Biological Control Program

2008 Annual Report

California Department of Food & Agriculture

BIOLOGICAL CONTROL PROGRAM

2008 SUMMARY

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CALIFORNIA DEPARTMENT OF FOOD AND AGRICULTURE
PLANT HEALTH AND PEST PREVENTION SERVICES
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 Baldo Villegas, Dale Woods, and John P. Mattia (Orange, CT). Infestation of perennial pepperweed east of Susanville, California. (Photo courtesy of Lassen County Weed Management Area). Inset photo shows severe infection of perennial pepperweed by the plant pathogen, *Albugo candida*. (Photo by Villegas and Woods)

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Preface

M. J. Pitcairn

I am always encouraged to hear from individuals who let us know their appreciation for our efforts and the impacts of one of our projects. As a state program, our traditional immediate customers are the County Agricultural Commissioners who assist us in the release and establishment of new biological control organisms statewide. We also interact with several state and federal agencies that manage public lands and need help controlling invasive noxious pests. But, occasionally, we get notes from individuals that belong to small local organizations. Recently, we received thank you notes concerning the possible release of a new biological control agent on Russian thistle. This common but noxious weed affects California in many different ways: large tumbling plants cross highways causing traffic accidents, tumbling plants accumulate and clog irrigation canals transporting water and, large piles of dead plants accumulate in the Fall. The only way to effectively remove these tumbling irritants is to create large piles and ignite the piles on fire. These large pyres of burning plants result in unwanted ash and particulates in air so any efforts to reduce the abundance of these plants are greatly appreciated. For many of the exotic weeds that have become serious nuisances over large regional areas, the only hope of control may be through biological control. It is always good to receive small notes of appreciation for our work. We will do our best to keep it up.

In anticipation of a new biological control agent, we established several research sites to examine the impact of a new root beetle on yellow starthistle. This work is being done in cooperation with Lincoln Smith of the USDA Agricultural Research Service who performed the host specificity testing necessary to determine the insect's safety following its release. We expect approval of the permit request to be awarded in 2009 and releases to begin in 2010. Several sites in different climate areas of California were set up and initial densities of yellow starthistle were taken. A detailed summary of this activity is found in this year's annual report.

New in this year's report is a list of peer-reviewed publications produced by the Biological Control Program staff. Most of the pest organisms that are worked on by the Biological Control Program are new to California and little information is available. Program scientists perform critical field and laboratory studies in order to obtain the information necessary to develop a successful control project. For many projects, Program scientists participate in cooperative research efforts with scientists from the University of California and the United States Department of Agriculture. Many of these activities and results are published in peer-reviewed books and journals. A list of publications published from 2005 to 2008 is found at the end of the report.

These are but a few of the highlights presented in this year's report. I hope you enjoy our 2008 report.

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Insect Projects

Weed Projects

Light Brown Apple Moth Parasitism by *Trichogramma* spp. Egg Parasitoids

William Roltsch and Nada Carruthers[1]

The invasive light brown apple moth (LBAM), *Epiphyas postvittana*, was first reported in North America in February 2007, with populations concentrated in central coast areas of California. Plans are underway to eradicate this exotic pest relying greatly on sterile insect technique. Low densities of the target pest are critical to the success of sterile insect technique. Augmentative biological control using *Trichogramma* to facilitate sterile insect technique efforts is, however, under consideration. It would be employed in areas characterized by high moth densities. *Trichogramma* species native to California will be required for this task.

The objectives of this past year's work include: 1) the determination of whether *Trichogramma* species native to California will readily parasitize LBAM eggs; and 2) the determination of whether parasitism rates vary geographically and with regard to habitat (esp. plant species).

Figure 1. *Trichogramma platneri* collected from sentinel eggs.

A brief laboratory study was conducted during February and March of 2008 using the native species, *T. pretiosum* and the eastern North American species *T. minutum*, to determine the vulnerability of LBAM eggs to parasitism by these two native North American species of *Trichogramma*. Both species were obtained from commercial insectaries. Each species was exposed to 20 LBAM egg masses for 24 or 48hrs at 25^{o}C in small culture tubes. One female *Trichogramma* was placed in each culture tube and one male *T. minutum* was placed with each female *T. minutum*. However, because the *T. pretiosum* strain was largely uni-parental, males were placed with females only to a limited extent. Each treatment combination was limited to two replications because of limited availability of *Trichogramma*. Upon pooling data by species (i.e., ignoring exposure duration), on average *T. pretiosum* attacked 51% of the egg masses, and *T. minutum* parasitized 31% of the egg masses. Parasitism in one of the *T. minutum* tests was unusually low, which may account for the lower average percent parasitism than *T. pretiosum*. Relative to those *Trichogramma* that did parasitize LBAM egg masses, on average *T. pretiosum* parasitized 16 eggs per egg mass whereas *T. minutum* parasitized 20, producing one offspring per host egg.

Figure 2. Light brown apple moth egg mass; parasitized (black) and hatched eggs (clear) egg cases.

In the summer of 2008, field studies were initiated to identify naturally occurring parasitism of LBAM eggs. Field sites were established at residential and park sites in Santa Cruz and San Francisco counties. Sentinel LBAM egg masses on 20 plastic cards were stapled to

branch terminals for two days or more depending on seasonal temperatures. The plastic cards with eggs were trimmed down in size, placed in small culture tubes with a cotton plug and held at room temperature in a sealed plastic box at 70% RH. In addition to recording percent of egg masses attacked during each monitoring period, a physiologically-based exposure time was estimated (Figure 3, lower box). For each monitoring period, the exposure duration represents the percentage of time (in degree days) in which LBAM eggs were available for parasitism relative to the first half of LBAM egg development (i.e., 67dd ^{o}C). The first 67dd (total LBAM egg dev. time is 134dd ^{o}C) approximates the time in which eggs are vulnerable to parasitism. Parasite species determinations were made by Richard Stouthamer and John Pinto, U.C. Riverside.

Sentinel egg card field monitoring has shown that wild *T. platneri* and *T. fasciatum* are presently attacking LBAM eggs in some locations where LBAM is found in California. Given that the sentinel egg mass exposure time represents 60% or less of the time (see Exposure duration display box, Figure 3) in which the eggs are vulnerable to attack by *Trichogramma*, data suggest that more than 50% of the egg masses can be parasitized. The number of eggs attacked within an egg mass can be high as well, averaging 66% (±SE=6.4, n=24) at one study location in Santa Cruz. *Trichgramma* were uncommon at the San Francisco site. This field study represents the first time that *T. fasciatum* has been recorded in the United States. Formerly, it was reported in Central America, Mexico, and British Columbia. In addition, a third *Trichogramma* species has been collected and is awaiting identification.

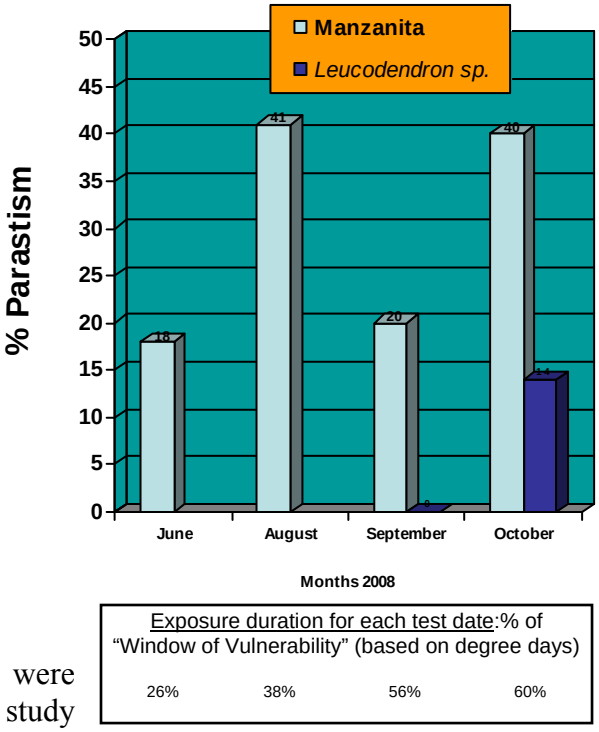


Figure 3. Percent of LBAM egg masses parasitized by resident *Trichogramma* species at one site in Santa Cruz on two plant species.

Although based on limited data, it is suggested that egg parasitism varies widely on a geographical basis, and among plant species. The prospect of successfully using *Trichogramma* in augmentative biological control to facilitate sterile insect technique is promising.

[1]USDA-APHIS-CPHST, Western Regional Research Center, Albany, California

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

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

Late larval instar

Since mid May 2008, populations of the light brown apple moth, (LBAM) *Epiphyas postvittana*, have been sampled regularly at two sites in each of San Francisco and Santa Cruz. The objective of the present study is to document seasonal patterns in the dynamics of LBAM populations as exhibited by larval densities and stage structure, and by larval parasitism by resident parasitoid species. At both sites in San Francisco (Golden Gate Park), the host plant of LBAM is the Australian tea tree (*Leptospermum laevigatum*, family Myrtaceae), with very small plants (approx. 1m in height) at site 1, and large plants, many years old, at site 2. At both sites in Santa Cruz, the host plant of LBAM is the indigenous shrub manzanita (*Arctostaphylos* spp., family Ericaceae), with low spreading plants at site 1, and small but taller plants at site 2. These were the only common host plants on which LBAM populations could be found in May 2008, and while LBAM has been found on a number of other host plants in San Francisco more recently, in Santa Cruz, manzanita and Pittosporum (*Pittosporum* spp., family Pittosporaceae) remain the dominant host plants.

LBAM populations were sampled every two weeks at each site during the main season, and once a month in winter (November through February). Leaf roll density was monitored by timed counts, the cumulative number of leaf rolls found within seven minutes for each of 22 plants at each site in San Francisco, 16-20 plants at Santa Cruz site 1, and 11 plants at Santa Cruz site 2. A sample of from 30-50 leaf rolls was collected from each site on each sampling date to (1) determine occupancy, (2) determine stage structure, and (3) determine parasitism from parasitoid cocoons and live LBAM individuals present. The proportions of leaf rolls occupied by 0, 1, 2 or 3 LBAM individuals was used to correct the leaf roll 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 leaf rolls were transferred to diet to rear through to adult for identification, and any parasitoid cocoons reared from the field-collected LBAM were allowed to emerge, were identified where possible, and were used to estimate parasitism by indigenous parasitoids.

Meteorus trachynotus

The results for parasitism are presented in Figure 1 for the two sites in San Francisco, and in Figure 2 for the two sites in Santa Cruz. In general, parasitism by indigenous parasitoids was greater in San Francisco than in Santa Cruz. The very high estimates of apparent parasitism at Santa Cruz site 2 should be treated with caution as they were based on very small numbers of occupied leaf rolls at this site (less than 10 larvae on each sample date in July and September onward). While 16 different parasitoid species were recovered from these sites, only a few caused notable levels of parasitism. The majority of parasitism at both sites in San Francisco was by *Enytus*

Enytus eureka

eureka (Ichneumonidae) and *Meteorus trachynotus* (Braconidae). In contrast at both sites in Santa Cruz, *E. eureka* was absent, and *M. trachynotus* was the only consistent parasitoid throughout the season, although a *Hormius* sp. (Braconidae) was significant at Santa Cruz site 2 early in the season.

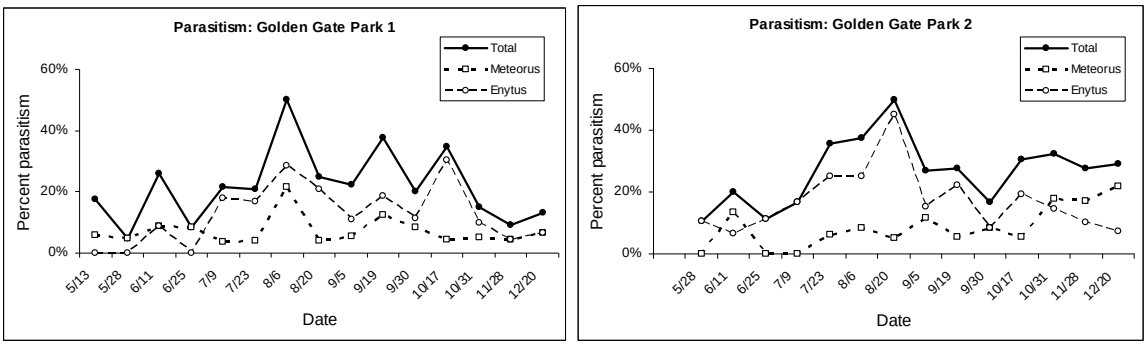


Figure 1. Parasitism of LBAM larvae at two sites in San Francisco, Golden Gate Park in 2008.

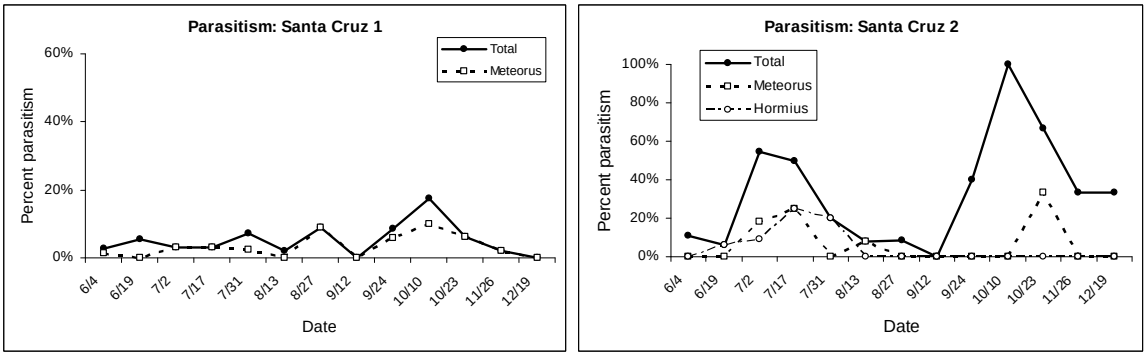
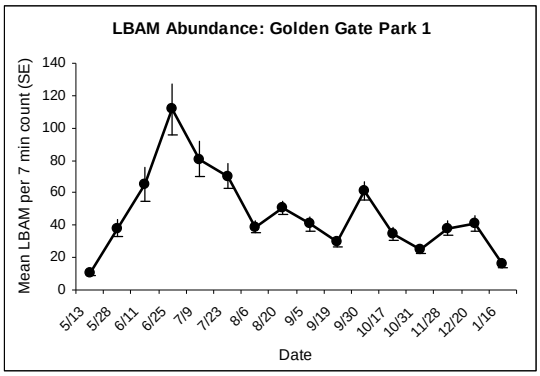


Fig 2. Parasitism of LBAM larvae at two sites in Santa Cruz in 2008.

Enytus eureka (Ichneumonidae) was the dominant parasitoid at the two sites in San Francisco. Parasitism through much of the summer by *E. eureka* was around 20%, although notably lower early in the season and again at the end of the year. In contrast, *M. trachynotus* was present at all four sites, but levels of parasitism were lower at 5-10% and showed no obvious pattern through the season. *Hormius* sp. (Braconidae) occurred only at Santa Cruz site 2 where its activity was similar to that of *M. trachynotus*.

The density of LBAM in San Francisco site 1 (younger plants) built to a peak in late June (Figure. 3), but subsequently showed a steady decline through the rest of the season. In contrast, there was a steady increase in density throughout the year at San Francisco site 2 (mature plants). The stage structure of the populations at both sites in San Francisco (Figure. 4) showed all larval stages present throughout the sampling period, with the exception of the winter months (November onward) when younger larvae appeared to be absent. Pupae were less commonly found but were present in late May and again in September. Similarly, 1st and 2nd instar larvae peaked in late May and again in early September.



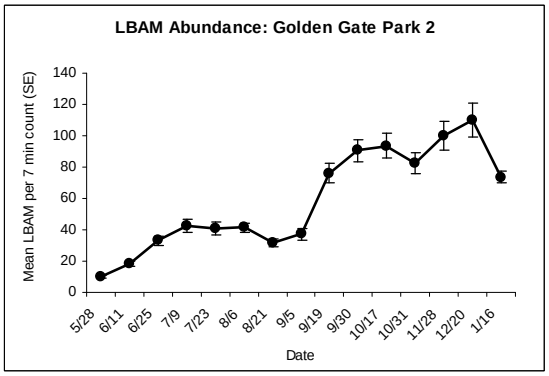
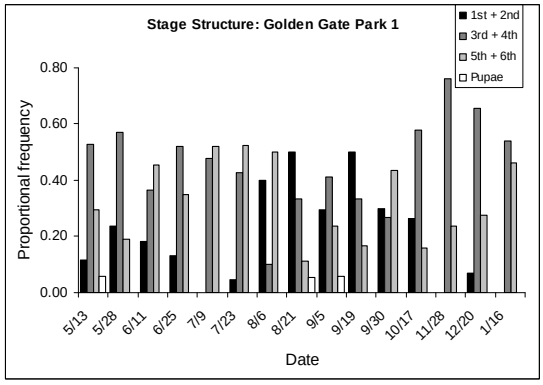


Figure 3. LBAM density on *Leptospermum laevigatum* at two sites in San Francisco, Golden Gate Park in 2008.



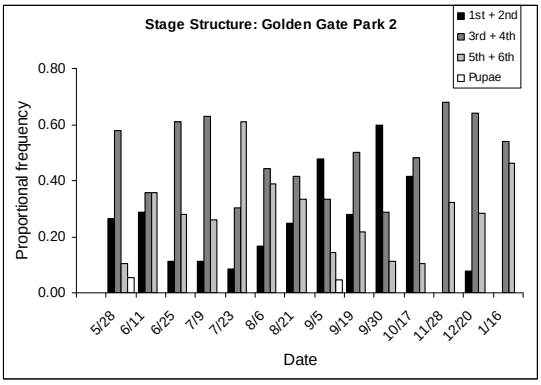
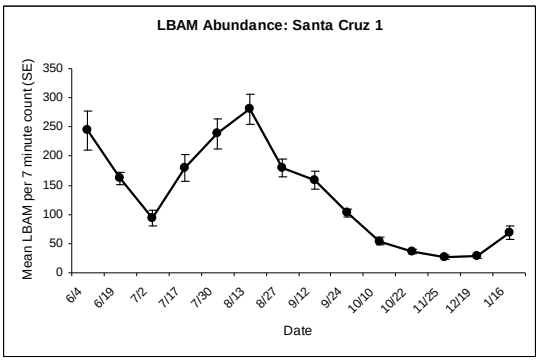


Figure 4. LBAM larval stage structure at two sites in San Francisco, Golden Gate Park in 2008.

In Santa Cruz (Figure 5), densities of LBAM were much higher at site 1 than at site 2, but at both sites populations peaked in early June and August, and declined in July and again in September and October. Part of the decline late in the season at site 1 may have been exacerbated by removal of some of the more heavily attached plants due to traffic safety issues at this site. The stage structure of LBAM in Santa Cruz (Figure 6) again shows all juvenile stages present throughout the period, with pupae present and peaks of 1st and 2nd instar larvae in May/ June and again in early October.

These patterns in the stage structure of LBAM populations in both San Francisco and Santa Cruz suggest that more extensive flights of adult moths occurred in May/June and September/October. Although not sampled specifically, the presence of adults and egg masses was also noted at the sites in both San Francisco and Santa Cruz in June and October. An additional flight of adult moths is expected in February/March as there are many large larvae present through the winter, particularly in San Francisco.



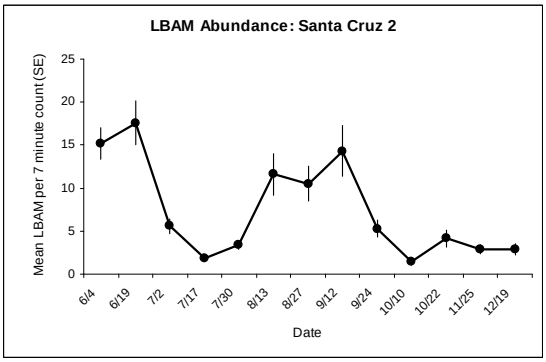
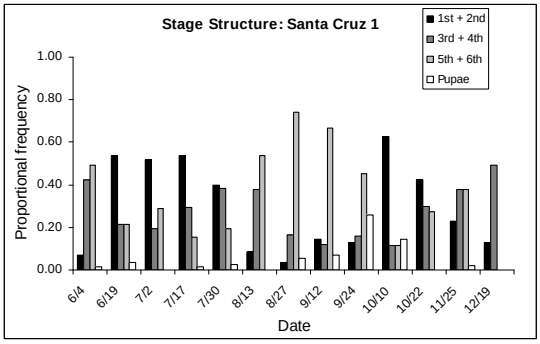


Figure 5. LBAM density on manzanita at two sites in Santa Cruz in 2008.



