Biological Control Program

Annual Report 2009

Photo by Kent Daane, University of California, Berkeley

California Department of Food & Agriculture

BIOLOGICAL CONTROL PROGRAM

2009 SUMMARY

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CALIFORNIA DEPARTMENT OF FOOD AND AGRICULTURE
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INTEGRATED PEST CONTROL BRANCH

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The CDFA Biological Control Program greatly appreciates the many biologists and agriculture commissioners throughout the state whose co-operation and collaboration made this work possible.

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Cover developed by Kris Godfrey, Baldo Villegas, and John P. Mattia (Orange, CT). Photo of *Anagyrus pseudococci* female, newly-emerged from a vine mealybug mummy. (Photo courtesy of Kent Daane, University of California, Berkeley)

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Preface

M. J. Pitcairn

In 1973, Ric Dunkel, a program supervisor with the Environmental Monitoring and Pest Management Branch of the California Department of Food and Agriculture, purchased two 55 foot trailers for use as laboratories for the newly-formed Biological Control Program. The trailers were parked in the back area of a piece of property located on Meadowview Road in south Sacramento owned by the Department. Over the years, three more trailers were added. Two were donated by other programs when they were no longer road worthy. The third trailer was leased from a private company. These five trailers served as the laboratory space for the scientific staff of the Biological Control Program for many years. They were well used and served us well but, over time, slowly deteriorated to a point where they no longer provided sound or safe working space. So, in 2009, four of the trailers were removed and, in their place, a new triple-wide modular unit was installed. Inside is a series of small individual laboratory rooms and a larger meeting room. The youngest of the original five trailers was moved to the side and completely remodeled to house the quarantine facility used by the program. These two units make up the new laboratories for the Biological Control Program.

Left Photo: New modular laboratory facility of the Biological Control Program (foreground); remodeled quarantine facility (lbackground).
Right Photo: Inside laboratory room of new laboratory facility.

Life within the Biological Control Program is only now returning back to normal. All of the supplies and activities that had been performed in the old laboratories had to be relocated during the replacement of the trailers. Most of the equipment was placed in temporary storage in another building on site. Some of the insect and plant cultures were lost. Many were restarted once the new facilities were in place but it has taken time to build the cultures up to what they were prior to the change in facilities. Despite these inconveniences, all of us are excited to be in these new and improved laboratory facilities and are looking forward to the next field season.

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Insect Projects

Weed Projects

New Developments in the Biological Control Program of Diaprepes Root Weevil in California

Loretta Bates[1], James Bethke[1], Gary Bender[1], Joseph Morse[2], Jorge Peňa[3], and Kris Godfrey

The arrival of Diaprepes root weevil, *Diaprepes abbreviatus*, in California raised great concern in the citrus industry as well as other commodities. It is a polyphagous weevil with a broad host range and is considered a major insect pest of citrus, woody ornamental plantings, and ornamental plant nurseries in Florida and beyond. The incompletely described host range includes avocados, stone fruit, grapes, and a number of landscape plants unique to California. It was found infesting parts of Orange, Los Angeles, and San Diego counties in 2005 and 2006. In 2006, the California Department of Food and Agriculture (CDFA) began an eradication program in the known infested areas of these three counties. Insecticides were a core component of the eradication program, however, the more effective insecticides have limitations in their registration which preclude their use in some of the treatment areas. In July 2008, the eradication program ended with a loss of funding. Biological control was considered early on. Research to determine if egg predators or parasitoids could be used as a part of the management program was initiated in 2007 and continued through 2009.

The wasp, *Aprostocetus vaquitarum,* was the first natural enemy released in San Diego County as part of this program. It is known to impart significant mortality to Diaprepes populations in southern Florida, and a laboratory colony is maintained at the University of Florida, Tropical Research and Education Center. This wasp is actually a predator, rather than a parasitoid. The female wasp places her eggs within the Diaprepes egg mass; the eggs hatch, and the wasp larvae begin to feed externally on the Diaprepes eggs within the mass. Each wasp larva requires more than one Diaprepes egg to complete development. The wasp larva eventually pupates within the Diaprepes egg mass. Shipments of *A. vaquitarum* were made to the University of California – Riverside Quarantine Facility from Florida beginning in 2006. Field releases were initiated in October 2007 and continued through 2009. In 2009, 1,124 *A. vaquitarum* adults were released at seven sites in two cities from April – November. The release sites were known infested sites that were not treated with insecticides.

Monitoring of the success of the releases began in March 2009 and continued through May. For each sampling date, young egg masses (less than 24 hours old) that were produced in a greenhouse in Encinitas were placed in an "egg trap" in a host tree. Each egg trap was comprised of a plastic drink cup in which the egg mass was suspended (Figure 1). The traps were left in the field for one or two days to allow parasitoids to find the egg masses. The egg traps were returned to the laboratory and the contents examined for the presence of parasitoids. The number of egg masses placed in the field varied on each date because the number of egg masses produced by the colony varied greatly over time. In total, 33 egg masses were exposed.

There was a concern that the egg trap may repel the parasitoid in the field, so several traps were sent to Florida for testing. The egg traps with egg masses were placed in the field paired with egg masses on sentinel plants. All egg masses were the same age. The egg masses were exposed in the field for two to three days. Adult *A. vaquitarum*

were attracted to, and oviposited in, egg masses on sentinel plants. The egg masses in the egg traps were not oviposited in. Future monitoring in California will be done using egg masses on sentinel plants.

Plans are being made to import and release two additional species of parasitoids of Diaprepes, *Fidiobia domincia* and *Haeckliana sperata*. These two parasitoids oviposit into the Diaprepes egg, and the parasitoid develops within the host egg. We currently hold a permit for importation and release of *F. dominica*. Efforts are being made to collect this parasitoid in Dominica and initiate a colony at the University of Florida, Tropical Research and Education Center in Homestead, Florida. The permit for release of *H. sperata* was delayed because additional host specificity testing is required to insure that this parasitoid is safe to release in California.

Figure 1. An egg trap placed in a lemon tree in San Diego County. This trap was designed to allow *A. vaquitarum* adults to enter and oviposit in the suspended Diaprepes egg mass, but not allow any hatching Diaprepes larvae to escape.

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More Releases of the Lygus Parasitoid, *Peristenus relictus*

Charles H. Pickett, Dale Spurgeon[1], Neal Hudson[1], Oleg Daugovish[2],
Tom Dorsey[3], and Marypat Stadtherr

Lygus spp., primarily *L. hesperus*, is a chronic problem to growers of cotton and many seed crops throughout central California. Although *L. hesperus* has been a key pest of strawberries along the central coast of California, it has not been until recently a serious pest to strawberries grown in Ventura County in southern California. To help control this pest, the nymphal parasitoid *Peristenus relictus* from southern Europe was released in the San Joaquin and Sacramento Valleys, as well as in the Monterey Bay Region from 1998 to 2004. Earlier surveys of *Lygus* spp. nymphs in California found this pest free of any nymphal parasitoids. *Peristenus relictus* became permanently established in the Sacramento Valley and Monterey Bay Region, but failed to establish permanent populations in the lower San Joaquin Valley.

Because a new population of *P. relictus* from Morocco became available to us, we decided to renew releases in the San Joaquin Valley. This population comes from a part of northern Africa with a much closer climatic match to the lower San Joaquin Valley than populations released during our first effort over 10 years ago. Releases were also initiated in Ventura County because *L. hesperus* populations have increased over the last three years, and are causing serious economic damage to commercial strawberries. A key factor in renewing releases at both locations has been the availability of permitted *P. relictus*. The New Jersey Department of Agriculture is culturing populations of *P. relictus* from both Morocco and Spain, for use by other cooperators.

Releases were initiated at the Shafter experiment station in the southern San Joaquin Valley on July 9, 2009. Three releases were made roughly three weeks apart for a total of 1,856 adults (mixed males and females). Five pre-release samples were taken beginning in May from a managed plot of alfalfa (total = 290 dissections of 2^{nd}-5^{th} instar nymphs). No parasitic larvae were found. On September 25, 2009, one post-release sample (n = 66) was taken and no recoveries of *P. relictus* were detected. Two releases were made in Ventura County for a total of 394 adult *P. relictus* (Morocco). Although we wanted to release the Spanish strain, none were available. One pre-release sample was taken in Ventura County on July 17, 2009 (n = 140 dissections of 2^{nd}-5^{th} instar nymphs). We hope to make additional releases at both locations in 2010.

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Insectary Plants for the Lygus Parasitoid, *Peristenus relictus*

Diego Nieto[1], Janet Bryer[1], Sean Swezey[1], and Charles H. Pickett

Although alfalfa has proven an excellent plant for harbouring both Lygus and *P. relictus*, the plant lacks flower nectary glands accessible to parasitoids. Nectar can greatly increase the longevity, and thereby reproductive output of adult female wasps. Potentially, the addition of a second plant species with alfalfa trap crop strips might boost the number of parasitoids, and parasitism itself, of lygus inhabiting these strips.

Five plant species were compared in replicated garden plots: alyssum, yarrow, poppy, California buckwheat, and alfalfa, to determine which supported the highest number of parasitoids relative to lygus. Results from summer 2008 collections show a range of responses (Figures 1-3). Lygus was consistently highest on alfalfa (Figure 1). However, the density of parasitoid larvae, and particularly parasitism itself, were not consistently highest on this plant (Figures 2-3). Parasitism was higher on buckwheat in July and August, and the number of *P. relictus* larvae was highly variable among plants. These results are noteworthy considering parasitism of lygus was not highest on the plant with the most lygus, i.e. alfalfa. Buckwheat appeared to support the highest ratio of parasitoids to lygus nymphs (i.e. parasitism). Other studies have shown that buckwheat has flower nectary glands that are more accessible to parasitoids, and they contain sugars of greater nutritional value than many other flowering plants. This comparison was repeated in 2009, but data are not yet available.

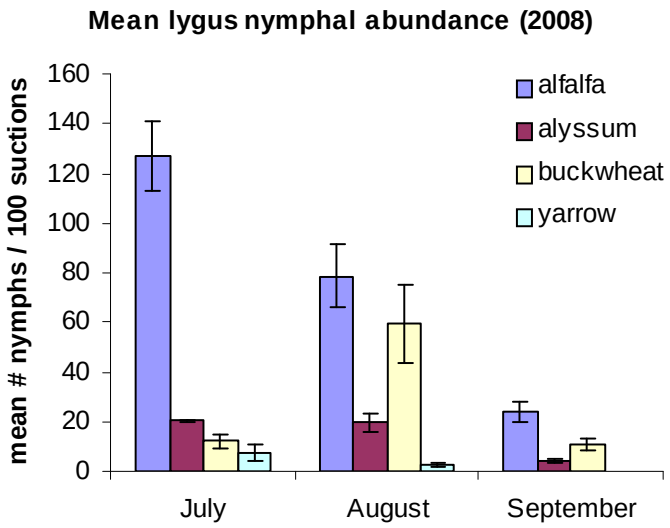

Figure 1. Mean Lygus bug nymph abundance in un-vacuumed alfalfa trap crops, alyssum, buckwheat, and yarrow. Eagle Tree Farm, Prunedale, CA.

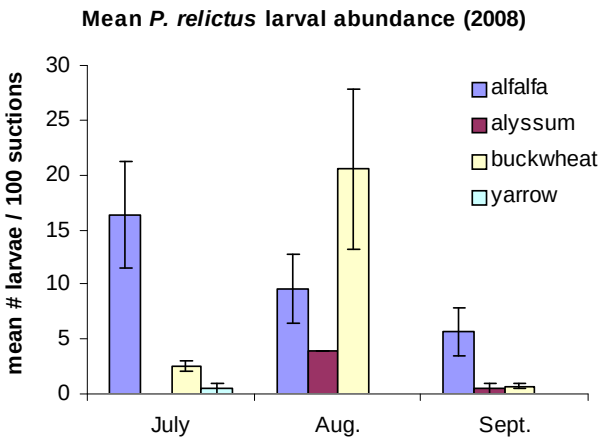

Figure 2. Mean *Peristenus relictus* larva abundance (from dissected lygus nymphs) in un-vacuumed alfalfa trap crops, alyssum, buckwheat and yarrow. Eagle Tree Farm, Prunedale, CA.

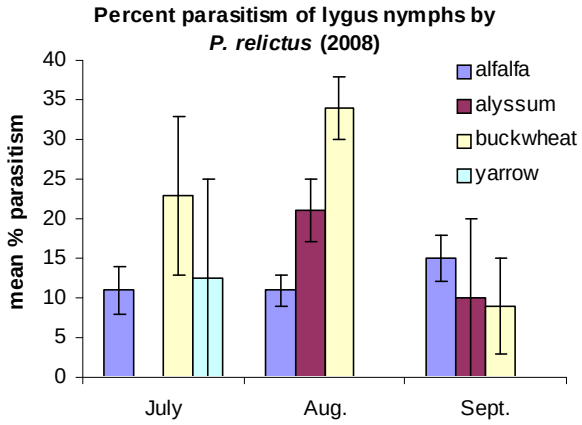

Figure 3. Mean percent parasitism of lygus bug nymphs by *Peristenus relictus* in un-vacuumed alfalfa trap crops, alyssum, buckwheat and yarrow. Eagle Tree Farm, Prunedale, CA.

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Management of Alfalfa Strips for Enhancement of the Lygus Parasitoid, *Peristenus relictus*

Diego Nieto[1], Janet Bryer[1], Sean Swezey[1], and Charles H. Pickett

In 2008, studies were conducted to determine how the imported lygus bug parasitoid, *Peristenus relictus*, can best be integrated into a strawberry system using managed alfalfa trap crops. Alfalfa is used to attract lygus bugs out of, or away from, strawberries, a high value crop in California. In organic strawberries, tractor-mounted vacuums are used to reduce lygus populations in alfalfa trap crops (Figure 1). Parasitism persisted in these vacuumed trap crops, both in terms of larval abundance (Figure 2) and percent parasitism (Figure 3). Average percent parasitism for August and September of 2008 was not significantly different between vacuumed (10.9 ± 2.3) and un-vacuumed (14.5 ± 2.7) trap crops (P = 0.30).

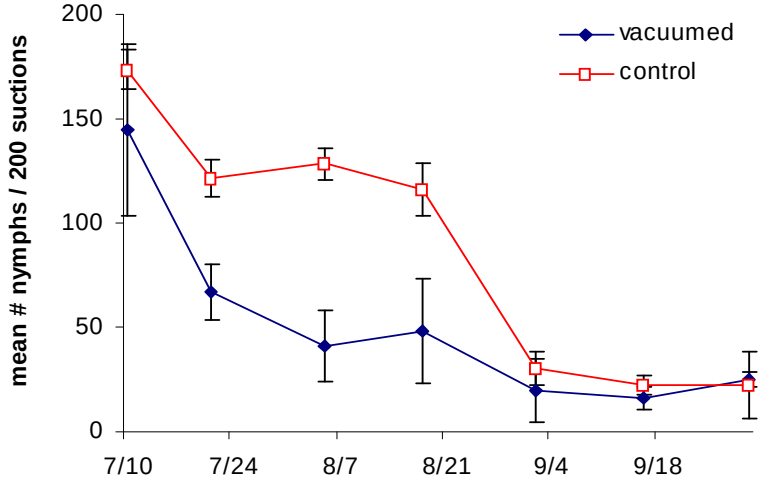

Figure 1. Mean lygus bug nymph abundance in vacuumed and un-vacuumed (control) alfalfa trap crops in an organic strawberry system. Control trap crops were heavily trimmed after the August 21 sample date. Data taken from Prunedale, CA in 2008.

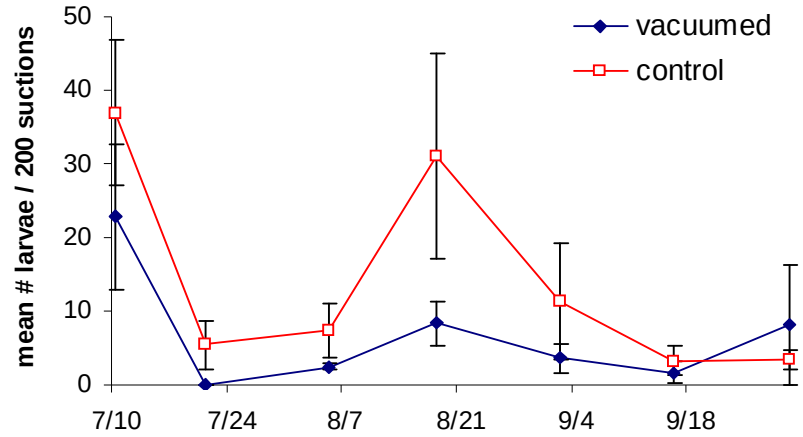

Figure 2. Mean *P. relictus* larval abundance in vacuumed and un-vacuumed (control) alfalfa trap crops in an organic strawberry system. Control trap crops were heavily trimmed after the August 21 sample date. Data taken from Prunedale, CA in 2008.

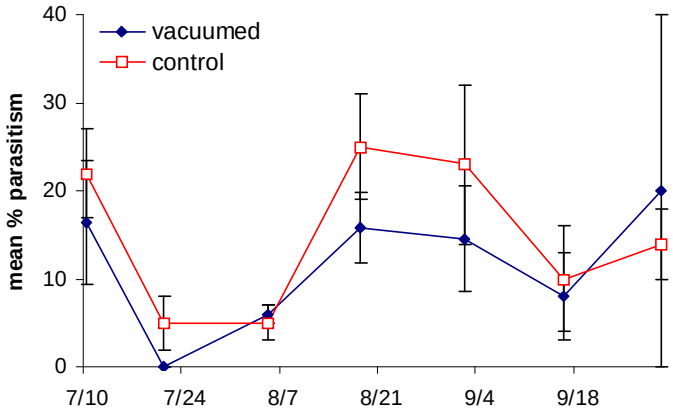

Figure 3. Mean percent parasitism of lygus bug nymphs by *P. relictus* in vacuumed and un-vacuumed (control) alfalfa trap crops in an organic strawberry system. Control trap crops were heavily trimmed after the August 21 sample date. Data taken from Prunedale, CA in 2008.

In this 2008 organic, trap-cropped strawberry system, alfalfa trap crops were separated by 49 strawberry rows. To determine the distribution of lygus bug nymphs and also *P. relictus* within these rows of strawberries, samples were collected based on their proximity to one of the two neighboring trap crops. Based on this sampling scheme, lygus bug nymphs were most abundant within the trap crops (T.C.) and adjacent strawberries (row 1 in Figure 4). Parasitism, both larval abundance and percent of nymphs parasitized, followed this lygus bug distribution pattern (Figure 5).

