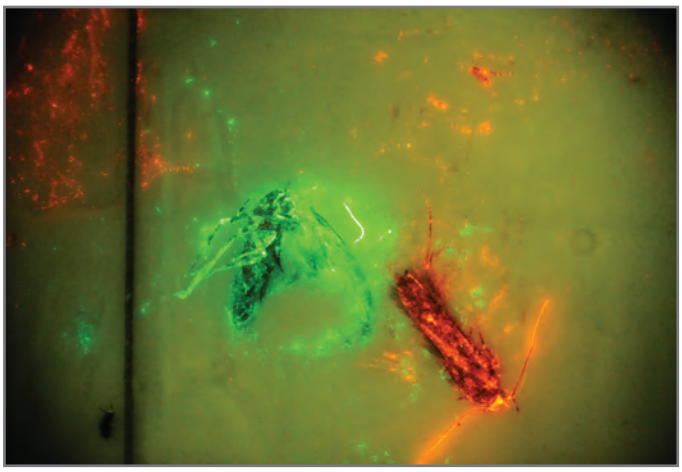


Figure 1. Pink Bollworm under microscope showing external dyes used to differentiate moths fed 0ppm Strontium chloride compared to 540ppm SrCl

The addition of various levels of strontium to the larval diet did not significantly affect diet pH, a critical factor in pink bollworm rearing. Pink bollworm were reared at 0, 90, 270, 540 and 720ppm on food grade $SrCl*6H_2O$ for 5 generations. No significant differences were found for developmental time (egg to adult), egg production (production (one-way ANOVA, $P > 0.0.9983$)) or percent hatch (one-way ANOVA, $P > 0.4953$), individual pupal weight was significantly reduced by 720ppm but was unaffected by other doses production (one-way ANOVA, $P > 0.0003$) , pupal production (one-way ANOVA, $P > 0.8113$) or eclosion rate (percentage of successful metamorphosis of adult from pupal case); nor were there significant differences found for adult production, longevity, mating, sex ratio, fecundity, or deformity. In addition, there was no significant effect on 14 day adult survival nor on male response to pheromone in a wind tunnel. Adult moths were held in cages, fed sugar water and sampled at various ages for strontium retention (Chart 1).

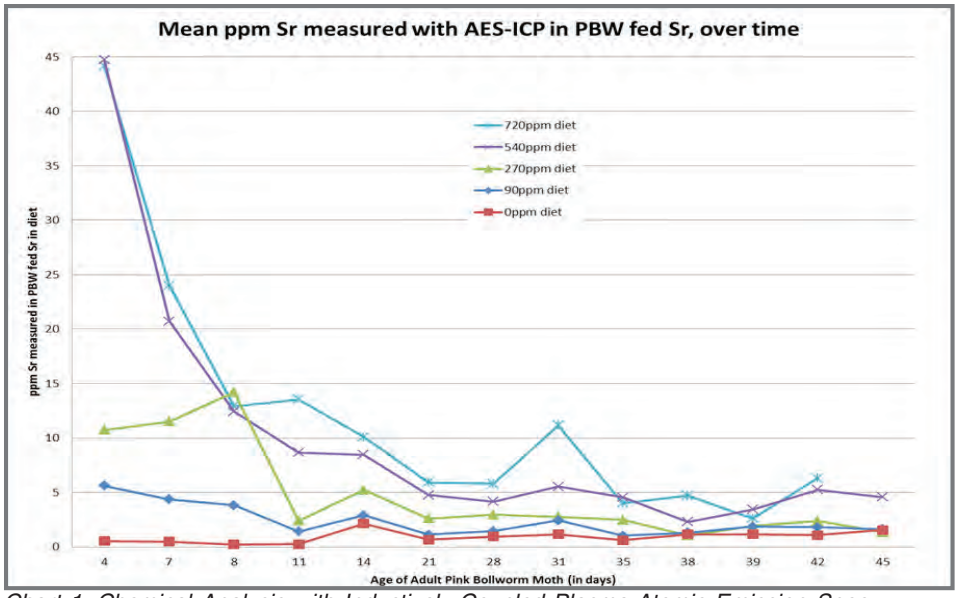


Chart 1. Chemical Analysis with Inductively Coupled Plasma-Atomic Emission Spectroscopy, at Arizona State Agricultural Chemistry Lab

Adult moths were sampled at 0, 7, 14, 21, 28, 31 and 42 days post-eclosion, frozen and held for ICP-AES analysis (Chart 1). At 0 to 7 days, treatments of 270, 540 and 720 ppm separated from each other and from 0 and 90ppm. As the adults aged (21+ days), the 270 ppm treatment was no longer consistently separate from 0ppm. Additionally, the 720 and 540 ppm no longer separated from each other but remained separate from the lower ppm treatments and from 0ppm. There was no laboratory-evidenced benefit from feeding > 540ppm. The PPQ pink bollworm Rearing Facility subsequently fed 540 ppm strontium chloride, in the diet, to all mass-reared moths (3.2 billion moths from April to October, 2012, ~17M/day) for the 2012 season. Moth production was unaffected.

Further lab and field tests were conducted throughout the cotton growing season. Externally-dyed moths fed 0 and 540ppm strontium were released by air and by hand over genetically modified cotton fields (genetically modified cotton produces a Bt toxin fatal to pink bollworm larvae). These releases were conducted in Blythe, California, in May (aerial release; Figures 2 and 3) and July (hand release). An additional hand release occurred in Peoria, Arizona in August. Both are Eradication Program non-release areas so the only pink bollworm present was either from the test or wild (unlikely to be wild as no wild pink bollworm have been caught in this area for the last 2 years). In all tests, the 0ppm strontium male pink bollworms responded significantly better to pheromone baited monitoring traps than the 540ppm strontium moths.

Figure 2. Custom built funnel built by and installed in AEO release aircraft. PBW were held in ice chests and dumped down the funnel and out the belly of the plane as the pilot flew over the field.

Female pink bollworm fed either 0 or 540 ppm strontium were placed in small cages (2x3") inside of sticky Delta traps in field cages (10x20x6'), in a cotton field. Each field cage was populated with 300 male pink bollworms (all fed 540ppm strontium). The traps were collected daily for 5 days and the males in each trap were counted. The small cages containing the female moths were brought inside during the day (pink bollworm mate at night) and the female moths were fed sugar water and placed back outside each night. There were no significant differences in numbers of males attracted to traps indicating that dietary strontium had not affected the female moth's production of pheromone to attract males (paired t-test, P = 0.4017).

Analysis for strontium using ICP-AES was also conducted in pupae, late instar larvae and for body parts of adults. Moths reared on 540ppm Sr and aged in traps were also tested. From these analyses we learned several things: 1) That strontium is readily measured in the immature forms of pink bollworm, even when fed at 270 ppm; 2) Strontium is lost between life stages (larvae to pupae to adults); 3) In adult pink bollworm, strontium is primarily stored in the abdomen and excreted over the life of the adult, as is red dye; 4) Strontium is very stable in dead moths (such as those held in traps, barring predation).

Strontium in field trapped, dye-depleted moths (pink bollworm with no visible red dye) from the eradication program areas were also analyzed using ICP-AES. There was sufficient strontium in 24 of 29 suspect moths trapped in AZ to confirm that they were dye depleted, mass-reared pink bollworm. However, using the ICP-AES method left us unsure about the origins of the remaining 5 of the 29 trapped (17%). Sample quality was a limitation because the abdomen is critical. Moths found in traps may have been damaged or partially eaten by predators leaving insufficient

Figure 3. John Claus, CPHST Phoenix, with ice chests full of moths, ready to fly to Blythe, CA, to pour moths out a funnel over cotton fields.

sample material. Dissection of genitalia is also a problem as it directly removes part of the abdomen and allows loss of abdominal contents. Strontium in field trapped moths from a non-program area (Torreon, Mexico), and strontium in moths cage-reared on cotton, okra and kenaf were also analyzed with ICP-AES. Strontium was undetectable or found at very low levels in moths not deliberately fed strontium.

These analyses revealed that food grade $SrCl*6H_2O$ is affordable ($7,925/season @17M/day) and non-toxic at 540 ppm. The ICP-AES method was successfully developed to analyze a single moth from a trap (reliable if abdomen is present). ICP-AES analysis was timely (one day) and affordable ($15/sample). As a result of the Eradiation Program's experiences in 2012, strontium will continue to be a part of the 2013 Pink Bollworm Rearing Facility mass-rearing diet.

Additional method to identify wild versus mass-reared pink bollworm. Another method to separate wild from mass-reared pink bollworm was the implementation of a high-performance liquid chromatography (HPLC) instrument. This method was developed in Albany, CA and the instrument was provided by the Gulfport CPHST lab prior to being transferred to Phoenix.

The new HPLC method is capable of detecting and measuring red dye in a single trapped moth. The method is quantitative (rather than "presence/absence") and can measure much smaller quantities of dye than are detectable visually using a dissection microscope and the human eye.

