CPHST LABORATORY FORT COLLINS 2007 ANNUAL REPORT

CPHST Fort Collins Laboratory 2007 Annual Report

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REPORT SUMMARY 2007 ANNUAL REPORT

This is an overview document that highlights the many diverse activities at this Plant Protection & Quarantine (PPQ) Laboratory. You should see this report as an attempt to provide a "snapshot" of our high visibility work. Our mission is to develop and transfer scientifically-based methods, innovative tools, and state-of-the-art technologies to PPQ and other state and federal agencies to reduce risk levels associated with new and established problem species. In 2007 the laboratory staff made advancements in three major areas; weed management, identification technology and pest detection and survey, as follows:

WEED MANAGEMENT- *Biological control of weeds using insects***.** Scientists documented Canada thistle rust mite population fluctuations over an entire growing season in northern Colorado. Native thistles were also sampled for mite occurrence. A study of bindweed mites indicates that, while they are able to initiate galls on some native bindweed species, other factors may prevent this mite from increasing to significant population levels. Endemic herbivores of hound's-tongue were cataloged, and *Diorhabda carinulata* (formerly *D. elongata*) continued to be redistributed. PPQ funded CABI Bioscience to conduct pre-release research on seven weed targets (Russian knapweed, hound's-tongue, yellow toadflax, hoary cress, garlic mustard, dyer's woad and perennial pepperweed). Artificial diets for several potential biocontrol insects were also investigated in 2007.

WEED MANAGEMENT- *Biological control of weeds using pathogens***.** Eighteen field sites were surveyed in CO and WY for the presence of diseased Canada thistle and perennial pepperweed and the pathogens were identified. *Alternaria cirsinoxia* was identified and was highly efficacious on Canada thistle. The fungus, however, also was shown to cause disease on sunflower and safflower, which will limit its usefulness in a biological control program. An unreported race of *Albugo candida*, a white rust pathogen, was identified through the perennial pepperweed surveys. Host specificity and efficacy studies with the Canada thistle and perennial pepperweed pathogens will continue in 2008.

WEED MANAGEMENT- *Chemical control of weeds***.** Field plots were used to evaluate three herbicides (Escort, Journey and Habitat) with two adjuvants; Escort had the best efficacy on common tansy. The potential of broadcast seeding for site restoration after herbicide treatment was investigated; seeding without any ground disturbance did not increase grass germination nor establishment rates. An herbicide and cover crop study for Benghal dayflower control in corn was also undertaken in 2007. Early season applications of herbicide followed by post-harvest cover crops showed promise and the potential for a second harvestable commodity. Herbicide tests on onionweed showed that Escort was superior to Telar and LandMark at onionweed control, with negligible injury to non-target vegetation.

WEED MANAGEMENT- *Geospatial technology.* The effectiveness of using remote sensing tools to map salcedar distribution over time was examined for three years at two different spectral resolutions. In addition, graph theory was used to examine the spatial arrangement and connectivity of four different major crops to enhance our understanding of pest movement across landscapes.

WEED MANAGEMENT- *Predicting invasions.* Using a unique set of standards and characteristics, including epigenetics, our lab will create a peer reviewed and effective predictive model for species' potential invasiveness previous to their introduction.

IDENTIFICATION TECHNOLOGY- *Identification tools***.** The Identification Technology team released five identification tools and conducted a total of 10 Lucid tool building workshops for over 75 participants at six locations across the U.S.The Robotic Automated Pest ID project successfully demonstrated a proof-of-concept, and is now being considered for five applications.

PEST DETECTION AND SURVEY- Survey and reference Guides. An update is provided on commodity-based reference manuals and survey guidelines for use by the Cooperative Agricultural Pest Survey (CAPS) program.

Biology of the Canada thistle rust mite

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Canada thistle, *Cirsium arvense* (Asteraceae), has become a widespread exotic weed throughout North America. More than 40 U.S. states have reported Canada thistle infestations, and as many as 3.2 million hectares are infested. Canada thistle occurs in annual and perennial crops, lawns and gardens, and in noncultivated habitats, including forests, pastures, rangeland, and riparian areas. In crops, Canada thistle causes economic losses by reducing yields and through resulting expenditures on chemical and cultural control; yield losses in crops can exceed 70%. In other habitats, *C. arvense* reduces the abundance of forage grasses and native plants through resource competition and, perhaps, allelopathy. Cattle production on thistle-infested pastures and rangeland is further reduced because livestock rarely consume the spiny weed and usually avoid areas with Canada thistle.

Canada thistle management options include cultural, chemical, or biological control techniques. Cultural and chemical tactics may be effective in some cropping systems but are rarely applicable in large wildland infestations. Biological control has provided large-scale, cost-effective management of perennial weeds when other management tools are not feasible. Since the 1970s, seven insects and a rust fungus have been deliberately or accidentally released as Canada thistle biocontrol agents in the U.S., but none are reliably effective, and several may have negative impacts on native plants. A potential new agent is the eriophyiid mite, *Aceria anthocoptes* (Eriophyidae), a European native that apparently was accidentally introduced into the U.S. Preliminary studies suggested that *A. anthocoptes* is host specific, and may be able to vector Canada thistle pathogens.

Aceria anthocoptes (**Fig. 1**) is a free-living mite that feeds on epidermal cells, primarily on leaf undersurfaces and inflorescences. Under laboratory conditions, feeding damage may lead to browning and curling of thistle foliage and plant stunting, but these symptoms are rarely seen in the field. However, almost nothing is known about the biology and host utilization patterns of *A. anthocoptes* under field conditions. Ochoa *et al*. (2001) documented *A. anthocoptes* populations in seven eastern and midwestern states, but its status in the western U.S. is unknown. Begun in

2005, my objectives in this project are to: (a) describe mite biology under field conditions in northern Colorado; (b) document potential utilization of native *Cirsium* species, and (c) locate mite populations in Colorado and adjacent states.

Figure 1. Aceria anthocoptes *(E. Erbe, USDA-ARS).*

Seasonal biology. Work was conducted at the "ARS farm" site, northeast of Fort Collins, CO, between early April and late November 2007, while living aboveground Canada thistle tissue was present. At one- to two-week intervals, ten Canada thistle plants were selected along arbitrary transects. Plant height, crown width, and flowering status were recorded during most sampling visits. Ten leaves and five flower buds or flowers (when present) were collected from each plant and returned to the laboratory. Vegetation samples were weighed and then processed to extract mites. Samples were washed for ca. 5 min in a 0.5% sodium hypochlorite solution, and the rinseate vacuum-filtered onto filter paper disks. Disks were examined under a dissecting microscope and all mites and other collected arthropods counted.

In 2007, *A. anthocoptes* was present from mid-May through mid-November at the ARS farm site (**Fig. 2**). Generally, mite populations exhibited a bimodal pattern in 2007 as in 2005 and 2006, with peaks occurring in July and September–October. Mites were found in appreciable numbers on Canada thistle plants beginning with bolting and continuing through the growing season until winter senescence; only a

 20 n O 120 140 160 180 200 220 240 260 280 300 320 100 Julian date

Figure 2. Mite abundance on Canada thistle at ARS farm site.

Mean no. mites (± SE)

Figure 3. Mite abundance on Canada thistle as a function of cumulative degree-days.

few mites were found on young thistle rosettes in spring 2007. Seasonal patterns in mite abundance are less clear when expressed as a function of cumulative degree-days in 2005, 2006, and 2007 (**Fig. 3**), suggesting that accumulated heat units are not the primary factor responsible for mite abundance patterns.

Within-plant distribution. On July 16, 2007, five large Canada thistle plants were harvested at the ARS farm site. Plant heights, maximum crown widths, and flowering status were recorded. Plants were then divided equally into upper, middle, and lower height levels. Each plant sample was further separated into leaf, flower, and stem / branch components. Each tissue sample was weighed and processed to extract and count mites, as described above.

Figure 4. Mite abundance on different parts of Canada thistle plants.

Mites were most abundant in the upper third, and least abundant in the lower third, of harvested plants (**Fig. 4**). *Aceria anthocoptes* was also more abundant on Canada thistle leaves than on stems/branches or flowers (there were very few leaves and no flowers in the lower parts of all sampled plants). Thus, leaves collected from the upper part of Canada thistle plants appear to be suitable sampling units for assessing mite populations.

Utilization of native *Cirsium* **thistles**. In 2007, mites were sampled from seven native thistles at 14 sites in northern Colorado (**Table 1**). At each site, leaf and flower samples were collected from five thistle plants, then weighed and processed to extract and count mites as described above. *Aceria* mites were collected from 6 of 7 native *Cirsium* spp. Mites collected from Canada thistle and native thistles are superficially very similar. However, it is not yet clear if mites collected from native thistles are, in fact, *Aceria anthocoptes* or, perhaps, another *Aceria* mite species. Mite samples are currently being examined by taxonomists in the U.S. and Europe to determine their identity.

Note: For additional information from this project, please see the 2006 Annual Report.

Table 1. Mean number of mites collected from native Cirsium *thistles and Canada thistle at sites in northern Colorado (RMNP: Rocky Mountain National Park, Larimer and Boulder Counties; PNG: Pawnee National Grassland, Weld Co.; DP: Douglas Pass area, Garfield Co.).*

Post-release assessment of native bindweed utilization by the field bindweed gall mite

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Introduction. Field bindweed, *Convolvulus arvensis* (Convolvulaceae), is an invasive weed native to Europe and Asia that was first recorded in North America in 1739 and has since spread throughout most of the U.S. Field bindweed is an aggressive perennial weed with an extensive root system, and can outcompete crops and other desirable forbs and grasses for moisture and nutrients. It is a pest in a variety of disturbed habitats, including pastures, overgrazed rangeland, annual and perennial agricultural fields, lawns, gardens, roadsides, and railroad rights-ofway. *Convolvulus arvensis* infests a wide variety of crops, and may cause yield losses of 45% to 100%.

Several herbicides may effectively control field bindweed in cropping situations, but control of established plants is often difficult and expensive due to the weed's deep and persistent root system. Cultivation may control field bindweed if it is repeated several times during a growing season over three or more years; this strategy is impractical in many infested habitats. Infrequent cultivation may actually increase field bindweed populations by facilitating vegetative reproduction. Classical biological control was recognized as a potentially useful management tool, beginning in the 1970s. Two biocontrol agents, a gall mite *Aceria malherbae* (Acari: Eriophyidae) and a defoliating moth *Tyta luctuosa* (Lepidoptera: Noctuidae), have been released in the U.S.

Only one native U.S. plant was included in the pre-release host specificity testing conducted in the 1970s, and several states have expressed concerns about possible nontarget

utilization by *A. malherbae* of native plants in the genus *Convolvulus* and the closely-related genus *Calystegia* (two and 16 native species, respectively, in the U.S.). The objective of this project is to determine if *A. malherbae* can survive and induce leaf galls on selected native bindweeds.

Methods. Test plots were established at the "ARS farm" site, northeast of Fort Collins. Seven plots were established in summer 2005 and 2006; replacement plants were added in spring 2007, when needed. Each plot consisted of 16 test plants, including three native species and field bindweed (control), each replicated four times and ran-

Figure 1. ARS farm bindweed test plot.

domly assigned to a planting location (**Fig. 1**). Four native bindweeds are represented in the various plots, depending on availability: Texas bindweed*, Convolvulus equitans* (occurs across the southwestern U.S., including Colorado); island false bindweed, *Calystegia macrostegia* (found in California); chaparral false bindweed, *Calystegia occidentalis* (occurs in California and Oregon); and Pacific false bindweed, *Calystegia purpurata* (found in California). Test plants were grown from seeds in the greenhouse prior to outplanting.

Each test plant was inoculated with mites by wrapping quantities of galled field bindweed stems (containing *A. malherbae*) around stems of the test plant. The amount of "inoculum" was arbitrary, but was generally proportional to the size of the plant. Plots were inoculated in September 2006; replacement plants were inoculated in August 2007. In late August and early September 2007, all plants were examined and relative gall abundance recorded using the following index: 0 (no galls); 1 (galls present – low density); 2 (galls present – moderate density); or 3 (galls present – high density). Five to 10 shoot tips were collected and returned to the laboratory, where they where examined

Figure 2. Frequency of Aceria malherbae *galling on test plants.*

Figure 3. Proportion of test plants with various galling scores.