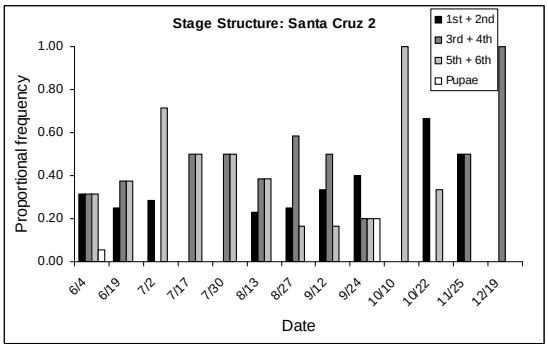


Figure 6. LBAM larval stage structure at two sites in Santa Cruz in 2008.

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Laboratory Rearing of Three Species of Leafrollers

Syed Khasimuddin

One possible tactic in the biological control of the light brown apple moth in California is to make use of natural enemies already present in the state attacking leafroller species that are related to the light brown apple moth. In order to evaluate these species as potential natural enemies of the light brown apple moth, they need to be evaluated in field settings and laboratory cultures on their original leafroller hosts. We report here on the laboratory rearing of three species of leafrollers.

The intended usage of laboratory reared leafrollers include: 1) to expose immature leafroller stages in the field to naturally occurring parasitoids. These would then be brought back to the laboratory for mass production of such parasitoids; and 2) to provide non-target host material for pre-release testing of light brown apple moth parasitoids in quarantine. The three species reared in the laboratory included *Platynota stultana*, the omnivorous leafroller, *Argyrotaenia franciscana*, the orange tortrix, and *Pandemis pyrusana*, the apple pandemis moth. Rearing of each of these species is described herewith.

Platynota stultana:

Continuing cultures from 2007 were bulk-reared on an artificial diet (Standard Oriental Fruit Moth diet- Citrus supplied by "Bio-Serve" – Product # F9649B) in square clear plastic "lunch boxes" with airtight lids (28.5 oz. capacity). The culture was placed in an environmental chamber with 22 ^{o}C and 35% R.H under a 14/10 light/dark cycle. During the first two generations, the diet in the containers was constantly infected by mold that hampered a normal development of the culture. Upon consulting with Dr. Lucia Varela of the University of California, the use of the "lunch boxes" was discontinued and small clear plastic cups (3.25 oz.) with airtight lids were used. Slabs of the pre-prepared diet, not exceeding one square inch, were supplied in each cup. Adults were kept in soft plastic cups (16 oz.) supplied with a 10% honey solution and a wax paper for oviposition. The open end of the cup was secured with a fine mesh cloth held in place with a rubber band. Adults oviposited on the walls of the cup as well as the wax paper. Survivorship of the different life history stages was improved significantly during the latter half of the year by using smaller rearing containers. All life history stages have been preserved as reference specimens.

Argyrotaenia franciscana:

Eggs of this species were received from Dr. Varela during June 2008 and a culture started in the laboratory with ambient conditions of temperature and humidity (22^{o}C ± 2 ^{o}C; 25% R.H). The diet and rearing containers were the same as for omnivorous leafroller described above. Under these conditions, the duration from egg to adult seems to average out to 42 days. All life history stages have been preserved as reference specimens.

Pandemis pyrusana:

Pupae of this species were received from Washington State University (WSU) under APHIS permit # P526P-08-01936 (CDFA permit # 34-07-08). A culture was initiated with the emerging moths in the "lunch box" containers, described under omnivorous leafroller, in the laboratory with the same diet as for the other two species. The culture did not survive beyond one generation. Another attempt was made with fresh pupae from WSU received during May 2008. The culture still did not get beyond one generation. It was decided to discontinue rearing of this species.

Field Search for Leafrollers and their Natural Enemies

Syed Khasimuddin

Attempts were made to collect leafrollers in the field to recover parasitoids that could be mass-produced for potential release against the light brown apple moth. These attempts were restricted to counties and areas that did not report any findings of light brown apple moth or were not quarantined. Collections made in the field (leaf rolls) were brought back to the laboratory and individual leaf rolls were placed in 1-oz. clear plastic cups with artificial leafroller diet and held for possible adult/parasitoid emergence. These attempts are summarized in the table below.

Date	Location	County	Host plants	Leaf rolls collected	Results
4/8/08	Sierra foothills research center	Yuba	apples, pears, valley oak, live oak	57	10 moths (Gelechiids**) 4 parasitoids identified as *Microgaster* sp.*
4/17/08	Chualar	Monterey (non-quarantined area)	grapes	14	One moth identified as orange tortrix
4/24/08 6/9/08	Hollister	San Benito (non-quarantine)	apricots, apples, cherries	5	Five leafrolls that yielded one orange tortrix
7/1/08	Winters	Yolo	apricots. prunes, cherries, grapes, peaches	0	No signs of leafroller
8/19/08	Lakeport	Lake	pears	0	No signs of leafroller

* - USDA-ARS Identification Lab. in Maryland

** - Marc Epstein, Pest Detection/Diagnostic Laboratory, Sacramento.

Citrus Leafminer Phenology in San Diego County and the San Joaquin Valley

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

The citrus leafminer (*Phyllocnistis citrella*) is a serious pest of citrus and can exacerbate citrus bacterial canker when the disease is present. Citrus leafminer is continuing to spread throughout California. It can be found in the desert valleys of southern California, along the southern coast, and is gradually moving northward through the San Joaquin Valley, as well as developing isolated populations in Stanislaus, Alameda, Contra Costa, Santa Clara, and Santa Cruz counties. The isolated populations are the result of accidentally moving citrus leafminer on nursery stock. In parts of southern California, citrus leafminer has been established for a number of years, and native gracillariid parasitoids have been attacking the leafminer larvae for some time. Very little is known about the native gracillariid parasitoids that may attack citrus leafminer larvae in the San Joaquin Valley. Therefore, this study was initiated in 2006 to compare the phenology of citrus leafminer along the extreme southern coast of California with the southern end of the San Joaquin Valley, and to survey the parasitoids attacking the leafminer in the San Joaquin Valley. This report includes assessment of development observed in 2008.

The phenology studies were conducted at five locations in San Diego County, three locations in Tulare County, and three locations in Kern County. Pheromone-baited traps that attract male moths were used at all locations. The starting date of the trapping and the number of traps placed varied with location. In San Diego County, two traps were placed at opposite ends of each site on March 3, 2008 and were replaced at approximately monthly intervals through December 11. At three locations, one trap remained in trees from December 2007 through early March 2008. Very few moths were found in these three traps through this time period. For two of the Tulare County locations and at all locations in Kern County, one trap was placed at each end of the block on February 26, 2008. At one of the Tulare County locations, two traps were placed at opposite ends of the block. Traps were replaced at approximately monthly intervals until November 24, 2008.

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, returned to the laboratory, and held for the emergence of any parasitoids.

The phenology of citrus leafminer varied with location in the state. In San Diego County, male moths were caught in traps beginning in early March and continued to increase in number throughout the summer and into the fall (Figure 1). There were some differences in the time that decline of numbers developed among the sites, probably due to the characteristics of the site. Some sites were located in nurseries, while others were in commercial citrus. In general, the number of male moths caught in traps in 2008 was similar to that found in 2007.

In the southern San Joaquin Valley, more moths were trapped in Kern County than in Tulare County (Figure 2). Also, the densities of moths trapped in the fall were 2 – 10 times higher in 2008 than in 2007. This increase in trap catches reflects the establishment of the citrus leafminer after its initial invasion. For the San Joaquin Valley, there appears to be a small, barely detectable flight of moths in late February through early April. A dramatic increase in moth density occurs from July through October and November (Figure 2).

Surveys of native parasitoids in Tulare and Kern counties recovered no parasitoids or evidence of parasitoids. However, larvae that had been preyed upon by unknown arthropod predators were found in October at several sites (Table 1). A small number of empty mines were found at one Kern County site in pruned plant material in February 2008, suggesting that citrus leafminer can bridge periods of limited flush using sprouts within the tree. The majority of mines were found in October and November (Table 1).

The results of these studies suggest that the seasonal phenology of citrus leafminer varies with the location in the state. Some of the phenological differences may be due to weather factors. For example, southern coastal areas are slightly warmer or or have temperature conditions more favorable for citrus leafminer throughout the year than the San Joaquin Valley. However, the numbers of citrus leafminer in the San Joaquin Valley are increasing, and this insect can survive periods of limited flush.

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 in 2008. 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
Sept 24	Kern 2	3	0	2	1	0
	Kern 3	5	5	0	0	0
Oct 22	Tulare 1	8	2	2	6	0
	Tulare 2	5	5	0	0	0
	Kern 1	14	7	1	5	1
	Kern 2	33	23	2	4	3
	Kern 3	16	12	2	1	1
Nov 24	Tulare 1	4	1	1	2	0
	Tulare 3	14	14	0	0	0
	Kern 1	19	1	19	2	0
	Kern 3	4	1	3	0	0

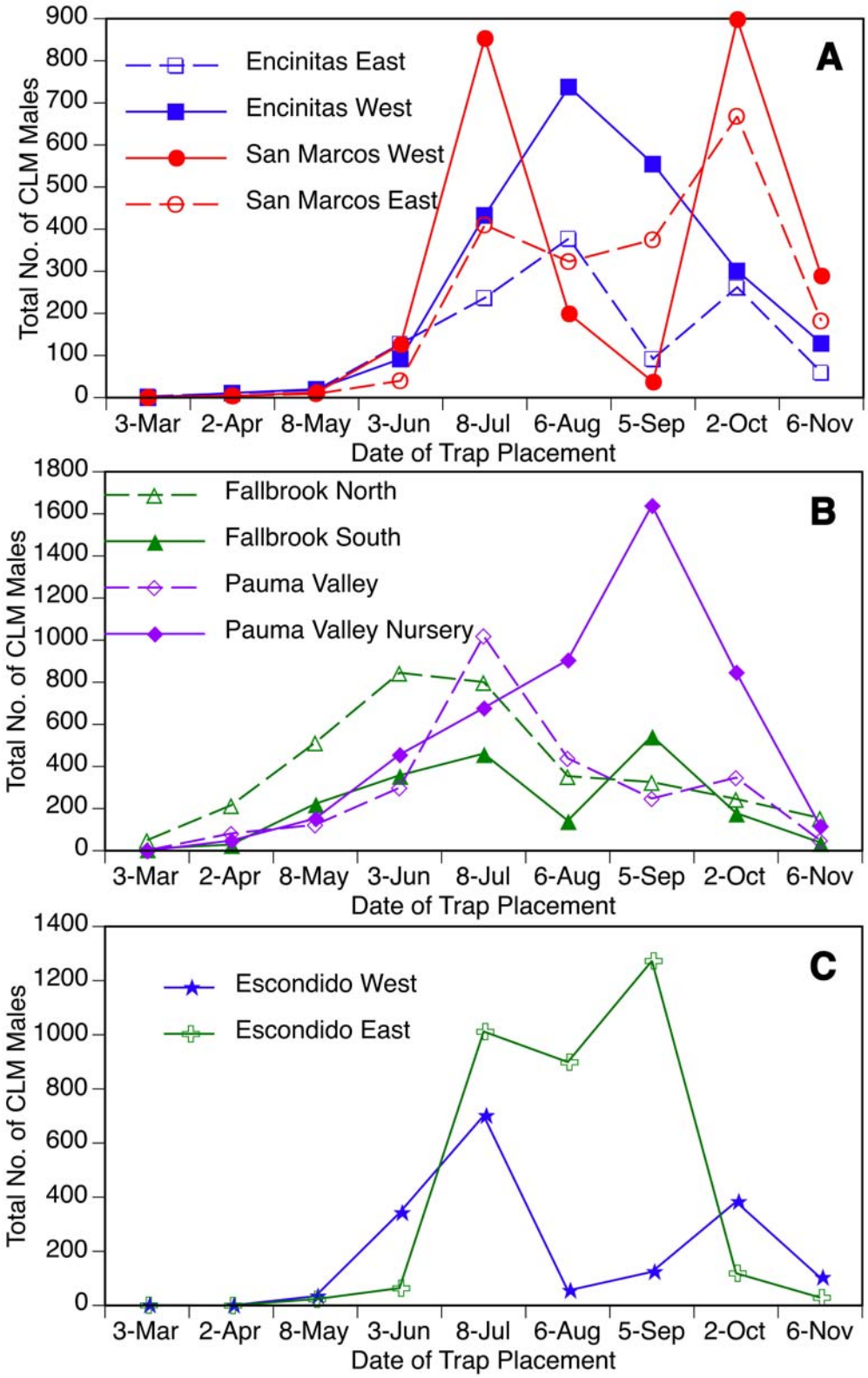

Figure 1. The total number of male citrus leafminer moths caught per pheromone trap placed in Encinitas and San Marcos (A); Fallbrook and Pauma Valley (B): and Escondido, in San Diego County from March through December 2008.

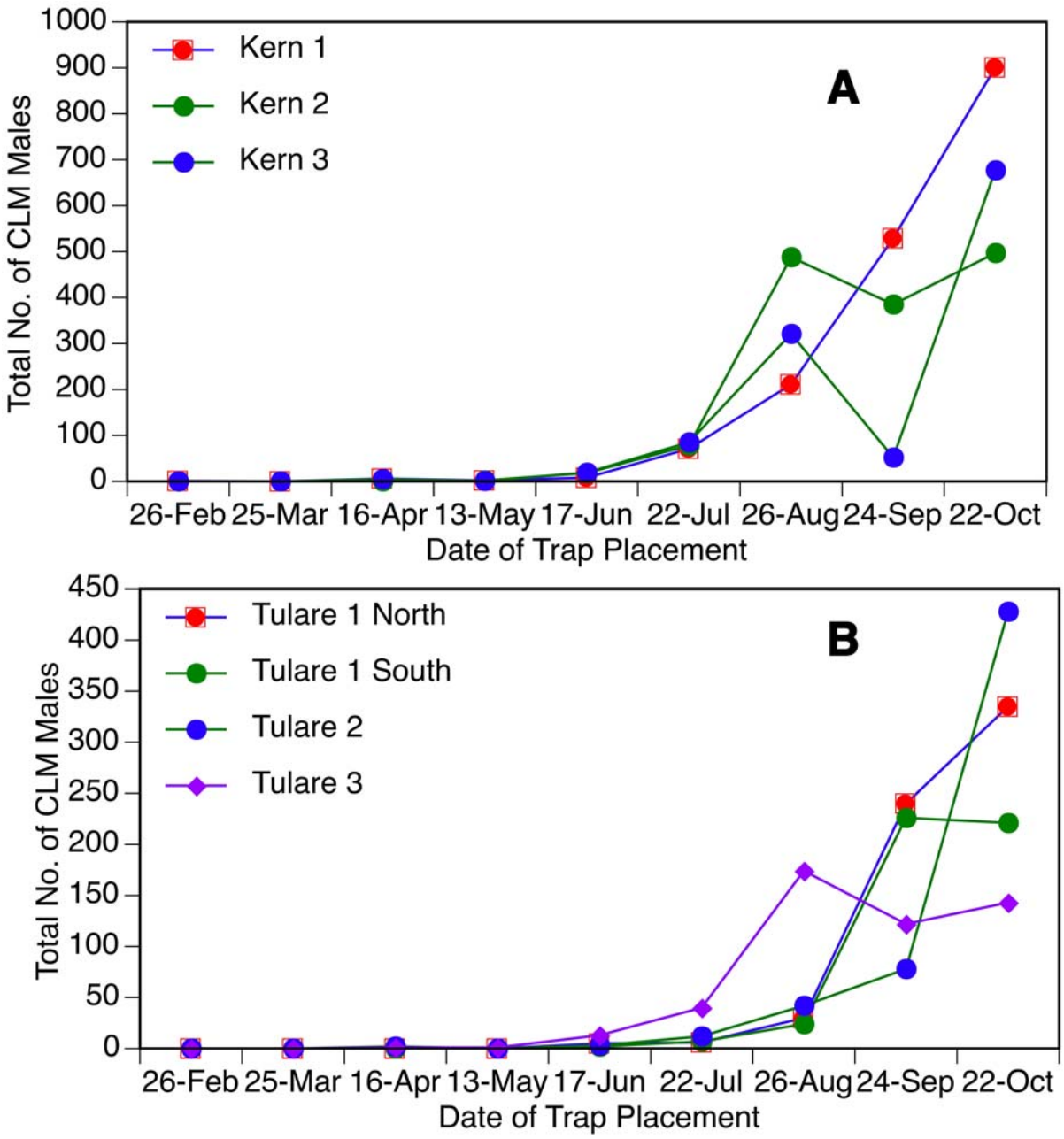

Figure 2. The total number of male citrus leafminer moths caught per pheromone trap placed in Kern County (A); and Tulare County (B), from February through November in 2008.

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Foreign Exploration for Parasitoids of the Olive Fruit Fly

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Many countries falling within the natural distribution of wild olive, *Olea europaea cuspidata* have been explored at least once during this project for parasitoids of olive fruit fly, *Bactrocera oleae* (Gmelin). One of us (CHP) returned to Namibia in April 2008 to expand the gene pool for parasitoids collected in 2007 and to train a co-operator in collecting. During 2007, woodlands of wild olive were discovered in a particular region of the country, the Kartsveld (19° south latitude, 1400 m), a unique shrub/tree savannah vegetation zone in north-central Namibia, delimited by the towns of Otavi, Grootfontein, and Tsumeb. The area is blanketed by a variety of tall shrubs to small trees, and receives about 500 mm (20 inches) of rain a year, although rainfall is highly variable between years. We had found a number of locations with abundant wild olive trees in 2007, but only a few trees with fruit. In 2008, we found ripe olives on trees that in 2007 had been fruit-free, and the opposite was true as well. For example, at the site near the Tietz ranch outside of Otavi, we made our best collection in 2007. In April 2008, no ripe fruit were available. However, we found trees with immature fruit (Figure 1). A small number of these trees went on to produce fruit in October 2008, springtime in Namibia. The entire country had unusually heavy rains during their summer, February to April 2007, which may explain this fruit set. Nevertheless, it shows that fruiting on these trees is variable and can occur at one, maybe two times of the year, or not at all. A 0.6 kg collection of fruit made by our co-operator 18 October 2008 from a site just outside Otavi failed to produce any insects.


Figure 1

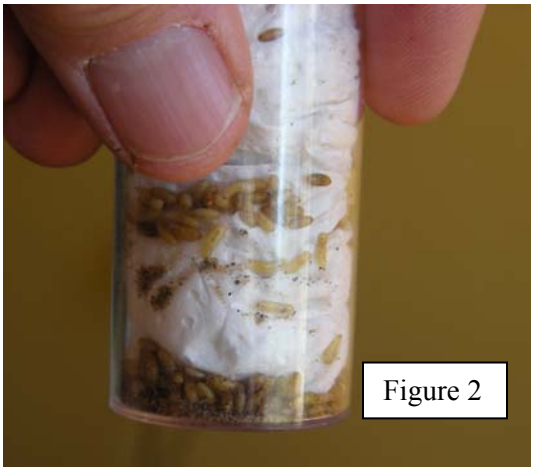
Figure 2

Some of the best collecting over the last four years was accomplished on the April 2008 trip to Namibia. A record number of fly puparia, over 5300, were returned to the USDA ARS EBCL. This was accomplished by rearing out some fly puparia while travelling in Namibia, shipping mainly fly puparia rather than fruit (Figure 2), and with help from our resident collector, Dr. John Irish. We had from three previous trips, a good concept of the best collecting areas within the country, and time of year to collect. As in the previous two collections, *Psyttalia concolor* (Namibia) was the dominant parasitoid collected, followed by *P. lounsburyi* (Table 1). Results from previous field collections suggest that *P. concolor* is a generalist parasitoid with little specificity for olive fruit fly. Collections from Namibia, however, indicate that the population in this region of Africa is unique, i.e. we are dealing with an isolated biotype with distinct preferences. *Psyttalia concolor* has made up only a minor component of the parasitoids collected from Kenya and South Africa. Although it has been the dominant species in Morocco and Canary Islands, it

attacked only a small percentage of flies (Table 2). In Namibia, however, it has been the dominant species on each of three collecting trips made in April and May (Table 1). Furthermore, this population has attained some of the highest levels of parasitism of olive fruit fly of all parasitoids collected from olives, varying from 26 to 36 percent parasitism (Table 2).

Table 1. Species composition of braconid parasitoids reared from field collected wild olives (percent), 2003-2008.