Bibliography

Akey, D. H. and D. W. Burns (1991). "Analytical consideration and methodologies for elemental determinations in biological samples." Southwestern Entomologist(Supplement No. 14): 25-36.

Burns, D. W. (1984). ICE-AES analyses of Mediterranean fruit fly, pink bollworm, and boll weevil (Elemental). United States, Arizona, Arizona State University. PhD.

Burns, D. W., M. P. Murphy, et al. (1983). "The Evaluation of Internal Elemental Discriminators for Pink Bollworm by Inductively Coupled Plasma-Atomic Emission Spectrometry." Applied spectroscopy 37(2): 120-123.

Comparison of 20, 22.5 and 25 kilorad irradiations for released pink bollworm

Ernie Miller, Stacey Archuleta, John Claus, Guolei Tang, Anna Lowe, Michelle Walters and Eoin Davis

We investigated the benefits of increased levels of irradiation on pink bollworms (PBW) to find an acceptable trade-off between radiation level, survival of the first generation of offspring of treated moths (F1 progeny) and competitiveness. The PBW Eradication Program suspected that F1 progeny of sterile released moths were appearing in monitoring traps. These F1 moths are not fertile and could not start a new population of damaging pests. However, their outward appearance is identical to that of a wild, fully fertile, moth and their presence in traps could lead to expensive false positive response measures.

Historically, mass-reared pink bollworms are treated with 20 kilorad (krad) of irradiation just prior to release. Dose effects on F1 progeny were studied in 1984 and readdressed in early 2012. The PBW Rearing Facility reared eggs produced by 20x20, 22.5x22.5 and 25x25 krad treated adult

Figure 1. Pink bollworm with deformed wings, incapable of flight. Incidence of offspring deformity increases with parental radiation dose.

moths. The eggs were placed on diet and given time to develop. F1 progeny were counted and, if possible, mated to produce F2 (second generation) progeny in the lab. The numbers of insects, by life stage, were counted, per cell of diet. Quality indicators including the number of deformities, flight ability, and longevity were recorded. Results indicated that the current PBW Rearing Facility colony, not treated by radiation, produced 9 times the progeny compared to 1984, reflecting advances in rearing methods. Similarly, in 1984, a 20x20 krad cross produced one F1 progeny per 1000 parent females. In 2012, they produced 21 progeny per 1000 parent females. These are very small numbers of progeny but still important because so many millions of irradiated moths are released. The laboratory results convinced the PBW Eradication Program to adopt an increased dose of 25 krad (versus 20 or 22.5) to initiate the 2012 release season, to be followed by additional testing of the field competitiveness of the 25 krad moths. This decision was not taken lightly since radiation dose impacts insect quality and lengthens exposure time in the irradiator to obtain the higher dose; thus potentially delaying moth shipments to all the field locations.

Cotton field. Photo taken in Wellton, Arizona.

Competitiveness factors, including flight ability, female calling, mating, longevity, and male response to pheromone, were measured in cage and field studies. The first cage studies were conducted early season to measure male moth response to pheromone in traps. The pheromone mimics that produced by a female moth when she calls a mate. Large net cages (6'x6'x24') were placed over 3 rows of cotton. Two treatment levels of male moths were compared (20 and 25 krad). 50 males of each treatment were dusted with fluorescent dye and released in field cages (both treatments in each cage). After 24 hours, two pheromone baited traps were placed in each cage and checked daily until no additional moths were captured for 3 consecutive days. Seventeen replicates were completed. There were no significant differences in the number of 25 vs. 20 krad male PBW recaptured, indicating that the increase in radiation dose did not affect the male moth's ability to respond to pheromone (t-test with matched pairs, $P > 0.3454$). The second cage study was conducted to measure female moth ability to successfully mate with a male under field conditions. Female moths treated at 20 and 25 krad were placed in one- gallon mating stations and collected the following morning. Each female was then dissected to determine if she had mated by the presence of a male spermataphore in her bursa copulatrix. Males only were released in each cage to provide a male population in the cages. There was no significant difference between numbers of males attracted by either of the two groups of females (t-test with matched pairs, $t > 0.1478$). Using a similar protocol as described above, females were placed in mating stations in a commercial cotton field to again compare mating ability of females irradiated with 20 and 25 kr doses of irradiation. In these studies both irradiated male and female moths were released to simulate a native PBW population in a cotton field. Results again indicated no significant difference between numbers of males attracted by either of the two groups of females (t-test with matched pairs, $t > 0.3397$).

Open cotton bolls. Photo taken near Snook, TX

Once the cotton crop emerged, open field releases of moths (both sexes), treated at 20 and 25 krad were conducted. This study also compared the male moths' ability to respond to pheromone baited traps but in the realistic setting of open cotton fields. Three studies were conducted in Blythe, CA (two releases) and Queen Creek, AZ (one release) during the span of the cotton season (May into September). Considered overall, there were no significant differences in the number of 25 vs. 20 krad male PBW recaptured (t-test with matched pairs, $P > 0.9349$). 25 krad is now the standard irradiation treatment at the PBW Rearing Facility for the PBW Eradication Program.

Development of a new active ingredient in bait and evaluation of a new formulation of the traditional active ingredient in bait for controlling grasshopper and Mormon cricket on rangeland*

R. Nelson Foster, K. Chris Reuter, Lonnie R. Black and Larry E. Jech

Abstract. Baits have a long and useful history in grasshopper and Mormon cricket control. Developing and testing new products, such as Agrisel's Carbait 5 and Wilbur-Ellis's (W-E) Stiletto, is necessary as part of an ongoing effort to provide tools needed to mitigate rangeland pests. The new carbaryl bait, Carbait 5, produced high levels of mortality against *Phoetaliotes nebrascensis*, placing it in the susceptible category and indicating the bait should perform as well as other formulations of carbaryl bait currently used in the USDA (2002) sponsored Rangeland Grasshopper / Mormon Cricket Suppression Program. The new active ingredient, chlorantraniliprole, showed activity against grasshoppers, although mortality occurred slower than with carbaryl baits and at significantly lower levels of control occurred on adults compared to 5th instars. It is also noted that formulated, tested and then stored chlorantraniliprole bait appears to produce lower mortality in this test. It is still to be determined if: 1) the final dose of chlorantraniliprole bait should be increased; 2) the interval of time the bait must be available for acceptable control should be increased. Another toxicant, permethrin, does not appear to be acceptable in bait formulations.

Figure 1. Spreader mounted on ATV for testing.

Introduction. Baits have been utilized for grasshopper control in the Unites States since 1885 when wheat bran bait containing arsenic, sugar and water was used in the San Joaquin Valley of California (Coquillet 1886). Since then baits have evolved considerably, relying on combinations of numerous toxicants and carriers in wet and dry formulations (Foster 1996). As a result of inexpensive and highly effective insecticide sprays developed in the 1940's, large scale use of baits had generally disappeared by 1950. However, baits based on wheat bran and carbaryl remain an important tool to control grasshoppers and Mormon crickets today (Foster et al. 1979). While lower concentrations of active ingredient (AI) and application rates are now used, no major changes in the bait components used for grasshoppers and Mormon cricket has occurred since the late 1970s. Even though additional carriers have continued to be evaluated as alternatives to wheat bran, over the years none have been shown to be superior. Bait formulations today still rely on carbaryl as the active ingredient and are based on wheat bran, apple pumice, food waste or other carriers (Foster et al. 2006).

A concern for the long term availability of carbaryl based baits has renewed the interest in finding environmentally and economically acceptable alternatives. The following study was conducted to evaluate a new formulation of carbaryl bait for activity and to develop the lowest effective dose of a new AI, Coragen, in a bait formulation for grasshopper and Mormon cricket control on rangelands.

Objectives

1) Evaluate a new formulation of carbaryl bait (Carbait 5–Agrisel USA Inc.) for activity and utility against grasshoppers and Mormon crickets on rangeland.

2) Compare new bait formulations against existing standard carbaryl baits for efficacy, speed of mortality and overall utility.

3) Develop the lowest effective dose of Coragen in a bait formulation for grasshopper and Mormon cricket control on rangelands.

4) Determine if new baits elicit species specific varying levels of susceptibility (sensitive, vulnerable, non-susceptible) similar to existing wheat bran, apple pumice or food waste carrier based carbaryl baits.

Materials and Methods. Carbaryl-Agrisel Studies. Agrisel® Carbait (Agrisel, USA Incorporated, Suwanee, Georgia) was tested on a ten acre plot, in a field trial conducted on the Severson ranch near the town of Alexander, North Dakota. The Agrisel was applied at a target rate of 10 lbs. per acre using an ATV (Figure 1). The Agrisel Carbait 5 carrier is wheat and corn based material that is formulated at 0.05 lbs. AI per pound of carrier.