Figure 4. Relative occurrence of live Aceria malherbae *mites on test plants.*

under a dissecting microscope to detect the presence of live mites. Sickly or dead plants were not examined.

Results. Galling was observed on all test plants, including all four native bindweeds. However, galling was much more frequent, and gall index scores were significantly higher, on *C. arvensus* than on native plants (**Figs. 2 and 3**). Living mites were observed on field bindweed and two native species (*C. macrostegia* and *C. purpurata*) but not on *C. equitans* and *C. occidentalis*. Interestingly, mites were more frequently observed on *C. macrostegia* and *C. purpurata* than on field bindweed, though no effort was made to quantify relative mite abundance (**Fig. 4**).

This experiment demonstrates that *A. malherbae* can survive and initiate limited gall formation on at least some native *Convolvulus* and *Calystegia* species when grown in a common garden with field bindweed. However, previous results from this experiment suggest that mites do not successfully overwinter on the native bindweeds, and their occurrence in 2007 may be a result of the 2007 inoculations and/or local movement from populations on field bindweed. Thus, low-level utilization of native bindweeds may occur only when these plants grow in close proximity to field bindweed on which *A. malherbae* populations persist.

A detailed report of the *Aceria malherbae* host utilization experiment will be submitted to California Department of Food and Agriculture (CDFA) in 2008. It is possible that additional replicates of these field garden plots will be established in spring 2008, using additional native plants.

Endemic herbivores of the exotic weed hound's-tongue in western Colorado

Hound's-tongue, *Cynoglossum officinale* (Boraginaceae), is a biennial or short-lived perennial forb native to eastern Europe and western Asia that reproduces by seeds. In the 1800s, it was accidentally introduced into North America and is now a widespread exotic weed. Hound's-tongue has been reported in more than 40 states, but is primarily a pest in the western U.S.; it is listed as a noxious weed in six western states. It invades disturbed, dry rangeland, pastures, roadsides, and abandoned crop fields. Hound'stongue reproduces by seed, and the barbed fruits are spread by humans and domestic and wild animals. The fruits themselves may cause skin and eye injuries to livestock and degrade the quality of wool. Hound's-tongue is also toxic to most domestic grazing mammals. The weed is an effective competitor for scarce water on dry sites and may displace native grasses and forbs.

Current management options for hound's-tongue include several herbicides that may be effective against small infestations but are rarely cost-effective over large areas. Properly-timed mowing may control very small hound's-tongue patches, but this technique is poorly suited to large infestations in inaccessible areas. Because of the logistic and ecological constraints presented by large wildland infestations, and the relatively low economic value of infested rangeland, biological control was acknowledged as a potentially viable and cost-effective management strategy for *C. officinale*, as it has been for other perennial weeds in stable, non-agricultural habitats.

Currently, no biological control agents have been released in the U.S., but PPQ is a partner in a consortium funding the discovery of, and pre-release research with, prospective biocontrol agents. The weed biocontrol research process often includes a pre-release assessment of endemic native or introduced herbivores that attack an exotic weed. This work may identify potential biocontrol agents that already have been introduced into the U.S., or endemic natural enemies that could themselves be promising biocontrol agents. Hound's-tongue natural enemy surveys have been conducted in Canada but not in the U.S. This project initiates a pre-release natural enemy survey at several sites in western Colorado.

Methods. Two sites were selected for study in the Douglas Pass area in northern Garfield County, Colorado. The

"lower" site was sampled on June 14 and July 25, 2007 and was located in a disturbed area frequented by grazing cattle; the "upper" site was located in a relatively undisturbed, open Douglas-fir–aspen forest habitat, and was sampled on June 14, 2007. Both sites had fairly large (>1 ha), contiguous hound's-tongue populations.

At each sampling visit, 20 plants were arbitrarily selected at 2-5 m intervals along a transect through the middle of the infestation. The height, crown width, number of stems, and flowering/fruiting status of each plant was recorded. The type and extent of insect feeding was noted (**Table 1**), and each plant was observed for 2 min to note any visible arthropods. Finally, each plant was shaken over a collecting tray and dislodged organisms were captured for subsequent identification. Five whole plants, including roots, were collected at each site for closer examination and dissection in the laboratory. When present, flowers and/or seeds were examined separately and then held in Berlese funnels to extract any arthropods.

Results. On June 14, plants at the "lower" site were mature and most (80%) had produced fruits, while those at the higher-elevation "upper" site were either in the rosette stage (35%) or starting to bolt (65%). The parental plants sampled on June 14 at the "lower" site were dead on July 25, and new, progeny rosettes were sampled on that date. No plants had bolted by July 25.

At least half the monitored plants had some visible insect feeding during all three sampling visits (**Table 2**). This damage consisted almost exclusively of small feeding holes, though one plant sampled at the "lower" site on June 14 had larger, irregular feeding; no skeletonizing or leaf mining was observed. Almost all of the feeding was very limited in nature, consisting of a few holes on (typically) basal, rosette leaves; four plants sampled at the "upper" site on June 14 had more extensive feeding. Samples collected from each plant have not yet been analyzed. However, flea beetle adults, Lepidopteran larvae, and grasshopper nymphs were among the arthropods observed on hound's-tongue plants during the three sampling visits, all of which could cause the feeding damaged recorded.

Figure 1. Powdery mildew on hound's-tongue leaves, Garfield Co., CO.

Dissections of whole plants revealed no internal ("mining") or external feeding damage on hound's-tongue leaves (aside from a few small feeding holes), stems, branches, or roots. No flower or fruit damage was observed on any plant. Flowers and seeds held in Berlese funnels produced a few thrips (common, generalist feeders on flowers), but no other arthropods.

Among the most common hound's-tongue "herbivores" observed was the powdery mildew fungus, *Erysiphe cynoglossi*, which produces characteristic white spots on foliage and stems (**Fig. 1**). While powdery mildew was not present at the "upper" site, two plants (10%) and 16 plants (80%) showed symptoms at the "lower" site on June 14 and July 25, respectively. *Erysiphe cynoglossi* has previously reported from the western U.S., Canada and Europe.

These preliminary 2007 results show that hound's-tongue experienced only minor feeding damage from endemic arthropods at the two sites. We do not yet know if this is typical among hound's-tongue populations in the region; we plan to sample herbivory at additional hound's-tongue sites in Colorado and, perhaps, adjacent states in 2008. A survey in British Columbia reported more damage from several foliar and root pathogens than was observed at these Colorado sites, though insect herbivory appears similar.

Distribution of *Diorhabda elongata* **for the biological control of saltcedars**

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Saltcedars, *Tamarix* spp. (Tamaricaceae), comprise a group of shrubs or small trees native to Europe and Asia that have become important invasive weeds of riparian and other habitats in the western U.S. Presently, saltcedars are estimated to infest more than 610,000 ha in more than 20 states. Saltcedar infestation results in the gradual elimination of native woody plants, forbs, and grasses. The creation of partial or total *Tamarix* monocultures reduce bird and mammal populations, increase the frequency and severity of wildfires, alter streamflow patterns, and increase flood risks. The major economic impacts of *Tamarix* infestations are based on water losses in the arid western U.S., since evapotranspiration from saltcedar stands is much greater than that from a comparable native plant community. A recent study estimated that saltcedar-based losses exceed \$100 million annually.

Management options for saltcedars include cultural strategies (e.g. burning, mowing, mechanical pulling, and root plowing) and herbicides. Generally, *Tamarix* suppression resulting from these treatments is temporary, even if treatments are repeated. Cultural and chemical tactics in most saltcedar infestations are expensive, labor-intensive, and limited to accessible areas. Both strategies may also have considerable negative impacts on nontarget plants and animals, soil and water quality, and stream flow. Biological control was recognized as a viable management strategy for invasive saltcedars. The first insect imported as a biocontrol agent in the U.S. is the Asian leaf beetle *Diorhabda elongata*1 (Coleoptera: Chrysomelidae). Research has demonstrated that *D. elongata* is a highly host-specific insect that may cause significant defoliation of saltcedar stands and, ultimately, saltcedar mortality while having little impact on native plant and animal communities. In 2003, USDA-APHIS-PPQ Western Region sought to begin general, implementation releases of *Diorhabda elongata* in selected states north of 38° N latitude. After some delays, PPQ issued release permits in July 2005, allowing field releases of *D. elongata* to begin.

In May 2007, newly-emerged *Diorhabda* adults were collected near Humboldt Lake in western Nevada (**Fig. 1**). Collected beetles were sorted and packaged by the Colorado Department of Agriculture (CDA) biological control laboratory in Palisade, and shipped to project cooperators

Figure 1. Collecting Diorhabda *adults in Nevada (May 2007).*

for field release. In May and June 2007, nearly 70,000 beetles were released at 28 sites in eight states (Idaho, Iowa, Montana, Nebraska, Oregon, South Dakota, Washington, and Wyoming). Four of the 2007 releases established new insectaries, while the remainder (n=24) augmented insectary sites established in 2005 and/or 2006.

Figure 2. Location and status of saltcedar leaf beetle insectary sites (2007).

From 2005 through 2007, 48 *Diorhabda* insectary sites have been established in 10 states (**Fig. 2**, **Table 1**). Based on 2007 data, half of the release sites – and 71% of sites whose population status is known – have established beetle populations (**Table 2**). Of the 34 sites whose establishment status is presently known, half (n=17) had a single beetle release and half had releases over two years. Establishment rates were the same (12 of 17, or 71%) whether single or multiple beetle releases were involved. Thus, a single beetle release appears adequate to establish *Diorhabda* populations at favorable locations.

Pre- and post-release monitoring of beetle impacts on saltcedar plants and possible effects on plant communities, continued in 2007 at most of the 48 sites. It is still too early to draw any conclusions from these data. However, at least five of the 24 sites with established beetle populations (21%) reported some visible saltcedar defoliation.

In 2008, *Diorhabda* and vegetation monitoring will continue at all 48 release sites. We are not planning additional beetle releases unless new states and / or agencies become involved in the program.

¹A recent taxonomic revision of the genus *Diorhabda* (January 2008) reclassifies the beetles used in the PPQ redistribution program as *Diorhabda carinulata*. This beetle is native to central Asia, including China and Kazakhstan.

Pre-release research and development efforts for PPQ weed biocontrol targets:

project updates

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Introduction. Before classical biological control can be implemented against an exotic weed target, potential biological control agents must be identified, studied for possible effectiveness, and screened for host specificity (i.e. their risk to nontarget U.S. plants is assessed). Prerelease research and development is conducted in the native range of the weed, typically in Europe and Asia. CABI Bioscience (Delémont, Switzerland) and the USDA-ARS European Biological Control Laboratory (Montpellier, France) conduct this work for PPQ weed biocontrol targets.

This report summarizes 2007 research and development efforts addressing seven weed targets identified by PPQ for which biocontrol agents have not yet been released in the U.S. All were identified during PPQ target canvassing efforts in 1997 (Russian knapweed), 2000 (hoary cress and garlic mustard) and 2005 (hound's-tongue, dyer's woad, perennial pepperweed, and yellow toadflax). This work has been conducted by the CABI Bioscience, though ARS is collaborating in pre-release research addressing hoary cress and Russian knapweed.

Russian knapweed, *Acroptilon repens* **(Asteraceae).** The initial biocontrol agent to be considered for U.S. release is the bud gall wasp *Aulacidea acroptilonica* (**Fig. 1**). A petition for U.S. release was submitted to PPQ and the Technical Advisory Group (TAG) in 2005, and TAG recommended approval for field release in March 2007. APHIS submitted an environmental assessment in November 2007 that will be published for public comment in early 2008. We are hoping that a PPQ permit will be issued in time for initial field releases of *A. acroptilonica* in Wyoming during summer 2008. In 2007, the *A. acroptilonica* colony that has been successfully established on potted Russian knapweed plants at the CPHST Mission (Texas) laboratory's quarantine facility was maintained and augmented by additional insects collected in Turkey and Uzbekistan. Insects from this colony will be used to initiate field releases once the agent is permitted for this purpose.