Country	*Psytallia concolor*	*Psytallia lounsburyi*	*Utetes africanus*	*Bracon* spp.	*Psytallia ponerophaga*	*Diachasmimorpha* spp.
Morocco, 2004	100	0	0	0	0	0
Canaries, 2004	100	0	0	0	0	0
Pakistan, 2005	0	0	0	0	100	0
Reunion, 2004	0	0	0	0	0	100
Namibia, 2004	45	0	12	28	0	0
Namibia, 2007	57	23	5	15	0	0
Namibia, 2008	70.2	22.1	6.6	1.1	0	0
RSA, 2003	6.3	29.4	45	18.9	0	0
RSA, 2004	3.2	21.2	68	7.7	0	0
RSA, 2005	0	54.0	46.0	0	0	0
[1]Kenya ‘A’ 2004	0	95.4	4.6	0	0	0
[1]Kenya ‘B’ 2004	0	100.0	0	0	0	0
Kenya, 2005	0	62	38	0	0	0
Kenya, 2007	1.1	92.5	7.4	0	0	0
China	0	0	0	0	0	100

[1]Collections from ‘A’ Mt. Kenya and from ‘B’ Mt. Elgon, otherwise all other Kenyan collections are from Mt. Kenya.

Table 2. Parasitism of *B. oleae* from single or multiple collections of wild olives.

Date	Country	No. olive fruit fly puparia	Parasitoid sp. and # collected		% flies parasitized[1]
May 2004	Namibia	694	*P*. nr. *concolor*	108	26.2
			P. lounsburyi	0	--
			U. africanus	22	5.3
			Bracon spp.	54	12.0
May 2007	Namibia	507	*P*. nr. *concolor*	274	35.0
			P. lounsburyi	79	13.0
			U. africanus	14	2.6
April 2008	Namibia	5338	*P*. nr. *concolor*	1134	36.0
			P. lounsburyi	364	11.6
			U. africanus	113	3.6
			Bracon spp.	121	3.8
Oct 2004	Canary Islands	965	*P*. nr. *concolor*	38	2.3
Nov 2004	Morocco	318	*P*. nr. *concolor*	38	10.6
May 2005	Rep. South Africa	272	*P. lounsburyi*	57	15.0
			U. africanus	48	13.0
Sept 2006	China	427	*D. longicaudata*	11	2.5
Feb 2003	Kenya, Mt. Elgon	5291	*P. lounsburyi*	302	9.0
Feb 2004		100	*P. lounsburyi*	21	35.0
Nov 2002	Kenya, Burguret Forest	1560	*P. lounsburyi*	433	33.0
			U. africanus	21	2.3
Sept 2003		11,000	*P. lounsburyi*	7828	80.0
			U. africanus	302	3.0
May 2007	Kenya, Ontulili Forest	31,800	*P. lounsburyi*	2202	54.0
			U. africanus	23	1.2
Fall 2007	Kenya, Burgurt Forest	31,800	*P. lounsburyi*	7281	23.0
			U. africanus	582	1.8
Oct,2005	Pakistan	460	*P. ponerophaga*	176	28.0

[1]Based on number of adult insects emerging from fruit and fly puparia.

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Releases of *Psyttalia* spp. into California for Control of Olive Fruit Fly

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Efforts to permanently establish populations of new, exotic parasitoids specific for olive fruit fly in northern California were continued in 2007 and 2008. While making these releases, cage studies were conducted simultaneously to determine differences in fecundity rates between the candidate parasitoids: *Psyttalia lounsburyi* (one strain each from South Africa, and Kenya), and *P*. nr. *concolor* (from Namibia). The number of release sites has increased over this time to include seven counties: Butte, Fresno, Napa, San Luis Obispo (for details on this county see Wang et al., this volume) Solano, Sonoma, and Yolo.

Field cages were placed on ends of branches (Figure 1) in early summer for controlled studies to compare parasitism rates of *P. lounsburyi* (strains from South Africa, Mt. Kenya) and *P*. nr. *concolor* collected in Namibia. One to two weeks prior to releases flies were added to cages, followed by parasitoids ca. one week later. We added ca. one adult female fly per 5 fruit. Enough adult female parasitoids were added to reach a minimum ratio of one per two fruit, or a minimum of 20 per cage. Additional parasitoids were released directly into trees for permanent establishment. These came from our own cultures and from cultures maintained by Arnaud Blanchet at the European Biological Control Laboratory, and from field collections on Mt. Kenya by Peris Machera. Not all stains/species of parasitoids were available every year. Therefore in 2006 only *P. lounsburyi* from South Africa was tested and in 2007 only the strain from Mt. Kenya was available. In 2008, *P*. nr. *concolor* (Namibia) and *P. lounsburyi* from South Africa were tested, but the latter was available only late in the year and in low numbers.

No significant differences were noted in parasitism rates between the different release sites within the 2006 and 2007 season (Table 1). However, the number of parasitoids emerging from olives in cages increased a whole order of magnitude from 2006 to 2007 in Yolo, Butte, and Solano counties sites (no recoveries were made from cages in 2007 in Napa and Sonoma County, see below). Overall, the percent parasitism increased from ca. 3.5% in 2006 to ca. 50% in 2007. One possibility for this increase was the higher number of host larvae available to the parasitoids in 2007 at the monitored sites. Although the mean number of larvae per olive remained about the same going from 2006 to 2007, the total number of larvae available to parasitoids in each cage increased from three per cage to about 50 per cage (Table 1). Also, honey was streaked onto the top surface of leaves inside cages in 2007, but not in 2006. This likely helped in the over doubling of adult survivorship between the two years (Table 1). Lastly, the results may have varied between years because two different strains were used: parasitoids in 2006 came from West Cape, South Africa, and in 2007 they came from the Burguret Forest on Mt. Kenya. The latter may have a higher affinity for olive fruit fly resulting in higher oviposition rates. This explanation is supported by emergence data from parasitoids collected on Mt. Kenya and Mt. Elgon in Kenya, and from various locations within West Cape, South Africa. *Psyttalia lounsburyi* has repeatedly been the dominant braconid emerging from olive fruit fly infested wild olives in Kenya. Of nine collections made from Mt. Kenya and Mt. Elgon between 2003 and 2007, *P. lounsburyi* has averaged 80% of the individual parasitoids collected (range: 19% to 100%); most of the remaining individuals were *U. africanus*. Three years of continuous field data from West Cape, South Africa showed that the dominant parasitoid attacking olive fruit fly in wild olive trees is *Utetes africanus,* followed by *P. lounsburyi* (Vaughn Walton et al., unpubl.

data). From 2005 to 2007, a total of 7,322 parasitoids were collected from fruit. Of these, *U. africanus* made up 71.3%, and *P. lounsburyi* made up 23.4%, followed by 1.7% *P. humilis* (a part of the *P. concolor* species complex), 1.8% *Bracon* spp. and 1.6% Eurytomidae.

Psyttalia concolor (Namibia) was released for the first time fall 2007. A total of 111 females were released into cages at the Student Experimental Farm at UC Davis, and 95 surviving adults were liberated into tree canopies. Most notable, these parasitoids were not released into cages until 15 November 2007, yet some emerged from olives collected over one month later, showing that adults can survive (95 of 111) and oviposite under cool, fall conditions. Approximately 371 female *P.* nr. *concolor* (Namibia) were released directly into trees at the Spring Mountain site in Napa County, while 501 and 301 female *P. concolor* were released at the UC Davis and Butte County sites, respectively.

In 2007, no parasitism was reported for parasitoids released into cages at sites in Napa and Sonoma counties (Table 2). The most likely cause for these results was low host availability in caged olives. While this value varied from 0.71 to 1.05 in caged olives at sites in Yolo, Butte, and Solano counties, the mean larvae per olive at sites in Napa and Sonoma varied from 0.06 to 0.24. Because the latter sites were located in areas with milder conditions, no flies were added to cages at the beginning of the study. Sting marks on fruit indicated a much higher larval population was present than actually existed.

Figure 1. Cage used to house released parasitoids in California

Table 1. Summary of caged releases, 2006 and 2007, *P. lounsburyi*.

Site	Year	Mean parasitism (SE) %	Mean larvae per olive[3]	# fly hosts per cage (SE)	Ratio: # released flies to olives	Mean # female parasitoids released per cage	Mean # olives per cage	Total # parasitioids liberated from cages	Survivorship of adult parasitoids %
Butte, Leuders	2006[1]	0.9 (2.8)	0.30	1.83 (3.41)	0.94	9.33	20	140	19.2
Napa	2006[1,4]	9.5	1.70	--	--	n.a.	--	--	--
Sonoma	2006[1]	n.a.	--	--	--	--	--	--	--
Yolo, UCD	2006[1]	5.6 (14.0)	0.62	4.28 (3.1)	0.77	11.8	27.9	106	25.8
Butte. Leuders	2007[2]	52.0 (12.6)	1.04	52.2 (71.4)	0.20	23	44	116	74.9
Napa, Spring Mt,	2007[1,2]	--	0.20	--	--	15	38.7	--	0
Solano, Wolfskill	2007[2]	23.5 (8.6)	0.71	26.75 (9.3)	0.21	19	36.0	97	75.4
Sonoma, Stone Edge	2007[1,2]	--	0.06	--	--	15	45.5	--	0
Sonoma, Hanzell	2007[1,2]	--	0.24	--	--	10	21.5	--	0
Yolo, UCD,	2007[2]	49.1 (31.1)	1.05	49.8 (8.9)	0.20	26	49.2	123	79.5

[1]Original strain collected from West Cape Province, South Africa
[2]Original strain collected from Mt. Kenya
[3]Based on number of emerging puparia, adult flies and parasitoids
[4]No measure of variance available

The USDA/UC Wolfskill Experiment and four locations in the San Luis Obispo area were added as new release sites in 2008. Much higher numbers of *P*. nr. *concolor* were released in 2008 than in 2007 (10,235 vs. 95), while fewer *P. lounsburyi* (510 vs 582). Good collections of the former from Namibia in spring of 2008, plus help from EBCL's culture aided in this effort. However, releases of *P. lounsburyi* were dependent on field collections coming from Kenya, which did not materialize due to very low parasitism. In 2008 collections came from the Ngare Ndare Forest, while pervious collections came from the Burguret Forest. Although excellent within-season recoveries have been made from the coastal releases (see Wang et al., this volume), to date poor recoveries have been made of *P*. nr. *concolor* from inland sites. Releases were initiated in July, the earliest so far; however, most sites experienced low population densities of flies. Caged comparison studies were initiated much later in the season, November, because we were waiting on collections of *P. lounsburyi* coming from Kenya. Cold temperatures most likely greatly reduced survivorship and developmental rates. Field samples are still being processed.

Table 2. Number of parasites released for control of olive fruit fly, 2008.

County	Site Name	Total Released (♂♀)	
		P. concolor (Namibia)	*P. lounsburyi* (Kenya)
Solano	Wolfskill	565	0
Yolo	UC Davis	901	30
Butte	Leuder's	250	0
San Luis Obispo	multiple sites	6200	0
Fresno	KAC	500	300
Napa	Spring Mountain	1119	180
Sonoma	Hanzell	700	0
Total		**10,235**	**510**

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Field Evaluation of Introduced Olive Fruit Fly Parasitoids in Central Coast California

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The olive fruit fly, *Bactrocera oleae* (Rossi), has become a major pest of olive, *Olea europaea* L., in California. Current research efforts emphasize long-term management practices, and biological control is a part of this program. Two African parasitoid species, *Psyttalia* nr. *concolor* (Szépligeti) (Fig. 1) and *P. lounsburyi* (Silvestri) have recently been imported into California and are approved for release in this state. We evaluated the effectiveness of these two parasitoids through field-cage tests and open releases of *P.* nr. *concolor* to hopefully establish this parasitoid in San Luis Obispo (SLO), California.

Fig. 1. Olive fruit fly parasitoid, *Psyttalia* nr. *concolor*, probing host fly larvae on fruit.

Four non-commercial olive tree sites that were unsprayed and never harvested were selected for this study in SLO (Fig. 2): (1) Broad St. (a private parking lot with 10 small Mission-type olive trees); (2) California Polytechnic State University (Cal Poly) Campus (at two locations about 500 m apart: five large Mission trees near the student dormitory and about 20 large Mission olive trees within the hills — the fruit on the hillside trees were relatively smaller, matured later, and were less infested by flies than those fruit on the trees near the student dorm; (3) Avila Beach (a private property with 10 small Mission trees); and (4) Righetti Ranch (a private property with 10 large Mission or Sevillano trees). The four sites were at least five miles apart from each other.

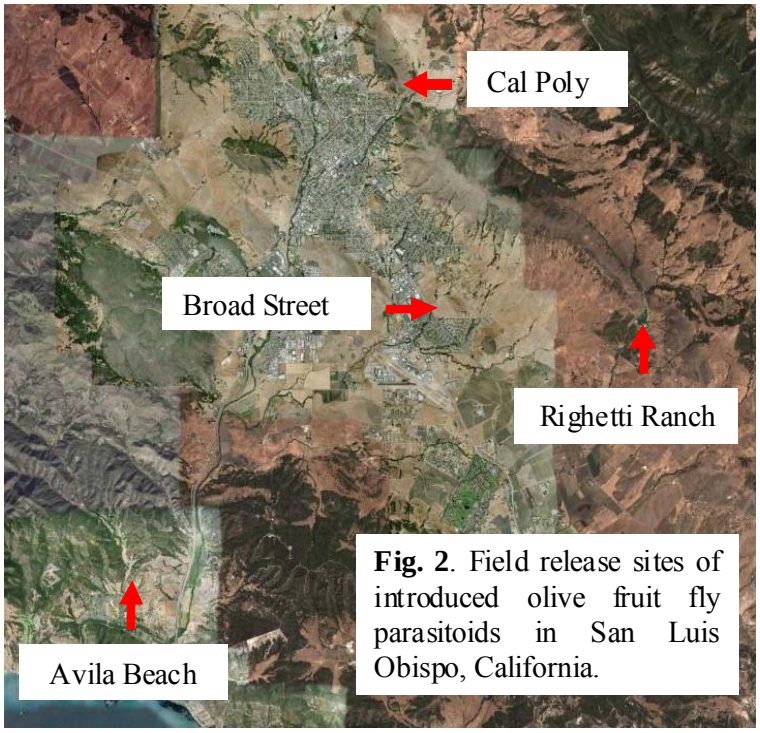

Fig. 2. Field release sites of introduced olive fruit fly parasitoids in San Luis Obispo, California.

Four field-cage tests with *P.* nr. *concolor* were conducted at three sites described above and throughout the major fruit seasons (Table 1). Each test consisted of 10 small cylindrical screen cages (45 cm long X 25 cm diameter) except the Cal Poly hillside test, which consisted of five large screen cages (61 cm long X 48.3 cm diameter) to enclose more olive branches due to

relatively low numbers of fruit at this site. Each cage enclosed 80-100 fruit. All field-cages were established before the concurrent open-field releases of parasitoids in the same sites. For the earliest test (i.e., 22 August 2008), the cages were set-up three weeks prior to the release of parasitoids and five gravid 2-week old female *B. oleae* were released into each cage to establish the fly infestation due to low levels of natural infestation by *B. oleae* in the early fruiting period. For all other cage tests, all fruit were heavily infested by naturally accruing flies and no fly inoculation was needed. The majority of parasitoids used in this study were provided by the USDA-ARS European Biological Control Laboratory (Arnaud Blanchet, Walker Jones), Montferrier, France. The original *P. lounsburyi* colony was started from parasitized *B. oleae* collected in wild olives in Kenya, while the colony of *P.* nr. *concolor* was originally established with field collections of wild olives in Namibia. John Andrews oversaw the importation of the parasitoids at UC Berkeley Quarantine Facility.

Table 1. Field-cage evaluation of *P.* nr. *concolor* in San Luis Obispo, California

Study site	Set-up date	Temp (°C)	Host density per cage	Parasitism by *P.* nr. *concolor*	Parasitism by *Pteromalus* sp.
Broad St.	22 Aug.	18.7 ± 0.4 a	161 ± 16 a	32.4 ± 5.8 ab	0.0 ± 0.0 a
Cal Poly (dorm)	28 Aug.	19.2 ± 0.5 a	120 ± 24 a	18.5 ± 4.3 b	21.5 ± 2.7 b
Cal Poly (hills)	4 Sept.	18.4 ± 0.3 a	146 ± 23 a	64.3 ± 13.8 a	0.0 ± 0.0 a
Righetti Ranch	7 Oct.	18.0 ± 1.3 a	154 ± 29 a	42.5 ± 5.3 a	0.7 ± 0.3 c

* Value (mean ± SE) followed by different letters within the column are significant different (ANOVA, $P < 0.05$).

Fifteen mated, one-week old female *P.* nr. *concolor* individuals were released into each cage, with each also provided with water and honey. A 30 cm X 30 cm piece of cardboard was placed over each cage to reduce direct sunlight on the cages. After two to three weeks, cages were removed and fruit returned to the laboratory for emergence of parasitoids and flies. Dead puparia were reconstituted in water for one day, and then dissected under a microscope to determine the presence or absence of immature parasitoid cadavers and pharate adults. Parasitism was estimated based on the number of emerged and dissected wasps and flies.

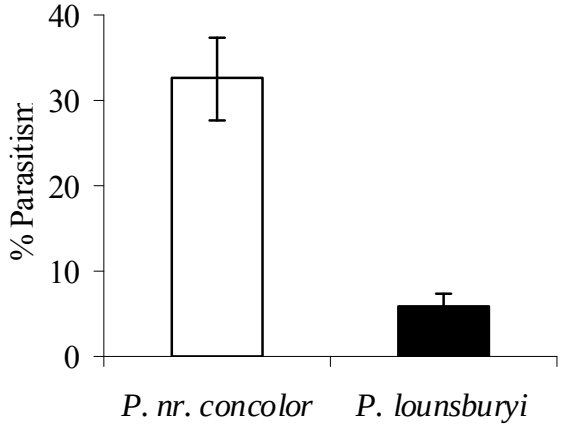

Fig. 3. Comparison of parasitism (mean ± SE) between two introduced olive fruit fly parasitoids in San Luis Obispo, California.

Field temperatures and host density were similar among different test sites (Table 1). Parasitism was lower at the student dorm site than the hillside site at Cal Poly (Table 1). Prior to release of any imported olive fruit fly parasitoids, *Pteromalus* sp. nr. *Myopitae*, a generalist, ectoparasitic parasitoid was reported to attack *B. oleae* larvae in SLO. At the student dorm site, parasitism by *Pteromalus* sp. was higher than all other sites (Table 1). Our laboratory tests showed that *Pteromlaus* sp. attack both unparasitized fly larvae and larvae that were previously

parasitized by *P.* nr. *concolor*. This might have reduced the parasitism by *P.* nr. *concolor* at this site. The size of fruit on the hillside site was smaller due to lack of management, and this might have also led to increased parasitism because fly larvae would be more susceptible to parasitism.

To compare the effectiveness of *P.* nr. *concolor* with *P. lounsburyi*, a field-cage test was conducted at the Righetti Ranch on 22 October. Test conditions for each species consisted of 10 screen cages and used the similar methods as described above. All 20 cages were placed in one tree, and 15 mated, one-week old female parasitoids were released into each cage, with water and honey provided for the parasitoids. The contents of the cages were collected after a three week exposure, and parasitism was estimated based on the number of emerged and dissected wasps and flies. There were 44.6 ± 6.3 fruit per cage and 1.93 ± 0.64 larvae per fruit in the *P.* nr. *concolor* test, and 36.0 ± 3.0 fruit, and 2.32 ± 0.56 larvae per fruit in the *P. lounsburyi* test. Parasitism by *Pteromalus* sp. was < 1.5% in both tests. Parasitism by *P.* nr. *concolor* was about six-fold higher than that by *P. lounsburyi* (ANOVA, $F_{1,18} = 26.3$, $P < 0.001$) (Fig. 3).

P. nr. *concolor* individuals were released into open tree canopies at the four different sites (Fig. 2). The number of parasitoids released was based on quantities received in each shipment and the number available from laboratory colonies at the time of the releases. Immediately prior to each release, fruit were randomly collected from the trees that were used for the field releases to monitor the fly population and parasitism by extant parasitoids. Collected fruit were placed in plastic containers (11 cm high X 11 cm diameter) covered with organdy cloth and held in the laboratory at 25 °C (under this temperature most fly eggs develop into pupae within two weeks) for emergence of flies and parasitoids. To suppress mold formation and allow pre-pupal larvae to drop to the bottom of the container, a metal grid was fitted into the container (2 cm above the bottom) and the fruit were placed over the metal grid. Fly pupae were collected once weekly. The puparia for the first two weeks represented the host stages that were likely attacked by the parasitoids in the field and were used to estimate the field parasitism. The emergence containers were held for several months, enough time for fly larvae or parasitoids of any age inside the fruit to develop to adults or exit the fruit (e.g., immature *Pteromalus* sp. always remain inside the fruit if not emerging).