Results. Pre-count assessments of the population were carried out and applications made the following day. Table 1 shows species composition for pretreatment species list. The pretreatment population was substantial and damaging with 39 grasshoppers per m^2 counted before treatment. The population was composed of 2 susceptible species comprising more than 92% of the population with most of the population consisting of 5th instar through adult (≈51 %) at the time of census. Although the population was relatively old in the current field application trial, the material worked quite well. The treated plot was paired with a no treatment control. The plots were sampled shortly before the bait was

Table 1. Pre-count species and age structure for Carbait5 and untreated plots, McKenzie Co. North Dakota, 2012.

	1	2	3	4	5	M	F	Total
Agrisel Carbait 5								
Melanoplus femurrubrum	1	15	46	58	61	4	2	187
Melanoplus sanguinipes	0	0	3	17	74	13	12	119
Other	0	1	11	9	2	0	1	25
Total	1	17	60	84	137	17	15	331
Percent of Population	0.30%	5.14%	18.13%	25.38%	41.39%	5.14%	4.53%	
Untreated								
melanoplus femurrubrum	0	3	25	23	29	0	0	80
Melanoplus sanguinipes	0	0	10	15	24	4	5	58
Phoetaliotes nebrascensis	0	11	17	6	1	0	0	35
Other	0	3	8	8	13	3	8	43
Total	0	17	60	52	67	7	13	216
Percent of Population	0.00%	7.87%	27.78%	24.07%	31.02%	3.24%	6.02%	

applied and 1, 2, 4 and 7 days post treatment. On day 7 the resulting mortality was 81% indicating the Agrisel Carbait 5 is a good candidate for USDA/ APHIS/ PPQ treatments and following label directions will give good control, especially with susceptible species.

Chlorantraniliprole-DuPont studies

A 16 oz. cup trial was conducted in late May 2012 to determine the effect of aging on "in house" formulated Coragen baits. We tested previously formulated bait against a freshly formulated batch of bait using the same concentration and carrier. The objective was to determine if the active ingredient became less effective following a short storage period. If the bait loses efficacy with time, the combination of toxicant and carrier may give inconsistent results depending on the storage life.

The cup application rate was equivalent to a 10 lb. per acre field application. A high rate is used to ensure the grasshopper is able to find the bait in the cup or cage. The grasshoppers had been collected on the Apache San Carlos reservation and were late 4th and 5th instars and young adult *Melanoplus sanguinipes,* which are susceptible and provide a good bioassay for the baits. The chlorantraniliprole bait does not provide immediate or high levels of control with *M. sanguinipes* as seen with carbaryl based baits.

A second study was conducted with grasshoppers caged on grass planted in 4 inch square pots. This is meant to simulate a more natural system that offers a choice between forage and baits. In the first tests the aged chlorantraniliprole appears to be slightly more effective in the cups but slightly less effective when the bait is placed in a more natural situation where there is forage available. The difference is well within limits of experimental design and error. Chlorantraniliprole does not appear to be as readily accepted as carbaryl, as seen in other trials.

Bait cage studies in western North Dakota, 2012

Nine bait formulations were tested using late instar and early adult *Melanoplus femurrubrum*, a species known to be a readily controlled with bran and carbaryl baits. Cages were placed in a hayed meadow near the ten acre Carbait5 test. Five individual grasshoppers were placed in cages that were then treated with bait at a field equivalent rate of 10 lbs. (0.034 gm. per cage). The surviving grasshoppers were counted on one, two, three, seven, eight and nine days post treatment (Table 2).

These results are similar to other results for species that are known to be susceptible to carbaryl based baits. The lower levels of control seem to indicate that the combination of toxicant and carrier were not effective. The W-E 2% may require higher application rates to achieve desired levels of grasshopper population mitigation. The amount of toxicant in the Coragen 0.25% bait may not be at a high enough concentration to provide adequate control. The permethrin toxicant at the dose provided does not appear to be suitable for grasshopper control.

Studies of *Phoetaliotes nebrascensis* response to carbaryl baits.

Phoetaliotes nebrascensis were exposed to Agrisel Carbait 5, Ecobait 5% active ingredient level in 16 oz. cup cages. The two baits produced equivalent mortalities over the course of the experiment. This places *P. nebrascensis* in the susceptible category (Onsager, et al. 1996-1999). In another trial the *P. nebrascensis* were exposed to Ecobait 2% and two levels of Coragen, 0.25 and 2 oz., on flaky wheat bran. As seen previously, the Ecobait provided quick control while neither dose of Coragen gave adequate control. Studies conducted with *M. femurrubrum* in North Dakota with Coragen at these levels seemed to indicate the femurrubrum was controlled with the higher, 2 oz. dose, while the lower amount and aged Coragen appear to be less effective.

Table 2. List of entries in the cage bait trial near Alexander, North Dakota, McKenzie Co. 2012.

Bait	Toxicant	% Concentration	Carrier	7 Days Post Percent Mortality
Agrisel Carbait 5	Carbaryl	0.05	wheat + corn	90
Coragen 0.25%	chlorantraniliprole	0.25	wheat bran	73
Coragen 0.25% 'old"	chlorantraniliprole	0.25	wheat bran	60
Coragen 2.0%	Carbaryl	2.00	wheat bran	90
Ecobran 2%	Carbaryl	2.00	wheat bran	88
Ecobran 5%	Carbaryl	5.00	wheat bran	92
Wilbur-Ellis 5%	Carbaryl	5.00	food waste	86
Wilbur-Ellis 2%	Carbaryl	2.00	food waste	77
Wilbur-Ellis Stiletto	permethrin	0.05	food waste	58

It may also indicate that *P. nebrascensis* may be more able to sense the Coragen, and reject the bait combination. The higher dose did provide a slightly higher level of control.

Conclusions. Carbaryl-Agrisel Studies. Agrisel® was tested in a field trial conducted in McKenzie County and was found to produce acceptable control, quickly reducing the grasshopper population from near 40 grasshopper/m^2 to less than 8/m^2. This would result in a grasshopper population that while still substantial, is considerably less damaging than the population before treatment.

Cup Cage Trials 'new' vs. 'old' Coragen. It had been observed that Coragen formulated, tested and stored seemed to be less effective compared with freshly manufactured bait. Using *M. sanguinipes*, late instars and early adults in 16 oz. cups and soon thereafter in grass pots with caged grasshoppers resulted in no mortality higher than 50%. In one test, the 'old' Coragen was slightly better and in a test conducted on potted grass the results were reversed. These tests were conducted using the 0.25% dose which may not be a lethal dose at the concentration tested regardless of whether the bait was aged or fresh.

Field bucket cage studies. Agrisel provided very good control against *M. femurrubrum.* Its use and resulting field mortality against rangeland grasshoppers and Mormon cricket should not be different than other formulations of carbaryl bait currently used in the USDA sponsored rangeland grasshopper / Mormon cricket suppression program.

The new AI, Coragen, continues to show activity against grasshoppers. The doses of Coragen bait tested may be too low at the 0.25% level and may need to be increased to the 2% range. The interval of time bait must be available for acceptable control is still to be determined. It appears that the Coragen bait may lose potency if stored for long periods of time. This raises the question about how long and under what conditions the bait could be stored. Stiletto, a new product from Wilbur-Ellis, was tested in field cages and found to produce marginal mortality. It appears that the concentration of the active ingredient, permethrin, is too low or is a repellent. The remaining baits that use carbaryl as the active ingredient continue to perform on rangeland. Tests with *P. nebrascensis* and other species indicate that the individual species may be susceptible to carbaryl based baits but that other toxicants such as chlorantraniliprole may have limited utility under some conditions.

*A complete detailed report is available upon request from the CPHST Phoenix Laboratory.

Acknowledgements. The authors wish to thank Frances Wagner, Peacock Industries, Saskatchewan, Canada; Jim Glatzhofer, Wilbur-Ellis, Fresno, CA; David I. B. Vander Hooven and Dean Hoy, Endrus Processing LLC. Upper Sandusky, OH, for supplying untreated and carbaryl treated bait for comparison. Special thanks are extended to Dewey Murray who traveled, located and captured grasshoppers and Mormon crickets used in the study. Thanks are also extended to North Dakota USDA, APHIS, PPQ for mechanical support.

Cooperators

John D. Cantlon, DuPont Company, Lakewood, CO

Renae Heick, Agrisel Incorporated, Suwanee, GA

Bryan Isgrig, Wilbur-Ellis Company, Fresno, CA

Dewey Murray, USDA, APHIS, PPQ, Phoenix, AZ

References

Coquillet, D. W. 1886. Report on the locust of the San Joaquin Valley, California. Washington, DC: U.S. Commission on Agriculture Annual Report: 1885: 289–303.

Foster, R.N. 1996. Baits for Controlling Rangeland Grasshoppers: An Overview. In: Cunningham, G.L., and M.W. Sampson tech cords. Grasshopper integrated pest management user handbook. Tech Bull. 1809. Washington, DC: U.S. Dept. of Agriculture, Animal and Plant Health Inspection Service: II.11-1—II.11-2.