Host specificity experiments with the stem gall midge *Jaapiella ivannikovi* were completed by CABI in 2006; a petition for U.S. release was submitted to TAG in early 2007 and is currently under review. Galls containing this

Figure 1. Aulacidea acroptilonica *gall on Russian knapweed plant (CABI).*

insect were collected in Uzbekistan and sent to the Montana State University quarantine facility in August 2007 to establish a quarantine colony.

CABI conducted pre-release research with the root-mining moth *Cochylimorpha nomadana* in 2007 that will be continued in 2008. Host-specificity tests with the shoot-mining weevil *Lixus strangulates* in 2007 showed that it can successfully develop in safflower; thus, this insect will not be further considered as a potential biocontrol agent for the U.S. CABI also continued agent exploration efforts in Iran in 2007, and biological studies and host-specificity test with promising agents will continue in 2008.

Estimated year of first agent release in U.S.: 2008

Hound's-tongue, *Cynoglossum officinale* **(Boraginaceae)**. In 2007, CABI continued host specificity experiments with the most promising prospective agent, the seed-feeding weevil *Mogulones borraginis*, and U.S. *Cynoglossum* species and other native Boraginaceae. 2007 experiments concentrated on the native plants *Cynoglossum grande* and *Hackelia californica*. Despite some rearing challenges, this research can hopefully be completed in 2008, leading to submission of a field release petition to PPQ and TAG later in 2008.

Estimated year of first agent release in U.S.: 2009 or 2010

Yellow toadflax, *Linaria vulgaris* **(Scrophulariaceae)**. In 2006 and 2007, molecular and host-plant analyses confirmed that the stem-galling weevil previously known as *Rhinusa hispida* actually represents two species: *Rhinusa pilosa* on yellow toadflax (**Fig. 2**) and *R. brondelii* on Dalmatian toadflax. Host-specificity testing with *R. pilosa* continued in 2007; results showed that this weevil can oviposit and induce minor gall formation on several related North American plants, though utilization was much less than that on yellow toadflax. Only one native plant (*Sairocarpus virga*) supported very limited larval development.

Figure 2. Rhinusa pilosa *galls on yellow toadflax plant (CABI).*

Taxonomic and life history research with stem weevils in the genus *Mecinus* continued in 2007. Populations of the stem-mining weevil *M. janthinus* can be collected from yellow toadflax in Europe, and this appears to be a different insect than the Dalmatian toadflax-adapted strain now widely distributed in the U.S. Also, *Mecinus heydeni* is a stem-galling weevil that appears to be preferentially associated with yellow toadflax. Basic biology and host specificity research with *R. pilosa*, *M. heydeni*, and the yellow toadflax population of *M. janthinus* will be continued in 2008.

Significant taxonomic revisions among plants formerly included in the family Scrophulariaceae have occurred over the last several years. This has necessitated an overhaul of the test plant list used in host-specificity experiments with prospective yellow toadflax agents. A revised test plant list was begun in 2007 and should be completed in early 2008.

*Estimated year of first agent release in US***: 2010**

Hoary cress, *Lepidium draba* **(Brassicaceae).** The most promising prospective biocontrol agent is the stem-galling weevil *Ceutorhynchus cardariae*. Research to date shows that this insect may induce gall formation on several North American plant species, but only the target weed supports complete development. Host-specificity tests continued in 2007 and, hopefully, will be completed in 2008, after which a petition for US release will be submitted. Research on two other prospective agents, the stem-mining flea beetle *Psylliodes wrasei* and the seed weevil *Ceutorhynchus turbatus* continued in 2007, but work with these agents is still

Figure 3. Ceutorhynchus alliariae *adult (CABI).*

several years from completion; *C. turbatus* appears to be the most host-specific prospective hoary cress agent. Finally, CABI initiated host testing with the Russian rootmining weevil *Melanobaris* sp. in 2007 and will continue this work in 2008; this is the only known root feeder with potential as a hoary cress biocontrol agent.

Estimated year of first agent release in U.S.: 2009 or 2010

Garlic mustard, *Alliaria petiolata* **(Brassicaceae)**. Four weevils are under development as potential biocontrol agents in the U.S., with work conducted by CABI. In 2007, host-specificity experiments with the most promising agent, the root-mining weevil *Ceutorhynchus scrobicollis*, were completed and a petition for U.S. release was prepared. This petition will be submitted to PPQ and the Technical Advisory Group (TAG) in early 2008. Additional host specificity experiments were conducted with the stemmining weevils *Ceutorhynchus alliariae* (**Fig. 3**) and *C. roberti* and the seed weevil *C. constrictus* in 2007, emphasizing possible utilization of the native mustard *Rorippa sinuata*. In 2008, additional host-specificity tests with the

latter three weevils, along with *C. scrobicollis* rearing and field collection, are planned.

Estimated year of first agent release in U.S.: 2009

Dyer's woad, *Isatis tinctoria* **(Brassicaceae).** Biocontrol research and development efforts targeting this weed began in 2005. In 2007, CABI continued experiments examining the biology, host specificity, and impact of two promising agents, the root-mining weevil *Ceutorhynchus rusticus* and the stem-mining flea beetle *Psylliodes isatidis*. Research was initiated on additional prospective agents that were identified during exploratory collecting in Turkey, Russia, Kazakhstan, and Germany, including another rootmining weevil, *Aulacobaris licens*, and a second stemmining flea beetle, *Psylliodes sophiae*. In 2008, prerelease studies will continue with these insects as well as additional prospective agents identified during European and Asian collecting trips, including several seed feeders.

Estimated year of first agent release in U.S.: 2011

Perennial pepperweed, *Lepidium latifolium* **(Brassicaceae)**. In 2006 and 2007, exploratory CABI surveys for perennial pepperweed natural enemies were conducted in Russia, Ukraine, Turkey, and China. Among the first prospective biocontrol agents to be studied in 2007 are a root-mining weevil, *Melanobaris* sp.

Figure 4. Melanobaris sp*. on perennial pepperweed (CABI).*

(**Fig. 4**), a stem-mining flea beetle, *Phyllotreta reitteri*, a stem-galling weevil, *Ceutorhynchus marginellus*, a stemmining fly, *Lasiosina deviata*, and a leaf-spot fungus, *Septoria lepidii*. Basic biology studies and host-specificity experiments with these and perhaps other potential agents will continue in 2008, as will natural enemy surveys in the aforementioned countries and Iran.

*Estimated year of first agent release in U.S.***:** 2012

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

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Canada thistle (*Cirsium arvense*) is an aggressive, creeping perennial weed that competes with other plants for water, nutrients and space, reducing the grazing capacity and diversity of rangeland and natural areas (**Fig. 1**). This plant tends to establish in disturbed areas and in environments without continuous groundcover, but also occurs in other habitat types. Once plants are established, aggressive vegetative growth enables established populations to increase in density. Canada thistle reduces forage consumption in pastures and rangeland; for example, cattle typically will not graze within or near infestations. Additionally, Canada thistle may have an allelopathic effect on other plant species. Canada thistle is the primary common name for *C. arvense* in North America, but it may also be called field thistle or creeping thistle in other parts of the world.

Figure 1. Cirsium arvense *rosette and flowers.*

Cirsium arvense has become an introduced pest in North America, Australia, New Zealand, and South Africa. At present, Canada thistle may be found roughly between latitudes 37° N and 58° EN in North America. All Canadian provinces and about 40 U.S. states have reported *C. arvense* infestations. Nearly 3.2 million hectares (8 million acres) could be infested in the United States; the most significant infestations generally occur in the north-central and northwestern states, from Michigan west to Washington and south to Colorado and Nebraska. Canada thistle is a listed noxious weed in at least 28 states and is the most frequently-listed weed in the United States.

Cultural control tactics for Canada thistle include cultivation (tillage), mowing, burning, planting competitive plants, and grazing. Many of these options reduce Canada thistle populations in agricultural fields but are not suited for most other habitats. A variety of herbicides are currently labeled for use against the weed in most cultivated or uncultivated habitats: these include 2,4-D (and related materials), clopyralid, dicamba, and glyphosate. Due to logistical and environmental concerns, and growth habit (perennial with a deep and extensive root system), Canada thistle is difficult to control large wildland infestations chemically.

Biological control is currently being investigated to be included in an integrated pest management program for Canada thistle. Known biological control agents for Canada thistle include several arthropods: a stem and shoot gall fly *(Urophora cardui)*, a seed head weevil *(Larinus planus)*, a mite (*Aceria anthocoptes),* a stem mining weevil *(Ceutorhynchus litura)* and a foliage feeder *(Cassida rubiginosa)*. Little research, however, has focused on plant pathogens as potential biological control agents of Canada thistle. In Canada, 287 pathogenic fungi were isolated from Canada thistle. Seventy one endemic fungal isolates and one bacterial agent (*Pseudomonas syringae* pv. *tagetis*) were evaluated for biological control activity on Canada thistle. Eighteen isolates caused significant reductions in shoot emergence and root weight, and increases in chlorosis and/or death of Canada thistle, indicating that there may be potential endemic biological control agents already present on Canada thistle in North America. During surveys of endemic Canada thistle diseases in Montana, six genera of plant pathogens were collected (*Alternaria*, *Fusarium*, *Septoria*, *Puccinia*, *Sclerotinia*, and *Pseudomonas*). Only *Sclerotinia sclerotiorum*, which has a very broad host range, was selected for further study. The goal of this study was to survey Canada thistle patches for endemic pathogens and to assess their potential as biological control agents of Canada thistle.

In 2006 and 2007, 18 field sites in five counties of Colorado and Wyoming were surveyed for the presence of diseased Canada thistle (**Fig. 2**). A total of 115 plant samples dis-

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Figure 2. Plant Disease Survey sites in Colorado and Wyoming.

playing disease symptoms were collected, and plant pathogens cultured (where possible) and identified. A total of 5 fungal genera were identified including: *Alternaria* (**Figs. 3 & 4**), *Puccinia, Septoria, Fusarium, and Sclerotinia*. These results are similar to the genera identified in the Montana study described above.

Figure 3. Alternaria *symptoms on* C. arvense. *Figure 4.* Alternaria *symptoms on* C. arvense.

Currently, Koch's postulates (four criteria designed to establish a causal relationship between a causative microbe and a disease) are being conducted in the greenhouse to determine causality and efficacy. In host specificity testing on plants in the Asteraceae family, *Alternaria cirsinoxia* was highly effective on Canada thistle; *A.cirsinoxia* initially caused a foliar chlorosis, which then led to a foliar necrosis and plant death. However, sunflower and safflower, agronomically important members of the Asteraceae, also appear to be susceptible to *A. cirsinoxia*. Additional field visits are not planned. Inoculations with the other pathogens identified are currently pending for 2008.

Survey for white rust (*Albugo candida***) and other natural enemies of perennial pepperweed (***Lepidium latifolium***)**

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Perennial pepperweed (*Lepidium latifolium* L., **Fig. 1**), an introduced plant in the mustard family (Brassicaceae) from southeastern Europe and Asia, is invasive throughout the western United States. It is listed as a noxious weed in California, Colorado, Connecticut, Hawaii, Idaho, Oregon, Nevada, New Mexico, Utah, Washington, and Wyoming. The weed can establish in a wide range of environments and is a common problem in flood plains, irrigation structures, pasture, wetlands, riparian areas, roadsides, and residential sites. Perennial pepperweed can rapidly form large, dense stands that displace desirable vegetation. Populations easily spread along waterways and can infest entire stream corridors, riparian areas, or irrigation structures. Flooded streams often transport root sections from eroded stream banks causing new infestations to develop downstream. Once established, perennial pepperweed is persistent and difficult to control in crops, natural areas, and ornamental plantings. Perennial pepperweed also reduces forage quality in hay or pasture. The weed is now a serious pest on alfalfa farms, resulting in decreased cropland values and reduced sales potential.