Table 2. Pre-release sampling of olive fruit fly population and parasitism by extant parasitoids in San Luis Obispo, California

Release site	Sampling date	No. of fruit sampled	Flies per fruit	% Parasitism by *Pteromalus* sp.
Broad St.	22 Aug.	385	0.91	3.01
Cal Ploy (dorm)	28 Aug.	166	2.01	19.10
Cal Poly (hills)	28 Aug.	243	0.57	0.00
Avila Beach	7 Oct.	170	2.12	0.00
Righetti Ranch	7 Oct.	330	3.62	0.68

In the earliest release at Broad Street, olive fruit fly population density was lower, compared to the two October releases in both Avila Beach and Righetti Ranch sites where all the fruit were heavily infested by the flies (Table 2). At Cal Poly, fly population density was about four-fold higher at the student dorm site than the hillside site (Table 2). The highest parasitism by *Pteromalus* sp. was also recorded from the student dorm site, but no *Pteromalus* were found from Avila Beach and the foothill site (Table 2).

Two different methods were used to estimate post-release recovery of parasitoids and olive fruit fly: (1) direct sampling of fruit — fruit were sampled at random from the same trees in which parasitoids were released; and (2) collection unit samples. Fruit began to fall from trees as they matured and flies exited from mature fruit (they pupate inside fruit when fruit are not very mature) to pupate in soil. When this began, 5 – 10 screen cages with tops open (30 cm long, top diameter 30 cm) were placed beneath olive braches to collect dropping fruit and pupae. Dropped fruit and pupae were collected every two weeks. Number of emerged flies, wasps and dead pupae were recorded.

Table 3. Open field release and recovery of *P.* nr. *concolor* in San Luis Obispo, California

Release site	Release date	# Female wasps released	Post-release sampling and number of insects recovered					
			Date	# Fruit collected*	Fly	*P.* nr. *concolor*	*Pteromalus* sp.	Dead pupae
Broad St.	22 Aug.	200	4 Sept.	371	146	7	58	6
			7 Oct.	220	271	10	32	255
			4 Nov.	153	118	43	25	21
Cal Poly (dorm)	28 Aug.	600	4 Sept.	402	56	10	35	1
			7 Oct.	780	802	4	63	126
				CU	68	6	0	6
	4 Nov.	2500	12 Nov.	187	108	4	4	230
			21 Nov.	1095	385	34	8	755
Cal Poly (hills)	28 Aug.	500	4 Sept.	292	51	3	29	2
			7 Oct.	272	253	6	6	83
				CU	23	6	0	7
			22 Oct.	CU	179	8	2	114
Righetti Ranch	7 Oct.	1100	4 Nov	120	207	12	0	67
				CU	243	21	9	334

* CU: collection unit.

P. nr. *concolor* were recovered consistently at all release sites following the releases (Table 3). In particular, at the Broad Street site, although a low number of wasps (200 females) were released, increasing numbers of parasitoids were recovered through September to November, suggesting that the parasitoid had temporally colonized this site and developed for one to two generations by November. On 29 July, we found that the fruit matured earlier in the Broad Street site compared to other sties, and so the parasitoids were released earlier in the season there. Relatively, low numbers of *P. concolor* were recovered from the August releases at Cal Poly. This site was characterized by all large olive trees that bore abundant fruit. The number of wasps we released was probably too low to make any significant recovery from this site. Thus, 2,500 more female *P.* nr. *concolor* were released from 28 October to 4 November at the student dorm site, which resulted in recovery of 34 *P.* nr. *concolor* from 1,095 fruit collected on 21 November. Additionally, about 1,000 female *P.* nr. *concolor* were released at the Avila Beach

site on 7 October, but post-release sampling was not conducted. *Pteromalus* sp. were commonly recovered from all sites (Table 3). Pupal mortality was considerably high, but we suspected that a large portion of dead pupae were parasitized by *P.* nr. *concolor*.

In conclusion, both introduced parasitoids (*P. lounsiburyi* and *P.* nr. *concolor*) successfully attacked olive fruit fly in field-cage tests, and displayed varying potential to suppress the fly population, with *P.* nr. *concolor* being more effective than *P. lounsiburyi*. *P.* nr. *concolor* was recovered consistently from post-release samples at different sites. Abundant roadside and residential olive trees grow in the SLO areas, which may provide the best opportunity for this parasitoid to establish and spread from there. Post-release sampling will be conducted in the future to determine if the released parasitoids will overwinter and permanently establish in SLO.

Acknowledgements. We thank Martha Gerik for assistance, and Therese Kapaun for finding some release sites. We are grateful to Walt French, Scott Ritterbuck, Anne May and David Righetti for allowing us to conduct studies at their properties, and Victoria Yokoyama for kindly providing us field cages. Funds were provided by USDA CSREES Special Grants Program: Pest Management Alternatives.

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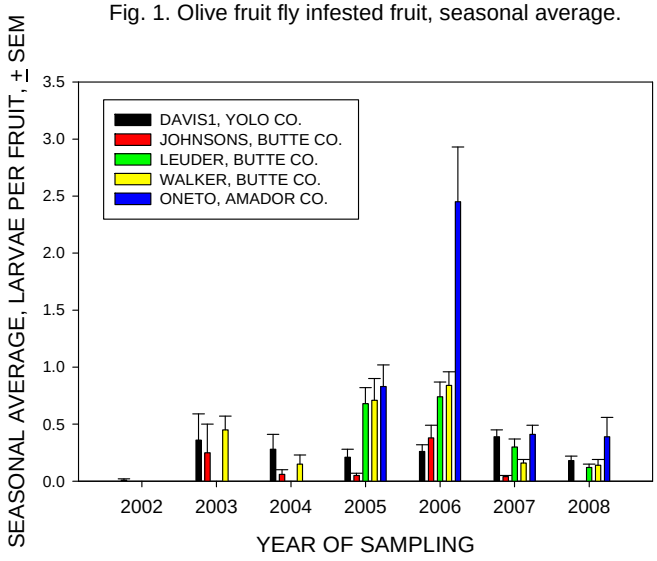
Olive Fruit Fly Larval Population Dynamics

Charles H. Pickett, Kent Daane[1], Marshall Johnson[2], Chia Moua, and Lue Yang

Olive fruit fly, *Bactrocera oleae* (Gmelin) (Diptera: Tephritidae), is the primary pest of olives in the Mediterranean basin, where the vast majority of the world's olives are produced. It is capable of infesting 100% of the fruit on a tree, rendering the harvest unmarketable. Following its introduction in 1998, this fruit fly pest became firmly established in olive-growing regions throughout California. In anticipation of releasing new imported parasitoids into California, several release sites were identified in California. We report on the seasonal population dynamics of olive fruit fly larvae in sites from the northern part of the state, and extant parasitoids found associated with these fruit.

Olives were sampled from three to five trees per site, monthly during the summer and fall. Only olives with visible fly oviposition marks were selected while fruit were green; after ripening, olives were selected randomly from all fruit within arms reach. Up to 50 fruit were collected per tree. Fruit were returned to CDFA's laboratory in Sacramento and placed in either 0.5 or two liter paper cans with a false bottom, depending on size of olive collection, and held for two months. The number larvae we report on, is based on the number of adult flies emerging from fruit. Therefore this number reflects only the number of larvae successfully developing to adults. The number and sex of flies, plus the taxa of parasitoids emerging from fruit were recorded for each sample. Adult flies were also monitored at sites using ChamP™ traps baited with ammonium bicarbonate and male pheromone lures, until release of parasitoids was initiated. One trap was placed in each tree from which olives were sampled. Use of these traps was discontinued in some trees where we began releasing parasitoids.

Fig. 1. Olive fruit fly infested fruit, seasonal average.



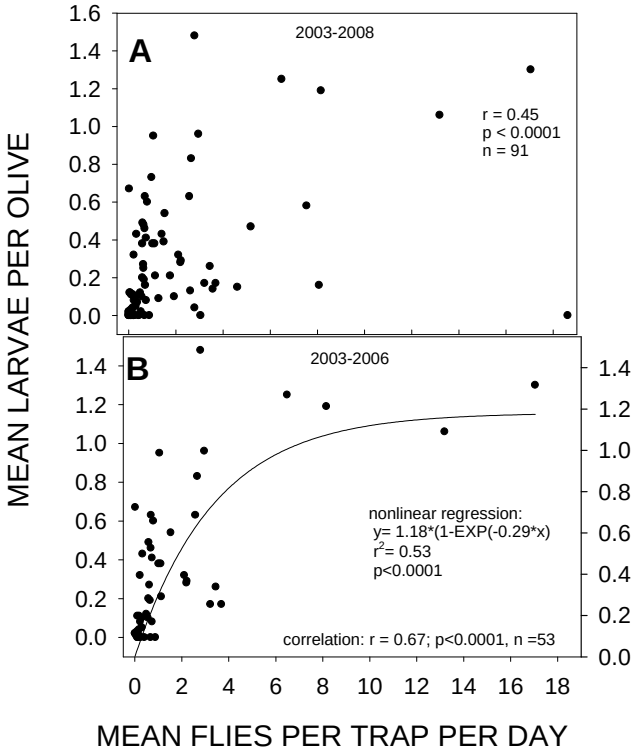
Olive fruit fly larval densities peaked in 2005 and 2006 (Fig. 1). Seasonal averages rarely exceeded one per fruit, except at one site, Oneto's farm in Amador County. This is an isolated patch of trees in the foothills of the Sierra Nevada, no longer in use commercially. A significant correlation was measured across all study sites, between trap catches of adult flies and larval densities when using monthly averages collected from 2003 to 2008 (Fig. 2A; r = 0.45, p<0.0001). When examining the same correlation within a single year, all but 2007 and 2008 had highly significant correlation coefficients between larval densities and number of adults on ChamP traps (Table 1). In the latter two seasons, there were several instances in which high numbers of adult flies were caught in ChamP traps, but little or no fruit were infested in the trees where traps were hung. For example at the Walker site, 2.8, 3.04, and 18.6 flies were recorded per trap per day with only 0.04, 0.0, and 0.0 flies emerging, respectively, from olive fruit. One explanation for this discrepancy is that the olive fruit fly population may have declined

regionally during these years (see Fig. 1). The traps may be drawing-in flies from long distances rather than reflecting the local population in sampled trees. By examining just years 2003 through 2006, however, an even greater correlation exists (increasing from $r = 0.45$ to $r = 0.67$; Fig. 2B). Fitting a non-linear, 2 parameter equation to these data, estimated values increase exponentially to a maximum of about 1.18 flies per fruit ($r= 0.72$; $p<0.0001$). These results suggest that during most years there was a strong relationship between trap catch of adult flies and fruit infestation. They also show that even if several eggs were oviposited into a single olive, rarely does more than one adult fly emerge from a single olive.

Table 1. Correlation values between density of olive fruit fly in olives and adult trap catches in respective trees, measured monthly.

Year	*r*	*p*-value	Number of monthly samples combined from all sites
2002	--	--	2
2003	0.91	0.0034	7
2004	0.74	0.0014	15
2005	0.83	0.0002	14
2006	0.657	0.0035	17
2007	-0.0157	0.94	19
2008	0.19	0.46	16

Fig. 2. Correlation between infested fruit and trap catch of adult flies, northern California, means by month. (A) all data between 2003 and 2008 and (B) only data between 2003 and 2006.



The most common parasitoid emerging from infested fruit was a species of *Pteromalus*. Two other taxa were also found but in much fewer numbers, individuals from Eurytomidae and Eupelmidae.

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Impact of Arena Shape on Parasitoid Oviposition

Charlie H. Pickett, Lue Yang, and Arnaud Blanchet[1]

An artificial medium for rearing olive fruit fly was developed several decades ago. It was developed to aid in artificial mass rearing of flies and other research needs particularly during late spring to early summer when olives are unavailable. More recently, the same medium was modified by Robertson (unpublished data), and adopted by us and cooperators at the USDA ARS European Biological Control Laboratory to maintain new parasitoid cultures specific for olive fruit fly. However, good production of parasitoids developing on olive fruit fly larvae growing in this medium has been difficult. Production has never equaled that obtained when using olives, and often results in a heavily skewed sex ratio favoring males. One attempt to increase parasitism of olive fruit fly larvae developing in this medium has been to create an oval shaped arena to mimic the shape of an olive. We report on a study to compare a ball-shaped stinging arena to one with a flat surface.

Design of Stinging Arenas

The flat Petri plate based stinging dish was made by hot gluing two-90 x 10 mm petri dish lids back to back, creating a double-sided petri dish. The edge of one side of the petri dish lid was sanded down to about 2-3 mm in height. This shortened height ensured that when the final chamber was complete, larvae would be closer to the glued lid and more accessible to parasitoids. Olive fruit fly larvae and larval diet are added to the shortened side of the petri dish lid, and then covered with stretched parafilm. The reverse side is not sanded so that a polypropylene lid can be snapped securely onto it (Figure 1a). A 10 cm x 10 cm x 10 cm plexiglass cage was constructed to house the Petri dish in a way that contains the parasitoids yet maximizes exposure to parasitoids (Figure 1b). A hole cut out on the bottom side of the cage which is slightly larger than the circumference of the stinging dish but smaller than the polypropylene lid. When the stinging dish is placed through that hole, the polypropylene lid prevents the entire sting dish from going through the cage and seals any open spaces where parasitoids might be able to escape. This setup provides for an easy exchange of stinging dishes.

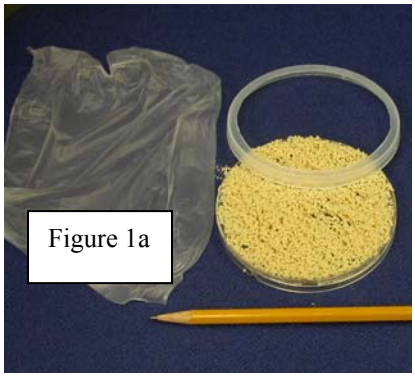

Figure 1a

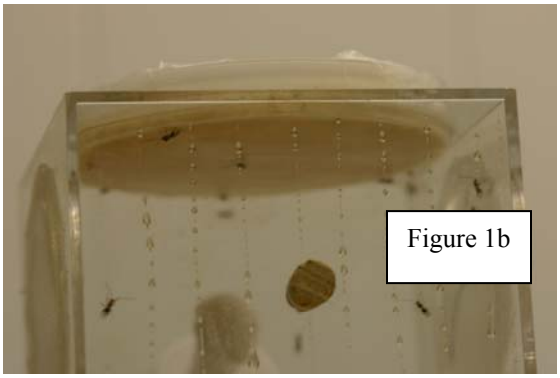

Figure 1b

Stinging Ball Arena

The core of the stinging ball is made of a small (ca. 3.81 cm diameter) foam ball. Artificial larval diet and larvae are thinly spread out on a piece of stretched parafilm. The foam ball is placed on top of the diet, and the parafilm with diet and larvae are then wrapped around the ball. The stinging ball is then suspended in a 10 cm x 10 cm x 5 cm cage (Figure 2) where it will be exposed to parasitoids.

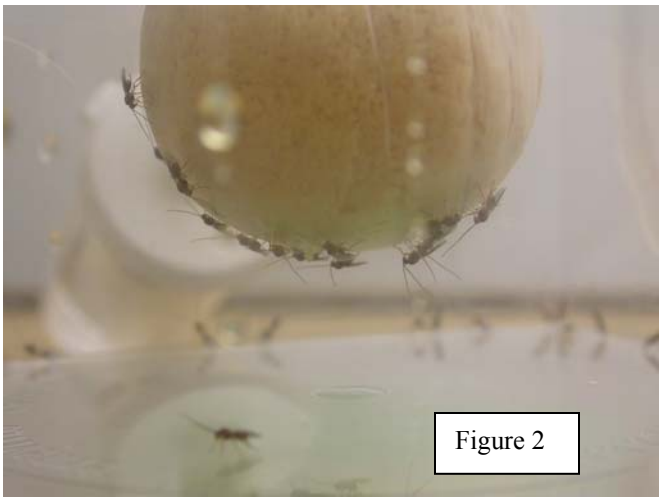

Figure 2

Experimental Design

Paired-replicates consisted of one ball arena and one Petri dish shaped arena, each placed in separate cages, side by side. After adding diet and hosts to each, 50 adult female *P. lounsburyi* were added to each cage. Parasitoids came directly from the USDA, ARS EBCL where they had been previously reared on Mediterranean fruit fly, *Ceratitis capitata*. The 'Kenyan' strain had been reared at EBCL for up to 30 generations prior to shipment to us, with parasitoids originating from collections made from the Burguret Forest in 2002, 2003, and 2005. The original host was *Bactrocera oleae*. The South African strain was collected near Stellenbosch in the West Cape region and shipped to EBCL during the same period. We received from EBCL the 15th or 16th generation. Room temperature was set at a constant 23 °C, and a 12:12 hr light, dark regime using florescent lights. The effect of arena shape on parasitism was measured three ways. For one day, every two hours, from 8:00 AM to noon, (A) the number of adults on the surface of the arena, and (B) the number of times they oviposited, was recorded for two minutes. Lastly, (C) the number of adult parasitoids and flies emerging from these arenas five weeks later was recorded. This process was repeated eight times for each of the two strains, winter/spring 2007.

Results and Discussion

Overall, the ball-shaped arena had a slightly greater impact on parasitoid activity and production than the flat Petri dish. This impact was more pronounced with the South African strain of *P. lounsburyi* than the Kenyan strain. More parasitism was recorded (at α=10%) for the ball using the South African strain, but no difference in parasitism was measured between the two shapes for the Kenyan strain ($p = 0.2488$; Table 1).

The ball appeared to be more attractive to the South African strain than the Kenyan strain, as shown by a higher number of South African adult parasitoids recorded searching on this surface (Table 2); equal numbers of adults were recorded on the ball and Petri dish arenas for the Kenyan strain. The Kenyan strain made significantly more attempts to oviposite on the Petri dish than the ball (Table 2); no difference was recorded for the South African strain. The Kenyan strain may have had more difficulty finding its host larvae on the Petri dish, forcing it to make more attempts than when on the ball. This difference may have resulted from Parafilm fitting tighter over the surface of larvae and diet on the ball than the Petri dish. It was easier stretching the layer of Parafilm on the ball-shaped arena than the Petri dish. A tighter fit would

create a denser layer of larvae close to the surface, than when slighter looser as with the Petri dish system. Although the South African strain did not attempt oviposition significantly more on the Petri dish, the trend was in this direction.

Table 1. Paired comparison (n=8), of the percent parasitism achieved with the two arenas. Two strains of *P. lounsburyi* on an arena in the shape of a ball versus a flat surface (Petri dish). Positive t-values indicate a stronger response to the ball than the flat surface.

Strain	t-value	Pr > |t|	Mean, ball	Mean, dish
South African	1.93	0.0944	37.14	27.29
Kenyan	1.21	0.2488	27.20	20.64

Table 2. Paired comparison (n=8), observational data. Two strains of *P. lounsburyi* on an arena in the shape of a ball versus a flat surface (Petri dish). Positive t-values indicate a stronger response on the ball than the flat surface.

	South African strain		Kenyan strain	
	t-value	Pr > |t|	t-value	Pr > |t|
# of adults on arena	5.15	0.0013	0.56	0.5950
# oviposition attempts	-0.84	0.4279	-4.76	0.0021

[1]USDA, ARS European Biological Control Laboratory, Montferrier, France

Biological Control of Diaprepes Root Weevil

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

Diaprepes root weevil, *Diaprepes abbreviatus*, 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. 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 began an eradication program in the known infested areas of these three counties. Insecticides are a core component of the eradication program; however, the more effective insecticides have limited registration labels and cannot be used in some of the treatment areas. In July 2008, the eradication program ended with a loss of funding. Research on biological control was initiated in 2007 to determine if egg predators or parasitoids could be used as a part of the management program. Foreign exploration began in 2008 to investigate whether strains or species of parasitoids could be found that may be better adapted to climatic conditions in southern California.

The wasp, *Aprostocetus vaquitarum,* was the first natural enemy released in San Diego County because 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. In 2006 and 2007, shipments of *A. vaquitarum* were made to the University of California – Riverside Quarantine Facility from Florida. Field releases were initiated in October 2007 and continued through 2008. In 2008, 4,859 *A. vaquitarum* adults were released at 11 sites in four cities from February – November. The release sites were known infested sites that were not treated with insecticides. Monitoring of the success of the releases will begin when a steady supply of Diaprepes eggs can be generated within the quarantine area.