Foster, R.N. C.H. Billingsley, R.T. Staten and D.J. Hamilton 1979. Field cage tests for concentrations of carbaryl in a bait and its application rates for control of Mormon cricket. Jour. Econ. Entomol. 72: 295-297.

Foster, R.N. and K.C. Reuter. 1996-1999. Evaluation of Rangeland Grasshopper Controls: A General Protocol for Efficacy Studies of Insecticides Applied From the Air. In: Cunningham, G.L., and M.W. Sampson tech cords. Grasshopper integrated pest management user handbook. Tech-Bull. 1809. Washington, DC: U.S. Dept. of Agriculture, Animal and Plant Health Inspection Service: II.2-1—II.2-7.

Foster, R.N., K. Chris Reuter, Lonnie R. Black, Bruce Helbig, Lane Kozel, and Luke V. Schuetzle. 2006. Report. Field Cage Comparison of Wheat Bran, Apple Pumice and Food Waste Baits Containing Carbaryl Against Rangeland Grasshoppers Species with Varying Susceptibilities to Standard Wheat Bran Baits.. USDA-APHIS-PPQ-CPHST-DS&PMSL. 12 pp. Sept. 2006.

Onsager, J.A., R.N. Foster and Larry Jech. 1996-1999. Bait Acceptance by Different Grasshopper Species and Instars. In: Cunningham, G.L., and M.W. Sampson tech cords. Grasshopper integrated pest management user handbook. Tech Bull. 1809. Washington, DC: U.S. Dept. of Agriculture, Animal and Plant Health Inspection Service: II.12-1—II.12-5.

USDA. Rangeland Grasshopper and Mormon Cricket Suppression Program. Final Environmental Impact Statement – 2002. 2002. USDA, Marketing and Regulatory Programs, Animal and Plant Health Inspection Service.

Confirming an effective field dose of Chlorantraniliprole (Prevathon®) for control of rangeland grasshoppers*

R. Nelson Foster, Larry E. Jech, K. Chris Reuter, Lonnie R. Black, Scott P. Schell, Alexandre V. Latchininsky, Daryl Hill, Bruce Shambaugh, Justin Gentle, Boone Herring, Derek Asche, Clay Plamer and Codie Coon

Introduction. The broad set of conditions under which grasshopper control on rangeland is implemented requires several tools that function differently to address diverse situations. The kit must also be readily available, economically viable, easily applied and reliable. The current treatment options for USDA/APHIS/PPQ sponsored grasshopper suppression efforts on rangeland are carbaryl (Sevin XLR), diflubenzuron (Dimilin 2L) and malathion (Fyfanon ULV) sprays and several carbaryl baits. Both malathion and carbaryl have been used for almost 50 years and diflubenzuron since ca. 2000. There are other treatments registered for grasshoppers on rangeland. However, certain label restrictions including: high volume per acre requirements, different volumes for crops and rangeland, grazing restrictions as well as cost make them less attractive. Additional options are actively being sought.

A new class of insecticides may offer some alternative solutions for rangeland grasshopper control. Prevathon; active ingredient, (AI), chlorantraniliprole; Rynaxypyr–DuPont, a member of the anthranilic diamide class of insecticides that provides control through a novel target, the ryanodine receptor which releases stored calcium from the muscle's sarcoplasmic reticulum. This impaires the regulation of muscle contraction and has been shown to exhibit a 500-fold differential selectivity toward invertebrate, over vertebrate receptors (Cordova et al. 2006). Uptake is through ingestion of treated material, as are all other approved USDA-APHIS grasshopper suppression program treatments (Pfadt et al. 1970, Lloyd et al. 1974, Foster et al. 2008). Prevathon demonstrates translaminar activity and rainfastness. After feeding on treated foliage, insects exhibit rapid cessation of feeding, lethargy, regurgitation, muscle paralysis and typically death within 1-3 days. (DuPont, Coragen Technical Bulletin).

A preliminary evaluation of Coragen (AI = chlorantraniliprole) for field activity against grasshoppers on rangeland showed promise as an alternative treatment candidate. The initial replicated field dose study, 2011, with Coragen showed 80 to 98% control of grasshoppers on rangeland but did not perform in dose rank order as expected, and only one dose was evaluated in the popular and more economical reduced agent area treatments (RAATs) 50% coverage (Larsen and Foster, 1996; Lockwood et al. 2000; Foster et al. 2000, Foster et al. 2010, Jech et al. 2011). The following study was conducted to further develop a better dose mortality relationship for both 100% and 50% coverage treatments of Prevathon as a new treatment alternative for suppression and control of grasshoppers on rangeland.

Figure 1. Prior to treatment applications, gallon jugs are attached by pilot Daryl Hill to the spray boom for static calibration of the aircraft. Aircraft owned by USDA/APHIS/ PPQ, A&EO, Edinburg, TX

Objectives.

1. Determine the lowest effective dose of Prevathon for controlling rangeland grasshoppers.

2. Specifically evaluate aerial applications of 4.0, 6.0 and 8.0 fl oz of Prevathon in a total volume (TV) of 32 fl oz per acre for control of rangeland grasshoppers.

3. Compare efficacy resulting from 100% coverage and 50% Reduced Agent Area Treatment coverage applications, a combination of applying a reduced dose with less than total coverage application to produce a more affordable treatment while still significantly reducing the pest population.

4. Compare efficacy resulting from industry recommended diluent mixes and combined canola and (COC) crop oil concentrate, (COC; methylated soybean oil), diluents mixes.

5. Compare all Prevathon treatments to the current most frequent treatment of choice in APHIS sponsored rangeland grasshopper suppression control programs, Dimilin and Sevin.

6. Evaluate Prevathon for ease in mixing, calibration, operational aerial application and clean up.

Results. The population overall average instar age was 3.8, i.e. between third and fourth instar ideal time for rangeland grasshopper control treatment. Seven spp. made up 90 % of the population, all except, *Melanoplus sanguinipes*

Table 1. Species composition, percent, and age found in pre-count sweep samples for thirty-six (36) plots sampled near Newcastle, Wyoming, 2012.

Species	Immature instars					Adults		
	1	2	3	4	5	M	F	Total
Ageneotettix deorum	-	20	453	1,545	742	2	-	2,762
Trachyrhachys kiowa	21	74	560	608	131	-	-	1,394
Opeia obscura	41	216	622	254	5	-	-	1,138
Phlibostroma quadrimaculatum	101	150	237	297	60	-	-	845
Aulocara elliotti	1	8	38	90	395	220	50	802
Melanoplus sanguinipes	57	55	170	207	199	5	4	697
Phoetaliotes nebrascensis	152	90	26	-	-	-	-	268
other spp.	40	35	111	265	305	66	61	883
Total	413	648	2,217	3,266	1,837	293	115	8,789

were pure grass feeding species, Table 1. Based on the number and mix of species, it was felt that most of the hatch was over and the density would likely result in heavy damage if left uncontrolled. Aircraft and systems were calibrated capturing the control agents and adjusting pressure until the delivery rate was within 1% of desired application rate Figure 1.

Initial conditions were favorable for rangeland and grasshopper population growth and development. Rainfall recorded during the experiment amounted to about one-third of the seasonal rainfall. In addition, June and July were about 5°F and 4°F above expected and can be characterized as a developing drought.

Analysis of variance applied the pre-count densities indicate there was no population differences among the plots before treatments were applied. When the oneway analysis of variance, (ANOVA), was applied to the intervals of 7, 14 and 21 days, all intervals had significant results (see Table 2). When the untreated is included in the analysis, mean separations were found at each of the time intervals. Note that dropping the 4th Dimilin replicate and recalculating the means give overall results for Dimilin that are more typical of results seen in other trials with Dimilin. The 21 day interval gives final mortalities that are comparable to those seen elsewhere.

All Prevathon treatments provide significant reductions within seven days post treatment for both solid and RAATs applications. The Dimilin and Seven treatments also provided excellent control, both initially and over the time course of the experiment. Initial primary percent and adjusted mortality (Connin and Kuitert, 1952) from the Prevathon 8 fl oz per acre solid and the RAATs treatments at the final sample period was 99.2 and 91.5, the 6 fl oz rate solid application and RAATs applications were -100 and 92.3%, the 4 fl oz treatment, solid and RAATs was 99.6 and 86.9% respectively. The solid treatments produced higher mortality while the RAATs treatments ranged 8 to 15 percent lower mortality. It appears the RAATs application provides comparable and acceptable mortality over the same time course with a substantial increase in savings.

The Sevin SL and Dimilin 2L were applied as RAATs treatments on June 22rd and 24th against the similar pre-count grasshopper population densities. The Sevin XL treatment ultimately resulted in lower overall percent control compared to other Sevin XLR applications, under similar conditions. Other factors may have included poor forage condition at the time of the treatment due to drought, resulting in much of the applied material missing plant foliage and being deposited on bare soil. The final samples collected from the standard diflubenzuron were collected 19 days after the application but were analyzed as 21 days post application. These differences ultimately did not affect the outcome or conclusions.