Controlling perennial pepperweed is difficult once it is established. Seedlings are easily controlled by hand-pulling or tillage, but these techniques do not control established

plants because shoots quickly resprout from vast root reserves. Root segments as small as 1 inch are capable of developing new shoots. Perennial pepperweed is a prolific plant, capable of producing 6 billion seeds per acre. Mowing and burning are not effective at reducing pepperweed stands, but they are helpful at removing accumulated thatch. Current research focuses on the use of herbicides for pepperweed control. Several postemergent herbicides control perennial pepperweed, but repeat applications are usually necessary for several years to treat resprouting shoots and seedlings. In addition, there are few pesticides registered for its use over or near water, so herbicides can primarily be used only in dry areas. Currently, there are no biological controls available for perennial pepperweed, although the search is underway for biological control alternatives. Perennial pepperweed is in the same family as mustard and canola and there is concern that a biocontrol

*Figure 2. White rust (*Albugo candida*) on perennial pepperweed.*

insect or pathogen would attack agricultural crops. Perennial pepperweed was ranked number 11 on the Western Region's top biological control targets from their most recent biological control canvassing effort.

A white rust disease (**Fig. 2**) has been identified on the leaves of perennial pepperweed plants across the United States, especially during wet years, but the reports on the efficacy of white rust are quite variable. Some reports state *Figure 1. Perennial pepperweed.* That *Albugo* reduces seed set and number while others

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report that the white rust provides little or no control. The primary goal of this project is to evaluate the efficacy of white rust on perennial pepperweed and to survey for other endemic pathogens of the weed in Colorado and Wyoming at a number of field sites. This includes recording disease incidence and severity as well as collecting seed from diseased and healthy plants.

Determining which natural enemies are already present in the United States and assessing their current and potential impact on the target weed is a logical first step in developing biological control as a viable management option for perennial pepperweed. Eleven races of *A. candida* cause white rust on a range of hosts within the Brassicaceae. There have not been reports as to which race causes white rust of pepperweed. A second goal involves a race determination of the *A. candida* on pepperweed, comparing it with the races that cause disease on mustard and canola using a host differential. A host differential is a set of plant hosts that, on the basis of disease symptoms, serves to distinguish between various strains or races of a given plant pathogen.

In 2006 and 2007, 18 field sites in five Colorado and Wyoming counties were surveyed for the presence of diseased perennial pepperweed (**Fig. 3**). A total of 202 plant samples displaying disease symptoms were collected, their plant pathogens cultured (where possible) and identified. A total of 3 fungal genera were identified including: *Albugo, Peronospora*, and *Phyllostitca* (**Fig. 3**)*.* Identification is pending for an additional three fungal pathogens. Currently, Koch's postulates (four criteria designed to establish a causal relationship between a causative microbe and a disease) are being conducted in the greenhouse to determine causality and efficacy. Several native *Lepidium* species and crop species within the Brassicacae were grown to begin the preliminary stages of host specificity testing. Host differentials for the 11 races of *A. candida* (white rust) were grown and inoculated. Results indicate that the race of *A. candida* affecting perennial pepperweed is a new or unreported race. Results were negative for races 1 (radish), 2A & 2V (brown and condiment mustards), 3 (horse radish), 4 (shepherd's purse), 5 (*Sisymbrium officinale),* 6 (*Rorripa islandica*), 7A & 7V (canola), 8 (black mustard), 9 (crop mustards – cauliflower, broccoli, etc.), 10 (white mustard), or 11 (*B. carinata)*. Results of this study will be presented at the annual meeting of the American Phytopathological Society in July 2008. Additional field visits are not planned. Inoculations with the other pathogens identified are currently pending for 2008.

*Figure 3. Left: Downy mildew (*Peronospora parasitica*) on perennial pepperweed. Right: Phyllosticta leaf spot. Inset, upper left: Downy mildew.*

Second year results of three herbicides combined with two adjuvants for control of common tansy

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Common tansy (*Tanacetum vulgare* L*.*) is a perennial, invasive forb introduced from Eurasia as an herbal medicine. It is widely distributed throughout the U.S. and listed as noxious in Colorado and South Dakota. Approximately 125,000 acres are infested in South Dakota. Common tansy is seldom controlled with a single spray treatment. Enhanced herbicide treatments may allow native vegetation to regain dominance in the infested sites.

The objectives of this study were to: 1) evaluate the aboveground responses of common tansy and native vegetation to three herbicides and two adjuvants, and 2) evaluate the germination and establishment success of broadcast seeding for site restoration. Eleven treatments in a randomized complete block design were applied on June 22, 2005 at Terry Peak Ski Resort in Lead, SD. Three herbicides (Escort, Journey and Habitat) were applied alone or in combination with two adjuvants (MSO Concentrate and Stimupro). MSO Concentrate is a methylated seed oil applied at 10% v/v, and Stimupro is a seaweed extract applied at 0.16% v/v, and contains liquid NPK nutrients (3-3-3). At these rates, the cost is \$18, \$45, and \$48 / acre for Escort, Escort plus MSO Concentrate, and Escort plus MSO Concentrate plus Stimupro, respectively. Herbicides were applied with a $CO₂$ backpack sprayer fitted with a six nozzle boom and applied at 75.8 L (20 gallons) / acre. Data on percent cover of four vegetation classes (common tansy, grasses, forbs and bare-ground) were collected in Sept. '05,

June '06 and Sept '06. Common tansy stem density was randomly sampled with 0.5 m² quadrats in Sept. '05, Sept. '06, and June '07. Foliar biomass samples for common tansy, grasses, and forbs were collected in June '06 (12 months after treatment, MAT) and Sept '07 (27 MAT) from randomly placed PVC quadrats (0.5 m2). Vegetation monitoring plots (5.5 m x 12.2 m, or 18'x 40') were divided into two restoration plots (2.8 m x 12.2 m, or 9' x 40') for split plot, broadcast seeding (10,000 seeds / m2) on Sept. 27, 2005. Within one randomly selected main restoration plot, seeding by hand with a mix of five grasses and two clovers was performed. This grass / legume mix, recommended by the Natural Resource Conservation Service, included: 1) Canada wildrye, 2) Annual Ryegrass, 3) Pryor Slender Wheatgrass, 4) Pierre Sideoats Grama, 5) Lodorm Green Needlegrass, 6) Alsike clover, and 7) Purple Prairie Clover. The plots were not disturbed by tillage, fire, or mowing before seeding.

ANOVA analysis of the split plot seeding treatments at 12 or 27 MAT revealed no difference in grass foliar biomass between the seeded and unseeded sections of each plot. Thus, broadcast seeding without any ground disturbance does not increase grass germination or establishment rates for any of the herbicide / adjuvant treatments. The ski slopes were excessively stony, which prohibited seed drilling or even light disking of each half plot. The success of the broadcast seeding might have been greatly improved with

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any kind of ground disturbance so that the seed could come into direct contact with the soil. Additional analysis of the June 2007 common tansy density data show that Escort, combined with the two adjuvants, provided long term control of common tansy (**Fig. 1-4**). The Journey applications produced common tansy densities equal to or greater than the control plots. Also, visual evaluation of the Habitat plots revealed that other invasive species, such as houndstongue and plumeless thistle, were infesting these plots. Common tansy stem density changed only slightly when comparing herbicide only treatments (**Fig. 1**). This graph does show that the Escort applications combined with the adjuvants provided long-term control at least two years after application.

Oven dry, common tansy foliar biomass was 616 g·m2 for the Escort Only and 19 g·m2 for the Escort / MSO Concentrate / Stimupro application (27 MAT). The forb biomass was slightly higher for the Escort applications when compared to the control. Also, the grass biomass was significantly increased by the use of the adjuvants at 27 MAT. Escort applied at 70 g·ha-1 (1 oz \cdot acre-1) combined with 10% MSO Concentrate and 0.16% Stimupro resulted in increased grass vegetation. Foliar grass biomass was 237 g·m-2 for the Escort Only and 909 g·m-2 for Escort / MSO Concentrate / Stimupro application at 27 MAT. The selectivity of Escort resulted in long-term control of common tansy and increased grass vegetation, which is critical for erosion control on these steep ski slopes with high snow melt.

Figures 2-4, from left to right. 2) Control plot with average density of 111 stems/m² for common tansy and approximately 3 *– 5 ft tall at 24 MAT. 3) Escort alone with average density of 60 stems/m² for common tansy at 24 MAT. 4) Escort + MSO Concentrate followed by Stimupro with average common tansy density of 3 stems/m² 24 MAT.*

Reducing benghal dayflower seedbank populations with herbicides in corn, combined with post corn harvest cover crops

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Benghal dayflower (*Commelina benghalensis*, BDF) is semi-tolerant of glyphosate and is rapidly spreading due to the increased use of conservation tillage. Current herbicide treatments in cotton or corn do little to reduce BDF seedbank populations because of delayed germination and the short term soil residuals for Dual Magnum.

The study objectives are to: 1) determine the efficacy of corn herbicides on established BDF plants and germinating seedlings over a growing season, and 2) determine the inhibition of BDF seedbank germination following the planting of the cover crops velvetbean (*Mucuna deeringiana*), sunnhemp (*Crotalaria juncea*), sorghum / sudangrass (*Sorghum bicolor* x *S. sudanense*), and pearl millet (*Pennisetum glaucum*). This is an Integrated Weed Management (IWM) study that combines early season BDF control with herbicides and the late season BDF control from cover crop shading. The cover crops were planted after the corn harvest and allowed to grow until Nov 9, 2007. The shade from these cover crops may inhibit germination and growth of the BDF seedbank. Also, purported allelopathic effects of velvetbean and sorghum may also inhibit BDF germination in the second growing season.

Analysis of the herbicide and cover crop effects included the interaction between the two study factors. There was no interaction between herbicide and cover crop effects on BDF cover (*p*=0.9678) or stem density (*p*=0.7806). Neither BDF cover nor density was reduced by herbicide treatments when compared to the control plots at 6.5 months after ap-

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plication. These results indicate that delayed, mid-summer BDF germination is detrimental to repeated corn cropping systems. The herbicide soil residuals did not stunt the cover crop foliar biomass when compared to the biomass for the control plots. Further analysis of the cover crop data shows that the competition / allelopathic effects of the cover crops did not reduce BDF cover, but did reduce BDF stem density. The lack of cover crop effects may be due to the limited time between planting on Sept 10, 2007 and the final BDF sampling at 60 days after the planting. The competitive effects of the cover crops may have only taken partial effect 20 – 30 days after planting, leaving only 30 - 40 days for competition and shading to reduce BDF cover or stem density.

Cover crop foliar biomass was sampled at 60 days after planting. ANOVA analysis shows the biomass ranking was sudangrass > sunnhemp = pear millet > velvetbean (**Fig. 1- 3**). The rapid growth of sudangrass and sunnhemp, even when planted on Sept. 10, 2007, offers a potential second crop within a single growing season. Sudangrass is a forage grass with yields that range between 2721 kg - 4536 kg (3 - 5 tons) / acre of dry matter. It can be ready for harvest about 45 days after planting. Sunnhemp forage is not toxic, although other *Crotalaria* species can be to animals. 'Tropic Sun' sunnhemp produces about 2268 kg (2.5 tons) / acre dry matter and about 22.7 kg (50 lb.) of nitrogen per ton of dry matter, according to USDA- NRCS. A University of Florida study found that a 3-month-old sunnhemp crop produced about four tons / acre of dry biomass and fixed 81.65 kg (180 lb.) / acre of N. Another University of Florida study found that sunnhemp fixed 112.5 kg (248 lb.) / acre of N, compared to about 13.6 kg (30 lb.) / acre for sorghumsudangrass, a non-legume.

The long-term effects of the cover crops, including nitrogen fixation by sunnhemp and potential weed control from allelopathic root exudates from velvetbean and sudangrass will be measured on cotton growth. Cotton will be planted

across this site in 2008.