Plans are being made to import and release two other species of parasitoids of Diaprepes, *Fidiobia domincia* and *Haeckliana sperata* in 2009. These two parasitoids oviposit into the Diaprepes egg, and the parasitoid develops within the host egg. Colonies of both parasitoids are being maintained and increased at the University of Florida, Tropical Research and Education Center.

Foreign exploration for natural enemies that may be better adapted to the drier conditions found in southern California began in the drier parts of Jamaica and Puerto Rico in 2008. In the surveys conducted in Jamaica, three species of parasitoids, *Quadrastichus haitiensis*, *Baryscapus fennahi*, and *Fidiobia citri*, were collected and sent to the containment facility at the University of Florida, Tropical Research and Education Center. The colony of *B. fennahi* was cultured in the containment facility for only two generations after which time it died out. The colony of *F. citri* is currently being reared (through four generations) in quarantine. An application to USDA-APHIS for permission to field-release this parasitoid is pending. This parasitoid has been released against other weevils in different parts of the United States, although the success of these releases (i.e., establishment) is unknown. These previous releases may help to expedite the

permit process. Two parasitoids were found from collections made in Puerto Rico, *Q. haitiensis*, and *A. vaquitarum*. These parasitoids were reared in quarantine until it was determined that these parasitoids were the same strains as what is currently available in Florida.

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Establishment of *Lygus* Nymphal Parasitoids in the Monterey Bay Region

Charlie H. Pickett, Ciprian Simon, Sean Swezey[1], Janet Bryer[1], Diego Nieto[1], Martin Erlandson[2]

The most serious insect pest of strawberries grown in the Monterey Bay region of California has been *Lygus hesperus* Knight (Hemiptera: Miridae). Until recently *Lygus* has solely been managed through applications of broad spectrum insecticides. Cultural and biological alternatives have not been considered useful. Importation of the nymphal parasitoid *Peristenus digoneutis* Loan (Hymenoptera: Braconidae) into the eastern United States during the 1980's, and more recently, the establishment of *P. digoneutis* and *P. relictus* in northern California has created interest in colonizing these parasitoids in the Monterey Bay region. Two braconid parasitoids (Hymenoptera) of European origin, *Peristenis relictus* and *Peristenus digoneutis*, were released into non-crop vegetation at four locations in the Monterey Bay region of coastal Central California for their permanent establishment and control of *Lygus* spp. At two sites, parasitoids were released into wild vegetation known to harbor *Lygus* spp. near conventionally-managed strawberry fields. Parasitoids were also released into *Lygus* spp. specific alfalfa trap crops intercropped in two different organic strawberry fields (Figure 1). *Peristenus relictus* has persisted for over five years since last released into the original release site of wild vegetation and for three years at the first organic strawberry field release site. At the latter site, populations of *P. relictus* were significantly correlated with *Lygus* spp. collected from alfalfa trap crops from 2005 to 2007 ($r^2 = 0.60$; $p < 0.005$), suggesting a strong density dependent relationship between the two. At this organic strawberry farm, mean densities of *Lygus* in strawberries have fallen significantly ($p<0.05$) from a pre-release seasonal high of 2.7 nymphs per 50 suctions (bug-vac machine) in 2003 to 0.8 nymphs in 2007. This decline may be due to a regional decline in the Lygus population. However, the interplanting of vacuumed strips of alfalfa may have also contributed to some of this decline.


Figure 1

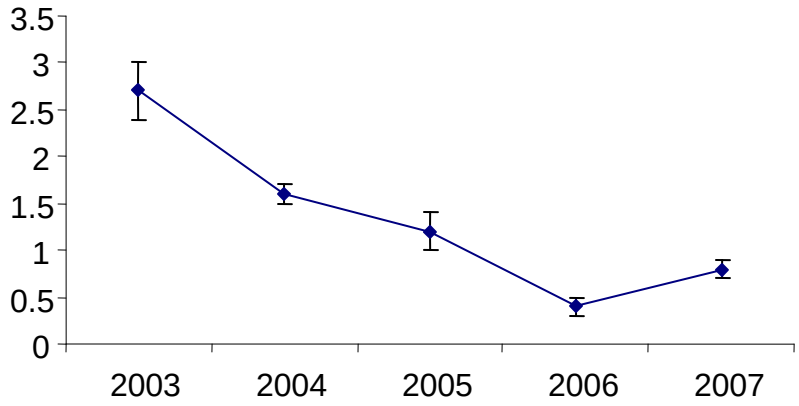

Figure 2. Lygus densities in strawberries at Eagle Tree farm, Monterey County.

Bordering wild vegetation composed of winter/spring annuals at three different sites supported both native *Lygus* spp. and *Closterotomus norvegicus*, an exotic mirid in California that is attacked by *P. relictus* in Europe. The relatively benign *C. norvegicus* dominated sampled vegetation in spring to early summer, when *Lygus* were nearly absent, allowing for the persistence and early build-up of *P. relictus* at the edge of the strawberry agroecosystem, with no detectable damage to strawberries. An overwintering population of *P. digoneutis* has not been found in the four release sites.

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[2]Agri-Food and Agriculture Canada, Saskatoon, Saskatchewan, Canada

Monitoring non-cultivated flowering vegetation to enhance the biological control of *Lygus hesperus* by the parasitoid *Peristenus relictus*

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

On the California Central Coast, *Lygus hesperus* Knight or the western tarnished plant bug (WTPB) is a key economic pest of strawberries produced for the fresh market. The WTPB parasitoid *Peristenus relictus* (Ruthe) (Hymenoptera: Braconidae) has been successfully established in organic strawberry systems interplanted with alfalfa trap crops. While effective biological control of WTPB has been recorded in alfalfa trap crops and adjacent strawberry rows, methods to further improve parasitism rates are still being developed. Among them is identifying flowering plants (mostly native perennials) present in the landscape that could facilitate further reductions of WTPB populations.

Flowers were sampled from 2006 to 2008 on an organic strawberry farm in Prunedale, CA. Data were collected from the following plants: sweet alyssum (*Lobularia maritime*), yarrow (*Achillea millefolium*) and buckwheat (*Eriogonum* spp.). Biweekly samples were taken during the flowering periods for each of these plants. Samples consisted of 100 one-second suctions from a hand-held vacuum (modified, reversed Stihl BG75 leaf blower). Once collected, WTPB nymphs were bottled in alcohol and shipped to the CDFA-BCP laboratory in Sacramento, CA for dissection.

From 2006 to 2008, both WTPB nymph abundance and percent parasitism of WTPB nymphs increased on flowering buckwheat (Figures. 1 and 2). This trend seems to correspond with the continuing growth of buckwheat, which produced more flowers in 2008 than in previous years. In fact, in 2008, percent parasitism of WTPB on buckwheat was greater than or equal to 25% on four different sample dates in July and August. Given these projections, future work should prioritize the role *Eriogonum* spp. play in reducing WTPB populations and whether *P. relictus* can utilize its flowers as a feeding source.

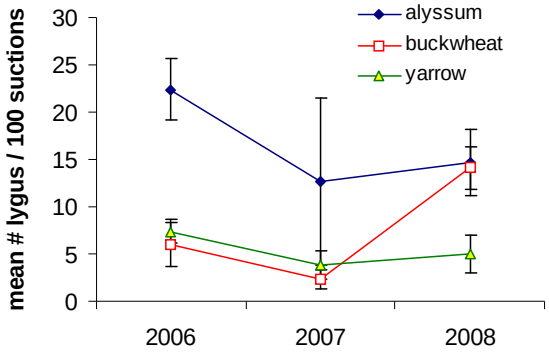

Figure 1. Mean WTPB abundance on flowering plants. Data collected at Eagle Tree Farm, Prunedale, CA from 2006-2008.

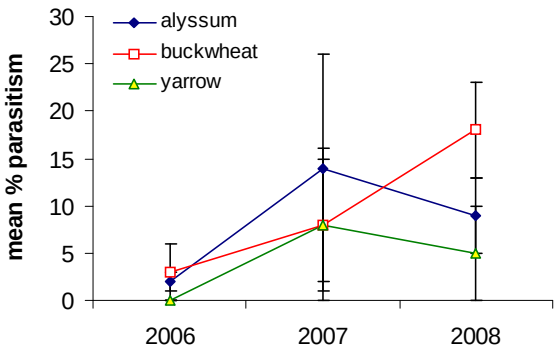

Figure 2. Mean percent parasitism of WTPB nymphs on flowering plants. Data collected at Eagle Tree Farm, Prunedale, CA from 2006-2008.

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Biological Control of the Solanum Mealybug, a Cooperative Project with Israel

Kris Godfrey, Gillian Watson[1], Raymond Gill[2], and Zvi Mendel[3]

The solanum mealybug, *Phenacoccus solani*, is an apparent native of North America and can be found throughout California. It is currently not a pest species in California, but causes extensive damage to peppers in Israel (Figure 1). During a 2004 field survey of a solanum mealybug population in southeastern Sacramento County, we found the parasitoid *Aenasius phenacocci* parasitizing the mealybugs (Figure 2). This was the first record of this parasitoid attacking solanum mealybug.

Figure 1. Peppers infested with solanum mealybug in production areas of Israel. (Photograph by Z. Mendel)

Figure 2. *Aenasius phenacocci* female parasitizing a solanum mealybug on peppers. (Photograph by Z. Mendel)

During July through September 2008, collections of solanum mealybug were made at four locations in California in an attempt to start a colony of *A. phenacocci*. Parasitoids were collected from an urban roadside in southeastern Sacramento County, a vineyard in Sonoma County, and a vineyard in Yolo County. The mealybug was also found in a citrus orchard in Tulare County, but no parasitoids were recovered in the Tulare County sample. A small colony was established at the Biological Control Program, Sacramento. Importation permits were obtained to send the parasitoid to Israel, and in September and November, two shipments were made. From these shipments, a colony has been established at the Volcani Center in Bet-Dagan, and releases will begin in 2009.

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Expansion of *Eretmocerus mundus* in Central California

Charlie H. Pickett, Dan Keaveny[1], Lia Chase, and Chia Moua

Several 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 parasites 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. Yearly monitoring has shown that 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, 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. Leaves with high rankings of whitefly infestations (over six nymphs per leaf) were placed into 0.5 liter 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. Species identification was based on adult females, using the shape of the anntenal funicle segment and setal arrangement on the mesoscutum. Since 2002, *E. mundus* has been the only released parasitoid recovered from wild vegetation and cotton. The percentage of total parasitism due to *E. mundus* in samples has slowly, but steadily increased to a high of 90% this last year, 11 years after the first releases were made (Fig. 1). Extant *Encarsia* have never been abundant, and the percentage of native *Eretmocerus* has decreased dramatically. In Kern County, there a consistent and steady increase in the numbers of *E. mundus* (Table 1). In contrast, it was not until 2006 before there a similar recovery from the remaining counties (Fresno, Tulare, and Kings). Initial releases were made into citrus groves bordering cotton fields at all sites. However, initial densities of *B. tabaci* in trees at the Kern site were two orders of magnitude higher than the others.

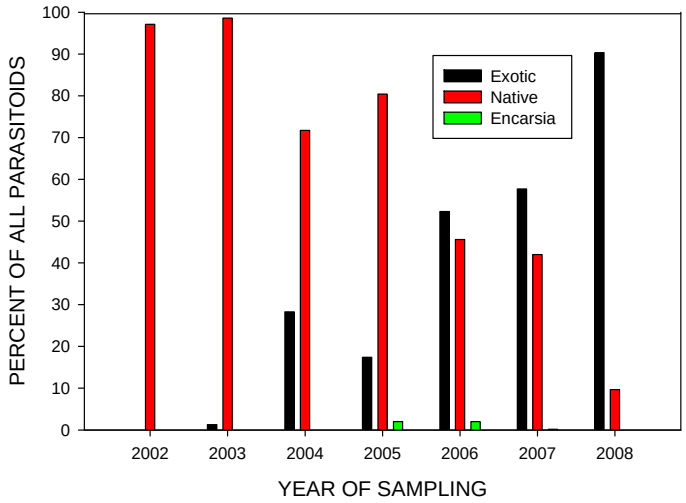

Fig. 1. Species compostion of parasitoids emerging from Bemisia infested cotton leaves, Central California.

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

Year of Collection	Percent of *Eretmocerus* that are *E. mundus*, by County (number of sites sampled)			
	Kern	Tulare	Kings	Fresno
2002	0.22 (11)	0.01 (9)	0.00 (1)	0.00 (7)
2003	0.05 (4)	0.00 (3)	0.00 (1)	0.00 (1)
2004	0.84 (6)	0.02 (14)	0.00 (1)	0.07 (10)
2005	0.80 (8)	0.00 (14)	--	--
2006	0.60 (56)	0.52 (49)	0.64 (11)	0.13 (16)
2007	0.49 (20)	0.54 (22)	0.73 (25)	0.16 (6)
2008	1.0 (3)	0.88 (5)	0.76 (6)	--

At this time, it appears that *E. mundus* is displacing the native *Eretmocerus*. While the actual numbers of *E. mundus* recorded are increasing, the native *Eretmocerus* are decreasing (Fig. 2). In fact, the highest level of parasitism, (Table 2) and number of parasites recorded, occurred during the first year of this post-release project. Over three adults per dry gram weight leaf were measured during 2002, of which almost all would have been native *Eretmocerus*. However, at this time *B. tabaci* was still at very high numbers throughout the central valley of California. *Eretmocerus mundus* appears to be continuing to increase its presence and impact, while *B. tabaci* is at much lower densities than during the 1990s (based on Pink Bollworm Program reports). Parasitism has continued to increase since 2005, when the exotic *E. mundus* started dominating the complex of *Eretmocerus* species, doubling from 4.2 to 8.5 %. Although a low figure for parasitism, most likely it will continue to increase, assuming the same type of pesticides are used in cotton. Furthermore, the regional spread of a parasitoid with a much higher specificity for *B. tabaci*, should have a significant impact on this pest in wild vegetation.

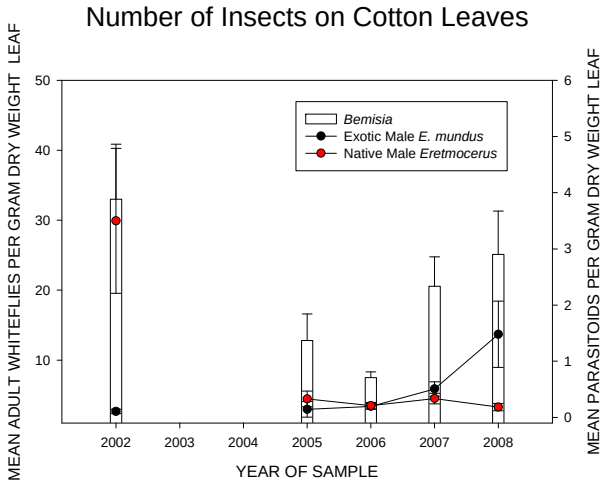

Fig. 2. Number of insects emerging from *B. tabaci* infested cotton leaves, central California.

Table 2. Parasitism of *B. tabaci* collected from heavily infested cotton leaves in the Central San Joaquin Valley, California.

Year of Collection	Percent Parasitism	SE	Number of Sites Sampled
2002	18.3	7.1	29
2003	--	--	0
2004	--	--	0
2005	4.2	3.9	26
2006	4.7	1.8	133
2007	6.9	2.9	74
2008	8.5	6.9	16

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

An Update on Avocado Lace Bug Seasonal Population Patterns in San Diego County

William Roltsch, Eduardo Humeres[1], David Kellum[2], Joseph Morse[1], Mark Hoddle[1]

The avocado lace bug, *Pseudacysta perseae* Heidemann) (Hemiptera: Tingidae), was found in San Diego County in late summer of 2004 (Figure 1). Monitoring of this new pest was initiated at that time including area wide surveys and subsequent monthly site monitoring beginning in July of 2005. These efforts have been described more fully in the 2006 and 2007 CDFA biocontrol annual reports. This report describes results from field monitoring of adult densities and damage through October 2008, at which time the project was terminated.

Beginning July 2005, five residential sites were sampled monthly. Sampling consisted of visual examination of 25 leaves in the lower canopy of selected avocado trees at each site, aided by the use of a headset magnifier (Figure 2). Mean population levels and leaf damage in the summer and early fall of 2008 were at their lowest of the three years (Figure 3). However, the late October sample was similar to that in 2007 in both numbers of adult lace bugs and damage. It is of interest to note that peak densities were observed during the month of October in 2005 and 2006, whereas the small peak in 2007 occurred in December, suggesting that heavy leaf damage in 2005 and 2006 may have influenced the timing of the realized annual peak population activity.

Figure 1.
Adult avocado lace bug.

In addition to the five sites sampled, new infestations at two sites in the coastal community of La Jolla were identified in the fall of 2006. Data from these sites, first sampled in February of 2007, were not combined with data from the other sample sites. These two sites were sampled to see if their densities would follow a pattern similar to population trends at other locations where population densities escalated and declined. In this case, we expected populations that were high in 2007 to subside in 2008. As of October of 2008, this was not found to have occurred. Adult densities and damage in 2008 were comparable to those in 2007.

Figure 2. Avocado lace bug leaf damage and field sampling.

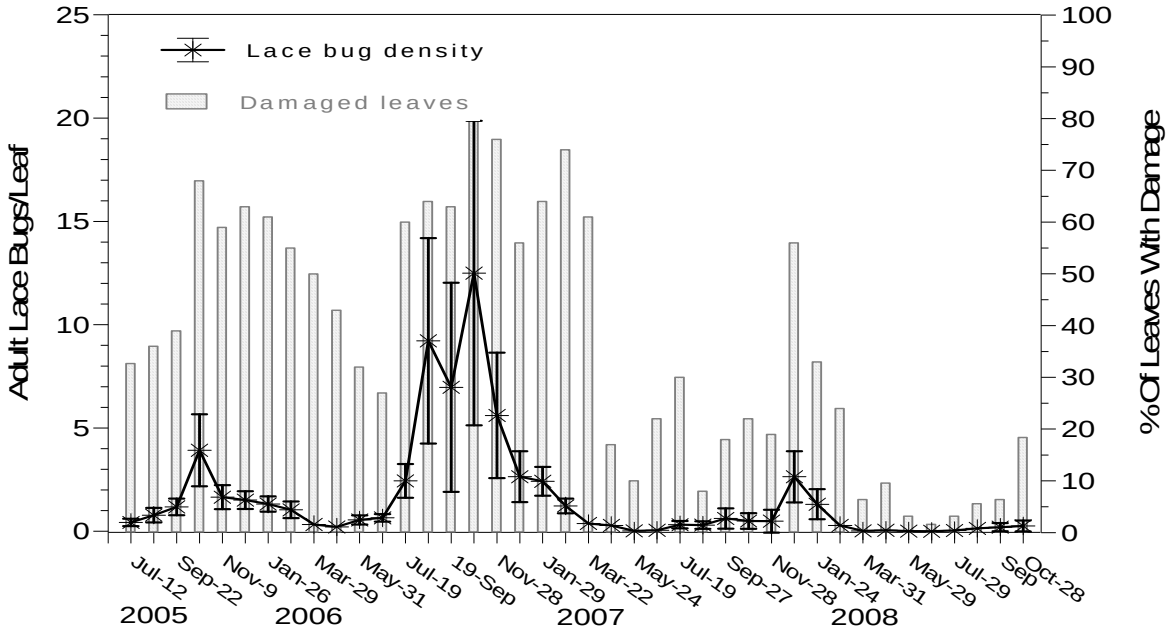

Figure 3. Seasonal changes in avocado lace bug populations and damage.

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[2]San Diego County Agricultural Commissioners Office, San Diego, California

Diffuse Knapweed Biological Control in California: 1976-2008

Dale Woods and Viola Popescu

Diffuse knapweed, *Centaurea diffusa*, has historically been one of the invasive exotic weeds of highest concern in the western North America. Consequently, it was actively targeted for biological control and over a dozen biological control agents have been released against this weed. Because California has a long standing eradication and exclusion effort, diffuse knapweed has not invaded successfully in many areas. It has established large populations only in remote locations, specifically the South Fork Mountains in Trinity County where eradication has been difficult. The biological control effort on diffuse knapweed within California has been limited to this area and has been comprised of two surges of activity. From 1976 through 1981, two insects, the seedhead fly, *Urophora affinis*, and the root beetle, *Sphenoptera jugoslavica*, were released in small numbers but no quantitative monitoring was initiated of either the released insects or the targeted weed. The second surge of biological control efforts began in 1993, involving introduction of several new biological control agents and included a plan to monitor their impact.