Repeated measures designs takes advantage of longitudinal data collections from the same plots over time. Analysis of the adjusted percent mortality indicated that there were differences among all treatments applied (P=0.0047) in the way the grasshopper population responded to the insecticides. Combining the Dimilin and Sevin means and comparing them to all the Prevathon treatments (Prob. <0.0001) indicates that there is a strong difference between the standard treatments, and the Prevathon treatments produce mortality equal to the current materials used by USDA, APHIS, PPQ rangeland grasshopper control program.

Analysis indicates that there is no statistically significant difference among the Prevathon treatments (F= 0.91) and this can be extended to methods of Prevathon application regardless of dose (RAATs vs. solid, P = 0.34) but it is noted that there is a numerical difference between the two rates on the final sample date (≈12.3%). This leads to the conclusion that the Prevathon when applied properly should give consistent control at a given dose, and the low dose at which control begins to fail needs confirmation.

Prevathon Treatments 2012 versus Coragen Treatment 2011. Prevathon Treatments 2012 versus Coragen Treatment 2011. A comparison based on the oneway analysis of variance of the pre-count densities for plots near Miles City, MT (2011) and those near Newcastle, WY (2012) revealed no significant difference between the pretreatment grasshopper population densities change through the course of the experiments, and the grasshoppers' populations responded in a like manner to the overall environmental conditions in both locations. A comparison of results with 'bridging doses' indicate that the treatments between the two were the same. This is a positive result, indicating the chlorantraniliprole should give consistent results when used properly.

Prevathon treatments compared with standard treatments. Repeated measure design when applied to the all treatment data for both years and adjusted for the changes in the untreated population indicated there is a significant difference among the treatments (Prob>0.002). A comparison of the means for the current standard treatments (Dimilin and Sevin) contrasted with the Coragen plus Prevathon also detects a difference among the two groups (Prob>0.0001). This is not totally unexpected since there are three different modes of action for the treatments being assessed. A comparison of the chlorantraniliprole applied in 2011 doses against the doses applied in 2012 using the bridging doses of the same or similar amount of active ingredient. For example, the 2012 dose of Prevathon at the 8.0 and 6.0 fl oz application was similar to the 2011 dose of Coragen applied at 2.0 and 1.5 fl oz in the solid applications. Also, the Prevathon 8 fl oz RAATs compares well with the Coragen 1.5 fl oz as and Coragen 2.0 fl oz RAATs. When these are set up and analyzed, using the specified contrasts for direct comparisons show no significant difference (Prob> 0.34), a good indication that the results for both years followed the same patterns of mortality and the chlorantraniliprole and untreated grasshopper populations behaved similarly across years and will have a high likelihood of producing the similar results elsewhere.

Summary. Aerial applications using the 4.0, 6.0 and 8.0 fl oz of Prevathon in a TV of 32 fl oz per acre for control of rangeland grasshoppers has been found to be very effective, providing 87% control at the lowest dose and 99% control when applied in a solid blanket at the 4 fl oz dose. RAATs produces a more affordable treatment while still significantly reducing the pest population 9 to 12% lower mortalities than the same rate applied at 100% coverage. It is possible to lower the volume of carrier which may diminish the cost but carries the added risk of control failure due to inadequate coverage. The lowest reliable effective dose of chlorantraniliprole for controlling rangeland grasshoppers may not yet have been reached. Comparing these treatments to the current most frequent treatments of choice in APHIS sponsored rangeland grasshopper suppression control programs indicates that chlorantraniliprole should work as well as Dimilin and Sevin. It mixes well, calibrates readily, and cleans up nicely following application. The lowest dose applied will be found in the Prevathon RAATs 4 fl oz (3.05 g. AI) per acre treatment. At this dose the actual amount of material applied is about 43% of the diflubenzuron dose (7.09 gm. AI). This may not be the final low dose to be tested, and the TV applied may also be reduced in future studies and compared with other materials again serving as operational controls.

Table 2. Mean percentage reduction and control of rangeland grasshoppers aerially treated with selected doses of Prevathon and standard treatments used by USDA, APHIS, PPQ. Tests were conducted on private range near Newcastle, WY in 2013.

Treatment	Mean percent reduction[1]									
	7 Days				14 Days				21 Days	
Prevathon										
8oz Solid	96	a	b		99			c	99	a
8oz RAATs	80	a			95		b	c	91	a
6oz Solid	93	a	b		89		b	c	100	a
6oz RAATs	88		b		87		b	c	92	a
4oz Solid	95		b		99			c	100	a
4oz RAATs	61		b		79		b	c	87	a
Dimilin Std 1oz RAATs	21		b		42		b		85	a
Sevin SL 12oz RAATs	37		b		66	a	b		67	a

[1] A one-way analysis of variance was conducted on the data. Means in column followed by the same letter are not significantly different (P ≤ 0.05). Adjusted data were determined by changes recorded in untreated populations (Conin and Kuitert 1952).

*A complete detailed report is available upon request from the CPHST Phoenix Laboratory.

Cooperators

John D. Cantlon, DuPont Company, Lakewood, CO

Bruce A. Shambaugh, USDA, APHIS, PPQ Cheyenne, WY

Boone, Herring, USDA, APHIS, PPQ, Cheyenne, WY

Justin A. Gentle, USDA, APHIS, PPQ Cheyenne, WY

University of Wyoming Laramie, WY

Bob Stoddard, Weston County, Wyoming rancher

Tuff Streeter, Airport Manager, Newcastle, Wyoming

Dean McClain, Ag Flyers, Torrington, WY

Steve Brill, Torrington, Goshen Co. Weed & Pest Control District, Torrington, WY

Hale Redding, Weston Co. Weed & Pest Control District, Newcastle, WY

Acknowledgements

The authors wish to thank John D. Cantlon, DuPont Company for supplying the material used in the test. Thanks are also extended to Tuff Streeter, Newcastle, Montana Airport Manager, for providing logistical assistance at the airport. Special thanks are extended to rancher Bob Stoddard for providing the rangeland for the experiment and for his support and cooperation during the study. Also Hale Redding, Weston Co. and Steve Brill, Goshen County Weed and Pest Control for their assistance with locating local cooperators, pesticide storage, sample storage and container disposal.

References

Connin, R.V. and L. C. Kuitert. 1952. Control of the American Grasshopper with Organic Insecticides in Florida. J.Econ. Entomol. 45: 684-687.

DuPont, Technical Bulletin . DuPont Coragen Insect Control

powered by RYNAXYPYR. Dupont Coragen Insect Control

Cordova, D., E.A. Benner, M.D. Sacher, J.J. Rauh, J.S. Sopa, G.P. Lahm, T.P. Selby, T.M. Stevenson, L. Flexner, S. Gutteridge, D.F. Rhoades, L. Wu, R.M. Smith and Y. Tao. 2006. Anthranilic diamides: A new class of insecticides with a novel mode of action, ryanodine receptor activation Pesticide Biochemistry and Physiology. Volume 84, Issue 3, March 2006, Pages 196-214.

Foster, R.N., K. Chris Reuter, Lonnie R. Black, Cheryl Huddleston, Daryl Hill and Tim Roland. 2008. Manner of pick-up of diflubenzuron by Ageneotettix deorum and Cordillacris occipitalis (Orthoptera: Acrididae) from aerially sprayed rangeland Report. USDA, APHIS, PPQ, CPHST Phoenix Lab. Phoenix, Arizona.7pp.

Foster, R.N., K.C. Reuter, K. Fridley, D. Kurtenbach, R. Flakus, R. Bohls, B. Radsick, J. B. Helbig, A. Wagner, and L. Jech. 2000. Field and economic evaluation of operational scale reduced agent and reduced area treatments for management of grasshoppers on South Dakota rangeland, 1997-1999. 40pp. In CD-ROM.

Foster, R. Nelson, Larry E. Jech, K. Chris Reuter, Lonnie R. Black and Daryl E. Hill. 2010. Preliminary field evaluation of aerially applied Coragen for control of grasshoppers on South Dakota rangeland. Report 10 pp. U. S. Dept. of Agriculture, Animal and Plant Health Inspection Service, PPQ, CPHST Phoenix Laboratory. Phoenix, AZ.

Jech, Larry E., R. Nelson Foster, K. Chris Reuter, Lonnie R. Black and Daryl Hill. 2011. Determination of effective field dose of chlorantraniliprole (Coragen) for control of rangeland grasshoppers. Report. 12pp. U. S. Dept. of Agriculture, Animal and Plant Health Inspection Service, PPQ, CPHST Phoenix Laboratory. Phoenix, AZ.

Larsen, J. and R.N. Foster. 1996-1999. Using Hopper to Adapt Treatments and Costs to Needs and Resources. In: Cunningham, G.L., and M.W. Sampson tech cords. Grasshopper integrated pest management user handbook. Tech Bull. 1809. Washington, DC: U.S. Dept. of Agriculture, Animal and Plant Health Inspection Service: II.6-1—II.6-6.