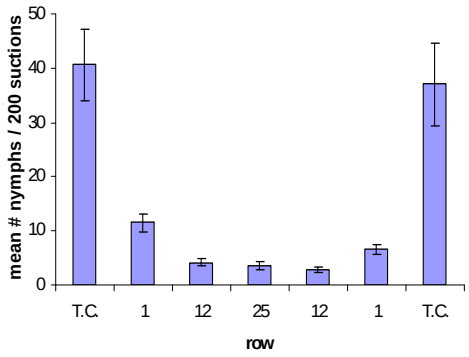

Figure 4. Mean Lygus bug nymph abundance in neighboring alfalfa trap crops (T.C.) and intermediate strawberry rows based on proximity to closest trap crop. Data taken from July-September, 2008 in Prunedale, CA.

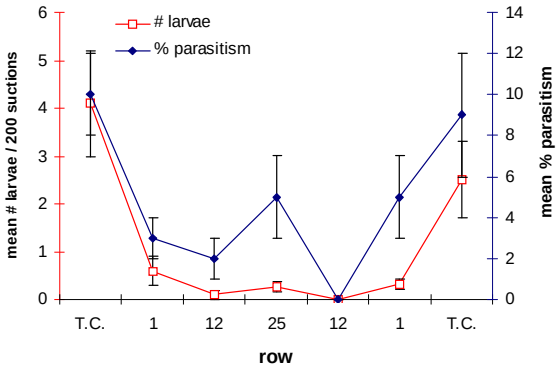

Figure 5. Mean *P. relictus* parasitism (both larval abundance and percent of nymphs parasitized) in neighboring alfalfa trap crops (T.C.) and intermediate strawberry rows based on proximity to closest trap crop. Data taken from July-September, 2008 in Prunedale, CA.

In conventionally-managed strawberries with trap crops, alfalfa is treated with Lygus-directed insecticides normally prescribed for use in strawberry rows. To determine if *P. relictus* could persist in this highly disturbed environment, parasitism in sprayed trap crops was compared with control (unsprayed) trap crops in 2008. While sampled parasitism declined to zero after two dibrom applications on July 28, and August 7, *P. relictus* activity (both larval abundance and percent of nymphs parasitized) recovered in September (Figure 6 A,B). For example, on September 18, 47% of recovered nymphs in sprayed alfalfa were parasitized.

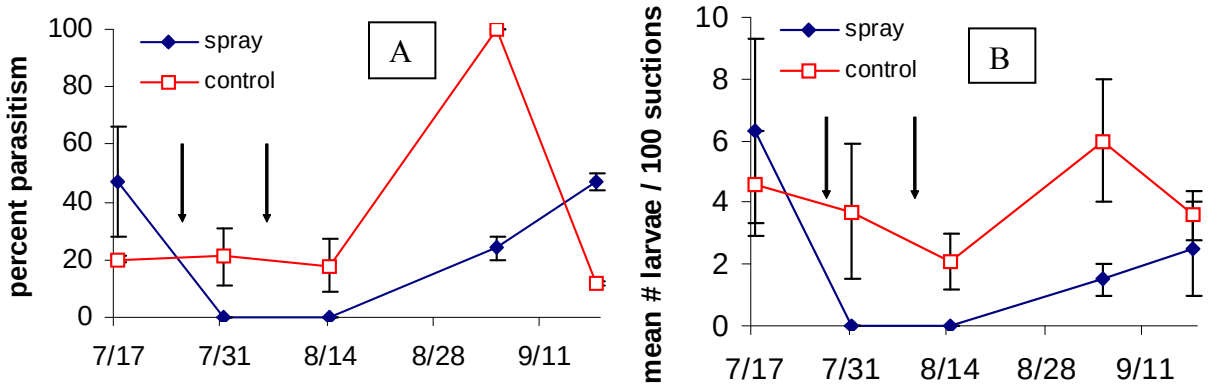

Figure 6. (**A**) Mean percent parasitism of Lygus bug nymphs by *P. relictus* and (**B**) mean *P. relictus* larvae in sprayed and unsprayed (control alfalfa trap crops in a conventional strawberry system). Arrows represent spray events. Data taken from Castroville, CA in 2008.

[1]University of California, Center for Agro-Ecology and Sustainable System, Santa Cruz, California

Parasitism and Predation of Light Brown Apple Moth Eggs

William Roltsch, Nada Carruthers,[1] Richard Stouthamer,[2] and Nancy Saechao

The invasive light brown apple moth, (LBAM), *Epiphyas postvittana*, was first reported in North America in February of 2007, with populations concentrated in the central coast of California. Plans are underway to manage or eradicate this exotic pest using sterile insect technique (SIT). Low densities of the target pest are critical to the success of SIT. Augmentative biological control using native *Trichogramma* species to facilitate SIT efforts by reducing high moth densities is under consideration.

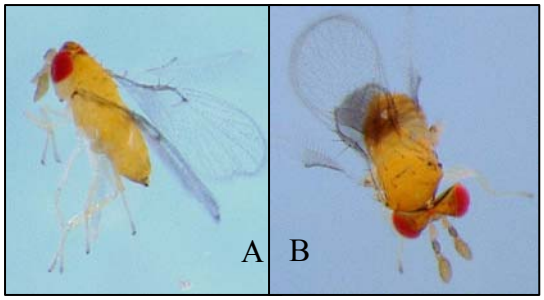

Figure. 1. A: *Trichogramma platneri* B: *T. fasciatum* collected from sentinel eggs.

The 2009 objectives included field studies to identify: 1) *Trichogramma* species seasonal impact on LBAM eggs; and 2) parasitism in relation to geographic variability and LBAM plant species.

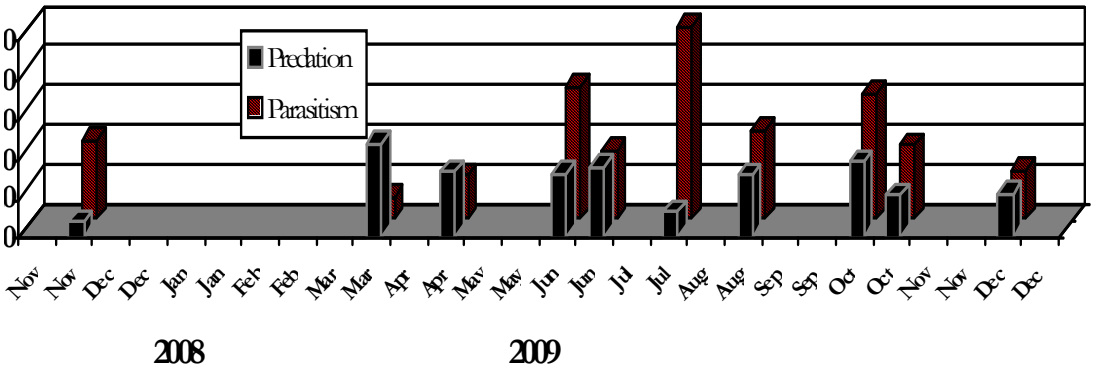

Figure 2. Parasitism and predation of sentinel LBAM eggs on manzanita at the high density LBAM site in Santa Cruz. Half month intervals.

Field studies were conducted to identify naturally occurring parasitism of LBAM eggs at several residential sites in Santa Cruz County and a park site in San Francisco County. Sentinel LBAM egg masses on plastic cards (approx. 1x3cm) were stapled to leaves of known LBAM hosts near branch terminals. In 2009, eggs were placed in the field for 67 degree days, which can be a variable number of calendar days, but represents 50% of the physiological developmental time of LBAM eggs. This corresponded to approximately one week during the warmest time of year, and two weeks during late fall to mid-spring. Sentinel eggs were placed at monthly intervals at one of the Santa Cruz sites and at Golden Gate Park in San Francisco County. Both sites were known to have perhaps the highest densities of LBAM in their respective geographic regions. Results at the frequently monitored site in Santa Cruz (Figure 2) showed that parasitism by wild

Trichogramma was low during much of the first generation of LBAM (eggs laid February to April), with an increase in late May and June coinciding with the second generation LBAM moth flight. Predation was relatively consistent between 20-40%, varying less across time than parasitism. Parasitism and especially predation data are conservative estimates of actual events because the in-field exposure period of sentinel eggs was only 50% of the average time it takes for naturally laid eggs to hatch. Parasitism in San Francisco was seldom recorded until September, at which point egg parasitism increased dramatically. On the shrub *Choisya ternate,* 54% of the egg cards were parasitized. On a tall *Myrtus communis* hedge, 29% of the eggs were parasitized. At the same time, no eggs were parasitized on the young Australian tea tree, *Leptospermum laevigatum*. This plant species has been monitored extensively for two years, and only one egg mass has been parasitized.

The relative contribution of different *Trichogramma* species varied over time and across locations. In Santa Cruz, parasitism consisted primarily of *T. platneri* in the spring and early summer, a combination of *T. platneri, T.* nr. *deon, T. pretiosum* and *T. fasciatum* in mid to late summer, followed by predominantly *T. fasciatum* and *T. platneri* during fall. *T. fasciatum* was particularly dominant late in fall. At the Golden Gate Park in San Francisco, egg parasitism in the fall was dominated by *T. fasciatum*, with the rare occurrence of *T. platneri.*

To provide a geographical characterization of parasitism in the Santa Cruz region, additional studies were carried out in April, June and October of 2009. Parasitism was assessed extensively (eight to ten sites on each occasion) in this urbanized area spanning approximately 10 km from Santa Cruz to Aptos. Paired sites (within a 0.5 km of one another) were selected; one with manzanita (*Arctostaphylus densiflora*) and one with *Pittosporum tobira*. These are two LBAM host plants in the region that are commonly infested. Studies demonstrated that parasitism levels were similar between the two common LBAM host plant species, however, highly variable from site to site (Table 1). Overall parasitism was approximately 10% in the spring, 25% in the summer (ranging widely from 0-60% across sites), and approximately 15% in the fall; once again varying widely from site to site (Table 1). As was detected at the frequently monitored site in Figure 2, predation was relatively consistent through the summer. However, the multi-site fall study indicated that predation increases considerably at that time of year (Table 2).

Figure 3. Light brown apple moth egg mass; parasitized (black) and hatched (clear) egg cases.

In summary, parasitism varied widely from site to site. Of particular significance, egg parasitism was very low in the spring generation of LBAM. Parasitism was similar across a number of LBAM host plants. However, it seems that on some LBAM host plant species, eggs are seldom attacked by *Trichogramma* egg parasitoids. Predation levels are less variable than egg parasitism and may be considerably higher than indicated by the

present data, because the egg cards were not exposed for the length of time that naturally laid eggs exist in the field prior to hatch.

Table 1. Geographic and seasonal variation of percent **parasitism** on two LBAM host plants in Santa Cruz County. Within each paired site listing, the first is for Manzanita, the second *Pittosporum*.

	Spring 2009		Early Summer 2009		Fall 2009	
Paired Sites	Manzanita	Pittosporum	Manzanita	Pittosporum	Manzanita	Pittosporum
Woodrow Av./ Woodrow Av.	21%	18	35	58	32	0
Ledyard/ E. Cliff Vill. Apt.	0	30	53	38	11	86
AJ Co. Park/ Porter Lot	7	0	5	5	0	0
Aptos Church/ Sea Cliff Inn	5	0	0	0	0	0
Deans Ln./ Sellars Ct.					12	8
Means	8.2	12.0	23.2	25.2	11.0	18.8

Table 2. Geographic and seasonal variation of percent **predation** on two LBAM host plants in Santa Cruz County. Within each paired site listing, the first is for Manzanita, the second *Pittosporum*.

	Spring 2009		Early Summer 2009		Fall 2009	
Paired Sites	Manzanita	Pittosporum	Manzanita	Pittosporum	Manzanita	Pittosporum
Woodrow Av./ Woodrow Av.	33%	21	35	50	16	44
Ledyard/ E. Cliff Vill. Apt.	42	36	36	17	68	64
AJ Co. Park/ Porter Lot	44	30	36	18	52	40
Aptos Church/ Sea Cliff Inn	30	29	21	20	52	20
Deans Ln./ Sellars Ct.					38	52
Means	37	29	32	26	45	44

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Seasonal Patterns of Activity and Larval Parasitism of Light Brown Apple Moth in Two Coastal Areas of California

Nick Mills[1], Linda Buergi[1] and William Roltsch

As part of a multi-year effort to understand endemic biological control of light brown apple moth (LBAM), populations have been sampled regularly at two sites in each of San Francisco and Santa Cruz. Urban sites were selected for monitoring as LBAM remains largely an urban invader at the current time, with a small incursion into caneberries along the central coast in 2009. In San Francisco, the host plant of LBAM is the Australian tea tree (*Leptospermum laevigatum*) and in Santa Cruz the host plant is an ornamental variety of the indigenous shrub manzanita, *Arctostaphylos densiflora*. LBAM populations are sampled every two weeks at each site during the main season, and once a month in winter (November through February). Abundance is monitored by timed counts, the cumulative leafrolls found within 5 min, for 22 plants at each site in San Francisco, and for 15 plants at each site in Santa Cruz. A sample of 50 leafrolls are collected from each site on each sampling date to (1) determine occupancy, (2) determine stage structure, and (3) determine parasitism. The proportions of leafrolls occupied by zero, one, two or three LBAM individuals are used to correct the leafroll estimates for LBAM abundance. The stage structure provides a measure of the seasonality of LBAM development, although both eggs and adults are missing from these samples. Live larvae and pupae from the leafrolls are transferred to diet to rear through to adult for identification. Parasitoid cocoons found from the field samples and those that emerge from the rearing of field collected LBAM are identified where possible and used to estimate parasitism by indigenous parasitoids.

The abundance of occupied LBAM leafrolls on Australian tea tree peaked in early 2008 in San Francisco (Figure 1a), but has now fallen to levels that are the lowest of any of the four sites sampled. In contrast, the abundance of LBAM on manzanita in downtown Santa Cruz has remained extremely high during the summer months, although falling to a much lower level in winter (Figure 1b). Average levels of abundance at the other two sites that were monitored were around 10 occupied LBAM leafrolls per 1 min count. The reason for the decline at site 1 in San Francisco is not known, but most likely influenced by increasing age of the young plants.

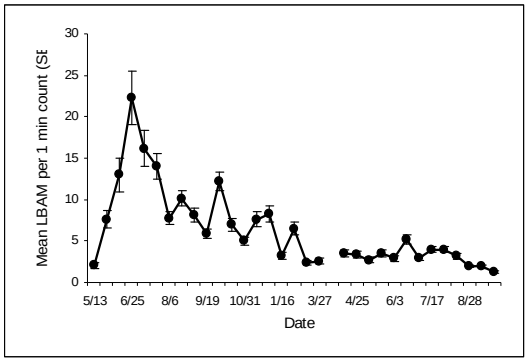

Figure 1a. Abundance of occupied leafrolls in **San Francisco** (site 1) in 2008 and 2009.

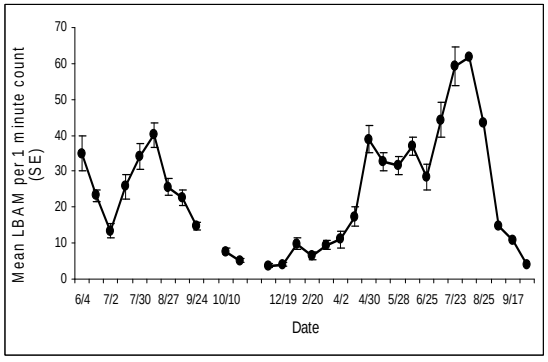

Figure 1b. Abundance of occupied leafrolls in **Santa Cruz** (site 1) in 2008 and 2009.

The pattern of seasonal phenology and voltinism of LBAM differs between San Francisco and Santa Cruz, as indicated by the stage structure of the populations. In San Francisco, young stage larvae (1st and 2nd) peak around late May/early June and again in August/September, with late stage larvae (5th and 6th) peaking in March and again in July/early August (Figure 2a). This suggests just two generations per year in San Francisco with peak flights in April/May and again in August. In contrast, in Santa Cruz, young stage larvae show peaks of activity in late March/early April, late June/early July, and October, with corresponding peaks of late stage LBAM larvae in January, late May/early June, and late August/early September (Figure 2b). This pattern suggests three generations in Santa Cruz with peak flights in February/March, June, and late September. In both areas, it is important to note that the generations are not distinct and that LBAM of all stages can be found almost throughout the year, although it appears to overwinter primarily as later stage larvae (3rd to 6th).

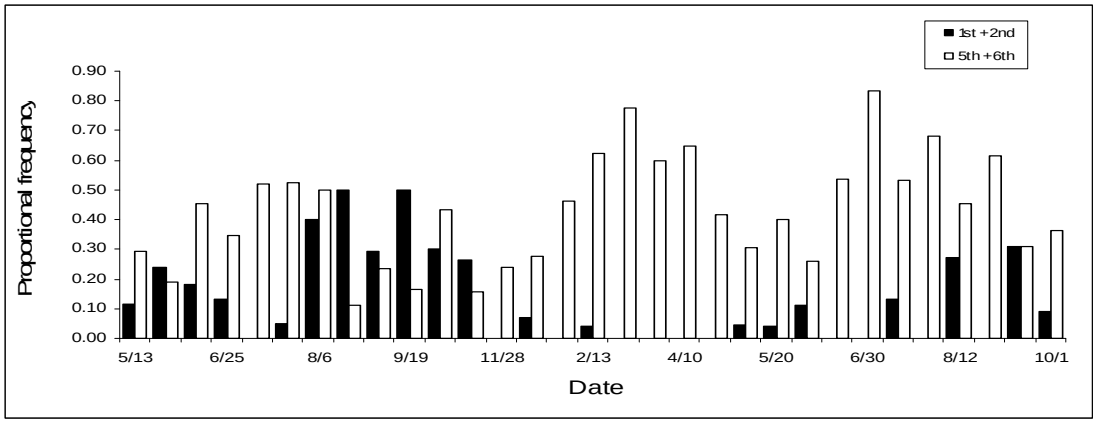

Figure 2a. The phenology and voltism of LBAM in **San Francisco** (site 1) as shown by the frequency of young (1st and 2nd) and late (5th and 6th) instar larvae on Australian tea tree.