*Figures 2 & 3, from left to right. 2) Sunnhemp (*Crotalaria juncea*) at 60 days after planting. 3) Sorghum / Sudangrass (*Sorghum bicolor *x* S. sudanense*) at 60 days after planting. Corn was harvested on Sept. 9 and cover crops planted on Sept. 10, 2007. Photos taken on Nov. 9, 2007.*

Effects of Escort, LandMark XP, and Telar combined with Conquer on onionweed efficacy and injury to pre-established grass revegetation

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Onionweed (*Asphodelus fistulosus*) is an herbaceous perennial in the lily family (Liliaceae) that has become invasive in Australian rangelands. Clusters of long, tapering, round, hollow leaves very much resemble chives or scallions. Onionweed is an introduced ornamental that has escaped cultivation into surrounding ditch banks and road sides. It seeds prolifically and can establish dispersed, local communities quickly. Onionweed occurs in six counties in Arizona, six counties in California, two counties in New Mexico and a few areas in Texas. An herbicide screening study is needed to determine the efficacy and selectivity of herbicide active ingredients on onionweed.

The main objective of this field study was to determine efficacy of three herbicides (described below) applied at two rates (except for Escort). A second objective was to determine the mixing rate effect of the adjuvant Conquer (methylated seed oil with emulsifiers) on onionweed control. Conquer was applied at either 12% or 24% v/v. The addition of Conquer may help slow droplet evaporation rates expected for low humidity conditions found in southern Arizona. A second spray adjuvant, Stimupro (a seaweed extract containing the growth hormones cytokinnins, gibberellins, and auxins with liquid nutrients NPK 3-3-3), was added to all treatments. The combination of foliar nutrients and growth hormones in Stimupro may increase

photosynthesis, which increases sugar production and can increase the translocation of herbicides. Stimupro was applied at 0.15% (v/v), and the cost is \$2.14 / acre at a spray volume of 57 L (15 gallons) / acre.

The herbicides tested were: Escort (metsulfuron methyl) applied at 59.1 mL (2 oz.) / acre, Telar (chlorsulfuron) applied at 59.1 mL (2 oz.) and 88.7 mL (3 oz.) / acre), and LandMark XP (sulfometuron methyl and chlorsulfuron) applied at 88.7 mL (3 oz.) and 133.1 mL (4.5 oz.) / acre. Two separate, but nearby sites were selected to test Conquer, a commercially available adjuvant, applied at 12% or 24% (v/ v) when combined with the three herbicides. With a spray volume of 56.8 L (15 gallons) / acre, Conquer would cost \$28 / acre at 12% (v/v). In 2007, the cost of Telar was \$17.50 / oz., LandMark XP was \$8.88 / oz., and Escort cost was \$13 / oz.

The two study sites were located at a quarry in Tombstone, AZ. The treatments were applied on March 8, 2007 as a broadcast application. All plants were actively growing at time of application. The backpack sprayer and four nozzle boom had an application rate of 15 gallons / acre with a 9 ft. (3 m) spray swath. The weather was: 13° - $21.^{\circ}$ C $(55^{\circ}$ -68° F), 19% - 27% relative humidity, and a windspeed of 7 MPH. In each plot 12 onionweed plants were pin flagged at

Figure 1. Effect of Escort, Telar, and LandMark on pinned onionweed survival at 8 months after application.

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the base of each plant. Pinned and unpinned onionweeds were counted on November 27, 2007 for survival status. Only plants with completely brown foliage were considered dead; i.e. an onionweed with only a single, partially green stem was considered alive.

Onionweed survival for Conquer applied at two rates showed no difference in survival ($p = 0.7441$). Due to the lack of effects with increasing Conquer rates, all data was pooled across eight blocks and analyzed for herbicide effects on onionweed survival. Further analysis for the pinned onionweed plants shows that all three herbicides did reduce onionweed survival when compared to the control (**Fig. 1**). Increasing either Telar or LandMark rates did not improve onionweed control. Herbicide effects on the unpinned onionweeds mirrored the responses of the pinned plants. The initial data collection found a total of 458 onionweeds (pinned and unpinned) within the eight Escort plots. By Nov. 2007 we recorded that all 458 onionweeds were dead within the Escort plots, which suggests that Escort has a very high efficacy for this plant species (**Figs. 2 & 3**). These herbicides are generally labeled for non-crop use (see specific labels for permitted uses). The effect of the herbicides on pre-established grass cover varied, with Escort having no effect on cover, while LandMark decreasing grass cover.

*Figures 2 & 3, from left to right. 2) Live onionweed in the Conquer only plot in left photo compared to, 3) a dead onion*weed plant in an Escort plot in the right photo. Photos taken on November 28, 2007.

Experimentally quantifying the invasive potential of plants (EQuIPP)

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Through the EQuIPP assessment, we will develop a series of predictive models (Screening for Plant Invasiveness – SPI) based on biological plant traits that can be used by APHIS officials to screen proposed species for importation. The SPI models will also: 1) provide scientifically-based information for revision of Q-37 regulations, 2) provide a second tier screening method to evaluate species in the NAPPRA "further evaluate" category, 3) streamline the weed risk assessment process, 4) reduce trade barriers for importation of horticultural and nursery stock, and 5) safeguard the natural and agricultural ecosystems within the USA. The models will be scientifically based, specific to growth form, accurate, quantitative, repeatable, transparent, and peer-reviewed.

The EQuIPP project is based on the interactive PEA hypothesis (Propagate, Escape, and Adapt; see **Fig. 1**) which circumscribes the three main biological processes that determine the ability of a plant species to become invasive. The three processes are: 1) the ability to propagate and disperse; 2) the ability to escape predation and exploit resources, and 3) the ability to adapt and adjust. These three processes interact in that one process influences changes in other processes, which ultimately can lead to greater fitness in the new environment. For example, re-

Figure 1. The interactive PEA hypothesis.

lease from enemies allows species to invest less energy in herbivore and pathogen defenses and more into propagule production, increasing its chance for local adaptation, and ultimately, greater fitness in its introduced range. The interaction between the ability to propagate and disperse and the ability to escape predation and exploit resources has been well studied and does not adequately explain plant invasiveness. Our interactive PEA hypothesis is different from other hypotheses on invasion in that we incorporated the ability of plants to adapt and adjust to the environment.

The ability of a species to adapt and adjust is the most complex of the three processes, which allows rapid modifications in plant morphology, physiology, and/or phenology to survive, reproduce, and colonize under new environmental conditions. Mechanisms for adaptation and environmental adjustment include epigenetic inheritance, phenotypic plasticity and genetic variation. These three mechanisms are the primary adaptive routes that enable a species to alter its phenotype when exposed to a new environment and/or stressors. Phenotypic plasticity may result in a change within one generation due to environmental cues. Epigenetic inheritance involves phenotypic change within one generation that is passed to offspring for at least one generation. Genetic variation sometimes produces phenotypic

change between generations, but often requires ten or more generations before a new phenotype emerges.

Epigenetic inheritance involves the ability of a species to rapidly express multi-generational plant modifications to a new environment. Epigenetics is the study of heritable changes in gene expression and function that cannot be explained by changes in DNA sequence (Bossdorf et al. 2008). These epigenetic modifications are based on molecular processes that activate, reduce, or disable the activity of certain genes. Epigenetic modifications can be heritable**,** and are rapid adaptation mechanisms involving molecular activation or silencing of genes that allow a species to change its physiology, phenology, and morphological traits in response to stressful environmental conditions. Phenotypes can be transmitted to succeeding generations even though the original stimuli are ab-

WEED MANAGEMENT PREDICTING INVASIONS

sent. Epigenetic inheritance is extremely important for understanding the ecology of invasiveness. An introduced plant may appear to lack invasive traits until it is exposed to stress, which causes a silenced gene to be turned on, allowing the plant to become invasive. Furthermore, traits that are no longer necessary and energetically costly maybe silenced, allowing more resources to be channeled to invasive traits. Of several epigenetic inheritance systems, DNA methylation is the most well-known and widespread in the plant kingdom. At least two published reports describe the shift of noninvasive cultivars to an invasive variety. Both variegated wavy leafed basketgrass, a variety of the invasive wavy leafed basket grass (Pohl 1981) and the Red Baron variety of cogongrass (Dozier et al. 1998) provide evidence of reversions from a non-invasive type to an invasive type likely through epigenetic inheritance. Epigenetic inheritance can be quantified at the molecular level with methods that can detect DNA methylation, and can also be evaluated with clonal propagules (or any other genetically uniform plant material) by exposure to a chemical that demethylates genes, and hence turns on once silenced genes.

In contrast, phenotypic plasticity is the ability of a particular genotype to exhibit different phenotypes under a range of environmental conditions. The phenotype is dependent on the environment and is not passed on to succeeding generations when the original stimuli are absent. A phenotype is the physical expression of a trait in an individual, and plasticity is the ability of a single genotype to produce more than one phenotype, usually in response to environmental variables (Ward et al. 2008). Phenotypic plasticity can be quantified as an index if biological parameters are expressed as a ratio with the associated resource parameters (light intensity, soil moisture, nitrogen concentrations in soil, etc.,Valladares et al. 2006). Phenotypic plasticity can be quantified at the whole plant level by exposing a genotype to a range of conditions.

Genetic variation oftentimes is not expressed in the phenotype, and selection only acts on heritable phenotypes, not silent changes in genes. However, a plant's ultimate phenotypic possibilities largely depend on its underlying genes and control of their expression. Genetic variation can increase via several mechanisms: repeat introductions, sexual reproduction, recombination, hybridization, polyploidism, and genetic mutation. Genetic variation, especially random mutation rates, fails to adequately explain the rapid adaptation of many invasive species to widely different abiotic and biotic conditions.

In addition to investigating the ability of invasive plants to adapt and adjust, we will also examine their ability to escape predation, compete for resources and the ability to propagate and disperse. Traits that enable plants to propagate and disperse include the ability of a species to have high fecundity and germination rates, extended seed dormancy, and effective seed dispersal, such as avian/animal transport, windblown seeds, or attachment structures for sticking to fur and / or clothing. Traits that enable plants to exploit resources are critical for invading certain habitats. For example, specialized structures such as a long taproot, allelopathy, drought, shade or freezing tolerance, nitrogenfixing ability, unpalatable foliage for insects or animals, or high light/water use efficiency may have a competitive advantage against native species.

This spring we will be initiating several pilot studies to experimentally quantify genetic variation, epigenetic inheritance and phenotypic plasticity for several paired invasive and non-invasive species. We plan to measure a variety of biological traits, including relative growth rate, specific leaf area, days to flowering, etc. These traits will then be correlated to their treatment variables. Lessons learned from these studies will be incorporated into larger studies with more paired species (invasive vs. non-invasive) in 2009. Ultimately, we plan to develop a biological database of traits for 20 – 30 invasive and non-invasive congeners that can be used to develop models specific to growth form, or life history strategy, to predict plant invasiveness.

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Mapping saltcedar distribution at a biocontrol study site

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This study evaluated the effectiveness of using remote sensing tools to map the distribution of saltcedar (*Tamarix* spp.) using airborne collected hyperspectral image (HSI) data. Several data sets were evaluated, including 1 and 2 meter resolution data. Also, several approaches to mapping those distributions were evaluated within the imagery [processing software, ERDAS IMAGINE \(http://www.leica](http://www.leica-geosystems.com)geosystems[.com\).](http://www.leica-geosystems.com)

It is rare within PPQ that a biocontrol initiative has this type of data available for program planning and management. Developing a consecutive year dataset contributes to the ultimate goal of having a saltcedar distribution data series that program managers will use to evaluate the performance of the biocontrol treatment.

Study description and methods

For this project, three years of data were flown (2002, 2003 & 2004) and were received georectified and mosaiced from the data source. All proceeding data preparation was performed using ERDAS IMAGINE's Spectral Analysis Workstation (SAW). Prior to atmospheric correction, "bad bands" were identified and removed from the analysis. Bands 1-3 (420.7-450.3 nm) were removed based on input from data provided. Band 37 (37.0 nm) was also removed.

The HSI data were collected prior to this analysis and subsequent field work. This means that the degree to which saltcedar trees have changed during those years was unknown. Previous studies have indicated that several distinct saltcedar spectra may be present for a study site

(Laes et al. 2003). Unfortunately, it is difficult to accurately know which saltcedar trees were green and which were senesced during the time of image capture. However, Anderson and others (2005) state that drawing the HSI with a certain band combination can visually show areas of saltcedar defoliation (**Fig. 1**). Therefore, the HSI data were drawn with three bands centered at 777.4 nm, 548.4 nm and 669.9 nm, displayed in red, green and blue, respectively. Drawing the HSI in this way allowed for effective display of both healthy and defoliated saltcedar trees.