In preparation for new insects which were field established in other states, we began ground surveys of diffuse knapweed in Trinity County. The surveys were intended to establish a starting point from which to evaluate impacts of the new biological control agents. During these surveys, we detected the presence of an unintentional biological control, the rust fungus, *Puccinia jaceae* var *diffusa*. The rust was present at very low levels. The rust has continued to be a part of the biological control impact in the area, erratically infecting between one and 62% of the knapweed plants. Although most infected plants remain vigorous, some plants become severely infected and debilitated (Figure 1). We began active release programs of additional biological controls in 1994 with releases of the knapweed bud weevil, *Bangasternus fausti*, and followed with the lesser knapweed weevil, *Larinus minutus,* in 1995. The knapweed UV seed fly, *Urophora quadrifasciata*, was not released as it had migrated to the state unaided and was reared from field-collected diffuse knapweed in 1995. Since that time, *U. quadrifasciata* has not been detected from the nearly 28,000 diffuse knapweed seedheads we have dissected in the laboratory. Consequently it is likely that the single rearing of *U. quadrifasciata* we reported in 1995 was a contaminant in the laboratory from other knapweed samples. Monitoring of insect distribution, research on impact of the biological control agents began immediately after the 1994 releases and continued for several years. It was discontinued from 2006-2007, then the sites were again evaluated in fall 2008.

Figure 1

The two seedhead weevils established readily at separate sites, built up substantial local populations, and have gradually spread to all sites in the area. One site was selected for intensive monitoring of plant density and bioagent activity. Several additional sites were selected for biological control agent activity monitoring only. *B. fausti* was the only weevil released at the intensively monitored site and within five years was attacking over 70% of the seedheads (Figure

2). The other released seedhead weevil, *L. minutus,* migrated to the area and steadily built in population density. Over the long term, it appears that *B. fausti* is being replaced by *L. minutus* as the predominate biological control at the intensively monitored site. This phenomenon was also observed at the other monitoring sites (data not presented). The gall fly, *U. affinis* which was released in low numbers beginning in 1976, seems to have attained only moderate population levels on diffuse knapweed in California even in the absence of competing seedhead feeders. By 1994, it was attacking only 18% of the seedheads when the weevils were released. It has been rapidly supplanted since that time and has not been detected since 2004. The population of diffuse knapweed plants has also been impacted by the combination of the two seedhead weevils. Diffuse knapweed seedhead density has declined steadily over several years (Figure 3) while the overall attack rate by the insects remained high.

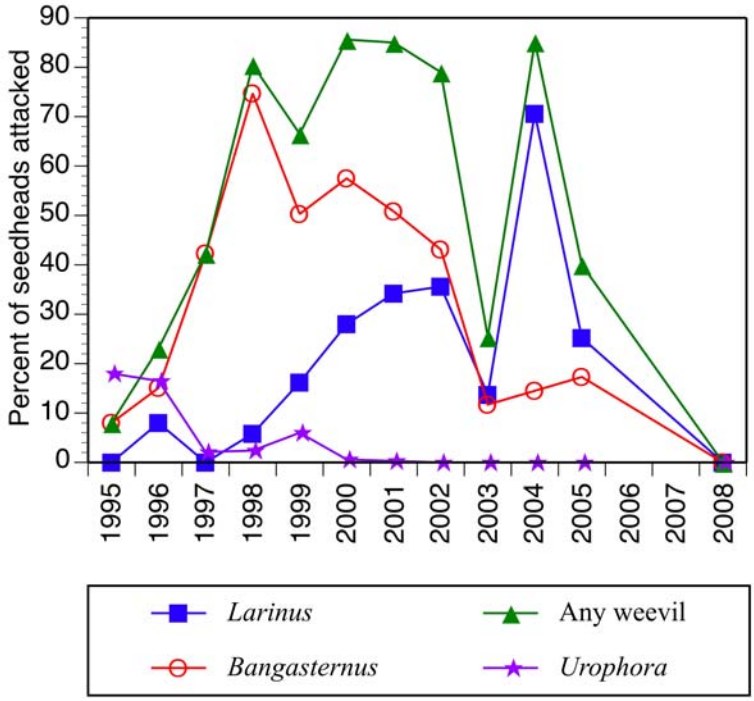

Figure 2. Attack rates of diffuse knapweed by the seedhead feeding insects.

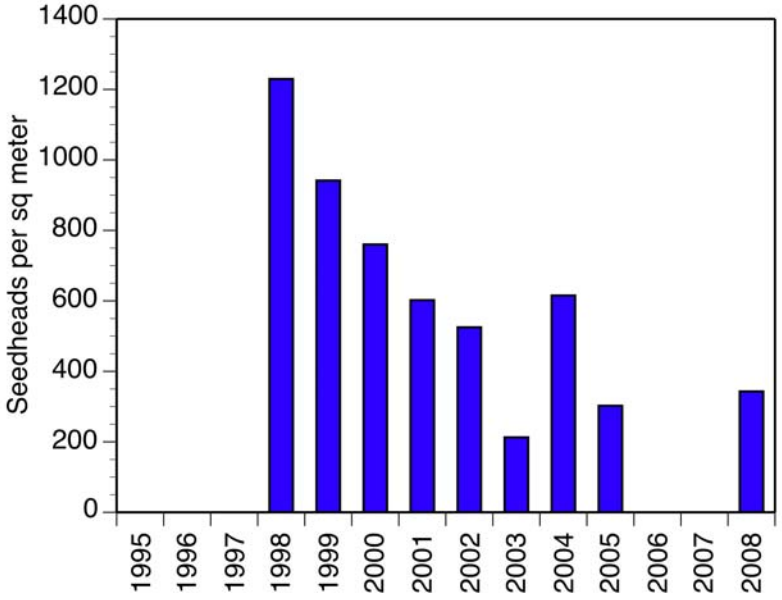

Figure 3. Density of diffuse knapweed seedheads in Trinity County. Not monitored in 2006-2007.

While the overall impression for control of diffuse knapweed has been very promising, additional factors have complicated the final analysis. Substantial cattle grazing damage occurred in 2003 and 2005. The grazing took place in mid summer and severely disturbed the weevil populations as many of the early season seedheads were destroyed with developing larvae inside. Grazing impacted both the populations of the weevils and also the density of diffuse knapweed seedheads. None the less, there was a substantial decline in knapweed seedhead density over the years. Unfortunately, wildfires in the early summer of 2008 destroyed over half of the biological control sites in the area. These sites were completely consumed along with neighboring foreasts. The remaining sites, including the intensively monitored site, were within a few hundred yards of active burning. Most of the diffuse knapweed biological control insects were in the adult stage at that time and seem to have been dramatically reduced by the fire. Less than 15% of the seedheads of surviving diffuse knapweed were attacked by biological control agents compared to over 70% in previous years.

Visual impacts of the change in diffuse knapweed density are also apparent when comparing vegetation cover (Figure 4). The dense green stand of bolting knapweed in summer 1994 is poorly recognizable as the dry fall stand in 2008 with very few plants and lots of bare soil apparent.

Figure 4. Before, and after, photos of diffuse knapweed in Trinity County representing 18 years of change.

Biological Control of Whitetops in California; a Future Project

Dale Woods, Michael Pitcairn, and Baldo Villegas

Whitetop is a common name applied to several invasive weed species. Two species, hoary cress and perennial pepperweed, are the target of biological projects which are still in the prerelease stages. Hoary cress, often referred to simply as whitetop, was, for many years, formally named *Cardaria draba*, but has been renamed, *Lepidium draba*. Perennial pepperweed, commonly known as tall whitetop, is named *Lepidium latifolium*. Hoary cress is the more problematic weed in many western states, but perennial pepperweed is the more problematic species in California.

In 2001, 'The Hoary Cress Consortium' was formed of several federal, state, regional and international groups to encourage development and implementation of biological control of this noxious weed and its close relatives. Participants in the consortium have been continuing that goal. Based on previous European field studies, a substantial effort is underway to find and develop biological controls for this group of related weeds. With funding from several western states (esp. Wyoming and Idaho) and federal agencies, several insects have been selected and are currently being evaluated.

CABI Europe-Switzerland is evaluating four potential agents: the gall forming weevil, *Ceutorhynchus cardariae*; the stem mining weevil, *Ceutorhynchus merkli*; the seed-feeder *Ceutorhynchus turbatus*; and the shoot-mining flea beetle *Psylliodes wrasei* as biological controls for hoary cress. The Biotechnology and Biological Control Agency (BBCA, Rome, Italy) has performed several explorations into the native range of perennial pepperweed for potential biological control agents. Promising agents are being tested by CABI for host specificity. Based on combined exploration results, CABI identified five prospects for perennial pepperweed and has initiated evaluations.

Releases of exotic insects as biological controls for *Lepidium* are still several years in the future because the nature of these evaluations will be complex. Several native species of *Lepidium* exist in the state and several commercial crops are genetically related. Host testing will have to address both of these issues.

As part of the larger goal of management of these invasive weeds, projects investigating native plant pests are also in process. One of these projects conducted by the USDA-ARS invasive weed lab in Reno, Nevada is to investigate the number, nature and significance of native and endemic insect species acting as pests of *Lepidium*. Their 2008 survey included several sites in California and Nevada and found a number of insect species, some of which were locally limited.

Plant pathogens are not currently being evaluated for importation as classical biological control agents but at least one, *Albugo candida*, has been identified in California on perennial pepperweed and is being evaluated. Preliminary evaluations are the subject of a separate report.

Preliminary Evaluations of the Impact of *Albugo candida* on Perennial Pepperweed

Dale Woods and Viola Popescu

Perennial pepperweed, *Lepidium latifolium*, also known as tall whitetop is an increasingly important invasive weed in California. Because it is difficult to control by cultural means or with herbicides, biological control is being considered as an alternative control option. A plant disease called white rust has been found in California attacking perennial pepperweed but the impact has not been evaluated.

White rust, sometimes called white blister, is a common disease in many areas of the world on a variety of species of plants, particularly crucifers. Currently the only identified species of the pathogen causing disease on brassicaceae is *Albugo candida*. This species has been described on a large variety of crops as well as a large number of weeds. In spite of this appearance of a wide host range, there is a substantial level of host specificity between isolates of *A. candida*. These have not been split into different species rather are currently divided into biotypes and races. The biotype developing on *Lepidium* may be host specific enough to consider it as a biological control agent. The historical name "white rust" is a poor choice of common name for this pathogen. White rust is not actually a rust disease like stem rust of wheat or even caused by a fungus. The causal agent is an obligate parasite belonging to the class Oomycota which is now classified in a new kingdom called the Chromista. Other important plant pathogens in this group include the downy mildews. White rusts tend to be severe if they occur on very young plants which sometimes die in severe attacks. Attacks on older plants usually manifest as a level or degree of debilitation. We have begun evaluations of this debilitating effect of *A. candida* on perennial pepperweed as a potential management tool to be combined with other management techniques, particularly mowing. Mowing of perennial pepperweed followed by herbicide application the following year has been shown to increase the level of control over either treatment alone. We have begun evaluating simulated mowing (clipping) followed by inoculation with white rust as a similar integrated control approach.

Two isolates of the pathogen were maintained on clonally reproducing populations of their original field-collected perennial pepperweed line. Plants of each field collected line (line W and line C), were grown in one gallon pots until they were six weeks old. Half of each population (W and C) were inoculated by their associated field collected *Albugo* isolate. The other half of each plant line was maintained as non-inoculated controls. Plants were rated for infection and the tops harvested for biomass at one month after inoculation. Ten days after the harvest, new growth included three to seven leaves per plant and plants were re-inoculated. The cycle of harvest and inoculation was repeated twice more at monthly intervals. Unfortunately, we did not count leaves prior to the initial inoculation therefore the first data is from one month post-inoculation.

For both plant accessions, repeated clipping of the tops alone (simulated mowing) did not negatively affect the non-inoculated (control) plants (Figure 1). Total biomass and leaf number of these non-inoculated plants recovered and eventually increased after each clipping. The addition of the pathogen to the clipping treatment negatively impacted the ability of the plant to recover from the clipping. Infected plants were able to produce new leaves following each clipping, producing roughly to the same number of leaves each month. Leaf number did not however, increase as it had in the non-infected plants. The new leaves on the infected plants were

also substantially smaller in size each harvest interval so the harvested biomass was greatly reduced in the infected and clipped treatments. This experiment will be repeated in the future and modified to refine the impact that the pathogen has on *Lepidium* when combined with clipping. Eventually, a field experiment will be established utilizing traditional mowing procedures.

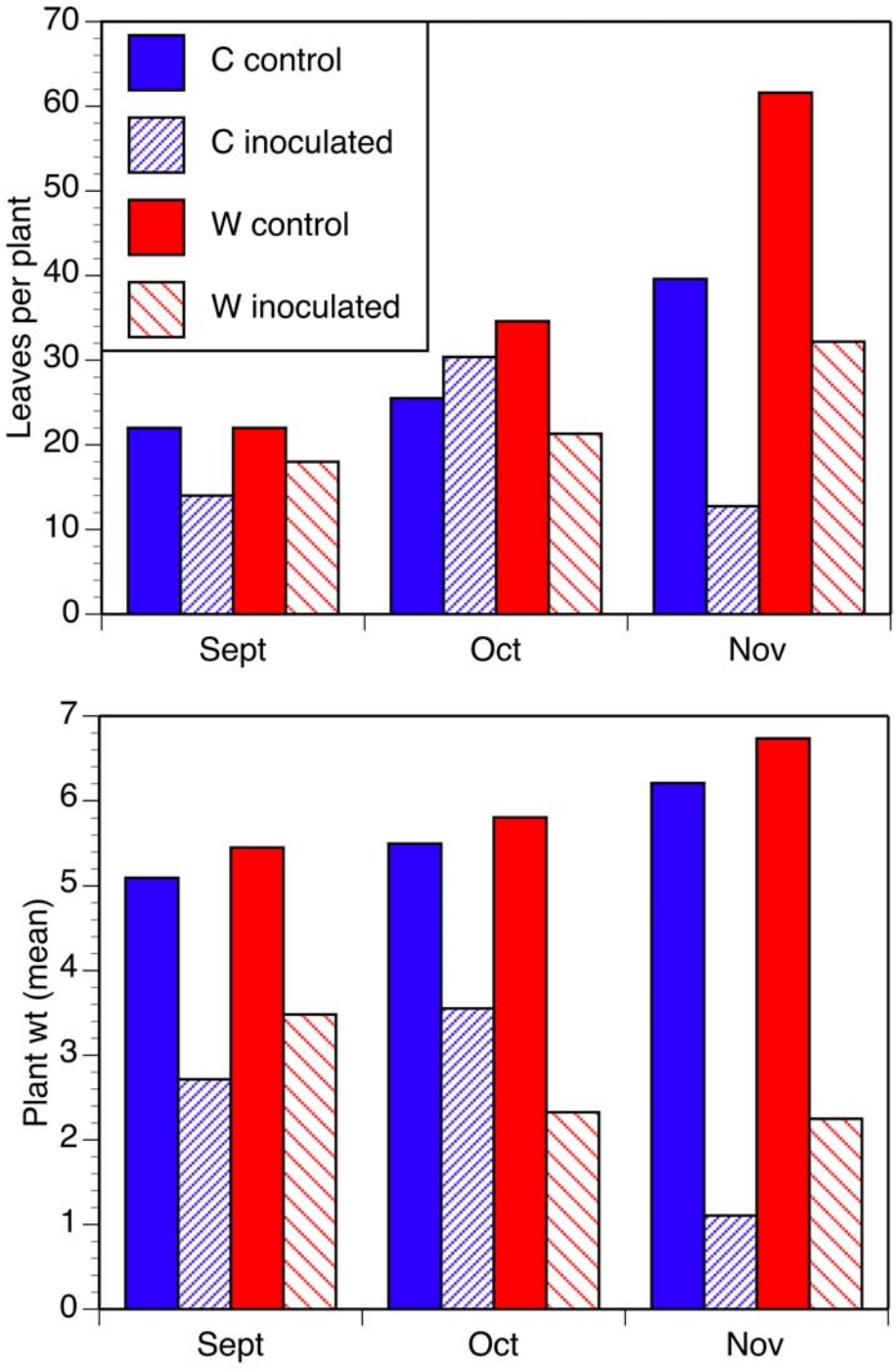

Figure 1. Impact of *Albugo candida* on number of leaves (top) and biomass (bottom) of perennial pepperweed

Biological Control of *Centaurea* Species by Imported Biological Control Agents

Dale Woods, Michael Pitcairn and Baldo Villegas

Classical biological control of weeds has an excellent record for safety, particularly in recent years. Most imported insects and pathogens have been well studied and have highly limited host ranges. Some imported agents do attack or feed on certain plant species other that the declared primary target weed species. Attack of plant species other than the original targeted species can be either a positive or negative event. In particular, the event can be positive when the additional species are equally important exotic, invasive plants and the attack was anticipated as part of the initial host specificity evaluation. This type of attack is often anticipated in the original host testing, but is not always a focus of the initial release strategy. Monitoring for unexpected attack beyond the targeted and expected species should be considered in all biological control programs as a quality control measure on the importation and release process. The California Department of Food and Agriculture has continued to monitor for these impacts for all agents we have released. Additionally, we have been evaluating approved biological control agents on exotic weeds other than their original primary target. In all cases to date, these were combinations that were reasonable extensions of the pre-release host testing knowledge base. This report summarizes our monitoring and partial evaluation of biological impacts of agents imported as biological controls of various species of *Centaurea*.

Biological control agents imported against yellow starthistle, *Centaurea solstitialis*

Urophora solstitialis – This gall forming agent has not been detected on any host other than yellow starthistle. This agent has been widespread in California for many years.

Bangasternus orientalis – A likely variant of this insect attacked other *Centaurea* species, including purple starthistle, in a pre-release field test in Europe. We have employed field cages to attempt establishment on Iberian starthistle, tocalote, and Sicilian starthistle without any success. Despite multiyear, statewide establishment of this agent, including on yellow starthistle populations adjacent to purple starthistle, we have never detected evidence of *Bangasternus orientalis* on any other host then yellow starthistle.

Eustenopus villosus – Pre-release host testing had indicated that the adult weevil feeds on a large number of *Centaurea* species as well as plants in closely related plant genera. In contrast, oviposition and larval development was very limited. We have detected field development of the weevil to adult stage on three other species of *Centaurea*; tocalote, Sicilian starthistle, and spotted knapweed.

Chaetorellia australis – Bachelor button was a known and expected host of this agent in pre-release studies. The peacock fly has established on bachelor button in several areas of the state and seems to require this plant species in order to survive for more than one year because it flowers two months earlier than yellow starthistle.

Larinus curtus – Attack has been limited to yellow starthistle only.

Puccinia jaceae – Pre-release host testing indicated bachelors button as a host. Greenhouse inoculations have been very successful but the rust has not been found in the field on bachelor button. An endemic rust species is widespread on bachelor button and will likely mask any potential infection by the introduced yellow starthistle rust. The endemic rust has a far greater attack rate and incidence.

Biological control agents accidentially released against yellow starthistle, *Centaurea solstitialis*

Chaetorellia succinea – This accidentally introduced species did not have a full quarantine evaluation, so the complete host range was unknown. We have found substantial attack on, and adults emerging from, three species of *Centaurea*; tocalote, Sicilian starthistle, and bachelors button. A highly limited attack was noted on a specific cultivar of safflower but no damage has been noted for several years.

Biological control agents imported against diffuse and spotted knapweed - most of the knapweed biological control agents were imported listing diffuse and spotted knapweed as the primary target species. Varying degrees of pre-release host testing occurred for each species on other *Centaurea* species.

Urophora affinis – This gall fly has been found at low levels attacking squarrose knapweed in California.

Urophora quadrifasciata – This gall fly has been moderately effective against squarrose knapweed, slightly effective against meadow knapweed, but has never been found in California attacking yellow starthistle. It has not survived repeated attempts to establish on purple starthistle.

Sphenoptera jugoslavica – This beetle has been very effective against squarrose knapweed.

Bangasternus fausti - This weevil has been very effective against squarrose knapweed. It has not survived repeated attempts against purple starthistle.

Larinus minutus - This weevil has been very effective against squarrose knapweed and moderately effective against meadow knapweed. It has not survived repeated attempts against purple starthistle.

Terrelia virens - Repeated attempts to establish this fly on squarrose knapweed and purple starthistle have not been successful.

