

# Biological Control Program 2006 Annual Report



California Department of Food & Agriculture



# **BIOLOGICAL CONTROL PROGRAM**

## **2006 SUMMARY**

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CALIFORNIA DEPARTMENT OF FOOD AND AGRICULTURE  
PLANT HEALTH AND PEST PREVENTION SERVICES  
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## Preface

M. J. Pitcairn

In our war against invasive species, the battle begins overseas. This observation is emphasized by reports on the foreign exploration efforts against the olive fruit fly and the avocado lace bug that occurred in 2006. Foreign exploration is the foundation of a biological control project for it is the natural enemies discovered through these efforts that are developed and released as biological control agents. Once the exotic pest is identified, the biological control scientist travels to the pest's area of origin and examines its populations in its native range. The climate and ecology of the region are compared with California and similarities and differences between a pest's native and introduced ranges can lead to important insights that result in a successful biological control program. During these trips, hundreds of individuals of the pest are collected and examined directly or held in the laboratory to rear any parasites living within the pest. Observations on the mortality of the pest due to the different natural enemies, variations in its distribution and abundance, and the interaction between the pest organism and its natural enemies are recorded. The results of these observations help identify those natural enemies that have the greatest potential as biological control agents.

The foreign exploration effort for natural enemies of the olive fruit fly has been very successful: over nine species or biotypes of species have been discovered that have potential as biological control agents. Host testing in quarantine has resulted in two species being approved for release; the others continue to be studied. The foreign exploration for natural enemies of the avocado lace bug has just begun and much work remains.

Significant progress has been made with the biological control of lygus bugs (*Lygus* spp.), a serious pest of strawberries. Two parasites have been release and appear to have built up high populations in the coastal counties of central California. Parasitism rates have steadily increased each year, results we find very encouraging.

In northeastern California, establishment of the crown weevil on Mediterranean sage appears to be successful. And, for purple loosestrife populations in the Sacramento and San Joaquin Valleys, efforts continue to establish the two leaf-feeding beetles in 2006.

These are but a few of the highlights of work that occurred in 2006. I hope you enjoy this year's report.

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## Release of Scuttle Flies for Biological Control of Red Imported Fire Ants

K. Godfrey, D. Morgan<sup>1</sup>, and A. Callcott<sup>2</sup>

Established populations of red imported fire ant (*Solenopsis invicta*) were discovered in California in 1998. Since then, the red imported fire ant has been found in four southern California counties and at scattered locations in the San Joaquin and Central Valleys. Some of these infestations were eradicated, whereas for other infestations, insecticidal treatments are continuing. These treatments are expensive and in some areas, may raise environmental and health issues. Alternative control measures such as biological control need to be investigated because in the southeastern United States, eradication efforts based solely on insecticides have failed. Research on biological control agents of the red imported fire ant conducted in South America and the southeastern United States since the 1980's have identified at least one agent, a scuttle fly, *Pseudacteon tricuspis* (family Phoridae), that has established and demonstrated effectiveness in the southeastern United States. The ability of this fly to establish and be effective in southern California is unknown. Therefore, a study was initiated in 2005 and continued in 2006 in Riverside County to investigate the establishment potential and effectiveness of this fly.

Studies on the ability of the scuttle fly to reduce densities of red imported fire ant continued at two new sites that were at least ¼ mile from sites used in 2005, in Lake Elsinore. On 28 March 2006, 6,600 fly pupae arrived at the Pierce's Disease Mt. Rubidoux Field Station in Riverside from Florida. The flies began to emerge from the pupae on 3 April, and by 13 April, 3,109 flies had emerged. Releases of the flies began on 4 April, at the two new locations in Lake Elsinore. A total of 2,910 flies were released at these sites.

To determine the success of the releases, sites where releases were made in 2005 and 2006 were monitored for fly activity. On 2 April 2006, before any new releases were made, the sites where the flies were released in 2005 were monitored for the presence of flies. No flies were found in this sampling, however, the sizes of the ant nests were smaller than in 2005, and the ants, less active. On 1 May, the new sites where releases were made in 2006 were monitored. At one of the release sites, no flies were found. At the other site, three flies were observed flying around the ant nests. The lack of recoveries at the release sites is not surprising considering that studies of this fly in the southeastern United States have shown that it can take up to three years to get detectable densities of this fly. The monitoring at these sites will continue for at least two more years.