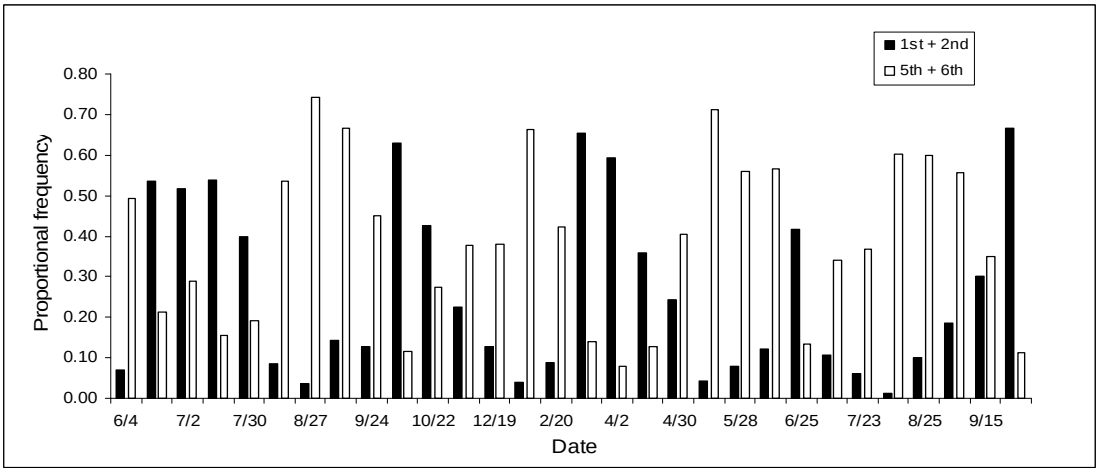

Figure 2b. The phenology and voltinism of LBAM in **Santa Cruz** (site 1) as shown by the frequency of young (1st and 2nd) and late (5th and 6th) instar larvae on manzanita.

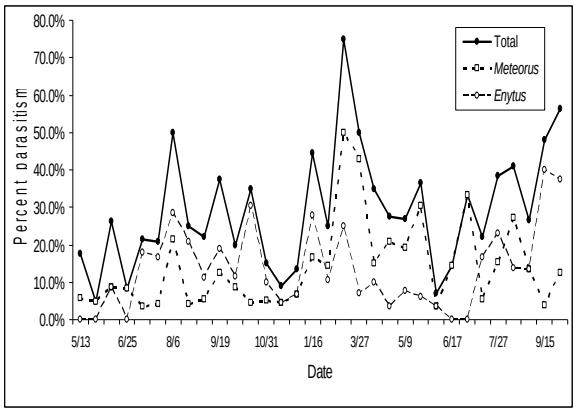

Figure 3a. Larval and pupal parasitism of LBAM in **San Francisco** (site 1) in 2008 and 2009.

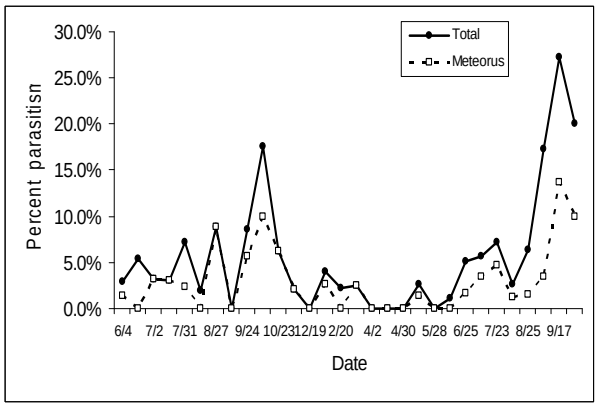

Figure 3b. Larval and pupal parasitism of LBAM in **Santa Cruz** (site 1) in 2008 and 2009.

Meteorus trachynotus

Parasitism at the four sites monitored was largely due to two larval parasitoids (*Meteorus trachynotus* and *Enytus eureka)*, although the latter species contributed little to parasitism in Santa Cruz. In San Francisco apparent parasitism has been as high as 75%, with *M. trachynotus* particularly active in spring, while *E. eureka* has dominated the parasitism from mid-summer (Figure 3a). In contrast, in Santa Cruz, parasitism has peaked at 27%, with *M. trachynotus* activity low in spring and greater at the end of summer (Figure 3b). For the two other sites monitored apparent parasitism has been around 40% for San Francisco (site 2) and 25% for Santa Cruz (site 2). The greater rates of parasitism in San Francisco are likely due to the greater activity of *E. eureka*, and the longer period of larval development (with only two generations per year) in this area.

Enytus eureka

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Foreign Exploration for Light Brown Apple Moth Parasitoids in New Zealand

William Roltsch

The light brown apple moth (LBAM), *Epiphyas postvittana*, was first detected in California in February 2007. As part of the overall response to this pest, a classical biological control project was initiated: identifying and collecting native Australian parasitoids of LBAM and conducting required host range tests for the eventual permitting and release of some species in California. This work is being conducted in cooperation with Dr. Nick Mills of the University of California, Berkeley (see report by Mills et. al.). The region of southeastern Australia, where LBAM is native, has been under a drought for several years, leading to conditions making it somewhat difficult to find targeted species at times other than in spring. Most of the known parasitoids of LBAM in Australia also occur in New Zealand as a result of fortuitous and project directed introductions of biological control agents subsequent to the initial introduction of LBAM into New Zealand in the late 1800's. With more moisture available during the New Zealand summer months (esp. Nelson area), New Zealand is a favorable location for collecting. A collecting trip was conducted in New Zealand in 2009. This report provides an account of collecting activities that took place during mid-summer in two climatically different agricultural areas of New Zealand.

Collecting was done in early February of 2009 at locations in the north end of the south island near Nelson, west to Motueka and east to Blenheim. A New Zealand contact [Peter Shaw, Plant & Food Research] provided a map of locations and plant types where LBAM could be collected, esp. on Scotch broom, *Cytisus scoparius* (L.) and gorse, *Ulex sp.* Additional collections were made from low density LBAM populations on ornamental plants at his research facility. Within this region, larvae were common and collections were made predominantly from Scotch broom along roadsides and riparian areas. In the north island, collections were made near the southern shore of Lake Taupo and in the Hawkes Bay area, which is the largest fruit production area of New Zealand. Collections were made in several vineyards and an abandoned apple orchard (2 ha). Assistance was provided in the Hawkes Bay area by Jim Walker and his colleagues (Plant & Food Research). Very few LBAM larvae were found at these sites. A large non-irrigated county park near Napier was also searched. The park consisted of large trees and shrubs, from which no LBAM were found. In the Hawkes Bay region of New Zealand, Scotch broom could only be found in the foothills and mountains surrounding Hawkes Bay above approximately 150m elevation. LBAM was intermittently common on Scotch broom (and to a lesser extent on dock, lupine and gorse) in the highlands surrounding Hawkes Bay region, well outside of orchard and vineyard farm areas.

Overall, LBAM densities at low elevations in Hawkes Bay were considerably lower (nearly undetectable in summer) than in the Nelson area. These two landscapes are different in terms of flora and moisture, with summers in Hawkes Bay being very dry (Figure 4). Agriculture in the Nelson area is more commonly associated with narrow valleys and riparian areas. Lastly, upon traveling to the interior of the north island, LBAM was collected from Scotch broom along the roadside on the south perimeter of Lake Taupo. LBAM larvae were very common on this plant at this location. At several

high elevation locations along Hwy. 47 south of Lake Taupo, Scotch broom was common, however, very few LBAM were present. In all, more than 300 larvae and pupae were collected and delivered to Evelyne Hougardy at the UC Berkeley quarantine facility.

Figures 1 & 2. LBAM collection location south of Lake Taupo. Scotch broom had numerous feeding sites typified by multiple stems and branches webbed together.

Figure 3. Late instar LBAM larva on Scotch broom sample.

The material collected produced 64 *Dolichogenidea tasmanica* (38 emerged) (Hym., Braconidae), seven *Goniozus* sp. (Hym., Bethylidae) (four emerged), 37 *Glyptapanteles demeter* (Hym., Braconidae) (32 emerged, many prior to arrival), three flies (Diptera, Tachinidae) and four hyperparasitoids. *Dolichogenidea tasmatica* was the dominant parasitoid. Because it was successfully being reared in a colony initiated from material obtained from Australia, it was not used in a colony. *Dolichogenidea tasmanica* was collected in all sample locations, however, it was most abundant in the Hawkes Bay and Lake Taupo samples from the north island. *Goniozus* sp. was the least common and colony initiation was not successful. *Glyptapanteles demeter* was very common in the samples obtained from the Nelson area in the south island, however, it was not successfully reared. Parasitoids of leafrollers often have very specific needs regarding conditions for mating and parasitization. As a result, they can be quite difficult to rear from small starting numbers.

Figure 4. Contrasting landscapes during mid-summer in New Zealand's primary fruit growing regions.

Table 1. Primary collection locations easily viewed using Google Earth.

Nearest City	Road	GPS Coordinates		Elevation	Plants
Nelson/Motueka South Island	Moutere Rd	-41.29593	173.057494	120m	Scotch broom
Nelson/Motueka South Island	Moutere Hwy.	-41.28803	173.04070	120m	Gorse
Nelson/Motueka South Island	Shaggery Rd.	-41.113937	172.954042	24m	Scotch broom
Nelson/Motueka South Island	Motueka Valley Hwy	-41.04860	172.87529	61m	Scotch broom
Picton/Blenheim South Island	Hwy 1	-41.405464 Est.	173.958709 Est.	12m	Scotch broom
Turangi North Island	Hwy. 47	-39.100036	175.542775	884m (no LBAM found)	Scotch broom
Turangi North Island	Hwy. 47	-38.98369	175.78387	369m	Scotch broom
Napier North Island	Taihape Rd.	-39.50826	176.60213	273m	Scotch broom/gorse
Napier North Island	"	-39.446003	176.48725	339m	broom
Napier North Island	"	-39.42058	176.41362	710m	broom
Napier North Island	Pakowhai Rd.	-39.60135	176.87204	2m	Various trees and shrubs
Napier North Island	Mere Rd.	-39.604000	176.732190	34m	Grape

Figure 5. Gorse plant in bloom. LBAM larvae found in soft terminal tissue.

Foreign Exploration and Host Range Testing of Light Brown Apple Moth Parasitoids

Nick Mills[1], Evelyn Hougardy[1], Kevi Mace[1]and William Roltsch

From a series of parasitoid survey trips made across the states in southeastern Australia we have been able to build a picture of the key parasitoids that attack light brown apple moth (LBAM) both within crop (grape and apple) and non-crop (plantain, white melilot, Hardenburgia, and broom) environments. The two dominant species that occur throughout the region are *Dolichogenidea tasmanica* (Hym.: Braconidae) and *Goniozus jacintae* (Hym.: Bethylidae). Other parasitoids that have been reared in some numbers are *Australoglytpa latrobei* and *Phytodietus celsissimus* (Hym. Ichneumonidae), *Bassus unimaculata* (Hym.: Braconidae), *Brachymeria phya* and *B. teuta* (Hym.: Chalcididae), *Eupsenella* sp. (Hym.: Bethylidae), and unidentified tachinids. The ichneumonids and tachinids have only been reared from crop plants, *Eupsenella* sp. has only been found from weedy host plants, and *B. unimaculata* has not been found in Tasmania. From these rearings we currently have four Australian parasitoids in culture in the quarantine facility at UC Berkeley; *D. tasmanica*, *G. jacintae*, *Eupsenella* sp., and *B. phya*.

Figure 1. LBAM leafroll on strawberry clover.

The three larval parasitoids, *D. tasmanica*, *G. jacintae*, and *Eupsenella* sp., can only be reared on LBAM larvae feeding on natural foliage, and we are currently using potted *Plantago lanceolata* in sleeve cages to maintain cultures of *D. tasmanica* that attacks 1st and 2nd instar host larvae, excised leaves of this same host plant in small plastic cups to raise *G. jacintae* and *Eupsenella* sp. A culture of *B. phya* is maintained using fresh pupae of LBAM in glass vials.

So far, host range testing has focused on *D. tasmanica*, as a key parasitoid that has been found to achieve up to 80% parasitism of LBAM larvae in New Zealand where it has been introduced. Parasitoids used in host range tests were reared on LBAM on either grape (*Vitis vinifera*) or plantain (*Plantago lanceolata*) in the UC Berkeley quarantine facility at 23°C, 25% RH and 16:8 L:D photoperiod. Naïve three day-old mated females were used in tests, unless otherwise noted. To reduce experience with the target host, parasitoids cocoons were harvested a few days before emergence and placed in glass vials in the absence of hosts and plant material. Emerging parasitoid females were collected daily and kept with an excess of males to ensure mating until used in tests.

Sequential no-choice tests are used, with individual parasitoid females exposed to the non-target species (= test species) and to the target species (LBAM) in sequence. This type of host range test evaluates the physiological host range of a parasitoid, and does not address the ecological host range which can be influenced by both parasitoid preference and behavioral and ecological compatibility. Because prior experience with LBAM may reduce response to lower ranked hosts, even if it is not present simultaneously during the test, the non-target host was presented first to naïve females. However, lack of oviposition experience could also reduce parasitism rates and therefore, for one test species (orange tortrix), a series of tests were undertaken with LBAM presented first to naïve females. Parasitoid females were held without hosts for at least an hour between the two hosts presented in sequence. For each sequential test, 10 1st and 2nd instar larvae of each host species presented on foliage. The number of hosts encountered and accepted for oviposition was recorded (= host acceptance). Each set of observations in a sequential test ended when all the hosts had been parasitized, after the parasitoid female had left the host patch, or after 15 min, whichever occurred first. A replicate consisted of a pair of observations (15 min max) with a single *D. tasmanica* female. Attacked host larvae were then transferred to new foliage and reared to the pupal stage or larval death. Timing of larval death was recorded as well as parasitoid cocoon production. Host larvae dying before reaching the pupal stage were usually too dry to be dissected to see if parasitism had occurred. Emergence, size and sex of parasitoid progeny, if any, were recorded. All tests were conducted in the at UC Berkeley quarantine facility at 23°C, 25% RH and 16:8 L:D.

Figure 2. *Dolichogenidea tasmatica* ovipositing.

Figure 3. *Goniozus jacintae*

Neonate host larvae were transferred to foliage several days before the tests and were used when the majority of target or test larvae have reached the second instar (about six days). The host plant used varied with test species; grape leaves for orange tortrix (OT) - *Argyrotaenia franciscana* and omnivorous leafroller (OLR) - *Platynota stultana*, and prune leaves for obliquebanded leafroller (OBLR) - *Choristoneura rosaceana* and apple pandemis (AP) - *Pandemis pyrusana*. It has not proved possible to be able to use test species that are indigenous non-crop tortricids. In California, these non-crop hosts are univoltine and have not proved amenable to rearing in captivity. Considerable time has been spent using black lights to capture adult female tortricids in the Bay Area over the

past year, in the hope that mated females would lay eggs and provide neonate larvae for testing. However, only males of non-crop tortricids come to light, and the occasional female that has been trapped this way has died within a day and failed to lay eggs.

No difference was found in the time spent in a patch or the number of host larvae attached between the two reciprocal sequences of host exposure for OT as a test host species. The mean number of LBAM larvae attacked in a sequential test was 3.8 (n = 24) when LBAM was presented first and 3.6 (n = 21) when presented second in the sequence, with 100% of the females attacking LBAM larvae in both cases. *D. tasmanica* appeared to attack a mean of 3.3 to 4.7 LBAM larvae during each sequential test (n = 15-24), with from 21-32 LBAM larvae producing cocoons of *D. tasmanica* from the set of tests for each target species, of which from 81.6-91.3% emerged with a sex ratio of from 76-86% male. All four test tortricid species were attacked by *D. tasmanica* in the sequential tests, which probed and apparently parasitized a mean of 2.0 larvae of OT (n = 21), 2.7 larvae of OLR (n = 24), 1.2 larvae of OBLR (n = 15), and 1.2 larvae of AP (n = 18). Of these apparent attacks, eight OT larvae produced cocoons of *D. tasmanica* 75% of which emerged and 100% were male, six OLR larvae produced cocoons with 50% emergence and 66% male, two OBLR larvae produced cocoons with 50% emergence and 0% male, and no AP larvae produced cocoons

Survey update - From a trip to Tasmania in November 2009, parasitism of LBAM larvae collected from strawberry clover was zero, matching an earlier observation of parasitism of larvae collected from white melilot in New South Wales in November 2008. In contrast, however, parasitism on shrubs such as Hardenbergia and Christmas bush (*Bursaria spinosa*) was found to be unusually high, suggesting that woody host plants are an important overwintering reservoir for both LBAM and its parasitoids. *Australoglypta latrobei* and *Meteorus* sp. were dominant species in the parasitoid complex.

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Spider Response and Fate Following the Consumption of Light Brown Apple Moth Larvae

William Roltsch and Nada Carruthers[1]

Like most leafroller species, the light brown apple moth (LBAM), is subject to predation and parasitism by a range of insect and spider species. Although it has been reported that spiders can be an important mortality agent of LBAM in its native Australia, a brief test was conducted to support or deny the assumption that the consumption of LBAM larvae is unlikely to exert ill effects toward spider species of North America.

Two hunting spider species were collected at two residences within the greater Sacramento region of northern California, including a species of Agelinidae (funnel web spider) on apple trees in a backyard in Elk Grove and Salticidae (jumping spiders) from roses in Orangevale. Male and female adult or near adult stages of both were used in the study. In the laboratory, spiders of each kind were held individually in transparent plastic containers (approx. 0.25 l). The lids of each container were perforated and each contained a small cotton ball moistened with purified drinking water. The room temperature was maintained at 24^{o} C. Treatments consisted of spiders provided with four, 3rd or 4th instar LBAM larvae or no larvae. Food was withheld from all spiders 48 hours prior to testing. Each treatment was represented by three containers containing individual spiders of each kind (i.e., six total containers representing three replications/spider family). Observations were recorded every 15 minutes for two hours, beginning at 11:50 am.

Two hour observation results: The three funnel web spiders in containers with larvae were stationary for 45 minutes as were all controls. At the one hour mark, one funnel web spider killed and continued to consume a larva through the second hour. A second agelinid killed a larva at the 90 minute point and continued to consume it up to the two hour observation and termination point. In both cases, the larvae were approximately 50% consumed at the two hour mark. The third funnel web spider remained stationary the entire two hour observation period. By comparison, the salticid spiders each killed a larva during the first 15 minutes. One of the three had completed the consumption of the larva (only exoskeleton remained) after 75 minutes, and at 90 minutes, it killed a second larva and completed consumption by 120 minutes. A second spider completed consuming its larval pray at 105 minute point and became stationary. The third did not completely feed on its larval prey, opting to walk inside the container (perhaps hunting) after the first 60 minutes.

Survivorship results: Observations were made during each of the following two days and at day five following the initial study. After 24 hours, all funnel web spiders in the LBAM treatment had consumed all four of the LBAM larvae in each container. In addition, all spiders in each treatment (i.e., LBAM or no LBAM) remained alive for 120 hours. Two of the three jumping spiders had consumed all four larvae in 24 hours; one larva was present in the third container. The last larva died by 72 hours, perhaps killed by the spider but not consumed. All jumping spiders in both treatments were alive after 120 hours. The spiders were subsequently held in a second laboratory in Sacramento and fed oblique banded leafroller larvae for the next month. All remained alive, and several molted. Results of this limited study support the assumption that there is no apparent

acute toxicity associated with the consumption of LBAM larvae by spiders, and toxicity is unlikely to occur with other members of the predator community. Furthermore, no negative long-term impacts on survivorship were observed.