Lloyd, J. E., R. E. Pfadt, and M. Ali. 1974. Manner of pick-up of ULV carbaryl by Aulocara elliotti from aerially sprayed rangeland. Jour. Eco. Ent. 67: 596-597.

Lockwood, J.A., S.P. Shell, R.N. Foster, C. Reuter, and T. Rachid. 2000a. Reduced agent-area treatments (RAAT) for management of rangeland grasshoppers: efficacy and economics under operational conditions. Int. Jour. Pest Manag. 46: 29-42.

Pfadt, R. E., J. E. Lloyd, M. Ali, and G. Sharafi. 1970. Manner of pickup of ULV malathion by grasshoppers from aerially sprayed rangeland. Journal of Economic Entomology. 63:1210-1214.

Continued operational scale evaluation of polyacrylamide polyvinyl polymer complex (Wilbur-Ellis EDT Concentrate®) as a replacement for oil diluents in Dimilin spray mixes to control rangeland grasshoppers*

Larry E. Jech, R. Nelson Foster, K. Chris Reuter, Lonnie R. Black, Daryl E. Hill and Dave Hirsch

Introduction. USDA/ APHIS/ PPQ sponsors and conducts, under certain criteria, cooperative control programs against damaging populations of grasshoppers on rangeland (USDA, 2002). Treatments used in these programs have been selected for minimal environmental impact, effectiveness, low cost, reliability and ease of use. The number of specific active ingredients that have been used in APHIS programs has remained relatively stable over the last 40 years. However, the formulations have changed and use patterns have evolved as the amounts of active ingredients have been reduced through experimentation. Diflubenzuron was added as an option in 2000, and acephate was dropped as an active ingredient in 2002. Our goal is to identify and develop additional treatment options while striving to further improve the economics of current treatments.

The objectives of this study were to compare grasshopper mortality under operational conditions using different treatment mixes. These mixes included: Dimilin 2L applied at 73.1 ml. per ha as a reduced area agent treatment (RAATs) application, a simple mix of Dimilin with polyvinyl polymer complex concentrate 1.3% by volume, a drift management adjuvant and water to confirm results of an earlier study with the original USDA/APHIS/PPQ recommended rate of 2.26 l. total volume per ha mix of Dimilin, 73.1 ml., crop oil concentrate, 730.75 ml., and water, 1461.5 ml. and a mix of crop oil 29.2 ml., canola oil, 263.1 ml. and water 263.59 ml. as used by some Weed and Pest Districts in Wyoming.

Methods and Materials. The current diflubenzuron standard formulation (Dimilin standard), mixed and applied at 31 total fl. oz. is used by USDA/ APHIS/ PPQ for grasshopper and Mormon cricket mitigation on federal, private and tribal rangeland in the western United States of America

(See Table 1 for list of agents and adjuvants applied). Another widely used formulation recommends using diflubenzuron with canola oil and crop oil concentrate with water and a CPHST Phoenix proposed simpler mix using diflubenzuron plus the anti-drift agent, EDT, along with water. These formulations were applied using RAATs (Foster et al. 2001) method to non-replicated operational scale sections (260 ha) in western North Dakota in McKenzie Co. near the town of Alexander.

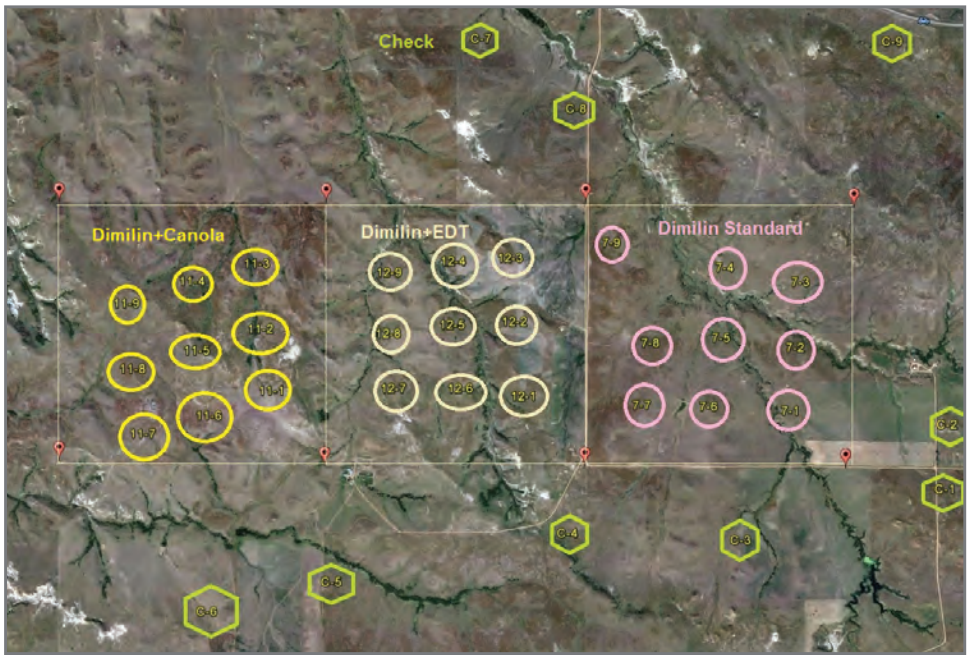


Figure 1. Satellite image of rangeland with location of survey sites near Sather Lake, McKenzie Co., North Dakota, 2012. Sites 7 (standard diflubenzuron) are on Severson ranch and Sites 11 (diflubenzuron with canola) and 12 (diflubenzuron with EDT) are on the Wambach ranch. Control (C) sites are located outside the treated sections

The study was conducted July 4-29, 2012 in McKenzie County of northwestern North Dakota ca. 17 km west and 22 km south of the town of Alexander, on the Severson and Wambach ranches which utilized mostly leased USDA FS National Grassland rangeland (Figure 1). The abundance of each species was determined from uniform sweep samples taken at each site consisting of 50 high and fast sweeps and 50 low and slow sweeps (Foster and Reuter, 1996). Nine sample sites were established in each block with nine untreated sites around the treated area. The age mixture was judged to be susceptible and corrective action would result in forage saved. The grasshopper infestation before treatment ranged from 15 to 23 grasshoppers per m2 over the 777 ha. Post treatment counts were conducted for 21 days and compared with untreated areas at least 0.4 km outside the perimeter of the treated area.

The grasshopper population data were expressed as percent mortality based on pretreatment counts in the same plot and were analyzed as such. Prior to analysis, the data was adjusted for the natural population change by the method of Connin and Kuitert (1952). An analysis of variance was performed on the primary data using Tukey's HSD multiple comparison test. The adjusted data were analyzed with a Kruskal-Wallis one-way analysis of variance.

All treatments produced mortality significantly greater than occurred in the untreated population (Table 2). Mean density in untreated populations was initially 15.7/m^2 and at the final sample date was 12.9/m^2. This was approximately an 18% decline in untreated grasshoppers due to natural population changes. After adjusting the data to account for this decline in the untreated population, the results indicate significant differences in levels of treated grasshopper mortality attributable to the treatments, were seen at 21 days post treatment, final mortality resulting from the treatments

Table 1. Volumes and adjuvants used in formulation of diflubenzuron for control of grasshoppers near Sather Lake, McKenzie Co., North Dakota, 2012.

Volume (ml.)				
diflubenzuron	Adjuvant 1	Adjuvant 2	Water	Total Volume
73.1	6.6 EDT[1]	-	797.2	876.9
73.1	730.8 COC[2]	-	1461.5	2265.3
73.1	29.2 COC	263.1 canola oil	8.0	949.9

[1]EDT is a polyacrylamide polyvinyl polymer complex

[2]Crop Oil Concentrate is a surfactant

Table 2. Results of treatments applied for grasshopper control at seven day intervals post treatment.

Mean percentage reduction[1]											
	7d				14d				20d			
Treatment	primary[2]		adjusted[3]		primary		adjusted		primary		adjusted	
Dimilin + EDT	65	a	61	a	80	a	78	a	87	a	86	a
Dimilin standard	72	a	69	a	82	a	79	a	88	a	86	a
Dimlin + Canola	68	a	61	a	87	a	85	a	93	a	92	a
Untreated	10	b			13	b			9	b		

[1]A Kruskal-Wallis one-way nonparametric analysis of variance was conducted on the data. Means in a column followed by the same letter are not significantly different ($P \leq 0.05$) as determined by an all-pairwise comparisons test. Adjusted data were determined by changes recorded in untreated populations (Connin and Kuitert 1952).

[2]Mortality unadjusted for untreated mortality

[3]Mortality adjusted using untreated population change

was not statistically different, ranging from 86% to 92% control. The Dimilin 2L used under operational conditions has produced mortality not significantly different than the formulation currently used by USDA/ APHIS/ PPQ to suppress damaging grasshopper and Mormon cricket populations on rangeland. Polyacrylamide polyvinyl polymer complex is used at a low rate (depending on the formulation). Without the need for crop oil concentrate the mix is simplified, the cleanup following application presents no issues, and the assigned signal word 'caution' raises no additional concerns.