The currently released suite of biological control agents on *Centaurea* have, to date, proven to perform within the expected range of host testing evaluations. Their intentional use on plant species closely related to the primary target (e.g. squarrose knapweed and tocalote) have been valuable outcomes that were within expectation of host range.

Impact and Long-term Population Maintenance of the Yellow Starthistle Gall Fly

Dale Woods, Michael Pitcairn, Donald Joley[1] and Charles Turner[2]

The gall fly, *Urophora sirunaseva*, was the second of the six insect species released as classical biological control agents to control yellow starthistle. The first introduction of *U. sirunaseva* into California was in 1984 with additional releases in succeeding years. Gall flies in general have a high degree of host specificity and continue to prove to be extremely safe. The yellow starthistle gall fly established well by 1989 therefore the CDFA in cooperation with the CACSA distributed the gall fly throughout the range of yellow starthistle in California. Beginning in 1993, we performed a series of field studies to evaluate the impact that the gall fly has on its host. These studies have three major thrusts: to determine initial establishment and attack rates per seedhead; to determine the impact that galls can have on the plant either as single galls or as clusters; and to determine if the gall fly can maintain high enough populations over many years to have an impact on plant populations. The details of some of these studies were recently published in the journal, Biological Control volume 47, pages 172-179 and are summarized below along with results from additional long-term population studies.

Establishment –

The gall flies established rapidly at all sites in California when they were first released. These releases occurred when there was little competition from other biological control agents for development sites within the yellow starthistle seedheads. In our best example, gall flies were released in 1990 at a site near Ukiah and by 1994, 55% of the seedheads had galls and the site supported 2.4 galls per seedhead. Other sites developed less rapidly and were less completely infested. For unknown reasons, some entire plants at each site always escape attack from the gall flies. Within the seedheads, galls usually develop as single galls or as small clusters of two to five galls, but some seedheads had as many as 14 galls (Figure 1).

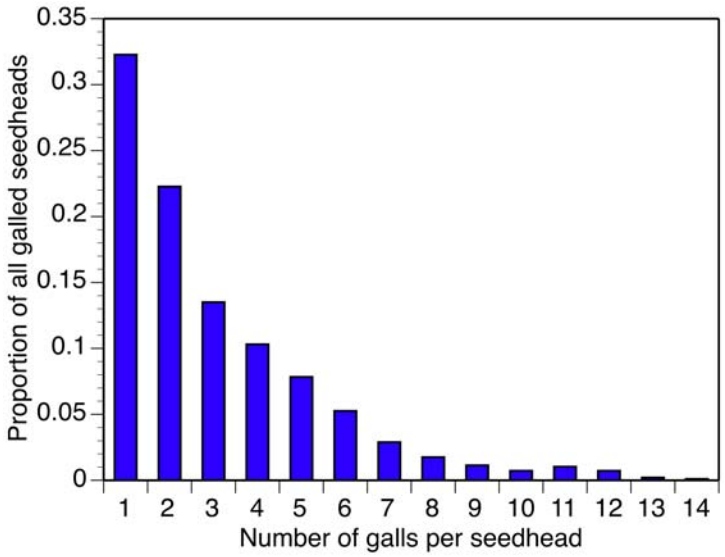

Figure 1: Frequency distribution of galls in galled seedheads at Ukiah 1993.

Impact –

The impact of the gall flies was studied for two years each at the Ukiah site and a site near Winters, CA. In order to quantify the impact of individual galls on seed production, we had to establish baseline data of seed production from non-attacked seedheads. Yellow starthistle plants vary widely in the number of seedheads they produce but also vary in size of seedhead. Most are between five and nine mm in diameter (Figure 2). Seedhead size is also highly correlated to the number of seeds produced in the seedhead (Figure 2). We were concerned that the gall flies might prefer larger seedheads than small so we compared galled seedhead at each size category to ungalled seedheads of the same category. We further compared the impact of increasing number of galls at each category. One example is shown in Figure 3 showing that increasing gall numbers reduces seed production at each size of seedhead. Based on multiple linear regression of the 5,812 seedheads evaluated at the two sites, a single gall was estimated to reduce seed number by between 2.1 - 2.9 seeds per gall. Individual galls were therefore estimated to cause a 5-11% decrease in seed production compared to ungalled seedheads depending on the year. The smallest seedheads were the most dramatically effected because they had a lower potential seed production and a relatively small number of galls could have a large impact.

Based on the fitted equations, 100% seed destruction could not be expected to occur at the Ukiah site, even at the highest densities of galls per seedhead. At sites with lower average seedhead size, complete seed destruction could theoretically occur if gall fly populations increased to extremely high levels.

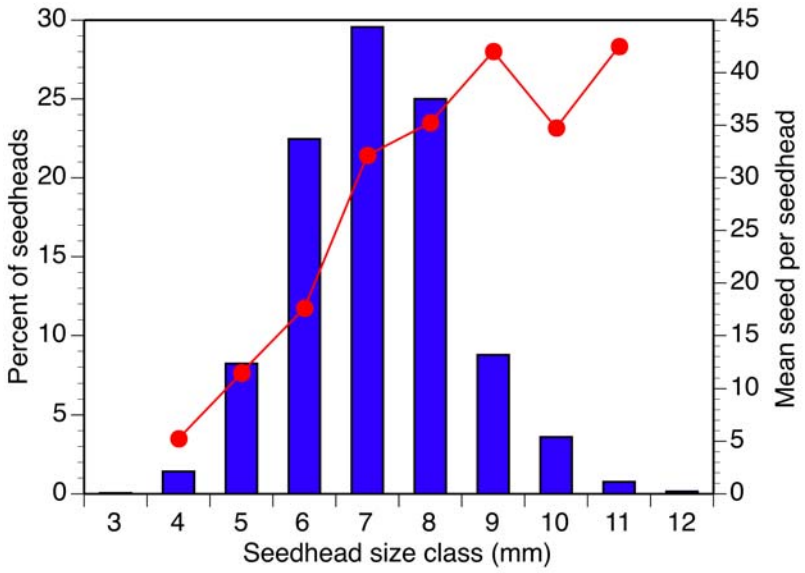

Figure 2. Size distribution of yellow starthistle seedheads (columns) and mean seed production at varopus seedhead sizes (line).

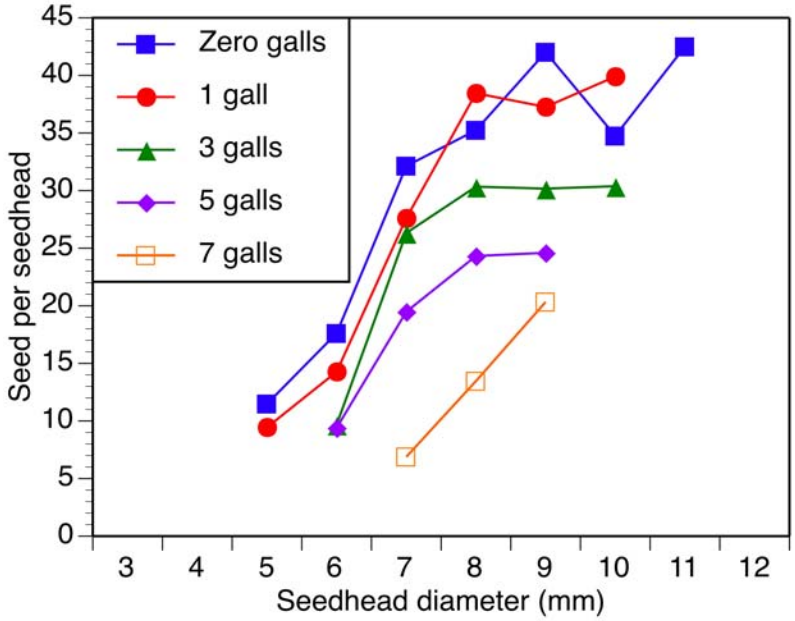

Figure 3. Impact of seedhead diameter and number of *Urophora* galls on mean seed production

Long-term followup –

Two generations of the fly were detected at the two sites that were intensely monitored. Thus, new galls were produced over much of the flowering life of the weed. With the ability to attack over most of the season and the potential to reach high number of galls per seedhead, the gall fly had the potential to have long-term impacts if it reached its full potential.

The Ukiah site saw slight increases from 1993 to 1994 in maximum number galls (from 14 to 15), seedhead attack rate (46% to 56%) as well as galls per seedhead. However, the follow-up sampling six years later showed the gall fly at less than half of the population level with only 21% of the seedheads attacked. The Winters site remained somewhat the same in 1996 as it was in 1995 but was not followed further.

Five additional sites have been monitored between 1995 and 2008 for long-term changes. These sites were not designed as *U. sirunaseva* study sites, and all have multiple species of seedhead attacking insects. Attack rates by *U. sirunaseva* were monitored at all five sites (Figure 4). The gall fly did not fair well at the Napa or Nevada County sites. Competing biological control agents have affecteded the gall fly at all sites but more so at these two. The hairy weevil, *Eustenopus villosus*, was released in high numbers at or near the time that *U. sirunaseva* immigrated. Larvae of the weevil consume developing galls of the fly along with seed and other plant reproductive tissue. The flies did better at the Yolo and Sonoma sites where they arrived before releases of the weevil. Both insects immigrated to the Solano site at about the same time.

All sites showed a dramatic decline in galls in 2001. Populations have rebounded since that time, but seem settled at lower levels than they were before 2001. The most likely

explanation for the 2001 population decline is a statewide weather pattern that interfered with yellow starthistle, the gall fly biology, or the timing between the two. Hairy weevil populations declined somewhat in 1999 or 2000 at the sites but were very high in 2001. The false peacock fly, *Chaetorellia succinea*, a common insect of yellow starthistle accidentally introduced in the early 1990s has invaded all sites. Populations increased to about 20% attack rate by 2001 and have since leveled off.

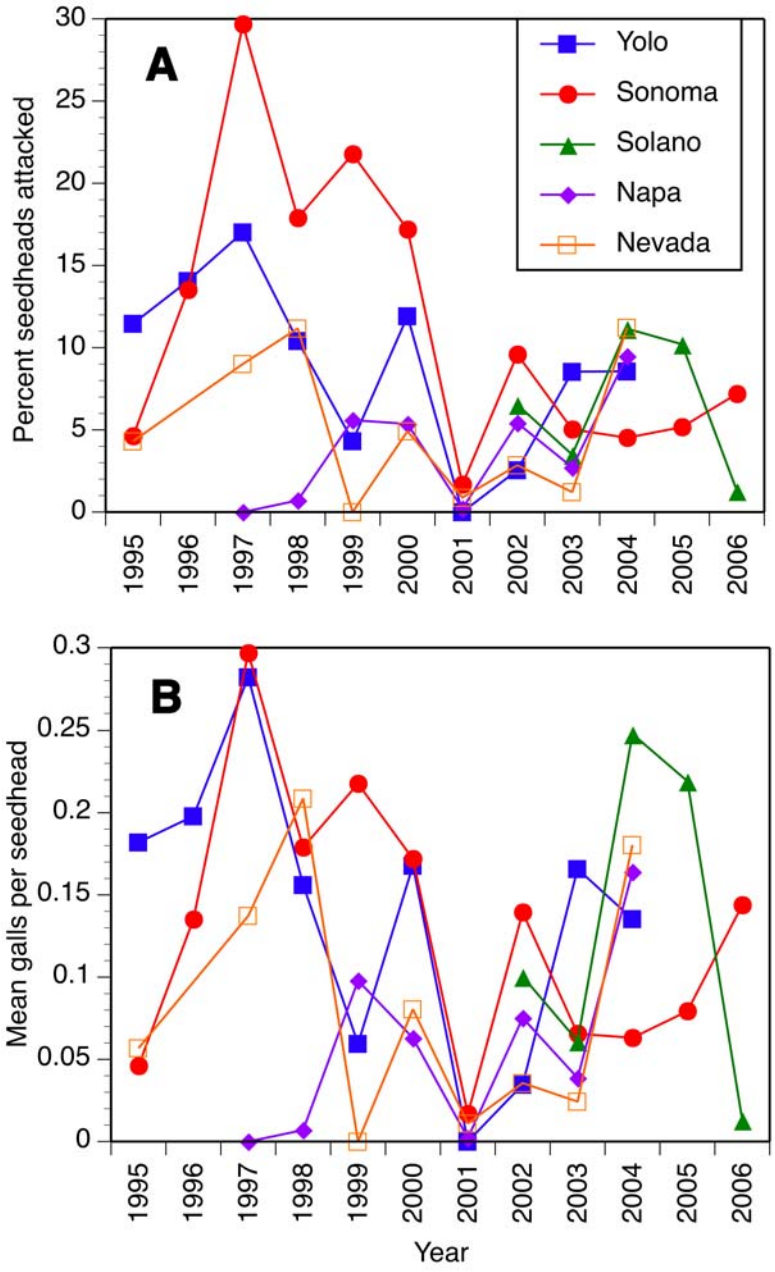

Figure 4. Attack rates of the gall fly on yellow starthistle at five long-term monitoring sites in California.

The gall fly does not appear to have progressed distinctly better at other sites in California. A statewide survey in 2001/2002 found *U. sirunaseva* galls at 61% of the 421 sites

surveyed and only eight sites had greater than 25% of the seedheads with galls. Additionally, we have only detected in-head gall densities of greater than eight on two other occasions out of 46,555 seedheads dissected statewide through 2006.

Even if the gall fly had maintained populations at the higher Ukiah levels, *U. sirunaseva* does not appear to have the potential to have a major impact on seed production by yellow starthistle in California as individual galls have a limited impact. Much higher population densities of *U. sirunaseva* are needed to significantly impact total seed production at the population level.

The larger seedheads of yellow starthistle will require extremely high levels of attack by *U. sirunaseva* to cause significant reduction in seed production on the population level. With an average reduction of 2.1 seeds per gall, an average of 14 galls per head would be necessary to achieve a 90% seed reduction at Ukiah. In contrast, our field sample average of 1.9 and 0.5 galls per head and would account for only 7.6% and 2.6% reduction of the seed produced at the Ukiah and Winters sites respectively.

Table 1. Maximum number of galls in a yellow starthistle seedhead at five long-term monitoring sites in California. At least 300 seedheads were evaluated at each site each year.

County	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
Yolo	5	8	4	4	2	5	0	2	7	5		
Sonoma	1	1	1	1	1	1	1	4	4	3	3	5
Solano								4	5	6	10	1
Napa			0	1	5	3	1	4	5	5		
Nevada	3		5	6	0	4	2	3	7	5		

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2. United States Department of Agriculture, Agricultural Research Service, Exotic and Invasive Weed Research Unit, Albany, California (deceased)

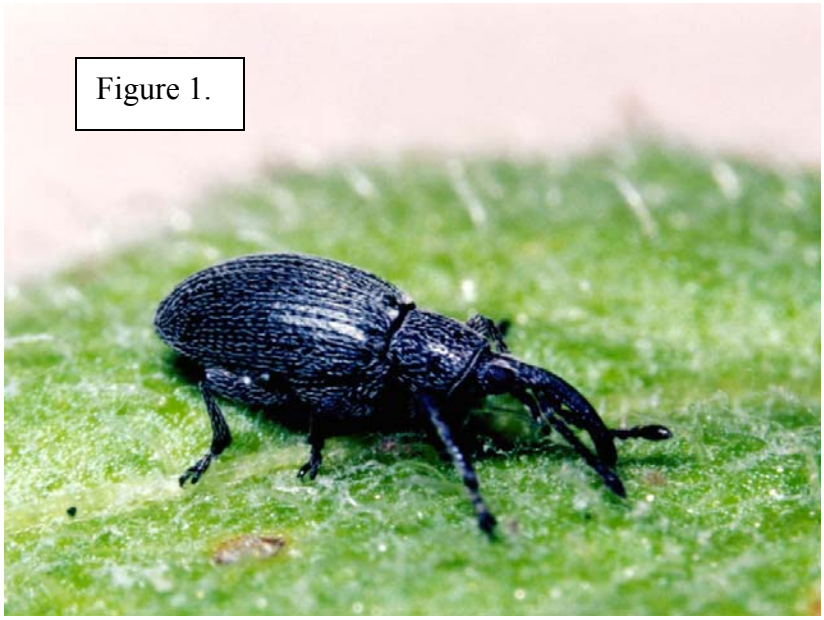
Preparation for the Introduction of a New Yellow Starthistle Biological Control Agent into California

Dale Woods and Lincoln Smith[1]

Six species of insects have been intentionally released as biological controls for yellow starthistle. Along with one accidentally introduced species, they all attack the seedhead of their target weed. Although marked control of yellow starthistle has been noted in some locations, the weed is not adequately controlled statewide. The introduction of a plant pathogen, *Puccinia jaceae*, was hoped to further impact starthistle by attacking a different part of the plant; the foliage, but has not yet proved to be a significant addition. In the past few years, final evaluations were completed on the newest addition to the biological control suite on yellow starthistle, the root feeding weevil, *Ceratapion bassicornae*. A proposal to field release the weevil has been submitted to regulatory agencies and final approval is anticipated in 2009.

The yellow starthistle rosette weevil, *Ceratapion bassicorne*, is native to Eurasia. Adults (Figure 1) feed on leaves and larvae develop in root-crowns of yellow starthistle. Host specificity testing has shown that yellow starthistle is its preferred host plant, but it can also feed on bachelor's button, causing small lumps in the stem. The insect has one generation per year. Adults lay eggs in leaves of rosettes in early spring. Larvae tunnel into the upper root and complete development in one to two months. Pupation occurs inside the plant, and adults emerge in May to June, as the plant is bolting. Most of the year the adults are dormant and hide in sheltered places such as tree bark. Larval damage stunts plants and can cause them to die. In Turkey, infestation rates of up to 100% have been observed.

Figure 1.



Field release of the weevil will have two goals. The first goal is to establish the weevil in the field in a manner to encourage natural population build-up so that further distribution from these sites can be obtained. The second focus is to establish the weevil at sites where its impacts can be evaluated and monitored. Future projects depend on field monitoring of current projects and transferring that knowledge. For *C. bassicornae*, six locations have been chosen to monitor those impacts. Each location has two sites, one where the weevil will be released and the second, a paired site, which will not receive weevil releases and act as a control, or comparison for the release site. These sites were established in 2008 in anticipation of releases, and will be jointly monitored by CDFA and USDA-ARS. They were selected to represent the diversity of habitats currently invaded by yellow starthistle. Preliminary evaluations of the yellow starthistle population at these sites are included in Table 1.

Table 1. Pre-release yellow starthistle populations at proposed *Ceratapion bassicornae* release sites. NC=not yet completed.