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Changes in Citrus Leafminer Phenology and Density as it Invades California

Kris Godfrey, Elizabeth Grafton-Cardwell[1], and David Kellum[2]

The citrus leafminer (*Phyllocnistis citrella*) is a serious pest of citrus and can facilitate citrus bacterial canker when the pathogen is present. Citrus leafminer was first found in California in 2000, and has continued to move throughout the citrus-growing parts of California. Found originally in the desert valleys of southern California, it has spread along the southern coast, moving northward through the San Joaquin Valley, as far north as Stanislaus County, and can be found as isolated populations in Alameda, Contra Costa, Santa Clara, Santa Cruz, and Solano counties. The isolated populations are the result of accidentally moving citrus leafminer on nursery stock.

The phenology of citrus leafminer varies in different parts of California and appears to be related to the amount of time citrus leafminer has been present in an area. In parts of southern California, citrus leafminer has been established for a number of years and is very new to others. Multi-year monitoring of these newer sites was established to understand how the phenology of citrus leafminer changes during invasion in California and how that influences the establishment of biological control.

In the desert valleys where citrus leafminer has existed the longest, nine species of parasitoids attack citrus leafminer. The predominant species are *Closterocerus utahensis* and *Cirrospilus coachellae*. Both of these parasitoids are generalists and typically attack gracillariid leafminers. Larval parasitism in the desert valleys ranges from less than 10% to as high as 70% (J. Heraty, University of California-Riverside, unpublished data). In San Diego and Ventura counties where leafminer has also existed for several years, three parasitoids, *Closterocerus utahensis*, *Pnigalio* spp., and *Chrysocharis* spp., have been identified attacking citrus leafminer (P. Mauk, UCCE – Southern Region, unpublished data). The parasitoids attacking citrus leafminer in the San Joaquin Valley are not well documented. This study was initiated in 2006 to investigate the phenology of citrus leafminer along the extreme southern coast of California and also the southern end of the San Joaquin Valley. The study also included surveys of parasitoids attacking the leafminer in the San Joaquin Valley. The study was completed in 2009.

Phenology studies were conducted at five locations in San Diego County from March 2007 through October 2009; at four locations in Tulare County from April 2006 through October 2009; and at three locations in Kern County from August 2007 through September 2009. Pheromone-baited traps that attracted male moths were used at all locations. Traps were replaced at monthly intervals during the study. The starting date of the trapping and the number of traps varied with location and the agronomic practices in place at each location. In Kern County, Kern 3 was lost due to orchard removal in the winter of 2008, and Kern 2 was lost in May 2009 due to orchard removal. During the winter months, traps were removed from the sites because moths do not fly during cold temperatures.

The survey of native parasitoids was conducted at all San Joaquin Valley locations each time traps were serviced. The survey consisted of examining five terminal branches on 10 randomly selected trees at each site. The first 10 – 15 leaves on the tip of

each terminal branch were inspected for the presence of leaf mines. Leaves with active mines were collected, and held for the emergence of any parasitoids.

The density and phenology of citrus leafminer varied with location in the state. In San Diego County, where citrus leafminer was first reported in 2000, large densities of moths were caught in traps from June through November or December from 2007 - 2009 (Figures 1-3). In Tulare County, where citrus leafminer was first reported in 2006, very low numbers of moths were captured during 2006 and 2007 (Figure 4). Large densities of moths were trapped in Kern County in 2007 – 2009, and in 2008 – 2009 in Tulare County (Figures 4-5). The peaks in density initially occurred from September through November, but as densities increased, the pattern in phenology was much like that in San Diego County (Figures 1-5). However, the densities of moths caught at peak times were always much greater in San Diego County than in Kern or Tulare County (Figures 1-5).

Surveys of the native parasitoids in Tulare and Kern recovered no parasitoids or evidence of parasitoids (Table 1). However, evidence of arthropod predation and several arthropod predators were recovered. As the density of citrus leafminer increased through time, more larvae were found from mid-summer on at some sites, rather than just in the fall. With the increase in larval density, the amount of predation increased.

The results from these studies suggest that the seasonal phenology of citrus leafminer changes with time after invasion. Early in invasion, the density of moths is very low and well synchronized with flush cycles of citrus. As the density increases, the pattern of phenology stabilizes, with more moths found year-round. The largest number of moths captured is during flush cycles. The populations of citrus leafminer in the San Joaquin Valley are beginning to achieve densities where they are an attractive prey source for generalist arthropod predators. More time is needed to attract generalist insect parasitoids.

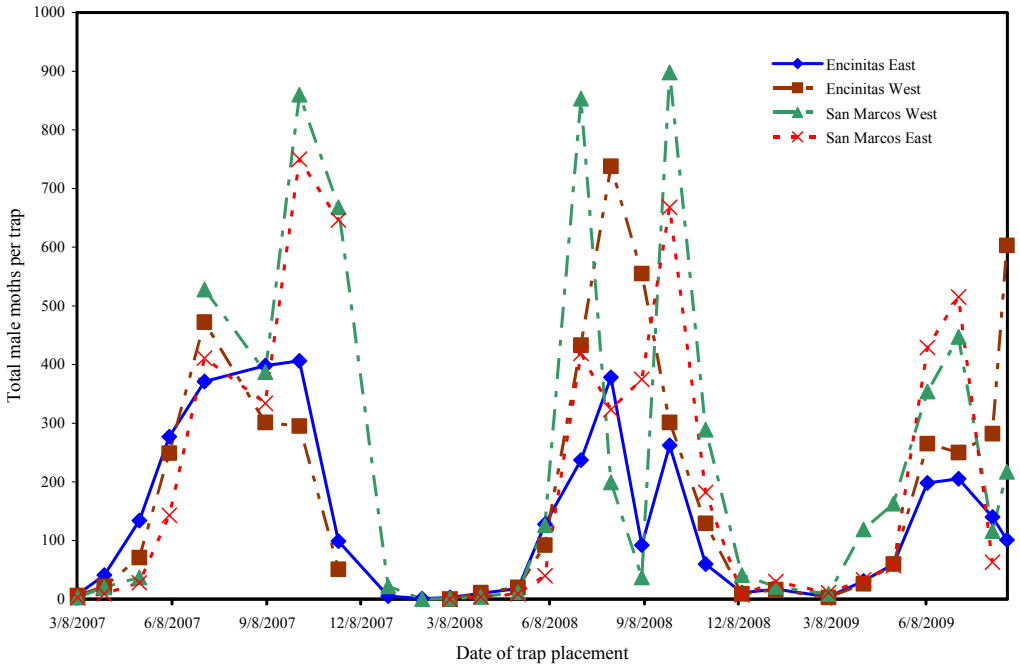

Figure 1. The total number of males moths captured in pheromone traps in Encinitas and San Marcos in San Diego County from March 2007 through October 2009.

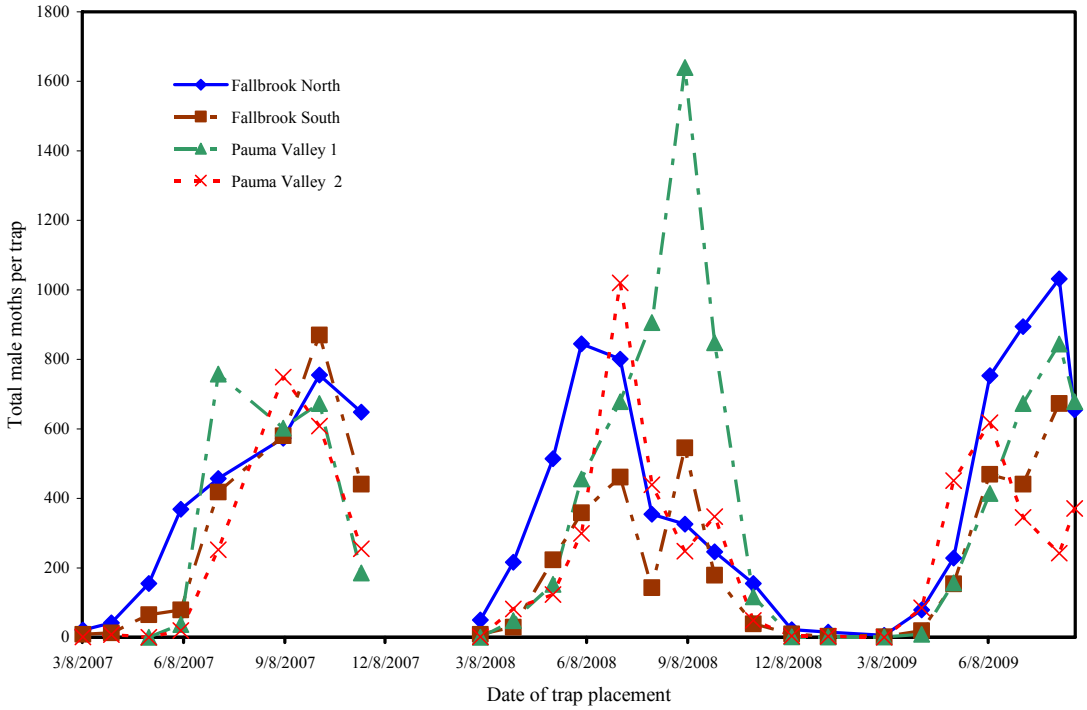

Figure 2. The total number of male moths captured in pheromone traps in Fallbrook and Pauma Valley in San Diego County from March 2007 through October 2009.

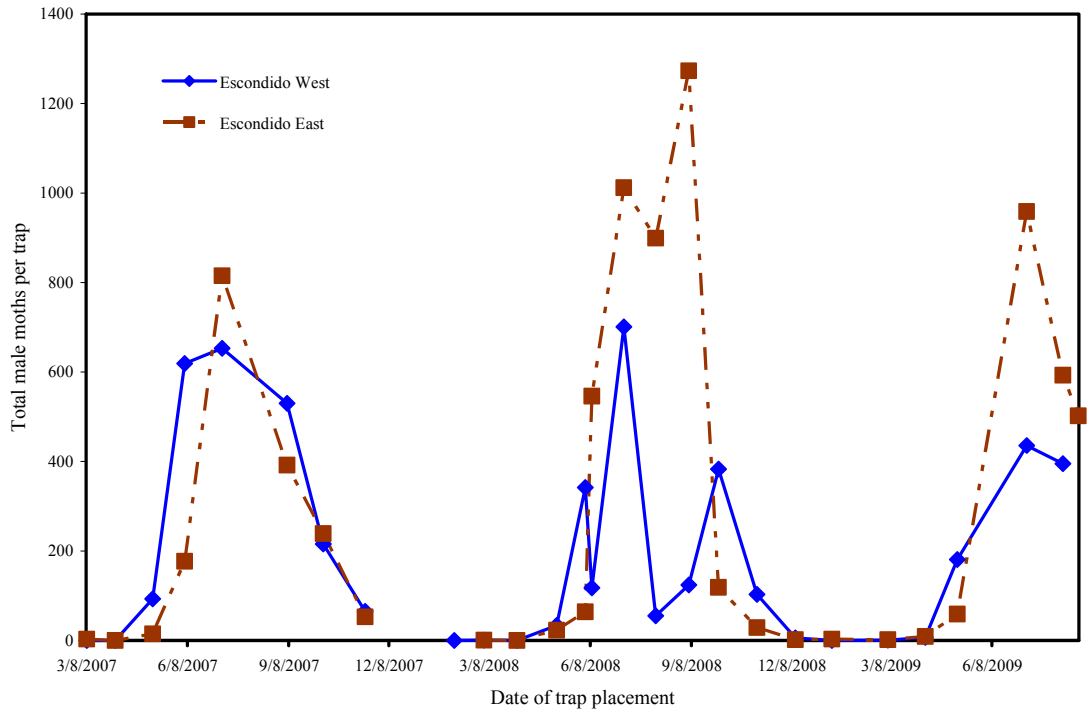

Figure 3. The total number of male moths captured in pheromone traps in Escondido in San Diego County from March 2007 through October 2009.

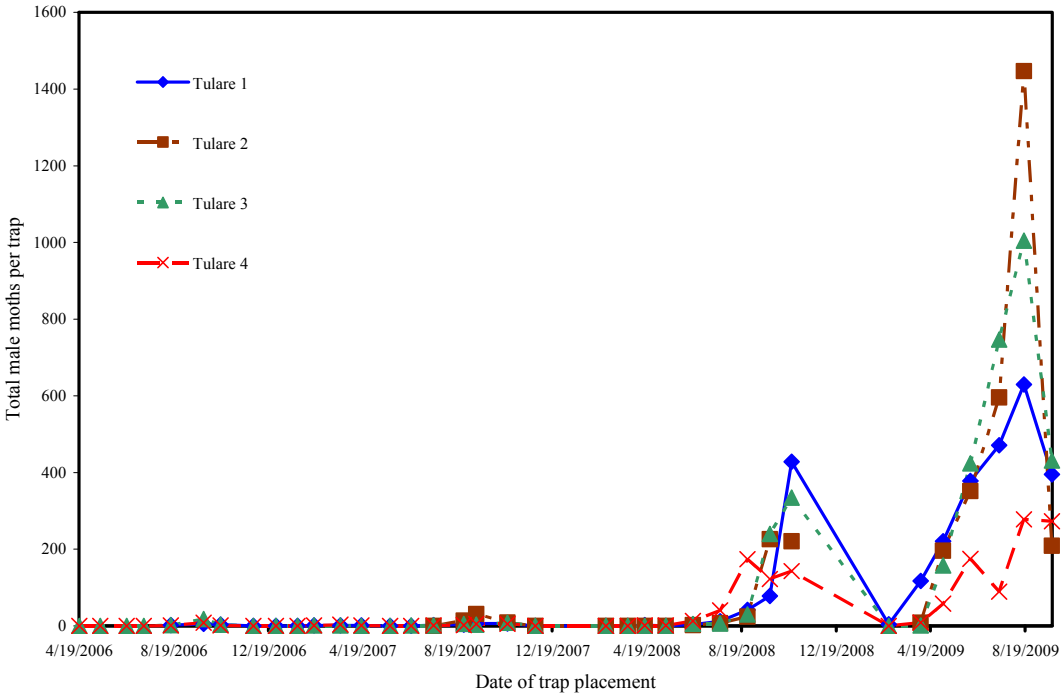

Figure 4. The total number of male moths captured in pheromone traps at 4 sites in Tulare County from April 2006 through September 2009.

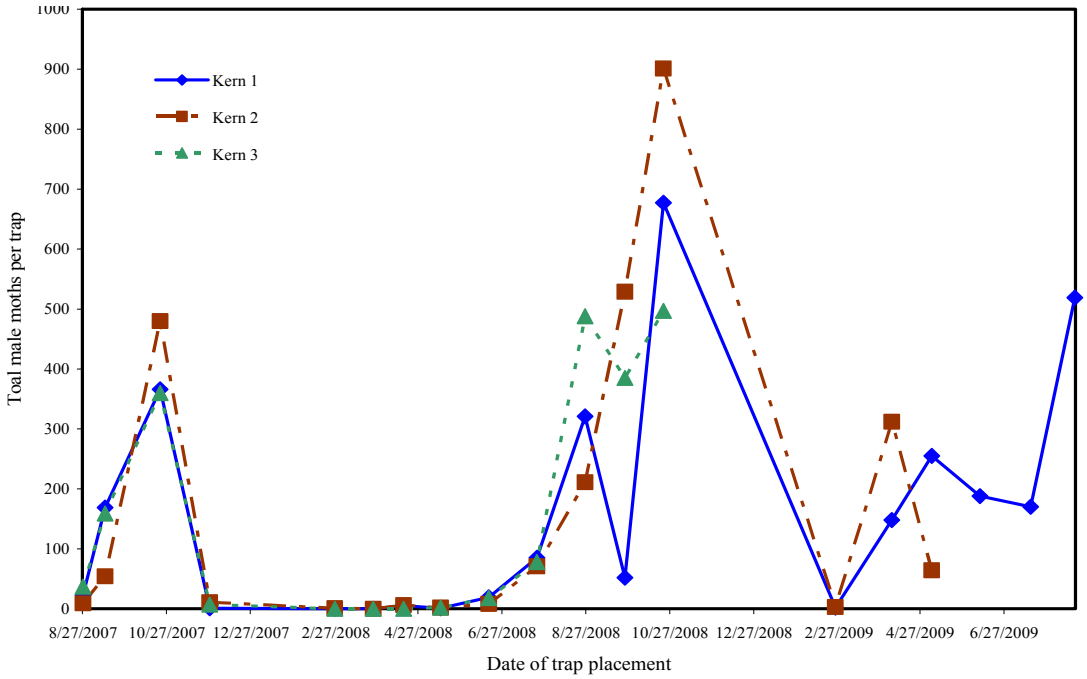

Figure 5. The total number of male moths captured in pheromone traps at 3 sites in Kern County from August 2007 through September 2009.

Table 1. The number of mined leaves in 50 branch terminal samples, emerged mines, larvae, pupae, and preyed upon larvae found in Tulare and Kern counties from 2006-2009. Results given for sample dates and sites with positive finds.

Survey Date	Site	No. of Leaves Mined	Emerged Mines	Live Larvae	Live Pupae	No. Preyed Upon
11/18/06	Tulare 3	1	0	1	0	0
7/19/07	Tulare 3	1	0	0	0	1
9/12/07	Kern 1	1	1	0	0	0
	Kern 3	1	2	0	0	0
10/22/07	Tulare 3	1	0	1	0	0
	Kern 1	1	1	0	0	0
11/27/07	Kern 1	7	1	8	1	0
	Kern 2	2	0	3	1	0
	Kern 3	2	1	0	0	1
12/19/07	Kern 2	13	2	3	4	0
	Kern 3	4	2	2	0	0
9/24/08	Kern 1	5	5	0	0	0
	Kern 3	3	0	2	1	0
10/22/08	Tulare 1	5	5	0	0	0
	Tulare 2&3	8	2	2	6	0
	Kern 1	16	12	2	1	1
	Kern 2	14	7	1	5	1
	Kern 3	33	23	2	4	3
11/24/08	Tulare 2&3	4	1	1	2	0
	Tulare 4	14	14	0	0	0
	Kern 1	4	1	3	0	0
	Kern 2	19	1	19	2	0
6/9/09	Tulare 2	12	0	5	3	1
	Tulare 3	22	0	9	12	2
	Tulare 4	1	0	1	0	0
7/16/09	Tulare 2	22	16	1	5	3
	Tulare 3[a]	30	9	2	18	4
8/17/09	Tulare 1	19	0	9	0	0
	Tulare 2	134	35	55	16	1
	Tulare 3[b]	55	22	8	0	4

[a]Chrysopid larva found on leaf with leafminer mines
[b]*Orius* sp. adult found on leaf with leafminer mines

[1]University of California-Riverside, Kearney Agricultural Center, Parlier, California
[2]San Diego County Agricultural Commissioner's Office, San Diego, California

Releases of Olive Fruit Fly Parasitoids

Charles H. Pickett, Daniel Wisheropp, Xin-geng Wang[1], Kent Daane[1],
Yael Argov[2], and Marshall Johnson[3]

During July 2009, cooperators at the University of California, Berkeley and University of California, Riverside (located at the University of California Kearney Field Station), along with CDFA made releases of two olive fly parasitoids, *Psytallia lounsburyi* and *P. concolor* (Namibia), the earliest seasonal starting date yet. A total of 7,600 *P. concolor* (Namibia) were released in San Luis Obispo, Solano, Sonoma and Yolo counties in summer/fall 2009 (Table 1, Figure 1). A large portion of these parasitoids were reared through contract by the Israel Cohen Biological Control Institute. They were able to rear a much higher number of *P. lounsburyi* than has been done in the past by ourselves and others. Adult parasitoids were released directly into the tree or into field cages. Recoveries of *P. concolor* (Namibia) have been made from coastal sites and for the first time, near Davis, California.