*A complete detailed report is available upon request from the CPHST Phoenix Laboratory. Mention of companies or commercial products does not imply recommendations or endorsement by USDA over others not mentioned. USDA neither guarantees nor warrants the standard of any product mentioned. Product names are mentioned solely to report factually on available data and to provide specific information.

Cooperators

Dan Largent (Boise, ID) Chemtura Corporation, Boise Idaho

Bob Eccles (Twin Falls, ID), Bill Bagley (San Antonio, TX)

James Glatzhofer (Fresno, CA), Wilbur- Ellis

McKenzie County Grazing Association, McKenzie County, ND

Timothy Abing, Acting District Ranger, USDA, Forest Service, Little Missouri National Grassland, McKenzie District

C. Severson and K. Wambach, Ranchers McKenzie County, ND

Acknowledgements

The authors wish to thank Dean McClain, Ag Flyers Inc., Torrington, WY for providing information and assistance in duplicating the Canola and Concentrated Crop Oil diluents formulation in aerially applied Dimilin treatments used by some of the Wyoming Weed and Pest Districts. Thanks are extended to Sidney, MT airport manager, William Henderson, for his cooperation in equipment storage and other logistical assistance. Special thanks are extended to the Severson and Wambach ranches for providing leased and private rangelands used in this study. Thanks are also extended to Sidney, MT USDA/ ARS researcher, David Branson, for allowing treatments to occur within a quarter mile of an established grasshopper monitoring research site. A special thanks is extended to Timothy Abing, Acting District Ranger for facilitating the research on Forest Service administered National Grasslands.

References

Connin, R. V. and L. C. Kuitert. 1952. Control of the American Grasshopper with Organic Insecticides in Florida. J. Econ. Entomol. 45:684-687.

Foster, R.N. and K.C. Reuter. 1996. Evaluation of rangeland grasshopper controls: A general protocol for efficacy studies of insecticides applied from the air. In: Cunningham, G.L., and M.W. Sampson tech coords. Grasshopper integrated pest management user handbook. Tech Bull. 1809. Washington, DC: U.S. Dept. of Agriculture, Animal and Plant Health Inspection Service: II.2-1—II.2-7.

Foster, R. N., K. C. Reuter, K. Fridley, D. Kurtenbach, R. Flakus, R. Bohles, B. Radsick, J. B. Helbig, A Wagner and L. Jech. 2001. Field and Economic Evaluation of Operational Scale Reduced Agent and Reduced Area Treatments (RAATs) for Management of Grasshoppers on South Dakota Rangeland, 1997-1999. In: Branson, David H. and Bethany Redlin (eds.) 2001. Grasshoppers: Their Biology, Identification and Management. 1st Edition. CD-ROM. US Department of Agriculture. Agriculture Research Service/Animal and Plant Health Inspection Service/University of Wyoming.

Foster, R. Nelson, Larry E. Jech, K. Chris Reuter, Lonnie R. Black, and Daryl E. Hill. 2011 Operational scale evaluation of EDT Concentrate as a replacement for oil diluents in Dimilin spray mixes to control rangeland grasshoppers. Report. July-November, 2011. 11 pp. USDA, APHIS, PPQ, CPHST Phoenix Lab, Phoenix, Arizona.

USDA. 2002. Rangeland Grasshopper and Mormon Cricket Suppression Program, Final Environmental Impact Statement-2002. USDA, MRP, PPQ, APHIS. Riverdale, MD.

Chris Reuter, USDA/APHIS/PPQ CPHST Phoenix Laboratory classifies grasshoppers collected to determine species and age before and after treatment in the field. There may be as many as 50,000 specimens collected during a typical trial.

Evaluation of ground applications of selected domestic strains of pathogenic fungi on four hectare (10 acre) and mini plots for control of rangeland grasshoppers (Orthoptera: Acrididae) near Dagmar, MT 2012*

Larry E. Jech, Nelson Foster, Stefan Jaronski, K. Chris Reuter, Lonnie R. Black, Donald W. Roberts, Rob Schlothauer and Chad Keyser

Introduction. Grasshopper and Mormon cricket outbreaks are cyclical and can cause extensive rangeland damage that may require intervals that include biologically effective grazing management to fully recover, especially during drought. Federal programs to mitigate pest outbreaks rely on traditional pesticides including organophosphate, carbamates, and insect growth regulators that require buffers, untreated areas near environmentally sensitive areas. Biopesticides are pathogens recovered from soil samples that can be used like pesticides for insect control. Partnering with USDA/ APHIS/ PPQ Western Region, USDA/ ARS/ Northern Plains Research Laboratory, Utah State University at Logan, and USDA/ APHIS/ PPQ/ CPHST Phoenix Laboratory has facilitated the discovery, development, and testing of a native pathogen suitable for use on western rangelands for grasshopper and Mormon cricket outbreak mitigation.

Materials and Methods. Four hectare plots, embedded in the southwest corner of 16 hectare buffers were treated with commercial and experimental isolates to replicate earlier studies. *Beauveria bassiana*, *Metarhizium brunneum*, *M. anisopliae* and *M. robertsii* were applied to a native rangeland site east of Medicine Lake near Dagmar, MT. The application rate was $1x10^{12}$ spores per acre in 7.5 liters / ha, of water. An ATV-mounted spray system modified to apply low volume (LV) and ultra-low volume (ULV) treatments was used for the application (Foster et al., 2011).

The grasshopper population was sampled weekly post-application using forty $0.1m^2$ aluminum rings arranged in four rows of ten) each. After counts were recorded, representative sweep samples were taken around the ring site (Foster and Reuter, 1996). Treated and untreated vegetation and insect sweep samples were collected and taken to the Northern Plains Research Lab for laboratory evaluation. Treated grasshoppers, 0, 3 and 6 days post-treatment were held on fungi-free forage for mortality and later sporulation in the laboratory under optimal fungal growth conditions to determine if a lethal dose had been delivered and pathogens recovered. Untreated grasshoppers were placed on treated vegetation for 72 hours, removed, and then observed for subsequent mortality. All cadavers were held for sporulation as a test for causal agent.

Since it is known that a grasshopper's ability to thermoregulate affects fungal pathogenicity, temperature observations were collected inside and outside the cages to assure exposure to ambient conditions (Figures 1 and 2) while allowing caged grasshoppers to behave normally.

Figure 1. Grasshoppers on thermally neutral cage near Dagmar, MT 2012.

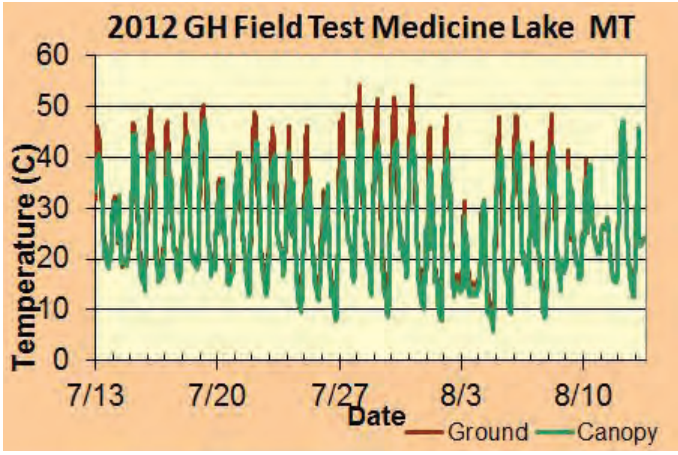


Figure 2. Results of temperature measurement within the cage using thermal surrogates.

Results and Discussion. Pre-count population ranged from 24.0 to 44.8 per m^2 with an overall average of 32.2 per m^2. The weather during application was clear to overcast with light, less than 16 kph, southerly winds. The field population of grasshoppers moved freely between the treated and untreated buffer (Foster et al., 2010). However, the long incubation period before onset of field mortality precluded definitive statistical analysis.

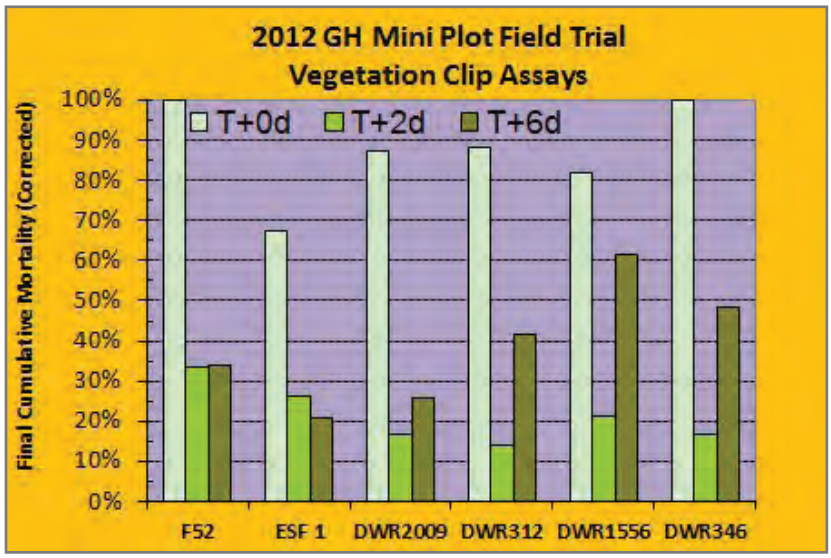


Figure 3. Bioassay result from grasshoppers exposed to forage treated with pathogens near Dagmar, MT

Mini-plots consisting of four replicated strips of pathogens were applied in a randomized plot design with four cages containing 5 grasshoppers each. This allows materials to be tested in a replicated trial. A problem with grasshoppers used to stock the cages precluded final analysis, but the experience gained will be used next field season.