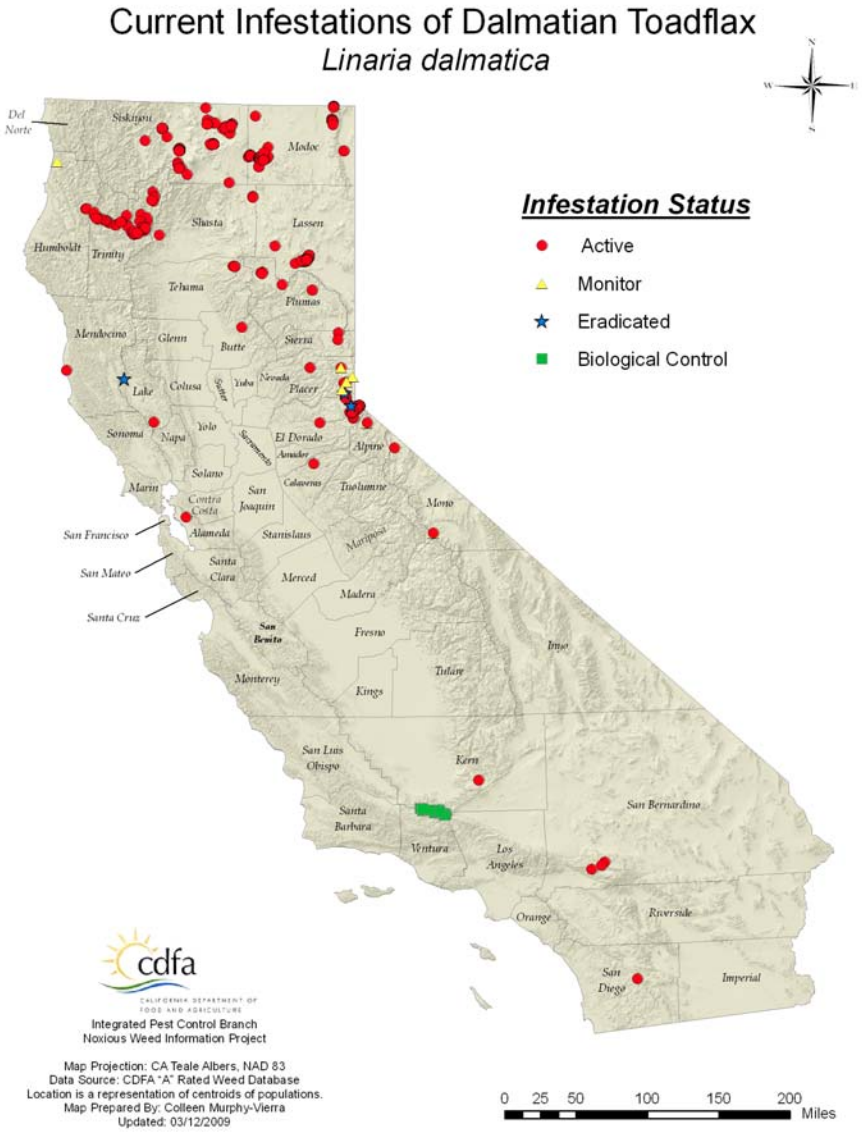
	Plant density (plants/m^2)	Plant height – mean – (cm)	Seedheads per plant -mean	% Seedheads attacked	Primary agent present
Siskiyou lake	291	17	1.6	70%	*E. villosus*
Siskiyou road	144	24	6.2	78%	*E. villosus*
Napa bowl	507	26	2.2	93%	*E. villosus*
Napa pike	718	34	3.1	88%	*E. villosus*
Glenn north	120	56	11.4	69%	*E. villosus*
Glenn south	275	53	17.9	61%	*E. villosus*
Yuba 1	57	69	12.7	NC	NC
Yuba 2	201	38	5.1	25%	*C. succinea*
Contra Costa 1	122	17	2.7	NC	NC
Contra Costa 2	435	10	3.0	64%	*E. villosus*
Monterey 1	123	38	6.0	NC	NC
Monterey airfield	41	46	97.7	66%	*E. villosus*

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

Biological Control of Dalmatian Toadflax in California

Baldo Villegas

Dalmatian toadflax, *Linaria dalmatica* (L.) Mill. (Scrophulariaceae), is a short-lived perennial that is native to the Mediterranean region. It can readily be established in disturbed areas, along roadsides, pastures and mountain meadows. In California, Dalmatian toadflax blooms from April through August and flowers produce very tiny seeds that are easily carried by the wind. A mature plant is estimated to produce upwards of 500,000 seeds which can last up to two years in the seedpods of dead plants. The plant also reproduces by lateral roots and by prostrate vegetative stems. Plants range from one to four feet in height. It was introduced into the United States as an ornamental plant in the 1800's. There are scattered infestations in California and a few of them are becoming increasingly hard to control due to the nature of the terrains where it has become established (Figure 1).



Biological control efforts against Dalmatian toadflax in other parts of the United States have been very effective due to the release of a toadflax stemboring weevil, *Mecinus janthinus* Germar (Coleoptera: Curculionidae) (Figure 2). This weevil was approved for release in the United States in 1995 and since then field releases have been made in Western United States and Western Canada. In many places, the weevil controlled the toadflax infestations within about five years. Consequently, numerous requests to initiate a biological control program against Dalmatian and yellow toadflax (*Linaria vulgare*) were made to the CDFA Biological Control Program. However, a major concern about initiating

a biological control program in California was the potential impact that *M. janthinus* weevils could have on native wild snapdragons which were known to occur in California. One species is tall snapdragon, *Antirrhinum virga* A. Gray. This native snapdragon occurs in chaparral communities in Napa, Sonoma, Mendocino, Lake, and Colusa counties located in northwestern California. These counties are just south of where the largest infestations of Dalmatian toadflax occur in California.

One expanding infestation of Dalmatian toadflax that was found to be nearly 400 miles from native snapdragons is located near Gorman, California at the corner of Kern, Los Angeles, and Ventura counties. The main toadflax infestation is within the boundaries of the Los Padres National Forest, the California State Park Off-Road Vehicle Park at Hungry Valley. The infestation has been there for a number of years and limited control actions have been taken by the California State Park and the Kern County Department of Agriculture. Despite these control actions, the toadflax infestation is expanding into prime wildflower habitats in mountain meadows and into the wild areas of the Los Padres National Forest (Figure 2).

At the request of the Kern County Department of Agriculture, a biological control program was implemented in May 2008. In this biological control program, 1400 *Mecinus janthinus* were released at three sites across a large mountain meadow located on the California State Park side of the toadflax infestation. The releases occurred on two different dates about two weeks apart. On May 20, 2008, a total of 200 weevils were released at each of the three sites. On the second date, June 4, 2008, another 200 weevils were released at each of the outside sites with the middle site receiving 400 weevils. The *Mecinus* weevils were obtained from Paul Brusven with the Nez Perce Tribe Bio-Control Center, Lapwai, Idaho.

In the fall, the three sites were monitored for initial establishment of the weevils. By this time, most of the toadflax plants were finished blooming. Weevil feeding signs were noted on stems of flagged plants where the weevils were released. One stem was opened at each site, and tunneling and live pupae were found indicating initial attack which hopefully will lead to recoveries of the weevils in the spring 2009. No additional releases of the weevils are planned in the area unless the weevils do not establish. The three release sites will be monitored for establishment and will be followed for three or more years to measure dispersal of the weevils throughout the infestation and in the control plots located in the Los Padres National Forest.

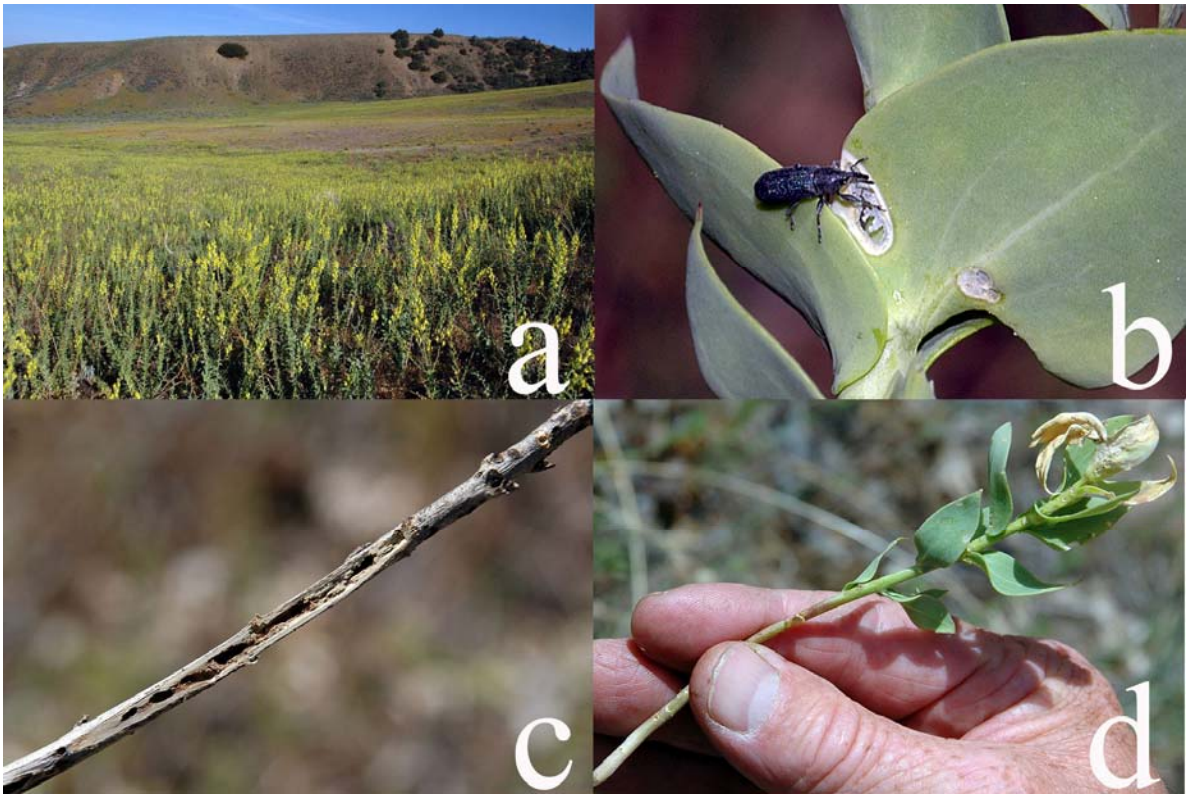


Figure 2. a) Dalmatian toadflax infestation at Hungry Valley State Park, Kern County, CA; b) *Mecinus janthinus* weevil with typical adult feeding damage on toadflax; c) toadflax stem showing stem damage and weevil emergence holes; d) weak and spindly toadflax stem produced by a plant that was heavily impacted by previous year's feeding by the weevil.

Biological Control of Saltcedar (*Tamarix parviflora*) in California

Baldo Villegas, Ray Carruthers[1] and Claudia Street[2]

The Crête biotype of the saltcedar beetle, *Diorhabda elongata* is well established in Yolo County from Guinda to Rumsey, California. This beetle was introduced by the USDA-ARS Invasive Weeds Research Unit (Albany, California) from the island of Crête where the climate is most similar to California's climate. The beetle feeds on both *Tamarix ramossissima* and *T. parviflora* which are the two most common saltcedars in California. In 2007, the implementation phase of this biological control program was turned over to the CDFA Biological Control Program by the Invasive Weed Research Unit. Two sets of releases were made in early September 2007, with no recoveries in the areas where the beetles were released. In 2008, releases of the beetle were put on hold in order study the beetle population in Cache Creek on *Tamarix parviflora*, specifically to determine the best collection and release strategies to insure successful establishment of the beetles in California.

Beetles emerge from their overwintering sites in early- to mid-April about the time that *T. parviflora* starts to bloom at Cache Creek (Figure 1a). The beetles can be collected and redistributed in April, but it would take considerable time and effort to collect the ideal 2000-3000 beetles per release. A second collection period for the beetles occurred in mid- to late-June when the F1 adults emerged from pupation sites at the base of the saltcedar plants. The beetles from this collection period were found to be easier to collect and the ideal release numbers were achieved in a relatively short period of time. A final collection period occurred in mid August when the F2 generation adults emerged to feed on saltcedar foliage. These beetles were also easier to collect and large numbers were collected in a shorter time period.

The beetles were mass collected using two methods. The first method involves "sweeping" the saltcedar foliage and branches with a sturdy sweep net when large numbers of beetles are seen. The repeated sweeping of the saltcedar foliage generally keeps the beetles at the bottom of the sweep net. In periods of inactivity or while looking for large numbers of beetles, the net can be folded or tied with a piece of string or flagging tape to prevent the beetles from escaping the sweep net. The second collection method involves looking for large numbers of beetles on the saltcedar foliage and "tapping" the saltcedar foliage and branches held over an open sweep net (Figure 1b). The beetles fall into the sweep net and repeated tapping of the foliage usually keeps the beetles in the bottom of the sweep net. The sweep net can be folded or tied during periods of inactivity. The sweeping and tapping of the saltcedar foliage can be continued until the beetles can no longer be maintained in the sweep net. The beetles are then transferred into pillow cases or into sleeves made of nylon chiffon that are placed over utility buckets (Figure 1c). Nylon sleeves were readily chosen during the second generation beetle collections as they were widely available and relatively inexpensive. We found suitable nylon sleeves at paint stores where they are being sold as paint strainers. These strainers have elastic in the open end and fit snugly over a paint bucket or other utility container. These paint strainers made it easy to store the beetles being transferred from sweeping nets and then to store several strainers in a small ice chest for easy transport to the release sites. Also, since the beetles are visible through the nylon strainers, they can be counted and monitored for activity. The goal in 2008 was to collect 2000-3000 beetles per release. All the beetles in one storage bag were released in one to two places in close proximity of each other at the base of one saltcedar plant or a clump of plants in order to concentrate the beetles and their swarming pheromone.

Monitoring of the sites was made at several intervals after the release of the beetles. During each monitoring period, beetle life stage was recorded. Feeding impact on the plant was also recorded by means of digital photographs (Figure 1d). Collections of the beetles were made at all three collection times – April (overwintering beetles), late June-early July (F1 adults), and August (F2 adults). No releases were made from the overwintering adults in April as it was difficult to collect sufficient numbers to move to other sites. The ideal redistribution collection period was found to be during the emergence of the F1 generation in late June. These beetles were easily mass collected and moved to release sites within the same day. These beetles do not have the tendency to swarm and fly away as occurred with the F2 generation. The F1 beetles stayed within the release area, fed on the saltcedar and laid eggs, resulting in successful adult and larval recoveries in October 2008. On the other hand, beetles collected from the F2 generation in late August did not lead to successful recoveries. One possible explanation is that the beetles from this generation are adapted for swarming and do not stay in the release area.

During 2009, redistribution workshops are being planned around the emergence of the F1 generation in mid lo late June. Counties and Weed Management Areas wishing to participate in these workshops will be contacted and redistribution workshops will be scheduled in June 2009.

Figure 1. a) *Diorhabda elongate* saltcedar leaf beetle; b) Cooperator John Watson (Cache Creek Conservancy) Mass-collecting saltcedar leaf beetles by tapping on infested foliage; c) Claudia Street mass-collecting of saltcedar beetles for redistribution releases; d) Claudia Street and Mike Pitcairn monitoring a beetle release site.

[1]USDA-ARS Exotic and Invasive Weed Research Unit, Albany, California
[2]Glenn County Resource Conservation District, Willows, California

Survey for the Milfoil Weevil, *Euhrychiopsis lecontei* in California

Baldo Villegas

The milfoil weevil, *Euhrychiopsis lecontei* Dietz (Coleoptera: Curculionidae), is a phytophagous insect associated with Siberian or northern milfoil, *Myriophyllum sibiricum* as well as other species in the genus *Myriophyllum* (Haloragaceae). The weevil is considered to be native to North America and has been found in 35 states in the United States. Prior to June 2007, the milfoil weevil was not known to occur in California. However, in July 2007, the weevil was found at Bon Tempe Lake and Lake Lagunitas in Marin County. These lakes are infested with Eurasian water milfoil, *M. spicatum*, a highly invasive species of *Myriophyllum*. The two lakes supply drinking water to several communities in Marin County making herbicidal treatments impossible. Biological control of Eurasian milfoil using the milfoil weevil, *Euhrychiopsis lecontei*, was suggested for these lakes and associated streams since the weevil could now be found in California. The proposed biological control program would require the importation of weevils from other parts of the United States into California in order to attain high weevil densities. The question of whether the weevil was truly a native California species or an "accidental introduction" came up and a survey was implemented in July 2008 to answer this question.

Prior to initiating the field survey, familiarity with the various species of *Myriophillum* species known in California was made by reviewing herbarium specimens in the CDFA Botany Laboratory Collection. Additional expertise was gained through taxonomic training sessions with Drs. Fred Hrusa and Dean Keltch. Additional herbarium specimen records from the Consortium of California Herbaria for the genus *Myriophyllum* were also evaluated. Nearly 300 herbarium records for the genus known to occur in California were studied. (Table 1).

Table 1: Herbarium records for *Myriophyllum* in California

Species	Common name	Records	Notes
M. aquaticum	Parrot feather	38	Invasive species; but unlikely to be a host of the milfoil weevil as most of the plant grows above the water surface.
M. heterophyllum	Twoleaf watermilfoil	2	Not native to California; no record of being a host to the milfoil weevil; weedy and invasive in the western states; limited to the desert region of CA.
M. hippuroides	Western milfoil	37	Native species; no record of being a host to the milfoil weevil.
M. quitense	Andean watermilfoil	6	Native species; no record of being a host to the milfoil weevil; limited distribution in CA.
M. sibiricum	Siberian milfoil	119	Native species; major recorded host to the milfoil weevil; also known as "Northern milfoil".
M. spicatum	Eurasian milfoil	57	Invasive species; recorded host of the milfoil weevil; present in many bodies of water in CA.
M. verticillatum	Whorled leaf watermilfoil	17	Native species; no record of being a host to the milfoil weevil.

Field experience with the milfoil weevil was gained by visiting Burke Lake in central Washington in June 2006. Ms. Jennifer Parsons (Aquatic Plant Specialist, WA Department of

Ecology, Yakima, WA) provided training for detecting the water weevils by snorkeling and seeing the weevils swim underwater and seeing them feed on the Eurasian milfoil plants. More recently, in July 2008, the two Marin County lakes infested with Eurasian milfoil were surveyed with Dr. Ray Carruthers and Ms. Skye Harper (USDA Invasive Weeds Center, Albany, CA). Additional training was obtained by discussing their monitoring techniques at their study sites. This experience was important in order to obtain a search image for the weevil and explore different ways to search for the weevils in lakes and streams in California.

In order to study the milfoil weevils further, bulk collections of Eurasian milfoil and some of the weevils were enclosed in large collection bags and brought back to the laboratory and stored in a refrigerator. In examining the samples a few days later, it was noted that the weevils will leave the bulk milfoil and migrate to the edges of the collection bags making them easier to see and collect. These collection techniques were incorporated into the overall collection scheme for this survey.

Site selection for the field survey was based on the known distribution of *M. sibiricum*, the main native host species for the milfoil weevil. The underlining premise was that if the milfoil weevil is a true California native insect, then, it should occur with its native primary host plant and potentially other native milfoil species. Survey routes were planned that included high elevation lakes likely to have Siberian milfoil as well as other native milfoil species.

All sites surveyed were accessible by car or located within a short hike from a main road. At each site, at least 10 minutes were spent inspecting the edges of the lakes, reservoirs, rivers, irrigation canals, etc. where milfoil might grow or float. If pieces of milfoil were found floating on the water, an aerial insect net was used to retrieve the floating milfoil to capture any insects that might be clinging on the floating plants. If the milfoil was found growing on the bottom of the survey site, the plants were pulled by the roots and deposited into the insect net, making sure that any insects clinging to the growing plants did not escape. If stands of milfoil were found growing at the site, then the stands as well as individual plants were "swept" with the insect net to capture any weevils present on the plants. The contents of the insect net were checked repeatedly to see if any weevils were being collected. Samples of the milfoil were placed in Ziploc baggies as well as in collection bags and kept in coolers for later inspection. Location coordinates (Latitude/Longitude and elevation) were taken using a Garmin 76cxs GPS Unit. Botanical specimens were collected whenever possible as the subsequent botanical identification was of utmost importance to this survey. Visits to the CDFA Botany Herbarium were done on a weekly basis in order to obtain species determinations for specimens kept in Ziploc baggies as plant press herbarium samples take longer to prepare and identify.

Over 90 sites were visited during July - October 2008. Of these, 51 sites were found to contain at least one species of milfoil. In some cases more than one species was found. In a few cases, specific determination of the milfoil could not be done as the diagnostic parts of the plants were not present. This was commonly the case between the two native species, *M. verticillatum* and *M. hippuroides*. Milfoil weevils were found at 11 sites containing its native host, *M. sibiricum*. This is in addition to Bon Tempe Lake and Lagunitas Lake in Marin County where the weevils were collected from Eurasian milfoil, *M. spicatum*. The 11 sites are well spread out in five northern California counties. One site each was located in Lassen, Placer, and Siskiyou counties, four sites in Plumas County, and four sites in Modoc County. Ten of the 11 sites contained only *M. sibiricum* which is the known host of the milfoil weevil. One site, located along Indian Creek in Plumas County had both *M. hippuroides* and *M. sibiricum*. At this site, the

west side of the creek had predominantly *M. hippuroides* while the east side of the creek was shallow and contained scattered plants of *M. sibiricum*. Since only a few weevils were collected at the site, it is assumed that they were likely present on the *M. sibiricum* plants found at the site.

At the conclusion of the field survey, all weevils collected were pinned and labeled in order to be properly identified by Dr. Andrew Cline with the CDFA Entomology Laboratory. He further contacted Dr. Charles O'Brien, a well known weevil expert, for final species identification. Three weevil taxa were collected and identified during this survey (Table 2). *Euhrychiopsis leconti*, the milfoil weevil was collected at sites in four counties containing the native species *M. sibiricum* in addition to the original two sites in Marin County which were infested with Eurasian water milfoil, *M. spicatum*. These collections represent the first records of these weevils from *Myriophyllum* in California.

Table 2: List of Milfoil Weevils Collected in California

Bagous californicus (Marsham)	Modoc, Plumas, Siskiyou
Euhrychiopsis lecontei (Dietz)	Marin, Modoc, Placer, Plumas, Siskiyou
Phytobius leucogaster LeConte	Modoc, Placer

Publications produced by Biological Control Program: 2005-2008

(names in **Bold** are part of the CDFA Biological Control Program)

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