Table 1. Number of parasites released for control of olive fruit fly, 2009.

County	Site Name	Total Released (♂+♀)	
		P. concolor (Namibia)	*P. lounsburyi* (Kenya)
Solano	Wolfskill	3,003	1,663
Yolo	UC Davis	1,638	2,075
San Luis Obispo	multiple sites	2,500	200
Sonoma		610	200
Total		**7,751**	**4,138**

Figure 1. Release of *P. concolor* (Namibia), at UC Davis, October 2009.

[1]University of California, Berkeley, California
[2]Israel Cohen Biological Control Institute, Beit Dagan, Israel
[3]University of California, Riverside, California

Parasitoids Attacking Olive Fruit Fly in Namibia

Charles H. Pickett, Arnaud Blanchet[1], John Irish[2], Alan Kirk[1], Kim Hoelmer[3], Kent Daane[4], and Marshall Johnson[5]

Several years of collections from Namibia suggest that the mix of parasitoids attacking olive fruit fly in this country differs from other regions where we have collected in sub-Saharan Africa (Table 1; see 2008 annual report). *Psytallia concolor* (ex. Namibia) has been the dominant parasitoid attacking olive fruit fly infesting wild olives in the Kartsfeld region of north central Namibia, which includes the towns of Grootfontein, Tsumeb, and Otavi (19° south latitude, 1400 m elev.). In other parts of Africa, this species has played a minor role or has been entirely absent, with *P. lounsburyi* and *Utetes africanus* being the dominant species. Although *P. concolor* was the only, and dominant parasitoid attacking olive fruit fly on the Canary Islands and in Morocco (2008 annual report), parasitism was typically less than 11%. These field data suggest that the population of *P. concolor* from Namibia may have a higher affinity for olive fruit fly, or at least greater survivorship in the olive tree habitat than other populations of this species. To further explore the role of *P. concolor* (ex. Namibia), we collected fruit monthly, three times over the olive fruit maturation period. This study reports on collections made from the Grootfontein 'median' site (Figure 1) and the 'Meteor' Rd. site in 2009 as well as collections from previous trips.

Figure 1. Grootfontein median site, April 10, 2009

Collections made from March through May 2009 showed that *P. concolor* continued to be the dominant parasitoid (Table 1). Collections in March, however, did not yield any parasitoids, perhaps because the fly population was so low at the two collection sites. Nevertheless, as in previous years, parasitoids and flies were both present in fruit in April and May, with *P. concolor* being the dominant parasitoid, followed by *P. lounsburyi*, *U. africanus*, and *Bracon* spp. *Psytallia lounsburyi* has been the dominant parasitoid in the Burguret Forest of Mt. Kenya, and *U. africanus* has been dominant on wild olives found in West Cape, South Africa. In South Africa, *P. concolor* has represented 0 to 6.3% of the parasitoids collected, and has never been reported attacking olive fruit fly in Kenya (Olive Fly Report, 2008). These results suggest *P. concolor* (ex. Namibia) differs in affinity for olive fly from other populations. Of the 762 fruit flies

collected, all were *B. oleae*, and three were *Ceratitis capitata*, a possible alternate host for *P. concolor* since this fly can attack fruit from a wide range of plant species. We hope to repeat this survey in 2010.

Table 1. Parasitoids emerging from *B. oleae* collected in Namibia, 2004 – 2009.

Collection Date	#puparia[1] or emerged flies[2]	Percent of all parasitoids (# collected)			
		P. concolor	*P. lounsburyi*	*U. africanus*	*Bracon* spp.
May 6, 2004	694[1]	58.6 (108)	0.0 (0)	11.9 (22)	29.3 (54)
May 6-13,2007	507[1]	74.7 (274)	21.5 (79)	3.8 (14)	0.0 (0)
April 18-21, 2008	5338[1]	65.6 (1134)	21.0 (364)	6.5 (113)	6.9 (121)
March 20, 2009	14[2]	0.0	0.0	0.0	0.0
April 16, 2009	848[2]	70.8 (148)	0.9 (2)	22.4 (47)	5.7 (12)
May 2, 2009	36[2]	75.0 (6)	0.0	0.0	25.0 (2)

In 2009, two collections sites: Grootfontein 'median' and the 'Meteroite' site

[1]USDA-ARS, European Biological Control Laboratory, Montferrier, France
[2]Windhoek, Namibia
[3]USDA-ARS, Beneficial Insect Introduction Research Unit, Newark, Delaware
[4]University of California, Division of Insect Biology (Dept. ESPM), Berkeley, California
[5]University of California, Department of Entomology, at the Kearney Agricultural Center, Parlier, California

Olive Psylla: Foreign Exploration 2009

Charles H. Pickett, Kent Daane[1], David Kellum[2], and Javid Kashefi[3]

The olive psylla, *Euphyllura olivine*, first reported in California in 2007 is currently confined to southern California but can potentially spread north into the olive production areas of California. When first surveyed, infestations were found within 15 miles of coastal communities in San Diego and Orange counties. A recent survey conducted by M. Johnson et al. (unpubl. data) has found that the psyllid has now expanded its distribution up to 30 miles inland: Fallbrook and Temecula. This survey did not find them in Palos Verdes, Anaheim, Burbank, Santa Clarita, Sylmar, Riverside, Sun City, Perris, or Hemet. In Europe and the Mediterranean Basin, the pest is composed of three closely related species, *Euphyllura olivina*, *E. phillyreae*, and *E. straminea,* with the former found mainly in the western half of the Mediterranean Basin. Because the olive psylla has the potential of damaging high numbers of olive flowers during spring months, we decided to investigate the potential for importing parasitoids of the pest. Surveys in southern Europe have found at least one species of encyrtid attacking olive psylla, *Psyllaephagus euphyllurae*. We report on a survey for extant parasitoids of olive psylla in San Diego County and a survey for parasitoids of the olive psylla in southern Europe spring 2009.

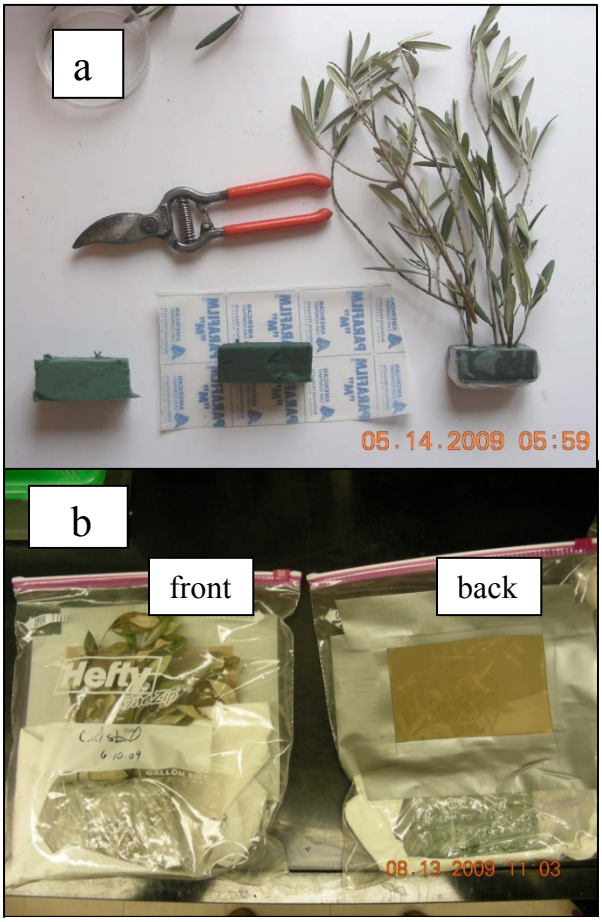

Figure 1. Materials (a) used to create bouquets (b) of olive cuttings.

On June 10, 2009, 10 trees were sampled in San Diego County for olive psylla and attendant parasitoids. Stem cuttings infested with high numbers of olive psylla nymphs were removed from five trees at the Presidio in San Diego, and from five trees sampled along public roads in Carlsbad. Cuttings were placed in cages constructed of (1 gal) plastic zip-lock baggies, modified with a screen opening to allow for breathing (Figure 1a,b). Bouquets of two to three, 0.2 m stem cuttings, were placed into water saturated floral foam, which in turn was wrapped in parafilm®. Two months later on August 13, 2009, cages were examined for the presence of parasitoid mummies (pupal case) and adults. Cages averaged 3.7 adult psylla (range: 1 to 10), and no mummies or adult parasitoids were found. We will repeat this in 2010. In his survey, Johnson et al.

(unpubl. data) found some generalist predators associated with clusters of olive psylla nymphs on stems of trees, but no mummies.

Drs. Kent Daane and Charles Pickett traveled to eastern Spain the first half of May 2009. With help from a local contact (Dr. Oscar Alomar, IRTA, Barcelona, Oscar.Alomar@irta.cat) we collected hundreds of olive stem cuttings from 20 locations infested with olive psylla nymphs. These collections came from northeastern Spain (Cataluña), the coastal island of Menorca which has a wild strain of olive tree, and south from the province of Murcia. The olive psylla, *Euphyllura olivina*, was easily found at most locations; lightest in the north and heaviest around Murcia (Figure 2), and on the island of Menorca. In the region of Murcia, a local contact Dr. Juan Antonio Sanchez (Instituto Murciano de Investigaciones y Desarrollo Agrario (IMIDA), juana.sanchez23@carm.es) and Charles Pickett, drove to several locations with commercial olive trees infested with olive psylla. He collected again several weeks after the initial visit and found what appeared to be *Psyllaephagus* sp. associated with infestations of olive psylla. Next, Charles Pickett spent two days collecting from six sites in the Montpellier area of southern France with Alan Kirk (USDA ARS European Biological Control Laboratory, France) and again found most trees infested with olive psylla. Two days were also spent collecting south and within 50 km of Thessaloniki, Greece where EBCL has a substation managed by Javid Kashefi. All examined olive trees from eight locations were infested with olive psylla, many with abundant populations (Figure 3). The psylla in this area were *Eurphyllura phillyreae*. Mr. Kashefi recovered adult psylla, syrphids associated with infestations, and psylla mummies (created by parasitoids).

Figure 2. Flowers and olive psylla on cuttings, Murcia, Spain.

Figure 3. Olive psylla nymphs feeding on olive stem, Thessaloniki, Greece.

Most of the olive cuttings from France and Greece, and some of the cuttings from Spain, were left at the EBCL laboratory in Montferrier for emergence. Only a few adult parasitoids emerged and have been

preserved in alcohol. Dr. Daane hand-carried the remaining cuttings to the UC Berkeley Quarantine and Insectary facility where he was successful in collecting live *Psyllaephagus* sp. However, he was unable to create a culture of the parasitoid.

We plan to repeat the collecting trip, starting a few weeks later when higher numbers of mummies should be present in collections.

We kindly acknowledge the help of Dr. Alessandra Rung, Plant Pest Diagnostics Laboratory, CDFA for identification of the psyllids, and Director of the European Biological Control Laboratory, Walker Jones, for use of laboratory space and help.

[1]University of California, Division of Insect Biology (Dept. ESPM), Berkeley, California
[2]San Diego County Department of Agriculture, San Diego, California
[3]USDA, ARS, European Biological Control Laboratory, Thessaloniki, Greece

Expansion of *Eretmocerus mundus* in Central California: 2002 to 2009

Charles H. Pickett, Dan Keaveny[1], Marypat Stadtherr, and Lia Chase

Five species of *Bemisia tabaci* parasitoids were released in the southern San Joaquin Valley from 1997 - 2000. They were released primarily into four study sites, one each in Fresno and Tulare counties, and two sites in Kern County. Typically, over 100,000 parasitoids were released weekly at each location with 4.05 million released in 1997, over 10 million in fall 1998, 3.2 million in 1999, and 124,000 in 2000. Additionally, much smaller numbers were released into several dozen sites over the same period of time. Since 2002, only one of the five parasitoid species that were released in large numbers remains, *Eretmocerus mundus* (Hymenoptera: Aphelinidae). We report on the current distribution and expansion of *E. mundus*.

The Pink Bollworm Program (CDFA), as part of a larger effort to monitor insect pests in cotton, selected cotton leaves within 10 m of pink bollworm traps. Samples were taken from Kern, Tulare, Fresno, and Kings counties. Only leaves with high rankings of whitefly infestations (over six nymphs per leaf) were placed into 0.5 l paper cans and held for at least two months, at about 25°C. The number of adult parasitoids and adult *Bemisia* emerging from leaves was recorded. The number of exotic *Eretmocerus* was based on males only, using the coloration of the pedicel in the antenna to discriminate exotic from natives. Species

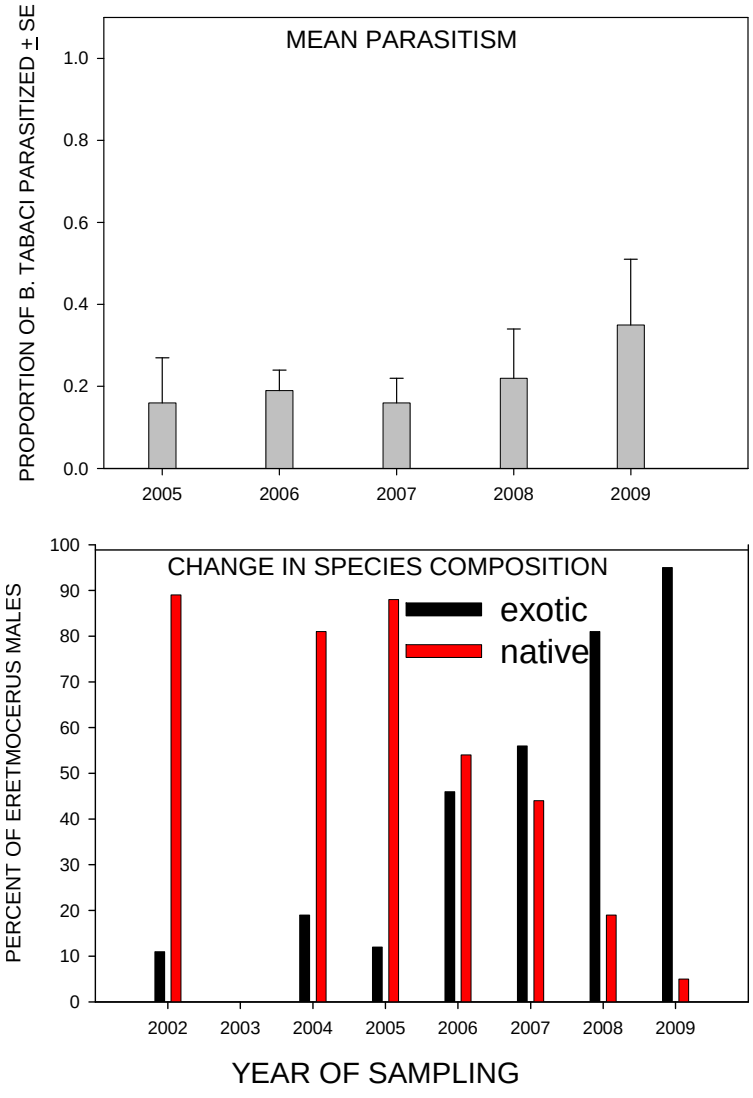

Figure 1. Change in parasitism and parasitoid species composition, Southern San Joaquin Valley, CA, 2002 - 2009

identification was based on adult females, using the shape of the antennal funicle segment and setal arrangement on the mesoscutum. Since 2002, *E. mundus* has been the only released parasitoid recovered from wild vegetation and cotton. While the percentage of *E. mundus* in samples has slowly, but steadily increased to a high of 95% this last year, the degree of parasitism (based on emerging adults) has also increased. Over the last three years, as the proportion of exotics has shifted to the majority, parasitism has steadily increased from 16 to 35% (Figure 1).

In only Kern County was there a consistent and steady increase in the proportion of *E. mundus* from first samples (Table 1). Beginning in 2006, there was a similar recovery from the remaining counties (Fresno, Tulare, Kings). Initial releases were made into *Bemisia* infested citrus groves bordering cotton fields at all three sites. However, initial densities of *B. tabaci* in trees at the Kern site were two orders of magnitude higher than the others.

Table 1. Presence of *Eretmocerus mundus* in cotton samples, by county.