Training data for healthy saltcedar were developed from information acquired during a field visit in July 2005 and GPS survey data provided by the USDA ARS Sydney, Montana office. The leaf bearing saltcedar library contained 68 spectral signatures of saltcedar features, and spectral signatures were chosen to represent a variety of saltcedar features throughout the site. In addition, the spectral signatures of other common vegetation in the study site were considered when determining the training data.

The Target Detection approach and Constrained Energy Minimization (CEM) algorithm within the SAW was used in the final classification. This combination was chosen due to its ability to minimize confusion with other vegetation types. An accuracy assessment was performed for each year's healthy saltcedar tree classification using field data collected in September 2005. To ensure correct accuracy assumptions, several measures were evaluated from the error matrix. The results are as follows. All three years' classifications yielded at least 80% of reference pixels being correctly

Figure 1. False color CASI image taken on August 28, 2003 with 1 m ground resolution. Healthy saltcedar shown in red: defoliated saltcedar shown in greyish-purple.

classified. Overall kappa statistic ranged between 0.5605 for the year 2002 to 0.8941 for the year 2003. A value near 1 suggests that there is an almost perfect degree of agreement between the classified data and conditions on the landscape (Landis and Koch 1977).

Change detection

Change detection provides a quantitative analysis of spatial distribution by identifying differences in the state of a feature or phenomenon by observing it at different times. The 2002 and 2004 saltcedar data from the Lovell, WY site has identical spatial and spectral resolutions, and using change detection to compare these two years will help determine the impact of biocontrol efforts.

Overall saltcedar distribution area for the study site is similar between the two years, with 87.58 acres for 2002 and 86.39 acres for 2004, which suggests the overall saltcedar representation in the site is unchanged. However, closer examination of specific areas within the site suggest differently.

In September 2004, USDA ARS colleagues captured GPS data at the Lovell site representing areas where saltcedar defoliation was occurring. These polygons were compared to the change detection results for validation (**Fig. 2**). These polygons also show a saltcedar decrease, validating the conclusions of the distribution data.

In some areas, saltcedar distribution appears to be increasing. This is likely due to the growth rate and colonization of saltcedar, where in favorable conditions it can grow 9 to 12 feet in a single season. It is also interesting to note that the majority of saltcedar decrease appears to be occurring in the northern half of the study site. This area appears related to the initial release site in the central-western portion of the study site. One may suspect that prevailing wind patterns from west to east might contribute to this pattern. Further investigation of wind patterns in the area is required to validate this assumption. Many saltcedar areas in the southern half of the site show increased saltcedar or no change.

Conclusion

This analysis and comparison of saltcedar distribution for the Lovell, WY biocontrol study site provides results that are valuable to both scientists and program managers. In most cases, a biocontrol effort solely depends on field observations to quantify the impact of a biocontrol effort. While this is a crucial piece of any biocontrol monitoring and assessment, it cannot provide quantifiable "measures of performance" such as area impacted. Incorporating weed distribution mapping using remote sensing analysis compliments field observations and offers program managers more tangible information when evaluating a biocontrol program.

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Figure 2. An example polygon produced by the saltcedar project in Lovell, WY.

Assessment of the Connectivity of the American Agricultural Landscape Using Graph Theoretics

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Introduction. Approximately two-thirds of the cropland in the United States is planted to just four major crops, raising questions about how connected cropland and crops are across the country and how this connectivity might influence, aid or abet the transmission of an escaped plant pathogen or pest. This study uses graph theory to examine the extent of corn, soybeans, cotton, and wheat, their spatial arrangement, and the natural clusters and breaks which may not be readily apparent in widely-planted crops to enhance our understanding of pathogen movements through the agricultural landscape. The method by which this is done also supplies an undirected geospatial network that may be used to roughly model the movement of a pathogen or pest through that landscape.

Many factors influence the ability of a pathogen or pest to move through the landscape, not the least of which is the availability of host for propagation and use as reserve areas. Pathogens that can move easily even when host availability is thin can find a readily connected landscape through which to move, while movement for pests that require a high density of hosts may be limited to a few places in the country where farmers routinely plant the same crop from farm to farm. The threshold at which the density of the host determines whether or not a pathogen can or cannot

Figure 1. In landscape ecology graphs, separate habitat patches are connected via a network (graph), with nodes at the centroids of the patches.

spread further from infected hosts is key in assessing the connectivity of the landscape.

Methods. Landscape ecologists often use graphs to examine the connectivity of disparate habitat patches surrounded by landscape types considered worthless or hostile to an organism such as an endangered species. In these studies, the habitat patches are represented in the graph by a node, or meeting point, usually located as the centroid of the patch, and connected one to another by an edge, or straight line representing the movement the organism might make between the patches (**Fig. 1**). When an edge between two patches is considered too long or too costly for the organism to traverse, that edge is "dropped", or removed, from the graph to show clusters of edges that are disconnected from each other (**Fig. 2**). This length or

Figure 2. In dropped-edge analysis, edges between patches that are too distant or too difficult for an organism to traverse are dropped out of the graph, leaving subgraphs of clusters of habitat patches, showing disruptions in connectivity.

cost value represents the movement threshold for that organism. The breaks in the graph show landscape ecologists where gaps in habitat exist and where conservation efforts such as purchase or restoration of additional habitat might benefit the species.

For this study, the graph is modified to represent the continuous cropped landscape, with nodes placed at the centroids of counties, for which crop acreage is available through NASS, and the edges connect each county to

every other adjacent county, including those that are adjacent only at corners (**Fig. 3**). A value representing the cost of movement along each edge is calculated from the inverse of the weighted density of the crop or crops in question for each county through which the edge travels. Then different cost values were chosen for threshold values and edges greater than that value "dropped" to show how connected the graph is for each of those thresholds (for example, **Fig. 4**). Statistics for the graph examine the connectivity (gamma index), number of patches remaining (Percentage of Landscape (PLAND)), and patch/graph fragmentation (landscape division (DIVISION), **Fig. 4**).

Results. The graphs for four crops studied show a range of patterns of connectivity and fragmentation at different cost threshold levels that offer clues into the susceptibility and resistance of the landscape to pathogens and pests (**Fig. 5**). Corn and soybeans are densely planted throughout the Midwest and Mississippi Valley, so much so that the ability of a pathogen to move through a large area of the country can be achieved by even those that require higher densities in order to continue spreading. A good example lies in the soybean graph, where the Mississippi Valley hosts do not disconnect from the upper Midwest until a very low cost threshold of 5 is reached. Wheat, which is planted in a different spatial pattern and concentrated in the Central

Figure 3. An example of a graph (network) built for a matrix situation, with nodes at the centroids of geopolitical areas and edges connecting adjacent areas.

Plains, the Northern Plains, and in eastern Washington State, shows that the graph breaks down at higher cost threshold values (closer to 15 or 20) and is therefore a little less connected and more easily fragmented. Cotton, which is planted in discrete regions across the southern tier of states, naturally lies in clusters that appear at higher cost thresholds, representing greater hurdles to pests and pathogens attempting to move between them.

The gamma index for these four also bear out this conclusion. The gamma index for a graph lies between 0 and 1, with connectivity greater the closer the number is to 1. For corn and soybeans, the gamma index remains in the .7-.85 range for most of the threshold values, until very low cost thresholds like .5 or .6 are reached. For wheat, the index slopes from higher numbers around .7 to lower numbers around .4 in steps, and shows that wheat is indeed

Figure 6. A network of hosts susceptible to Copitarsia *spp. analyzed with the Service Area tool in ArcGIS. By using equal-cost-unit service areas, the analyst can get a sense of the amount of time it could take for the movement of a pest to occur.*

Figure 5. Gamma index, PLAND, and DIVI-SION statistics for the four major crops planted in the continental United States.

less connected. Cotton begins at even lower numbers, about .6, and also drops to around .4 at the lowest cost thresholds, demonstrating that connectivity is not as great in this crop. PLAND and DIVISION statistics offer similar insights into landscape connectivity and fragmentation.

Determining the thresholds at which a graph fragments or holds together can be information of value to those responding to, or attempting to prevent, the spread of an escaped pathogen or pest. If the cost threshold beyond which a pathogen cannot spread can be discovered in field studies on that pathogen, a map can be created showing those areas most susceptible to it and the extent to which it may be able to spread. For example, if a type of wheat rust appears in Kansas and it is known that it has difficulty spreading in areas with a cost threshold of .4 or less, the area on the map in **Fig. 5** in dark and light blue may be the only areas in the country susceptible to that rust. The infected area might be more effectively quarantined from other areas of the country that fall into that threshold range. Conversely, if a pest can easily maneuver through areas with a high threshold cost, it may become apparent to policy makers and emergency responders that large areas of the country are susceptible and nationwide response and policies may need to be put in place.

Because the graph developed for this study was generated as an undirected cost network in geographic information systems software, the tools for undirected networks can be used to roughly model the spread of a pest or pathogen from one or more infection/infestation locations. In **Fig. 6**, a network built from the availability of all hosts at risk to infestation by *Copitarsia* spp. Has been analyzed to show the spread of the insect from three points at the Mexican border. Entry by the moth at the Big Bend area of Texas allows faster spread into the Great Plains than from points further east along the Rio Grande River. While

approximate, this kind of analysis can suggest places where trapping might be conducted or new investigations into pest movement might be warranted.

REARING & DIET DEVELOPMENT

DEVELOPMENT OF ARTIFICIAL DIETS

Artificial diets and the Light Brown Apple Moth (LBAM)
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Project: Light brown apple moth colony for western region research and control efforts

On the recommendations of the California Department of Food and Agriculture (CDFA), the LBAM colony was developed by CPHST in the laboratory located in Albany, California. CDFA made two collections of LBAM larvae in the Santa Cruz area in September 2007. Larvae (~180) were divided into four groups to test different approaches in rearing. Larvae were placed on two different diets: diet used in Hawaii for LBMB, and extruded pink boll worm diet supplied by the CPHST lab in Phoenix, AZ. Larvae were also placed in the small cages supplied with either bean or French broom foliage. Pupae were obtained from all treatments and were sexed and placed in cages ready for adult emergence (**Fig. 1**). The best survival of field-collected

Figure 1. Light brown apple moth adult (Todd Gilligan, [www.tortricid.net\).](http://www.tortricid.net)

LBAM larvae was on plants and the Hawaii LBMB diet. Three species of parasitoids were collected from fieldcollected material: two from pupae and one from larvae. Dr. Robert R. Kula from the USDA Systematic Entomology Laboratory (Smithsonian Institution) identified the specimens as one species of Meteorus (Braconidae: Meteorinae) and one species of Pimplinae (Ichneumonidae). The third parasitoid species was a brachonid fly. Insect production was lower in the early stages of the colony and has increased to meet present and future research demands.

In the CPHST laboratory CDFA is performing LBAM egg host suitability tests for commercially available Trichogramma species; CPHST is providing space and support. In addition CPHST provided LBAM eggs and rearing suggestions for two UC institutions to start their own LBAM colonies as well as one USDA rearing facility.

Project: Development of artificial diets for beneficial root and stem feeders

Our studies in the last year involved four different agents for the biological control of weeds: *Liocleonus spp.* for saltceder; *Cyphocleonus achetes* for spotted and diffuse knapweed; *Hadroplontus litura* for Canada thistle; and *Hylobius transversovittatus,* for the control of purple loosestrife.

The great majority of efforts were dedicated to developing efficient rearing systems for *C. achetes.* The artificial diet is still a work in progress, but strides are being made to allow continuous production of this insect. This system has now been used through multiple generations; however, the diet and overall rearing systems still need significant improvements to make them practical, cost-effective, and to provide insects that are up to appropriate quality control standards. To facilitate diet improvement, a self-selection technique was developed and tested on two root weevil species. Results were presented on a poster at the Entomology Society of America meeting in San Diego in December 2007.