Results from laboratory exposure indicate the vegetation samples collected immediately after application had received enough spores to induce high infection rates and subsequent mortality. Vegetation collected later resulted in lower infection rates, probably due to fungal pathogen inactivation due to ultraviolet light exposure (Figure 3).

Conclusions. Recent field work has provided improved application technologies and dealt with environmental, biological and behavioral factors that confound earlier experimental results and slowed development of biopesticides. Grasshoppers collected immediately after applications quickly developed a lethal infection, indicating the pathogens applied were infective in the field and secondary pickup is the main method of pathogen uptake. Subsequent forage samples did not sustain the high infection rates, indicating they were inactivated by field conditions, assumed to be due to ultraviolet light. Laboratory data based on vegetation and sweep samples collected before and after treatment has provided evidence that spores were infective at current application rates.

Acknowledgements. The authors would like to acknowledge project champions, Charles Brown, Bruce Schambaugh and Gary Adams.

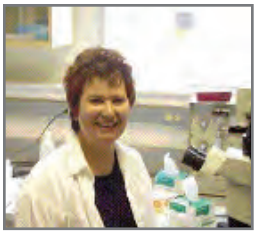
*A complete detailed report is available upon request from the CPHST Phoenix Laboratory.

Bibliography

Foster, R. N., Lonnie R. Black, K. Chris Reuter, Larry E. Jech and Dave Pierce. 2011. Design Enhancement of ATV mounted LV and ULV Liquid Sprayer for Grasshoppers and Mormon crickets on Rangeland. Report. June 2011. 13 pp. USDA, APHIS, PPQ, CPHST, Phoenix Lab, Phoenix, Arizona.

Foster, R. N. and K. C. Reuter. 1996. Evaluation of rangeland grasshopper controls: A general protocol for efficacy studies of insecticides applied from the air. In: Cunningham, G. L., M. W. Sampson tech. coords. Grasshopper integrated pest management user handbook. Tech. Bull. 1809. Washington, DC: U.S. Dept. of Agriculture, Animal Plant Health Inspection Service: II.2-1—II.2-7.

Foster, R. Nelson, Stefan Jaronski, K. Chris Reuter, Lonnie R. Black and Robin Schlothauer. 2010. Explaining Mycoinsecticide Activity: Poor Performance of Spray and Bait Formulations of Beauveria bassiana and Metarhizium brunneum Against Mormon Cricket in Field Cage Studies. Journal of Orthoptera Research, 19(2):B 303-313. 2010.



Nada Carruthers
Entomologist
Albany, CA
510-559-5790
nada.t.carruthers@aphis.usda.gov

Gericke Cook
Geographer
Fort Collins, CO
970-490-4489
gericke.l.cook@aphis.usda.gov

Tara Costanzo
Biological Science Technician
Fort Collins, CO
970-490-4474
tara.m.costanzo@aphis.usda.gov

Rich Hansen
Entomologist
Fort Collins, CO
970-490-4461
richard.w.hansen@aphis.usda.gov

Laura Hartmann
Biological Science Technician
Fort Collins, CO
970-490-4476
laura.a.hartmann@aphis.usda.gov

Lisa Jackson
Biological Scientist
Raleigh, NC
919-855-7549
lisa.d.jackson@aphis.usda.gov

Tom Kalaris
Modeling Coordinator
Fort Collins, CO
970-490-4462
tom.m.kalaris@aphis.usda.gov

Lisa Kennaway
Geographer
Fort Collins, CO
970-490-4463
lisa.f.kennaway@aphis.usda.gov

Craig Ramsey
Agronomist / Weed Scientist
Fort Collins, CO
970-490-4468
craig.l.ramsey@aphis.usda.gov

Amanda Redford
ITP Tool Developer
Identification Technology Program
Fort Collins, CO
970-490-4477
amanda.j.redford@aphis.usda.gov

fort collins laboratory staff

Julia Scher
ITP Resource Developer
Identification Technology Program
Fort Collins, CO
970-490-4465
julia.l.scher@aphis.usda.gov

Melinda Sullivan
Plant Pathologist
Fort Collins, CO
970-490-4469
melinda.j.sullivan@aphis.usda.gov

Lindsey Seastone
Biological Science Technician
Fort Collins, CO
970-490-4482
lindsey.m.seastone@aphis.usda.gov

Terrence Walters
ITP Coordinator
Identification Technology Program
Fort Collins, CO
970-490-4471
terrence.w.walters@aphis.usda.gov

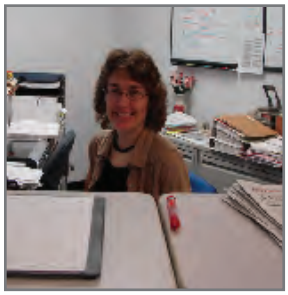
Patricia Smith
Laboratory Support Assistant
Fort Collins, CO
970-490-4464
patricia.m.smith@aphis.usda.gov

Rick Zink
Laboratory Director
Fort Collins, CO
970-490-4472
richard.t.zink@aphis.usda.gov

Christina Southwick
Biological Science Technician
CAPS Program
Fort Collins, CO
970-490-4473
christina.southwick@aphis.usda.gov

Fort Collins staff and on-site cooperators (Back Row, left to right): Lisa Martin, Terrence Walters, Rick Zink, Todd Gilligan, Melissa Tracy, Tara Costanzo, Patricia Smith, Rich Hansen, Amanda Redford, Deena Walters. (Front Row, left to right): Pritish Chamania, Gericke Cook, Debra Newman, Melinda Sullivan, Lindsey Seastone, Julia Scher, Sheryl Atkinson, and Lisa Kennaway. (Seated in front): Laura Hartmann. Not pictured: Nada Carruthers, Lisa Jackson, Tom Kalaris, Craig Ramsey, Luke Hartje, Lisa Peterson, Tyler Saxon, John Sheesley and Christina Southwick.

Lonnie Black
Biological Science Technician
Phoenix, AZ
602-431-3241
lonnie.r.black@aphis.usda.gov



Jane Larsen
Lab Support Assistant
Phoenix, AZ
602-431-3232
jane.b.larsen@aphis.usda.gov

John Claus
Biological Science Technician
Phoenix, AZ
602-431-3238
john.claus@aphis.usda.gov

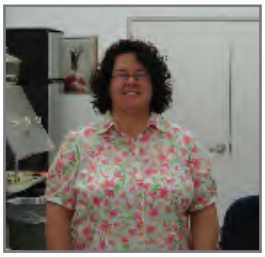
Anna Lowe
Biological Science Technician
Phoenix, AZ
602-431-3230
azlowe@aol.com

Nelson Foster
Entomologist
Assistant Laboratory Director
Phoenix, AZ
602-431-3245
nelson.foster@aphis.usda.gov

Nathan Moses-Gonzales
Biological Science Technician
Phoenix, AZ
602-431-3230
nmosesgo@me.com

Larry Jech
Supervisory Entomologist
Phoenix, AZ
602-431-3237
larry.e.jech@aphis.usda.gov

Chris Reuter
Biological Science Technician
Phoenix, AZ
602-431-3240
chris.reuter@aphis.usda.gov



Carey Laney
IT Specialist
Phoenix, AZ
602-431-3234
carey.laney@aphis.usda.gov

Henry Stewart
Maintenance Worker
Phoenix, AZ
602-431-3230
henry.l.stewart@aphis.usda.gov

phoenix laboratory staff

Guolei Tang
Biological Science Technician
Phoenix, AZ
602-431-3244
guolei.tang@aphis.usda.gov

Rick Zink
Laboratory Director
Phoenix, AZ
970-490-4472
richard.t.zink@aphis.usda.gov

Michelle Walters
Supervisory Entomologist
Phoenix, AZ
602-431-3233
michelle.l.walters@aphis.usda.gov

Phoenix Staff (left to right): Nelson Foster, Henry Stewart, Guolei Tang, Larry Jech, John Claus, Michelle Walters, Rick Zink, Carey Laney, Chris Reuter, Jane Larsen, and Lonnie Black. Not pictured: Anna Lowe and Nathan Moses-Gonzales.