Year	Mean proportion of parasitoids that are exotic, by county (number of locations sampled)				
	Fresno	Kern	Kings	Merced	Tulare
2002	0.00 (1)	0.26 (8)	0.00 (1)	0.00 (1)	0.02 (9)
2003	0.00 (1)	0.03 (3)	0.00 (0)	--	0.00 (0)
2004	0.13 (5)	0.72 (4)	0.00 (1)	--	0.04 (11)
2005	--	0.69 (2)	.	--	0.00 (9)
2006	0.4 (5)	0.53 (22)	0.65 (6)	--	0.36 (23)
2007	0.1 (2)	0.30 (5)	0.73 (16)	--	0.52 (17)
2008	--	1.0 (1)	0.75 (5)	0.98 (2)	0.75 (4)
2009	--	0.94 (6)	0.99 (3)	--	---

The number of parasitoids per gram cotton leaves shows the same pattern as parasitism. Except for 2006, there has been a steady increase in the mean number of parasitoids recorded from samples over the last three years (Figure 2), while the mean number of adult *B. tabaci* has been roughly the same. Since we have not been taking random samples of leaves, only those with high numbers of whiteflies, one would anticipate the mean number per leaf being constant through time. More importantly, the mean number of parasitoids over the last three years has been steadily increasing while the number of whiteflies has been similar, between 28 ± 7.34 (SEM) and 34.6 ± 12.1. And over the same period of time, their species composition has been shifting from native *Eretmocerus* spp. to the introduced *E. mundus.*

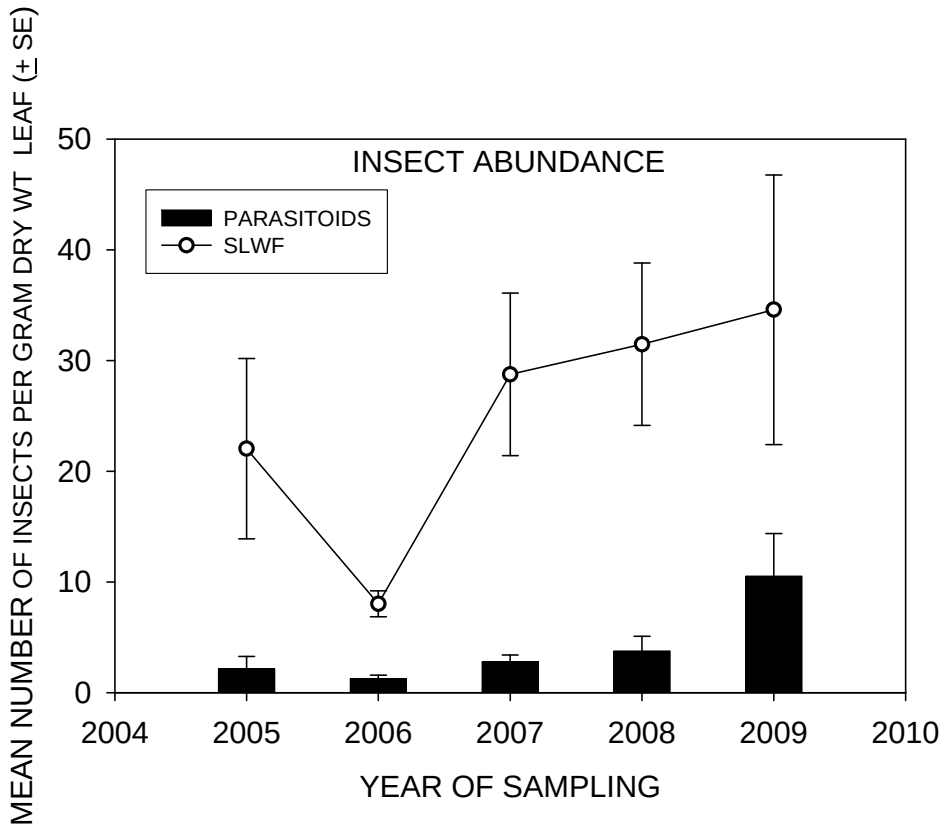

Figure 2. Number of parasitoids and whiteflies per gram dry weight cotton leaf. Southern San Joaquin Valley, California, 2005 through 2009.

[1]CDFA, Pink Bollworm Program, Shafter, California

Additional Projects in Early Stages or Without a Current Formal Report

Kris Godfrey

- Vine Mealybug – We made releases of the Sicilian strain of *Anagyrus psuedococci* at six sites in San Joaquin County. Each site received 140 females and 70 males between July 17 and October 1, 2009. A total of 840 female and 420 male parasitoids were released. The small number of parasitoids released was due to problems with rearing this strain.

- Cotton Aphid in Pomegranate Project – In late 2009, funding was obtained from the CDFA Specialty Crops Block Grant Program to study cotton aphid populations around the Lindcove Research and Education Center. The cotton aphid populations around the Center had increased in recent years because of the increase in pomegranate acreage in the area. Pomegranate is an alternate host for cotton aphid, and sexual reproduction occurs on this host in the winter. This study will look at the phenology of the sexual generation of cotton aphid and investigate several methods of control, including biological control using parasitoids and fungi. We will produce the parasitoids used for the studies and coordinate the studies using the fungi.

- Asian Citrus Psyllid – In 2008, Asian citrus psyllid arrived in California. The initial response was an insecticidal eradication program. However, biological control will play an important role in area-wide management of the psyllid. Funding was obtained from the CDFA Specialty Crops Block Grant Program to conduct the necessary host specificity testing for *Tamarixia radiata*, a parasitoid of the Asian citrus psyllid, so that field releases can be conducted in California. This project is a cooperative project among CDFA Biological Control Program, CDFA Pierce's Disease Program, the University of California – Riverside, and the Florida Department of Agriculture and Consumer Services.

Age Structure of Spotted and Squarrose Knapweed Populations in California: Implications for Biological Control Using Seedhead Feeders

Dale Woods and Viola Popescu

The majority of widely available biological insects for knapweeds are capitulum- or seedhead-feeding insects. There is very little data demonstrating successful weed control using only seedhead feeding insects. For a seedhead-feeder program to be successful with annual weeds, the attack level must be high enough to reduce annual seed production below the population replacement level. In addition to reducing annual seed production, a successful program must face the level of seedlings annually emerging from the seedbank and contributing to weed population maintenance. Consequently, high levels of attack must be maintained for several years. For weeds like yellow starthistle that have a relatively short seed survival in the soil, reestablishment of emerged plant populations depends primarily on annual seed production. Therefore, annual plant population maintenance in starthistle could potentially be greatly affected by the level of attack by seedhead agents. However, healthy plants, including most knapweeds produce an abundance of seed so the degree of attack necessary to affect plant populations may be quite large but the exact level is not precisely known.

Perennial weeds add an extra aspect to plant population maintenance in that they can produce viable seed at several points in their life to support ongoing populations. Annual seed destruction may have to be extremely high for many years to gain population control. Most, but not all of the invasive knapweeds are biennials or perennials. Spotted knapweed is often described as a biennial or short-lived perennial. Squarrose knapweed is also considered to be a short-lived perennial but actual expected lifespan is not known. The most successful biological control of knapweeds, specifically spotted and diffuse knapweed reported so far, has not relied solely on the seed-feeding aspects of biological control.

As part of our efforts to evaluate biological control of knapweeds in California, we have been following the status of individual spotted and squarrose knapweed plants in the field for several years. This has allowed us to estimate the life span of these two weeds and evaluate changes in population age structure of populations occurring over a several year interval. Seed-feeding arthropods are the primary active biological control on these weeds in California.

Spotted knapweed was studied at a fairly remote location along the Pit River in Shasta County. Permanent transects were established in 1994. Several individual plants were flagged for repeated annual monitoring beginning in 1999. Individual plants were marked with a small wire supporting a labeling tape ‘flag’ with a unique identifier. Each plant was assessed annually in the late summer/early fall. All plants flagged in 1999 were mature, bolted plants and were therefore at least one year old. Some degree of plant mortality occurred each succeeding year (Figure 1). Spotted knapweed is a fairly long-lived perennial at this site with the potential to last through at least nine years of seed production. Plants seem to achieve maximum reproductive levels about two to three years after their first bolt but may be prolific for several years (data not shown).

Beginning in 2002, the process of marking individual plants in the permanent transects was extended to all plants including rosettes. When rosettes were identified in the fall, they were likely already at least six months old, but some may have been 18 months old. Occasionally, the young leaves of a rosette dry completely during the summer and the plant appears dead or is not detected so rosettes re-emerging from the root in the late fall to early winter, often appear indistinguishable from the new seedlings. A new cohort of plants emerged each year and all plants were marked and monitored. Data from all years was combined and is shown in Figure 2. Mortality during the first and second years is extremely high so less than 10% of young rosettes are likely to survive. During the course of our experiments, no spotted knapweed plants have emerged and survived for more than 6 years. This is in contrast to the longer survival noted in plants that are already mature (Figure 1).

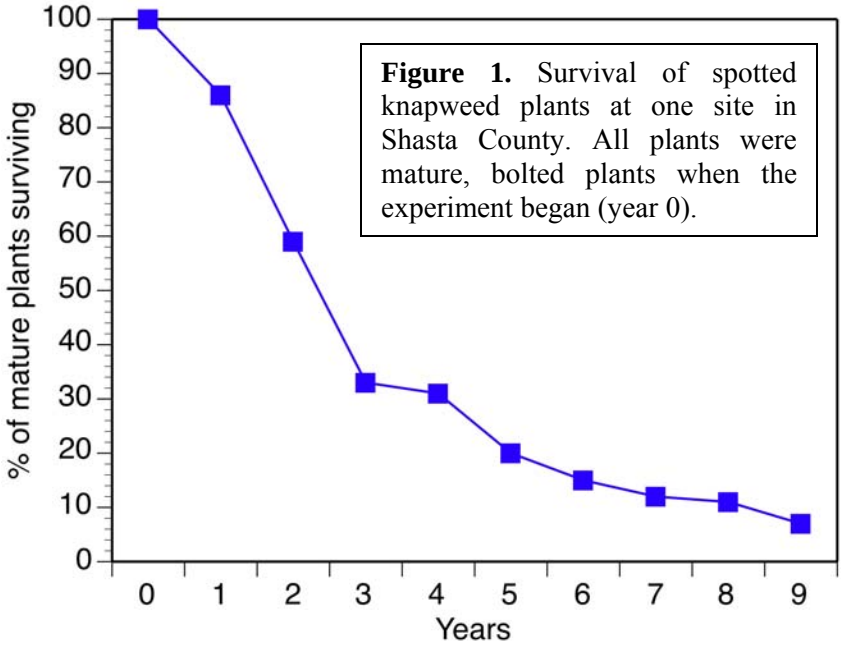

Figure 1. Survival of spotted knapweed plants at one site in Shasta County. All plants were mature, bolted plants when the experiment began (year 0).

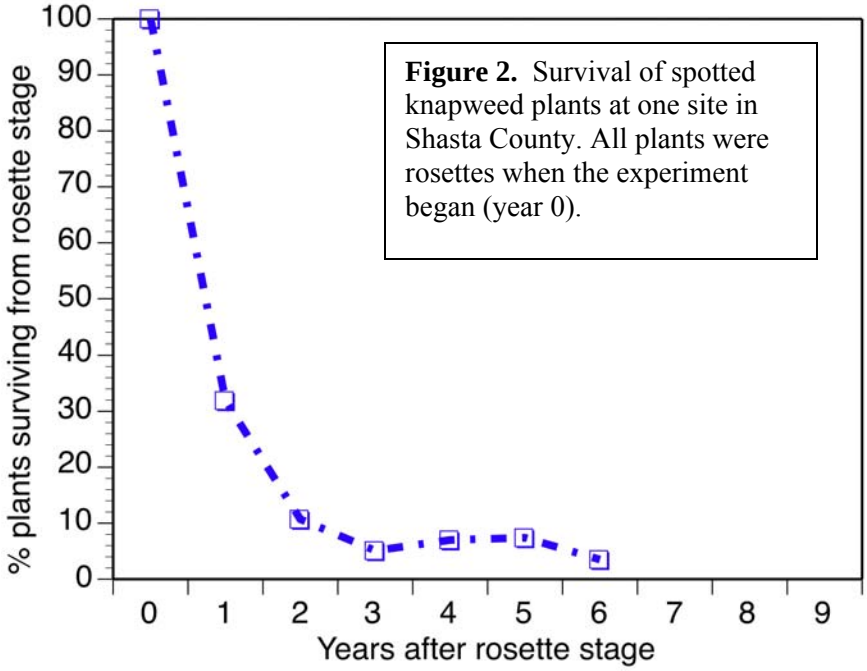

Figure 2. Survival of spotted knapweed plants at one site in Shasta County. All plants were rosettes when the experiment began (year 0).

Squarrose knapweed was evaluated in a similar fashion but was monitored in both the spring and fall. Three locations, with two to three subplots at each location were monitored. Two locations, Kane and Petersen, were in Shasta County, and the third, Pittville, was in Lassen County.

Squarrose knapweed seems to germinate in the winter or early spring. Seedlings are easily visible in May –early June when observations were made. Roughly 80% of the seedlings die within one year of emerging (Figure 3). Plant populations declined another 50% in the succeeding year. If plants survived to their second year, most remained alive for another three years. In fact, several plants at the two Shasta County sites are still alive so their lifespan is not yet clear. Results were very similar for all three sites. Some plants that were flagged at the beginning of the experiment were already rosettes or bolting plants, thus were of unknown starting age. The results from one of the plots at the Lassen County site, the longest running site, are shown in Figure 4. Seedling mortality was high during the first year. All of the initial seedlings were dead by two years thus the long surviving plants were already mature when the experiment started. Squarrose knapweed seems to be able to survive at least eight years under conditions at the Lassen County site. Plants at the other sites appear to be healthy enough to also approach this age.

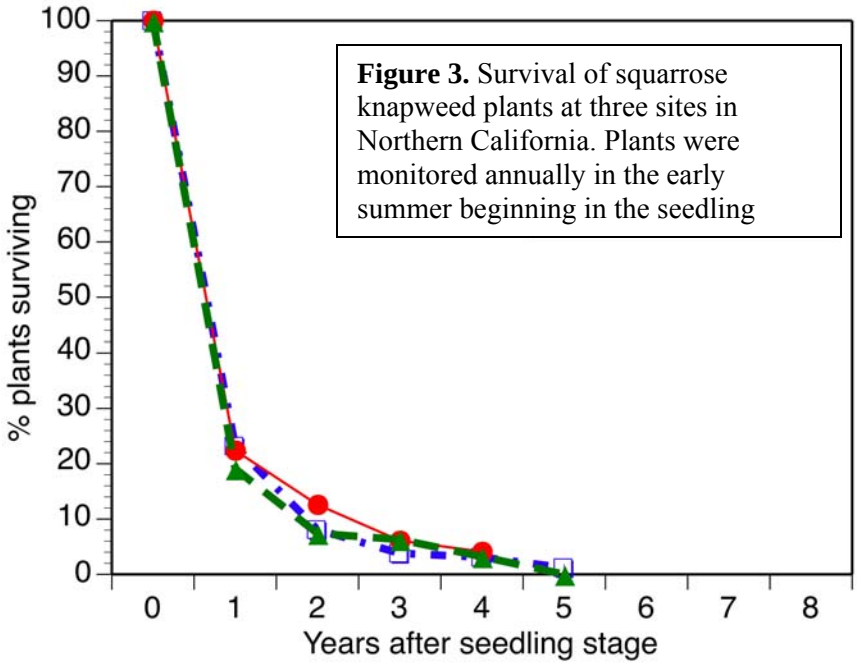

Figure 3. Survival of squarrose knapweed plants at three sites in Northern California. Plants were monitored annually in the early summer beginning in the seedling

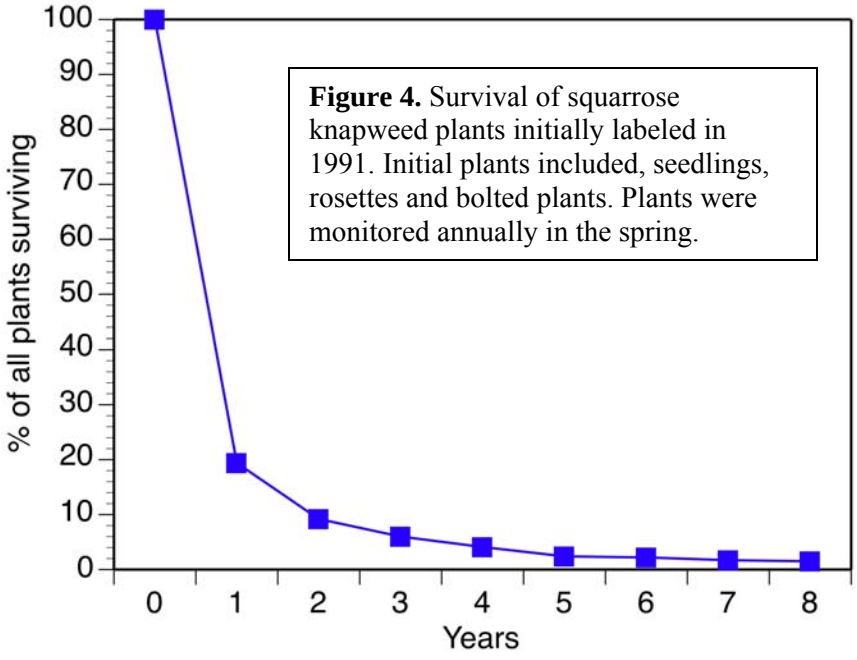

Figure 4. Survival of squarrose knapweed plants initially labeled in 1991. Initial plants included, seedlings, rosettes and bolted plants. Plants were monitored annually in the spring.

Successful biological control of either spotted or squarrose knapweed will be a long-term project. Plants are very longlived, (8+ years) and continue to produce seed for most of those years. Seed reduction of spotted knapweed due to seed-feeding biological control agents is less than 70% annually in our studies. Thus annual replacement of spotted knapweed populations is possible from escaped seeds alone, in addition to the existence of a reported long-lived seedbank in the soil.

In contrast, seed destruction of squarrose knapweed by seed-feeding insects has been over 99% for many years. Reestablishment of plant populations from annual seed production has become highly unlikely. Consequently, we are in the enviable position of waiting out the natural demise of aging squarrose knapweed plants that are only poorly replaced by a diminishing soil seedbank which itself is not replenished by annual seed production.

Yellow Starthistle Rust; Summary of Release, Establishment and Biology in California

Dale M. Woods, William Bruckart[1], Joe DiTomaso[2], Alison Fisher[3], Tom Gordon[2], Jon O'Brien[2], Mike Pitcairn, Viola Popescu, Lincoln Smith[3], Baldo Villegas, Andreana Yribe[4]

The introduction of the rust fungus, *Puccinia jaceae* var. *solstitialis*, as a biological control for yellow starthistle, was a milestone for modern biological control of weeds. Classical biological control of weeds has, to date, been largely reliant on the use of arthropods, and *P. jaceae* was the first plant pathogen released under the modern permitting system in the continental United States. As such, the pathogen was highly scrutinized and the approved release was highly anticipated. Therefore it is particularly important to be rigorous in follow-up evaluations of both safety and effectiveness of the pathogen. The original objective in selecting this pathogen was to release a safe pathogen that would provide stress to plants above or complementary to that created by the arthropod biological control agents. Pre-release testing is a good measure of likely safety but often not a strong indicator of potential impact in the field setting. This article presents a brief summary of some of the highlights, efforts, and research results obtained for *P. jaceae* since its introduction. Many of the details of this work have been published and can be located from references in our publication list.

Figure 1. Dense rust pustules from successful field inoculation of yellow starthistle

Pre-release Testing – Pre-release evaluations involved inoculations of test plants with the rust fungus in a microbial containment greenhouse. Over 65 plant species in 10 plant families were evaluated. Plant species closely related to yellow starthistle were emphasized. Extensive detailed studies were also completed with native *Cirsium* species and safflower including an open field test in Europe. With the exception of infection on bachelor's button, a declared noxious weed in several states, successful *P. jaceae* infections were limited to the target weed. The ability to evaluate the damage that the pathogen can have on yellow starthistle is limited in a containment greenhouse; however, infection did cause reduction in yellow starthistle leaf lifespan and root biomass in containment studies.