Work with *Liocleonus spp*. started by establishing a connection with cooperators in Europe, transferring resources, and reviewing literature. Sources of these weevils were identified and preliminary studies have been completed. The main difficulty in obtaining these agents is not in their collection, but in rearing them to adulthood, as it is difficult to transport entire root-masses of saltcedar. Work on this rearing system is planned to start in June of 2008.

The weevil *H. litura* was first released into North America in 1965 and is now established in Nebraska, Idaho, Montana, North Dakota, Oregon, South Dakota, Utah, Virginia, and Wyoming. Effectiveness studies of these stem and rosettefeeding weevils showed mixed results. *Hadroplontus litura* is commercially available and is regularly used in field releases. The objective of our preliminary study was to investigate the possibility of mass production of *H. litura* on an artificial diet to facilitate release efforts and reduce the costs of its production. Although no adult insects were produced on the offered diets, high survival after 8 days indicates that formulating an artificial diet for *H. litura* is feasible. For more conclusive results additional work is necessary.

Our studies with *H. transversovittatus* for the biological control of purple loosestrife is finalized. Technology has been transferred to three insectaries and production is on the way.

Identification Technology Program: Year in review

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Delivering Identification and Diagnostic Tools to PPQ and Cooperators

The entry, establishment, and spread of invasive pests are a constant threat to U.S. agriculture and native ecosystems. Globalization brings with it a wider variety of pests and new entry pathways due to increased importations and the ever-changing diversity of these imports. Rapid, accurate detection and identification of these pests before they can become established is a strategic objective for PPQ. The Identification Technology Program (ITP) team is dedicated to delivering to PPQ and its cooperators state-of-theart, technologically-based detection and identification tools to reduce pest entry, establishment, and spread within our borders. We design, develop, and deliver our electronic

Figure 1. Scale Insects, *a Lucid identification resource with interactive keys to scale families, mealybugs, soft scales, and other scales, was published on the Internet and on compact disc in 2007.*

resources and tools to be relevant to our diverse clientele who have varying levels of taxonomic knowledge and experience, a diversity of detection and identification responsibilities and needs, and are positioned off-shore, at portsof-entry, or within one of our 50 states.

Lucid Matrix-Based Identification Tools

Electronic matrix-based keys are an exciting, relatively recent identification technology that is fundamentally different from traditional, paper-based dichotomous keys; users can choose characters in any order, and can choose or ignore any character. The number of matrix keys produced worldwide is growing rapidly and may evolve to become the key format of choice for identifications.

CPHST has used Lucid**®** software to develop many matrix key-based identification tools ("Lucid tools") to help meet

PPQ's diverse identification needs. Lucid ([www.lucidcentral.org\)](http://www.lucidcentral.org) is the most widely used matrix key-building software, both nationally and internationally. A wealth of associated media, such as Html pages, drawings, photographs, and videos can be attached to the characters and taxa in Lucid tools, enabling them to be used not only for identification, but also for verification, information gathering, and as an image gallery. Including matrix keys as an integral part of a comprehensive web site (e.g., a site focusing on the study of a genus or specific commodity) enhances their value.

In 2007, the ITP continued to develop Lucid tools for use by both novices and experts located off shore, at ports-ofentry, and within our states, and to enhance and complement existing paper-

based and electronic identification resources. Tool development involves locating taxonomic experts to become authors as well as establishing collaborations among various academic institutions and governmental agencies. ITPproduced Lucid tools are peer reviewed and published on the Internet and/or on compact disc (CD). In 2007, CPHST delivered five Lucid tools to PPQ and its cooperators.

IDENTIFICATION TECHNOLOGY IDENTIFICATION TOOLS AND OTHER RESOURCES

Molecular Diagnostic Tool

- **Title:** *A Method to Distinguish between three mealybug lineages within the* Planococcus citri-P. mino*r species complex (Hemiptera: Coccoidea: Pseudococcidae)*
- **Authors:** Alessandra Rung, Sonja Scheffer, Gregg Evans, and Douglass Miller
- **Collaborators:** University of Maryland, USDA/ARS Systematic Entomology Laboratory, and USDA/APHIS/ PPQ National Identification Services

In 2007, the ITP delivered a diagnostic tool to PPQ to distinguish *Planococcus minor* (Maskell) from *P. citri* (Rissio). Based on morphology, the two species are extremely difficult to differentiate. *Planococcus citri* and *P. minor* are frequently intercepted at ports-of-entry, but only *P. citri* is established in the U.S. This is of special concern to PPQ because although *P. minor* does not occur in the U. S., it is one of the most commonly intercepted mealybugs at portsof-entry and is abundant in many areas of the Caribbean and Pacific.

A diagnostic method was needed that could be used to distinguish the two taxa in a timely fashion. In 2007, such a protocol was developed. The protocol employs polymerase chain reaction (PCR) of the 3' prime end of the mitochondrial gene Cytochrome Oxidase I (COI), followed by restriction fragment polymorphism (RFLP) analysis, and it

An Identification Aid Database Information

Title: *ID Source*

Collaborators: USDA/APHIS/PPQ's Western Region, Eastern Region, and National Identification Services and The University of Queensland

Expected Availability Date: Late 2008

The past decade has seen an increasing trend among taxonomists to produce identification keys (often matrixbased) and other identification tools using electronic media. These electronic keys are often found embedded in web pages on the Internet. Along with this trend, and undoubtedly due to the dominance of the Internet, there has been a proliferation of identification-themed websites, such as image databases and electronic floras.

A result of all of this activity is an abundance of electronic identification tools produced worldwide, many of them available on or accessible from the Internet. Some of these tools could undoubtedly be used to identify invasive species of interest to PPQ. However, although increasing numbers of electronic identification tools are continually being published worldwide, a means with which to comprehensively track and compile information about them is lacking.

can be used to rapidly differentiate among *P. minor*, *P. citri*, and a recently discovered taxon*.* The protocol is particularly useful because, unlike sequence analysis, it can be performed within a day, and it can be used to identify immature as well as adult individuals. This diagnostic tool is now available on the PPQ Western Region intranet.

PPQ individuals with identification responsibilities may regularly search the Internet for websites to assist them in their identification needs. However, it is time consuming to filter out those sites that are not pertinent. And, upon finding a candidate site or tool, it may not be easy to determine its relevance and usability, or how to access or purchase it.

In 2007, the ITP Team began the design and construction of a new identification resource to address these issues. ID

Figure 2. A major update to the popular Lucid identification tool Aquarium and Pond Plants of the World *was completed in 2007. Edition 2 was released on both the Internet and on compact disc.*

IDENTIFICATION TECHNOLOGY IDENTIFICATION TOOLS AND OTHER RESOURCES

Scher, was published on compact disc and also made available in

a new Lucid format called the Lucid3 On-line Player [\(www.lucidcentral.org\)](http://www.lucidcentral.org) in 2007.

Source will be an online searchable database of information and links to web-based identification keys and other electronic aids. ID Source will include only those aids selected as valuable for PPQ's identification and detection needs.

Populating the database will involve performing specialized search queries, along with research, filtering, and selection methods, to efficiently locate and gather useful sites and tools. ID Source is thus conceived as the first site of choice for users to consult, rather than a search engine. Tool information, such as title, authors, taxonomic group, and geographic/political region covered, and other information, will be clearly presented to enable visitors to quickly search for and assess which tools and sites they want to consult. ID Source will be available via the APHIS Intranet in 2008/2009.

Supporting Off-Shore Identification

In addition to the development of Lucid tools, which, as described above, are accessible to the international community, ITP supports the off-shore component of its three areas of focus (off-shore, ports-of-entry, and domestic) through participation in various international efforts. Three such efforts are highlighted below.

Barbados was the site of a training workshop, entitled *Mealybug and Scale Insect Identification*, for eastern Caribbean area surveyors and identifiers. The workshop was hosted by APHIS International Service Caribbean Area, USDA CSREES, Southern Pest Diagnostic Network, CPHST, Florida State Department of Agriculture, and Inter-American Institute for Cooperation on Agriculture. The recently-published ITP Lucid identification tool *Scale Insects – Identification Tools for Species of Quarantine Significance* was a significant component of the workshop. Instructors demonstrated its features and it was then used by the participants during a hands-on session to identify unknowns. Each workshop participant received a compact disc of the scale tool, as well as a copy of ITP's recently completed document "Best Practice Guidelines for Making an Identification Using Lucid Player."

Terrence Walters is the Lucid Project Coordinator for Quad (Quadrilateral Scientific Collaboration in Plant Biosecurity), comprising Australia, Canada, United States, and New Zealand. In this role, he directs the Quad Team on the development of Lucid tools to support the Quad's focus on plant protection and quarantine. In addition to ensuring the Quad community remains informed and updated on activities by the Lucid Team via the Quad website ([www.quadscoop.org\),](http://www.quadscoop.org) Terrence also oversees annual team objectives and tasks. During 2007, the following tasks were initiated or completed.

- 1. USA is collaborating with New Zealand on the development and enhancement of the Lucid tool *Identification Tool to Ants I: Ants of the Pacific Basin*. The tool will be made available to the Quad community in 2008.
- 2. New Zealand and Canada have initiated a Lucid tool to identify aphids of quarantine significance.
- 3. Identifiers from the Quad countries reviewed the beta version of ITP's Lucid identification tool *Wood Boring Beetles of the World: Wood Boring Beetle Families*.
- 4. Australia completed the Lucid tool *Mites of Quarantine Importance*.
- 5. New Zealand completed a major update to their *Plant Parasitic Nematodes* Lucid tool.

IDENTIFICATION TECHNOLOGY IDENTIFICATION TOOLS AND OTHER RESOURCES

Providing Training and Support to PPQ and Cooperators

2007 Workshops: How to Use Lucid Tools

A strategic objective for the ITP Team is to provide regular training for clients, cooperators, and collaborators in developing and using electronic identification tools and resources in support of pest survey and detection. "Lucid Builder" workshops cover the design, development, and delivery of identification tools and are held in even years. In odd years, the ITP Team holds "Lucid Player" workshops to train end-users in how to access and use these tools to make identifications.

The ITP conducted a total of ten on-site "Lucid Player" workshop sessions from May to September 2007. The workshop, entitled *Making the Most out of Lucid Identification Tools*, incorporated many suggestions from participants of the 2005 workshop and was essentially redesigned from square one. The workshop was held at six localities: the Los Angeles, Seattle, JFK, and Miami Plant Inspection Stations, University of Florida, and Florida A&M University. Over 75 individuals representing various PPQ work units and PPQ cooperators completed and received AgLearn credit for the hands-on workshop.

Figure 4. Co-Instructor Terrence Walters (standing) helping out one of the participants with an identification during the Making the Most out of Lucid Identification Tools Workshop *at the Miami Plant Inspection Station, Florida.*

Figure 5. Co-Instructor Julia Scher (standing) providing identification support to one of the participants during the Making the Most out of Lucid Identification Tools Workshop *at the JFK Plant Inspection Station., New York.*

Written evaluations were obtained from participants in all ten sessions. The ITP reviewed these after each session and modified workshop content based on participant suggestions as well the its own assessments. The workshop therefore evolved and improved over the course of the entire summer. Examples of some positive comments included the following:

Participant excerpts: "I was surprised how accessible and useful this program is to a field person [like] myself;" "Instructors were very informative and knowledgeable and good at conveying the topic about Lucid;" "This course should be agency wide;" "It is very informative and an asset to our work;" "The ease of its use is amazing;" and "[The course] opened up many possibilities and ideas within in my specialty."

A copy of the final workshop report, which includes agendas, list of participants, detailed reviews, data, and evaluations for all ten 2007 workshop sessions, is available from Terrence Walters or Julia Scher.

Delivering Automation Technology Based Tools for Survey, Detection, and Identification

Sorting through mountains of insects is tedious, difficult, and time-consuming work that places great demands on people and resources in the fight to protect our nation's agriculture and natural resources. The good news is that new technology now being developed by CPHST could soon begin turning the most tedious parts of this job over to machines.

Figure 6. RAPID Robotic Information Technology Agent (RITA) beginning to triage a collection of insects. RITA uses a vacuum pen to place specimens into designated containers.