Permit Process – A request for a permit to field release the pathogen was prepared based on quarantine and overseas findings from the risk assessment. The final permit for open field release was approved 25 years after the organism first entered containment as a candidate for biological control. The extensive delay in approval was due to many factors including both a detailed study of safflower susceptibility in containment and the lack of precedent within the regulatory system concerning release of plant pathogens.

First Release – The first release of *P. jaceae* var. *solstitialis* outside of containment was on July 8, 2003 when yellow starthistle plants were inoculated in the laboratory in Sacramento. The first open field release occurred the following day at an isolated location in Napa County. Infection was achieved at this site but the fungus did not spread or survive. The infected laboratory plants became the source of all future field releases as well as laboratory and field research in the western United States.

Figure 2. First field release of *P. jaceae* in the United States. Tall tent used for mature plants. Later releases used 8 inch tall black plastic tent over rosettes.

Rust Production in a Greenhouse – An extensive program of greenhouse-based production of rust spores was developed to grow, collect and store inoculum for future projects. Yellow starthistle plants were grown in pots in a greenhouse, inoculated with the pathogen and maintained until disease appeared. Spores were 'vacuumed' off of inoculated plants three times a week and frozen at ultra-cold temperatures. Frozen spores were accumulated and stored for several years. The program was in place from 2003-2006 and the spores became the basis of the state-wide distribution program.

Figure 3. Vacuum collection of spores from inoculated yellow starthistle.

Multi-year Rust Release Project – From 2004 to 2006, a large-scale statewide release program was in place in cooperation with county agriculture departments. A total of 176 releases of the rust were made in 40 counties of California. Releases covered the range of climates in the state where yellow starthistle exists in substantial populations. Each release was made in a one square meter plot in the midst of a larger starthistle infestation. Inoculations were highly successful with disease developing in as many as 93% of the sites during some years. Sites were monitored for several years to determine establishment and damage on yellow starthistle.

Releases in Other States – In 2008, the regulatory limits on the rust were amended so the rust can now be distributed outside of California. Shipments of the rust have been made to the Oregon Department of Agriculture and additional requests for spores are anticipated. Greenhouse-produced spores from Sacramento will be used to satisfy this demand.

Establishment – Establishment of a classical biological control agent is usually defined as multi-year, unaided survival and successful attack. The rust has clearly established in California but not widely so. In the two most successful locations, a field plot in Sacramento and one in southern Sonoma County, the rust has reappeared successfully each year from the first release in spring 2004 through the summer of 2009 for a total of five full years.

Re-emergence – A living host plant is required for *P. jaceae* var. *solstitialis* to grow and reproduce. Following infection, the fungus may produce several generations of spores on actively growing starthistle within a single season. From late summer through the winter, *P. jaceae* remains dormant as teliospores on infected plant debris, then infects

young plants and ‘re-emerges’ to initiate a new cycle of rust pustules. Re-emergence is the primary measure of the fungus survival success. Unfortunately, the yellow starthistle rust is demonstrating a poor degree of reemergence. Less than 21% of the inoculated sites had reemergence after one year. Re-emergence has declined each additional year to the point that less than 5% of the sites had reemergence after three years.

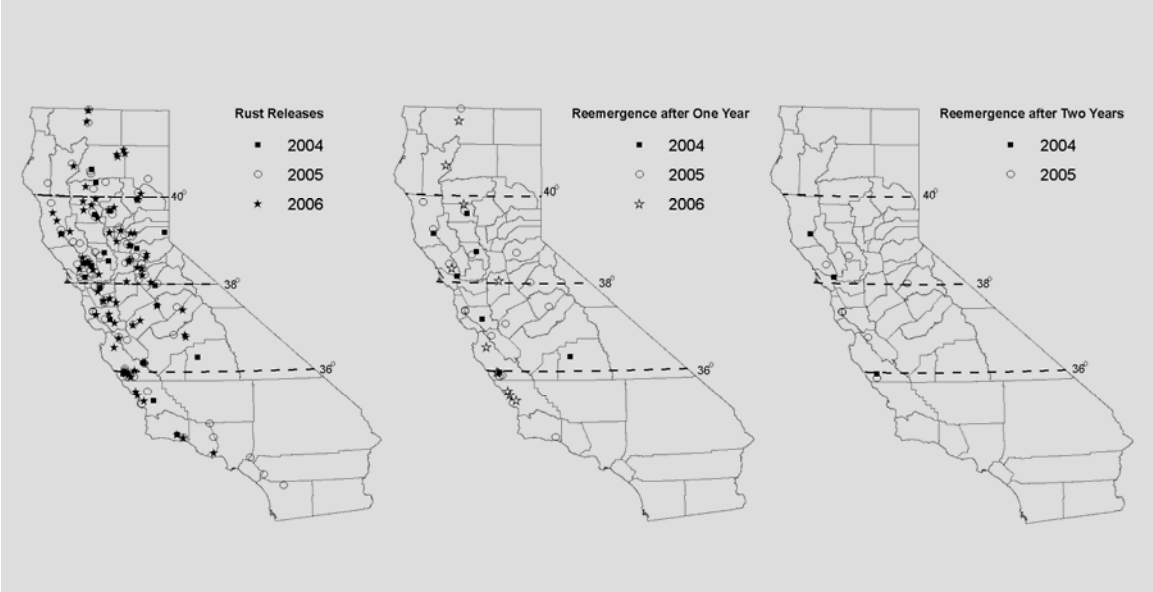

Figure 4. Re-emergence of the rust one- and two- years after field inoculations.

Spread – Most inoculated sites did not show any evidence of spread outside the inoculated area. In a few sites, the rust has spread rapidly and well. At one site in southern Sonoma County, the rust spread over the entire 37 acre yellow starthistle infestation within two years.

Biology – Studies on the biology of the rust and its environmental limitations are central to evaluating its success. The life cycle of rust fungi is somewhat complex with up to five spore stages, each having a specific function and appearance. All of the known spore stages of *P. jaceae* have been detected as naturally-occurring in the field in California confirming that the fungus is functional in our climate. The two spore stages that are the most visible and also critical for the success of the fungus, urediniospores and teliospores, have now been evaluated under California conditions in a series of experimental studies. The asexually-reproducing urediniospore is the form most commonly associated with the disease. This is the spore stage that was distributed during the release and distribution program. These spores were somewhat easy to produce and collect in our greenhouse production process. They are highly germinable and infectious if handled correctly. In order to ensure high infection rates during our field inoculations, we placed short plastic ‘dew’ tents over yellow starthistle plants immediately following inoculation to maintain high humidity and temperature. However, these are not always necessary as the rust can be successful even when a tent is not used. New generations of urediniospores are produced on plants in the field and continue spreading to new plants and leaves as long as the environmental conditions are acceptable.

The extended hot and dry conditions of summer in California limit new infections by urediniospores. Although we have demonstrated that infection is possible throughout much of the summer, for the most part, new infections decline rapidly as rain ceases, temperatures rise and dew periods shorten. Consequently, the time available for disease increase is limited. Urediniospores also have a very short (less than three weeks) lifespan under summer conditions in the Sacramento Valley. With summer conditions limiting both new infections by urediniospores as well as limiting their lifespan, disease expansion ceases well before yellow starthistle is mature. The telial stage of the fungus follows the uredial stage, potentially instigated by the early aspects of plant senescence. Teliospores of *P. jaceae* have now been shown capable of surviving adverse conditions of late summer and winter, and are in a germinable condition when new yellow starthistle seedlings are present (Dec-Mar).

The transition from urediniospore production to teliospore production may be the limiting factor for permanent establishment of the rust. In most of California, the low number of uredia that are produced means low production of telia and teliospores. Additionally, the host plant often makes a rapid transition from vigorous green tissue that supports uredia to dry and brittle. Leaf breakage eliminates most future fungal development as most infection is limited to leaf tissue. Teliospore production on leaves and stems has been inconsistent during the few years that it has been studied. Teliospores are thick walled to help survival during weather that is too harsh to allow disease development. With very limited (or declining) numbers of uredia produced over the season leading to limited (or declining) numbers of telia as the years go by, the initial inoculum load each season remains very low. Consequently, the disease cycle is severely limited and the rust performs poorly or dies out in many locations.

In spite of a generally poor performance statewide, the rust seems to be capable of functioning within much of the climate range of California where starthistle exists. Greenhouse and growth chamber studies have shown that infection can occur at temperatures below 10ºC and as high as 24ºC, temperatures representative of the winter seedling stage and the early summer maturing plant stage. Mature green leaves and stems seem to be able to support dense pustule development in certain regions including Sacramento and Davis on agricultural soils.

It is possible that the isolate of the rust that was introduced is not ideally suited to California's climate. Recent use of climate modeling software indicates that the isolate of *P. jaceae* was not selected from an area with ideal climate match to the climate of California. A rust isolate selected from other regions may be more effective in California.

A compatible relationship between host and pathogen is also essential for the success of the rust. Yellow starthistle seems to be generally susceptible to the isolate of rust released in California. All 62 accessions (collections) of yellow starthistle from around the state that were tested for susceptibility to the rust proved equally susceptible. Therefore, if environmental factors are not limiting, the disease can be expected to be successful statewide.

Impact – For the rust to be a successful biological control, it must also have a substantial negative impact on the plant itself. The rust has had little effect on plant mortality, biomass or flower production in yellow starthistle in natural oak woodland

settings or in monoculture in rich agricultural soils in the Central Valley. In spite of this general lack of impact, specific limited results of rust are apparent. The effect of rust infection on plant biomass and other parameters was greater when yellow starthistle was grown in competition with other weedy plants (wild oats) in a field setting but this effect was small and did not significantly decrease the competitive ability of yellow starthistle. Some biological factors, such a chlorophyll levels are reduced in severely attacked leaves. However, the rust infection does not affect the attack rate or damage by other insect biological control agents.

Preliminary results of an ongoing series of unpublished studies suggest that the rust is most damaging to young yellow starthistle, but infected plants also may recover. When larger amounts of rust spores were used to inoculate yellow starthistle seedlings, larger numbers of pustules developed. Since chlorophyll levels are reduced in severely attacked leaves, vitality of these leaves is likely reduced or lost. The lifespan of severely attacked leaves was reduced (Figure 5). Plants continued to produce new leaves at the same rate regardless of infection level, but the increased leaf loss in severely infected plants translated to reduced plant volume and biomass in young plant. This early damage was still apparent as the plants reach the end of their growth cycle and prepared to enter reproductive stage (Figure 6).

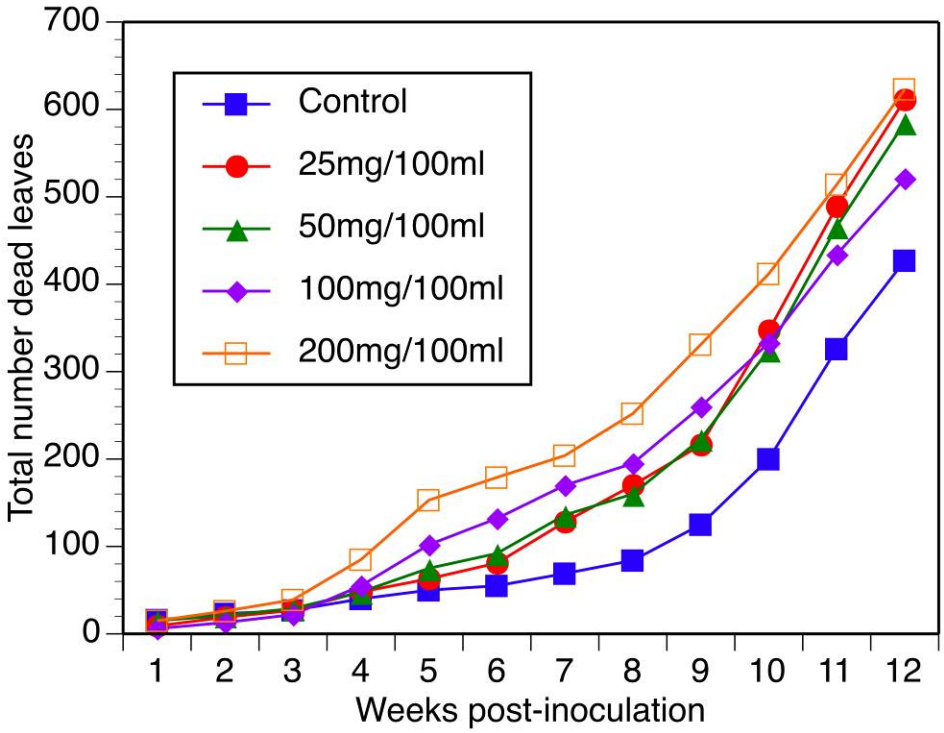

Figure 5. Concentration of *P. jaceae* inoculum on yellow starthistle leaf mortality.

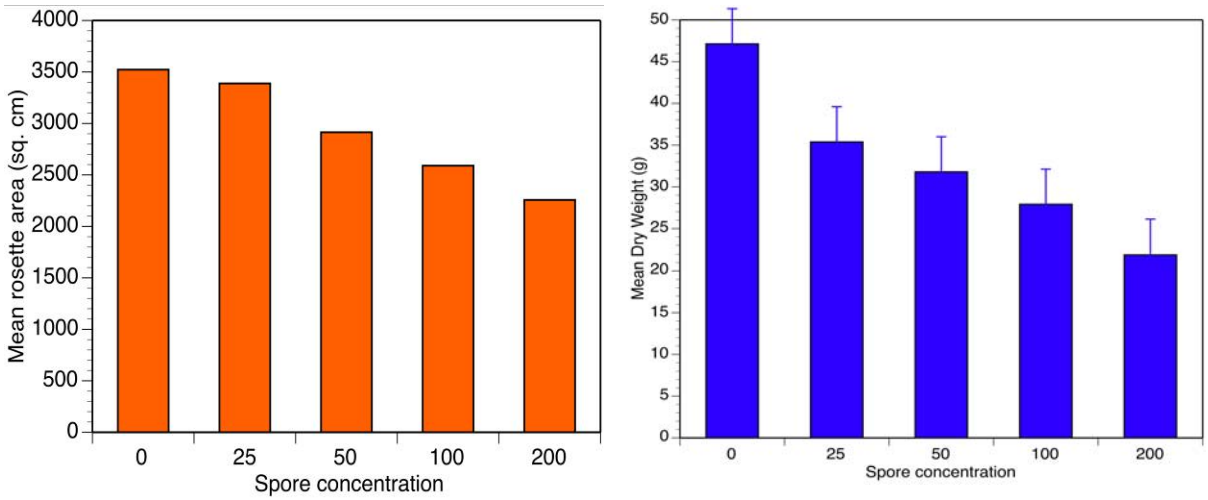

Figure 6. Concentration of *P. jaceae* inoculum on yellow starthistle rosette size (left) and weight (right) at initiation of bolting.

The rust does not affect certain plant variables such as the date that bolting initiates, or height of bolting stem at first bud. The rust carries some residual effect onto later plant development such as stem diameter, and total biomass of bolted plants. However, at full plant maturity there is no reduction of total plant biomass, the number of seedheads per plant, or the number of seeds per seedhead associated with infection by *P. jaceae*. Consequently, *P. jaceae* at varying rates of inoculum does not reduce reproductive capacity of yellow starthistle. The capacity of yellow starthistle to compensate for early impacts of infection severely limits the ultimate impact of *P. jaceae*.

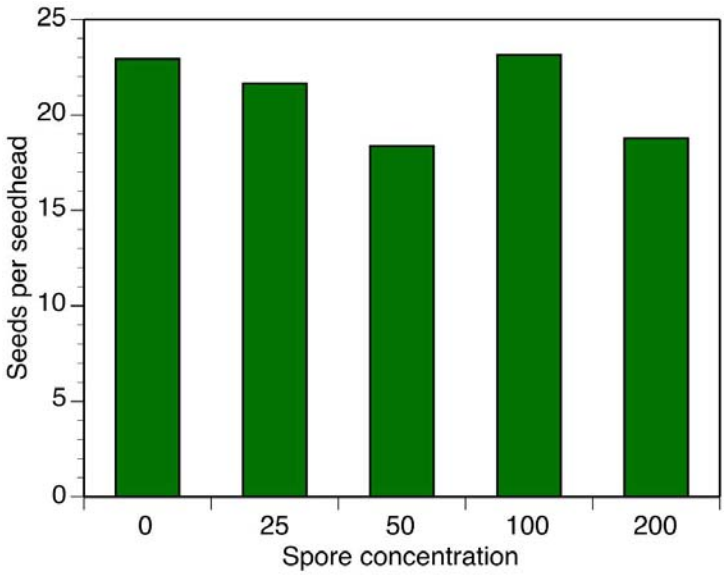

Figure 7. Spore concentration at inoculation on yellow starthistle seed production.

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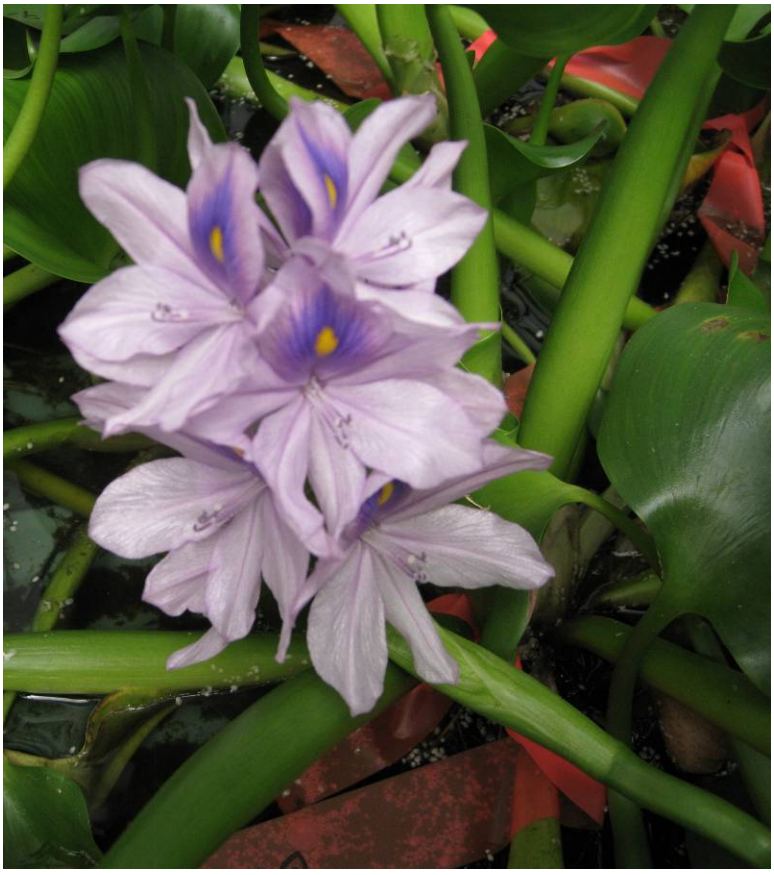
Endemic Diseases of Water Hyacinth in the California Delta

Thomas R. Gordon[1], Sharon Kirkpatrick[1] and Dale M. Woods

Water hyacinth (*Eichhornia crassipes*), a native of the Amazon River basin in South America, is probably the most troublesome aquatic weed in the Sacramento delta of California. Controls based on mechanical and chemical efforts are very expensive and controversial. Biological control represents an alternative that could be less expensive and less controversial. Two weevil species, *Neochetina eichhorniae* and *N. bruchi,* were released in the early 1980's but have failed to produce acceptable levels of control. They have, however, been considered more successful in other states and countries. One of the suggested differences in success between California and elsewhere has been the involvement of plant pathogens. Apparently, in the successful environments, endemic plant pathogens utilize feeding injuries caused by the weevils to attain egress to water hyacinth and then severely decay the plant. In 2007, we initiated a project, funded by the California Department of Boating and Waterways, to evaluate endemic plant pathogens for their impact on water hyacinth in California. Field surveys were conducted in 2007 and 2008, and pathogens were evaluated in the greenhouse during 2009.



Field surveys were both land based and water based. For the land based surveys, plants growing near the banks were collected with a long rake and inspected for symptoms. Similarly, surveys were conducted by boat to areas difficult to access from the bank. Although many areas were evaluated, the majority of plants appeared completely free of symptoms indicative of plant pathogens.

Water hyacinth plants displaying symptoms possibly attributable to fungal infections were collected from eight locations. All plant samples were examined in the laboratory and symptomatic parts (principally leaves and petioles) were excised for further scrutiny. Damaged tissue and immediately surrounding healthy tissues were immersed in 70% ethanol followed by 1% sodium hypochlorite to eliminate superficial microbes, and then placed on Petri plates containing a general growth medium. Plates were observed periodically over a period of about two weeks for fungal growth emerging from diseased tissue. Each distinctive growth form was recovered and isolated into pure culture as a hyphal tip or single spore.

Thirty-one isolates were obtained in this way and grown in pure culture. They were examined microscopically to determine if they were identifiable based on production of diagnostic spores and/or spore-forming structures. Those that did not produce spores or for which spores and spore-forming structures were not diagnostic of a known genus or species, were grown in liquid culture to obtain biomass for DNA extractions. Primers specific to the internal transcribed spacer region (ITS) (a conserved sequence found in all fungi) were used to amplify DNA via the polymerase chain reaction. Where the ITS sequence obtained matched a sequence in Genbank, the corresponding name was assigned to the unknown isolate. A total of 21 isolates were identified to species or to genus. The remaining isolates were not identified due to the absence of distinguishing morphological characteristics and failure of DNA amplification attempts. Six of the identified isolates were species of *Alternaria* and three were species of *Fusarium*, 10 other genera were represented by at least one isolate.

All 31 isolates were tested for pathogenicity on water hyacinth under greenhouse conditions. Plants were inoculated either with a plug of colonized potato dextrose agar or a suspension of spores obtained from cultures grown on the agar. Plug inoculations were accomplished by using a cork borer (0.8 cm^2) to remove tissue from a petiole on the plant to be inoculated and placing a colonized agar disk of the same size into the wound, which was then wrapped with parafilm to prevent drying. On a separate petiole, a wound was created using a pipette tip, into which 50 microliters of a spore suspension was injected. The quantity of spores delivered in each inoculation ranged from 6,000 to 50,000 as dictated by the numbers of spores produced by each isolate in culture. Each isolate was inoculated into three different plants, each of which was placed in a separate tub on a greenhouse bench. Wounded, non-inoculated control plants were included in each replication. Plants were rated based on the extent of lesion development at the site of inoculation using a 1-4 scale, with 1 corresponding to no lesion beyond what was evident on water controls, and 2, 3, and 4 corresponding to mild, moderate or severe lesions, respectively. Petioles with ratings representative of each category are shown in Figure 4.

Most isolates caused relatively little damage, regardless of the inoculation method, with necrotic tissue rarely extending more than a few centimeters in either direction from the site of inoculation. Twelve isolates that appeared to be among the most virulent were tested a second time. The agar disk method was used in the manner described for the first test and a second inoculation was performed by spraying a spore suspension onto leaves without wounding. The spray inoculation method was not effective, and no disease symptoms could be seen on any of the inoculated leaves. In the agar disk inoculations, only three of the 12 tested isolates induced lesions large enough to be considered pathogenic, and even these caused little damage. These isolates were retained in the event that it might be of interest to study them further.

Given the aggressiveness of the pathogenicity test that was used and the modest damage that was observed, it seems doubtful that even the most virulent isolates offer particularly good potential for use as biological control agents for managing water hyacinth. However, it is possible that altered application methods might enhance their capacity to cause damage. Insight into this question might be gained by comparative studies that include imported isolates that have been documented to cause severe damage to water hyacinth in other areas.

Figure 4. Lesion development following inoculation with plant pathogens. Rating scale; 1 (left) to 4 (right).

[1]University of California, Department of Plant Pathology, Davis, California

Evidence of Overwintering and Spread of *Mecinus janthinus* in California

Dale Woods, Baldo Villegas and Viola Popescu

The toadflax stem weevil, *Mecinus janthinus*, was released in California in May and June of 2008 in the Hungry Valley State Vehicular Recreation Area. This was the first permitted, intentionally released biological control insect for Dalmation toadflax in California. Releases were made at three sites within the infested area of the park. Each site received 400-600 adult weevils. Three additional nearby sites were selected as 'no-release' comparison sites. Weevil feeding signs (evidence of tunneling activity) was detected at the release locations in the fall of 2008 and again in the spring of 2009.

In late-spring (June 4) of 2009, all six sites were evaluated for evidence of weevil attack. Monitoring included in-field visual searching for signs of attack as well as a laboratory evaluation of field samples to determine the amount of attack. The 'no-release' control sites were free of any evidence of weevil attack. For the in-field evaluation, a walking transect was made in each cardinal direction from the release center at each site. Every two meters, a two minute detailed inspection of plants was made. Toadflax plants were inspected for evidence of weevil feeding, weevil emergence and the presence of the weevil itself. Maps were prepared of the apparent distribution of the weevils.

For the detailed attack studies, 100 intact stems were collected at each of the three release sites. Fifty stems were haphazardly collected within three meters of the release point. An additional 50 stems were collected from a circle 15 meters from the release site. The collected stems were all mature and dry, thus were from the 2008 growing season and represented the attack rate over the first summer (2008). The 2009 crop of stems had not yet bolted. With a single exception, all attacked plants were within three meters of the release points. With over 40% of the stems attacked within three meters of the result point and several larval mines (attacks) per stem, the weevils seem to have successfully overwintered in this southern location in California and are increasing in population.

Table 1. Attack of Dalmation toadflax stems by *Mecinus janthianus* in California.

		Distance from release point	
Site		3 meters	15 meters
East	Percent attacked	58%	1.7%
	Mean # mines	2.96	0.02
	Mean height of stem	63 cm	70 cm
Central	Percent attacked	41%	0
	Mean # mines	2.46	0
	Mean height of stem	63 cm	74 cm
West	Percent attacked	48%	0
	Mean # mines	1.62	0
	Mean height of stem	64 cm	70 cm

Puncturevine, *Tribulus terrestris* L. (Zygophyllaceae)

Baldo Villegas and Carolyn Gibbs[1]

Puncturevine, *Tribulus terrestris* L. (Zygophyllaceae), is native to the Mediterranean region and widespread in many parts of the world. It was first reported in California in 1903, and by 1912, it was considered a serious weed pest. Puncturevine infestations are a nuisance around homes, orchards, roadways, bicycle lanes, and recreational areas because of the spiny seedpods commonly called "goatheads." In 1956, the USDA-ARS and the University of California, initiated a cooperative biological control program on puncturevine and two weevils were introduced from Italy. The weevils, *Microlarinus lareynii* (Jacquelin du Val) [the seed-infesting weevil], and *Microlarinus lypriformis* (Wollaston) [the stem-infesting weevil], were introduced into California in 1961. The two weevils became widely established in California and have kept puncturevine at low levels in much of California. However, puncturevine is still considered a big problem in high elevation areas of California where the weevils have not been able to become established.

At the request of the Lassen County Agricultural Commissioner and the Lassen County Agricultural Board, an attempt was made to establish the two weevils in Lassen County in 2005. Approximately 2700 weevils were mass-collected in July from Tulare, CA and released at five sites in Lassen County and at one site in Shasta County. The sites were selected to improve the likelihood that the weevils could survive the cold winter temperatures common in the area. Sites were monitored for establishment of the weevils at least once per year. Urban sites and sites containing vegetation in addition to puncturevine were given the highest priority. In November 2009, a final monitoring survey was made to determine if the weevils had survived the winters. Of the five releases, only one population of the weevils established in the City of Susanville and it was found to be thriving. At this site, the seed weevil was found to be common and damage to the seedpods was greater than 50% (Figure 1). Damage caused by the stem weevil was absent from all the stems examined. This weevil was only found in some of the puncturevine crowns (Figure 2) suggesting that this weevil is much more cold sensitive than the seed weevil.

Figure 1: Seed damage by the seed weevil in Susanville, California

Figure 2: Emergence holes left by the stem weevil on the crown of a puncturevine plant.

Weevils can be established in high elevation areas of California if site selection guidelines are followed. Among the most important guidelines are choosing sites with moderate, undisturbed puncture infestations with nearby shelters where the weevils are protected during the winter months. Ideal shelters for the weevils are lawns, plant cover, and areas having a thick layer of mulch near the release site.

[1]United States Bureau of Land Management, Susanville, California

Mediterranean Sage, *Salvia aethiopis* L., (Lamiaceae)

Baldo Villegas and Carolyn Gibbs[1]

Salvia aethiopis L., (Lamiaceae) is native to the Mediterranean and western Asia. In California, it occurs in Lassen and Modoc Counties. In these counties, Mediterranean sage or "Medsage", is found infesting rangeland, roadsides, pastures, and meadows. It is strongly aromatic, biennial, and distasteful to cattle and horses. It grows two to three feet tall producing a stout taproot. Rosettes are produced during the first year averaging one foot in diameter but in well watered soils, may exceed two feet. During the second year, the plants produce a flowering stalk with numerous whitish flowers and many seeds. After flowering the plant dries, it breaks off from the taproot and tumbles across rangelands and roads spreading seed. The USDA-ARS started a biological control program against this invasive weed, and by 1969 began introducing biological control agents against Medsage. The weevil, *Phrydiuchus spilmani* Warner was imported from Italy and released in southern Oregon but failed to become established. A second weevil, *P. tau* Warner (Figure 1), was imported from Yugoslavia and released in Oregon in 1971 where it became established. From 1976 to 1980, collections of *P. tau* from southern Oregon were released by the CDFA Biological Control Program in cooperation with the Agricultural Commissioner's offices from Modoc and Lassen counties. Establishment was observed at several sites in Modoc County but no follow up was done to determine if the weevil has kept Medsage under control. From 2002-2005, surveys of Medsage infestations were made to determine where the weevil was established. Additional releases of the weevils were made in both Modoc and Lassen counties where the weevils were not found or where the weevils were never released.

Figure 1: *P. tau* feeding on Medsage leaf.

In November 2009, all release sites in Lassen and Modoc counties were surveyed for establishment of the weevils and to see if the weevils were impacting Medsage (Figure 2). The area between Oregon and Alturas along Hwy 395 was found to contain very few Medsage rosettes and the weevils were found at all the sites surveyed. These were the areas where most of the releases took place in 1976-1980. The area south of Tule Lake along Hwy 139 was found to have a higher density of Medsage rosettes than that found along Hwy 395 north of Alturas. No weevils were found during the survey indicating a need to re-introduce the weevils in the future. In Lassen County, the weevils appear to be impacting Medsage at variable levels from good control levels to none. The weevil either takes a long time to control Medsage or it is site specific in its establishment needs.

Figure 2: Damage by Medsage weevil.

[1]United States Bureau of Land Management, Susanville, California

Leafy Spurge, *Euphorbia esula* L. (Euphorbiaceae)

Baldo Villegas

Leafy spurge is a perennial plant native to Eurasia. It is a deep-rooted perennial and forms large patches through vigorous lateral root growth. The plant produces latex which can cause dermatitis to humans and grazing animals. If it is not kept under control, it can out-compete native grasses and forbs which are important components of productive rangelands and pastures. It is also very invasive in mountain meadows and riparian areas.

There are several infestations of leafy spurge in California and all are under eradication action. The infestation in the Scott Valley of Siskiyou County has been present for many years. Infestations in the area occur in irrigated pastureland as well as the riparian zone of the Scott and Klamath Rivers. The challenge in controlling this infestation is that most of the spurge is in very steep riparian terrain. A biological control project was implemented in 2001 by the Biological Control Program and the Siskiyou County Department of Agriculture with releases of two flea beetle species collected in the Medora area of North Dakota. The releases of the beetles were made within the riparian areas of the Scott and Klamath Rivers. No establishment took place due to the soil requirements of the flea beetles for more loamy soils away from riparian areas. Another release effort was made in June and July 2007 with releases of the flea beetle (*Aphthona lacertosa*) and the longhorned beetle (*Oberea erythrocephala*) collected from established populations in Oregon and released on leafy spurge populations in Siskiyou County. The releases were made at four sites containing loamy soils and located away from the edge of the rivers. One site was a working ranch with infested pastures and a wide flat riparian area near the Scott River.

Monitoring for establishment was made on an annual basis in May and June to see both the adult beetles and their damage. No destructive sampling was done to check for larval damage. All the surveys consisted of sweeping the spurge plants for adult beetles with a sweep net and observing their feeding damage on the plants. Both beetle species were found during 2008 and 2009 but only at two of the four sites. At a private ranch north of Fort Jones (Figure 1), the two beetles were found in scattered areas within the ranch but the best recoveries were found in loamy soils in the pasture and at a wooded area between the pasture and the Scott River. A smaller recovery of the two beetles was found near Indian Scotty Campground along the Scott River Road. However, the recoveries were limited to spurge plants along the road on loamy soil. No recoveries were made at two sites located along the Klamath River or on any spurge plants near the water line.

Figure 1: A release site north of Fort Jones, California that was ideal for establishing the biological control agents on leafy spurge.

Soil texture seems very critical to the survival of the two biological control agents. If additional sites can be found with loamy soils within the infested areas of Siskiyou County, releases of the two beetles could be made with good chances of survival followed by natural disperse to other spurge areas.

Saltcedar, *Tamarix parviflora* and *T. ramosissima* (Tamaricaceae)

Baldo Villegas and John Herr[1]

Several species of *Tamarix* or saltcedar (also commonly called tamarisk) are known to occur in California. In Northern California the prevalent species is *T. parviflora* while *T. ramosissima* is widespread from the San Joaquin Valley south into Southern California. One species of the *Diorhabda* leaf beetles, *D. elongata*, was established by the United States Department of Agriculture Agricultural Research Service's Exotic and Invasive Weed Research Unit in Albany, California. These beetles were introduced from the Greek island of Crête and released along Cache Creek in the Capay Valley of Yolo County starting in 2001. By 2006, the leaf beetles were declared well established and available for redistribution to other parts of California and the other similar areas in the United States where other *Diorhabda* species were not establishing.

In California, care was taken to map out the areas where the saltcedar beetles could safely be moved without migrating to nesting habitats of the federally endangered southwestern willow flycatcher, *Empidonax traillii* Audubon subspecies *extimus* Phillips, which will nest in saltcedar. In California, the releases were made in northern California in 2007 and 2008 season. In 2009 two sites in Kern County and one in Fresno County were evaluated for release and after consulting with colleagues with the USDA-ARS and University of California at Santa Barbara, releases were made in June and September on the prevalent saltcedar species growing there, *T. ramosissima*.

During 2009, unusual spring weather disrupted the emergence of the overwintering beetles, drastically reducing the buildup of the beetles along Cache Creek. As a result, laboratory reared beetles, consisting of eggs, larvae and adults, were obtained from Dr. John Herr (USDA-ARS, Albany, California) and released in Napa (n = 4,000) and Kern (n = 1,500) counties (Figures 1-3). Larvae and eggs were placed in sleeves enclosing individual branch terminals (sleeves were removed a few weeks later); adults were released in the open. Later, during a late-season build-up of beetles, approximately 11,000 beetles were mass collected and released at eight sites in western Fresno County near Coalinga, California. Each release consisted of 1000-2000 beetles. The releases were all open releases on *T. ramosissima* at all locations (Figure 4).

During 2009, all the release sites from previous years were surveyed for establishment. No beetles were recovered from any of the 2007-2008 release sites. The only recoveries noted during the monthly monitoring visits to the release sites were from the 2009 spring release that occurred in Napa County. No recoveries were made from the 2009 spring release in Kern County or from any other subsequent releases made in 2009.

Figure 1: Napa release site along Pope Valley Creek.

Figure 2: Attachment of a saltcedar bouquet infested with beetle eggs and larvae.

Figure 3: Kern County release site along an irrigation ditch near Lost Hills, California.

Figure 4: Fresno County entomologist Dr. Norm Smith releasing the saltcedar beetles at a site along Warthen Canyon east of Coalinga, California.

[1]USDA-ARS, Invasive Weeds Unit, Albany, California

Purple Loosestrife, *Lythrum salicaria* (Lythricaceae)

Baldo Villegas and David Kratville[1]

Purple loosestrife infestations occur in many parts of California. Various control methods are being used to control purple loosestrife as it grows along riparian areas at the water edge. In some areas, weed management areas are reluctant to use herbicides to control their infestations while in at least one other area public agencies have been prohibited from any herbicide controls. Mechanical controls are often used along herbicidal treatments in most infestations in California keeping purple loosestrife at variable levels of control. On the largest infestations located near Oroville in Butte County, McArthur in eastern Shasta County, Onyx in Kern County, and Sanger in Fresno County, biological control programs have been implemented with some success. Four biological control agents have been released in California for the control of purple loosestrife. Of these, the two *Galerucella* leaf beetles (*G. pusilla* and *G. calmariensis*) are impacting purple loosestrife.

In Shasta County, two leaf beetles have become well established. An area-wide survey was performed in Shasta County in 2009 and the beetles were found in all the areas surveyed. The two leaf beetles have also become established at one site near Palermo in Butte County. This is the third year of large releases being made at this site and it appears that the beetles are causing heavily defoliation to the purple loosestrife plants there. This is very important as previous efforts to establish the beetles have failed due to the fluctuating water levels at infestations near the Oroville Dam. Also this is important as it shows that these beetles are capable of surviving the hot weather associated with the northern Sacramento Valley area.

[1]CDFA, Integrated Pest Control Branch, Sacramento, California

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