Many insect survey and detection processes, where the same tasks are repeated over and over again, lend themselves well to the application of industrial automation techniques. These are the same basic techniques of automated material handling and image analysis that were so successfully applied to automated genome surveys.

Beyond the advantages of speed and relief from tedium, automation minimizes human errors and captures data instantly as part of the process. In developing tools, individual tasks are being addressed with the knowledge that each forms only a piece of a larger picture. Equipment to perform different tasks must, therefore, be modular with the capability of being linked at some future time. The advantage in this approach is that it allows for a multifaceted automation system to evolve as necessary hardware and software elements are developed. Therefore, a single component of the System can be immediately used rather than waiting for the rest of the system to be in place.

The RAPID (Robotic Automated Pest ID) project, initiated in 2005, strives to deliver tools and systems that will greatly reduce the volume of survey samples that must be hand processed and identified by automating the classification and sorting of these samples. RAPID utilizes the latest advances in image processing and analysis, remote sens-

ing, pattern recognition and industrial robotics technology to provide rapid, automated identification in support of targeted pest species detection. RAPID tools are being developed to support a wide variety of survey requirements including both insects and plant seed processing. RAPID is a federal / state collaborative effort among USDA/APHIS/PPQ Center for Plant Health Science and Technology, USDA Forest Service, and New Mexico State University's College of Agriculture.

The project is composed of several individual tasks and deliverables that are valuable standalone tools in their own right and in combination comprise the entire RAPID system. Below we discuss the principal components of RAPID, their use, and expand on the ultimate objectives for the entire RAPID system.

RAPID applications now in development or under consideration are given below:

- Biocontrol/IPM Obtaining reliable predator/prey statistics for alternative crop management approaches.
- Port Inspections Detection of federal noxious weeds seeds in imported spices and seeds.
- Pest Surveys Automation of a variety of CAPS and other established surveys could benefit from automation.
- Emergency Response Program Providing automated handling and sorting for the USDA's Potato Cyst Nematode (PCN) program.
- PPQ Cooperator Support Supporting USDA Forest Service (a project stakeholder) with a goal to "triage" wood boring beetle survey samples.

RAPID Tools in Development During 2007

Title: *SPS – Sample Processing System*

Technologies: Image processing and analysis, statistics, probability and classification theory

Functions: Performs classification or identification of objects in sample, maintains an extensive database recording all measured features of samples in addition to population statistics, survey data and images.

Title: *RITA – Robotic Information Technology Agent*

Technologies: 6-axis robotic manipulator, flexible automated parts feeding, robotics

Functions: Feeds samples from traps or other sources to SPS in continuous flow; robot uses vacuum pen to pick-up and sort samples, by taxa, to vials.

Title: *PISCES – Pest Identification Spectral Camera Experiment Station*

Technologies: Hyperspectral Imaging, remote sensing, image processing and analysis

Functions: Extracts hyperspectral reflectance "signatures" from samples using an imaging spectrometer. These signatures, which are not visible to the human eye, can then be used as features to refine classification of samples.

Title: *RID – Remote Identification System*

Technologies: Internet (Web) Applications, Relational Database, 6-axis robotic manipulator, image processing and analysis

Functions: Provides a highly flexible tool for remote (distance) identification of samples. The SPS makes an initial classification of samples and, working with RITA, locates the sample, picks it up and places it under a microscope. The remote identifier can control all aspects of tool including rotating the sample in 3-axis. The SPS database is fully integrated with RID, providing support and recording of ID results.

Figure 7. RAPID Remote Identification System (RID). The system provides a highly flexible, webbased tool to support local and remote (distance) identification of samples. Remote identifiers can control all aspects of the tool including rotating the sample (see monitor image) in 3-axis.

Significant Outreach Activities by the Identification Technology Program (ITP) for 2007

Presentations Given by the ITP Team

Title: *Building Matrix Keys Using Lucid* To: National Science Foundation PEET (Partnerships for Enhancing Expertise in Taxonomy) VI Conference Presenter: Julia Scher

Title: *Identification Tools and Beyond for PPQ* To: USDA/APHIS/PPQ's Eastern Region and Center for Plant Health Science and Technology Presenter: Julia Scher

Title: *CPHST's Identification Technology Program: Tools and Resources for Plant Inspection Stations* To: USDA's National Plant Inspection Station Conference Presenter: Terrence Walters

Title: *Robotic Automated Pest ID* To: USDA/APHIS/PPQ's Eastern Region, Western Region, and National Identification Services, and USDA/FS Forest Health Technology Enterprise Team Presenter: Jeff Drake

Title: *CPHST Fort Collins Laboratory's Identification Technology Program: Today and Tomorrow* To: USDA/APHIS/PPQ Western Region Presenter: Terrence Walters

Identification Tools Delivered by the ITP Team to PPQ and Cooperators in 2007

Aquarium and Pond Plants of the World, Edition 2

A Lucid interactive identification tool by Shaun L. Winterton and Julia Scher Collaborators: USDA/APHIS/PPQ Center for Plant Health Science and Technology and California Department of Food and Agriculture

Published on the Internet and on compact disc in 2007. The Internet version is available at: <http://www.lucidcentral.org/keys/> aquariumplants2/.

Scale Insects – Identification Tools for Species of Quarantine Significance

A Lucid interactive identification tool by Douglass Miller, Alessandra Rung, and George Venable Collaborators: University of Maryland and USDA/ARS Systematic Entomology Laboratory

Published on the Internet and on compact disc in 2007. The Internet version is available at: <http://www.sel.barc.usda.gov/ScaleKeys/> index.html. The CD is available upon request to Terrence Walters.

Cut Flower Exports of Africa

A Lucid interactive identification tool by Julia Scher Collaborators: North Carolina State University, and California Department of Food and Agriculture

Published on the Internet in 2006 [\(http://www.lucidcentral.org/keys/v3/cutflowers/\), bu](http://www.lucidcentral.org/keys/v3/cutflowers/)t in 2007 was published on compact disc. The CD is available upon request to Terrence Walters.

Note: Both *Aquarium and Pond Plants of the World, Edition 2.0* and *Cut Flower Exports of Africa* were made available in a different format—the Lucid3 On-line Player, which requires no special plug-ins in your browser or software on your computer, is deployed serverside, and supports the international Standard for Descriptive Data. The On-Line Player versions of both tools are available at: [http://www.lucidcentral.com/online_player/.](http://www.lucidcentral.com/online_player/)

Identification Tool for Weevil Biological Control Agents of Aquatic and Terrestrial Weeds in the United States and Canada

A Lucid interactive identification tool by Muhammad Haseeb, C. O'Brien, W. Flowers, and M. Kairo Collaborators: Florida A&M University and USDA Cooperative State Research, Education, and Extension Service (CSREES)

Published on the Internet in 2007: [http://www.famu.org/weeviltool.](http://www.famu.org/weeviltool)

Key to Species of Hylesine Bark Beetles of the Southeastern United States A Lucid interactive identification tool by James Baker and Steve Bambara Collaborators: North Carolina State University and Southern Plant Diagnostic Network

Published on the Internet in 2007: [http://www.lucidcentral.org/keys/v3/bark_beetles/Home_start_here.htm.](http://www.lucidcentral.org/keys/v3/bark_beetles/Home_start_here.htm)

Lucid Tools Initiated and/or in Development by the ITP Team During 2007

Identification Tool to Ants I. Invasive Ants of the Pacific Basin Collaborators: University of California Davis and New Zealand Ministry of Agriculture and Forestry Expected Completion: 2008

Grasshoppers of the Western United States, Edition 2.0 Collaborators: USDA/APHIS/PPQ Western Region and the University of Nebraska Lincoln Expected Completion: 2008

Wood Boring Beetles of the World: Wood Boring Beetle Families Collaborators: California Department of Food & Agriculture and Montana State University Expected Completion: 2008

Wood Boring Beetles of the World: Genera of the Bostrichidae and Buprestidae Collaborators: California Department of Food & Agriculture, Montana State University, and Harvard University Expected Completion: 2009

LBAM ID: Tools for Diagnosing Light Brown Apple Moth and Related Western U.S. Leafrollers (Archipini: Tortricidae) Collaborators: California Department of Food & Agriculture and Colorado State University Expected Completion: 2009

Pests and Diseases of Cultivated Palms from the United States and Caribbean Collaborators: Southern Plant Diagnostic Network, University of Florida, Florida A&M University, and Florida Department of Agriculture and Consumer Services-Division of Plant Industry Expected Completion: 2010

Identification Resource for the Fruit Fly Species of Anastrepha *I. The* Anastrepha daciformis, grandis, robusta, schausi*, and* serpentina *species groups*

Collaborators: USDA/ARS Systematic Entomology Laboratory, Smithsonian Institution, Commonwealth Scientific and Industrial Research Organization (Australia), and Universidad de Panama Expected Completion: 2010

Identification of Imported Dried Botanicals

Collaborators: USDA/APHIS/PPQ Delaware State Plant Health Director's Office and Delaware State University Expected Completion: 2010

PEST DETECTION & SURVEY SURVEY & REFERENCE GUIDELINES

Development of CAPS commodity-based survey schemes

CPHST STAFF: Melinda Sullivan (lead); Nehalem Breiter and Sharon Talley (support) CHAMPIONS: John Bowers (National Survey Coordinator), Kristian Rondeau (WR Program Manager Pest Detection), and Brian Kopper (ER Program Manager Pest Detection) CONTACT: Melinda Sullivan [\(melinda.j.sullivan@aphis.usda.gov,](mailto:melinda.j.sullivan@aphis.usda.gov) 970-490-4469)

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. In the past, the CAPS surveys have focused on surveying for one to a few organisms at a time. The purpose of the commodity-based manual is to increase efficiency by surveying for a suite of exotic pests at the same time, including those that may only be considered minor pests. The manuals also provide information on established pests that may be easily confused with exotic pests. By increasing survey efficiency, the odds of detecting a pest before it becomes established will be greatly enhanced. The reference manuals (**Table 1**) contain biological information about each pest; its host range, distribution information, and survey information in a single, user-friendly document. This manual will serve as a reference for survey specialists as they plan their cooperative agreements. Companion documents (survey guidelines) with specific survey and identification for a subset of pests within the reference document are also being prepared by CPHST (**Table 1**).

Small Grains Commodity-based Survey Soybean Commodity Survey Guideline Guideline Ω_d Ô **Survey Reference Cooperative Agricultural Pest Survey (CAPS)**

Manual	Date Prepared	Authors
Citrus commodity-based reference	July 2005	CPHST- Fort Collins, CO CPHST-Mission, TX
Citrus commodity-based survey guidelines	March 2006	CPHST - Raleigh, NC
Soybean commodity-based reference	February 2006	CPHST- Fort Collins, CO CPHST-Mission, TX
Soybean commodity-based survey guidelines	July 2007	CPHST-Fort Collins, CO CPHST-Raleigh, NC
Oak commodity-based reference	September 2006	Department of Entomology, University of Minnesota and the Northern Research Station, USDA Forest Ser- vice (Robert Venette)
Oak commodity-based sur- vey guidelines	September 2006	Department of Entomology, University of Minnesota and the Northern Research Station, USDA Forest Ser- vice (Robert Venette)
Grape commodity-based reference	July 2007	CPHST-Fort Collins, CO
Grape commodity-based survey guidelines	In process	CPHST-Fort Collins, CO CPHST-Raleigh, NC
Small grains commodity- based reference	February 2008	CPHST-Fort Collins, CO Department of Plant Pa- thology, Michigan State University (Ray Hammer- schmidt)
Small grains commodity- based survey guidelines	April 2008	CPHST-Fort Collins, CO CPHST-Raleigh, NC
Pine commodity-based reference	March 2008	Department of Entomology, University of Minnesota and the Northern Research Station, USDA Forest Ser- vice (Robert Venette)
Pine commodity-based sur- vey guidelines	April 2008	Department of Entomology, University of Minnesota and the Northern Research Station, USDA Forest Ser- vice (Robert Venette)
Corn commodity-based survey reference	In process	Department of Plant Pa- thology and Entomology, University of Nebraska (Robert Wright)

Table 1. Commodity-based manuals prepared or currently in process.

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