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## Colonization of *Lygus* Nymphal Parasitoids in the Monterey Bay Region

C. H. Pickett, D. Dugama<sup>1</sup>, S. Swezey<sup>2</sup>, J. Bryer<sup>2</sup>, D. Nieto<sup>2</sup>, and M. Erlandson<sup>3</sup>

*Lygus hesperus* Knight (Hemiptera: Miridae) is a serious pest to numerous field, fruit, and seed crops including strawberries and cotton. It is the number one insect pest problem to growers of strawberries in the Monterey Bay region of California. Currently *Lygus* is managed on most crops through applications of broad spectrum insecticides. The use of cultural and biological practices as alternatives to chemical control have not been considered useful. Importation of the nymphal parasitoid *Peristenus digoneutis* Loan (Hymenoptera: Braconidae) into eastern United States during the 1980's, however, successfully reduced *Lygus lineolaris* infesting alfalfa, a close relative of *L. hesperus*. *Peristenus digoneutis* has recently been reported attacking *L. lineolaris* infesting strawberries in New York. We have successfully imported and colonized both *P. digoneutis* and *P. relictus* (Ruhte), formerly *P. stygicus* Loan in Sacramento, California (see previous annual reports). Beginning in 2002 we began releasing the same parasitoids at locations in the Monterey Bay region. Although a native species of *Peristenus* has been found in this area (*P. nr. pallipes*), it has been found attacking only a closely related mirid *Closterotomus norvegicus*, and an early season species of *Lygus*, *L. shulli*. Today, there are four release sites: two of natural vegetation bordering or near strawberry production, and another two on organic strawberry farming systems.

Colonization of parasitoids requires a stable host/habitat. Strawberries are typically rotated every one to two years. An effort to colonize *Peristenus* spp. was initiated near the central coast of California because natural vegetation known to support *Lygus* bordered agricultural lands in the Monterey Bay Region. Two sites were established, first one near Castroville in 2002, then a second, near Harkins Slough (Watsonville). During this same period of time, one of us (SS) was experimenting with strips of alfalfa as a trap crop in organic strawberry production systems (named Eagle Tree and Elkhorn School sites). The same strips that helped reduce *Lygus* numbers through vacuum suctioning of alfalfa (Fig. 1), also supported within season releases of *P. relictus* (annual report 2005). We herein continue to report on progress of releases.



Fig. 1. Strawberries and alfalfa strips. Eagle Tree site.

Releases of parasitoids at the Monterey Bay region sites were made for two years. Releases of *P. relictus* and *P. digoneutis* were discontinued at the Castroville site in 2004 and at the Harkins slough and Eagle tree site in 2005. Limited releases of parasitoids were made for the last time at the Elkhorn School site in 2006 (1,060). Most of the parasitoids came from the Program's outdoor insectary of alfalfa in Sacramento.

The two commercial organic strawberry sites use one row strips of alfalfa interspersed every 40 rows of strawberries as a trap crop for *Lygus* (Fig. 1). At the Eagle Tree site, we released parasitoids into two 'release' strips, and monitored these and two additional non-release 'control' strips. Similarly, parasitoids were released into an edge, 'release' strip of alfalfa at the Elkhorn Slough site, and monitored in two other strips of alfalfa ( i.e. rows 40 and 80, interspersed between rows of strawberries, i.e. see Fig. 1).

Each month, beginning in April and ending by December, release sites were monitored for the abundance of *Lygus* and parasitoids. *Lygus* counts were made two ways depending on whether a sample came from alfalfa or strawberries. Alfalfa was sampled using a standard 37 cm diameter sweep net, taking 200, 180 degree sweeps walking down a strip. Strawberries were sampled using a gas powered vacuum (a modified Stihl<sup>®</sup> leaf blower) and taking two to four sets of ca. 400 suction of strawberry plants randomly selected while walking across a field. After each group of 300 suction, the contents of the organdy suction bag were emptied and *Lygus* nymphs placed into an alcohol filled vial. The number of nymphs was recorded and their bodies dissected for the presence of parasitoid larvae.

Two years since last released, parasitoids continued to be recovered in natural vegetation surrounding conventional strawberry fields at the Castroville site, the first release site in this region (Table 1). A neighboring field of conventionally grown strawberries was sampled for parasitized *Lygus*. Peaks of 50% and 5% parasitized nymphs were found in 2005 and 2006, respectfully. The high degree of parasitism in 2005 was surprising considering the berries were routinely treated with conventional pesticides. The lower degree of parasitism in 2006 may have been due to a drop in *Lygus* numbers, from 0.03 per vac in 2005 to 0.02 per vac in 2006, and the use of pesticides (one vac is approximately equal to sampling one strawberry plant).

Table 1. Trends in parasitism at the Castroville site. Shown is maximum parasitism for year of *Lygus* sampled from weeds.

<b>Year of Sample</b>	<b>Percent Parasitized</b>	<b>(n) number of individuals dissected</b>
2003	7	14
2004*	23	30
2005	29	54
2006	27	15

\*last year releases made into site.

*Peristenus relictus* has been recovered each year since first released at the Eagle tree site, from both the strips of alfalfa where they were first released (Fig. 2), and from nearby strawberries (Fig. 3). Parasitism was correlated with *Lygus* numbers sampled from alfalfa over these years, showing specificity by *P. relictus* for this host ( $r = 0.63$ ,  $p = 0.007$ ,  $n = 15$  sample dates). Sampling in these plots ceased mid-

summer 2006 because the grower disced the field. The lack of parasitism in the adjacent strawberries in 2006 is most likely due to the very low number of *Lygus* in the fields at that time. The strips of alfalfa beginning in 2006 were vacuumed as part of the normal operations on the farm. This was not done in 2004 and 2005 because we were making releases of parasitoids into these strips.

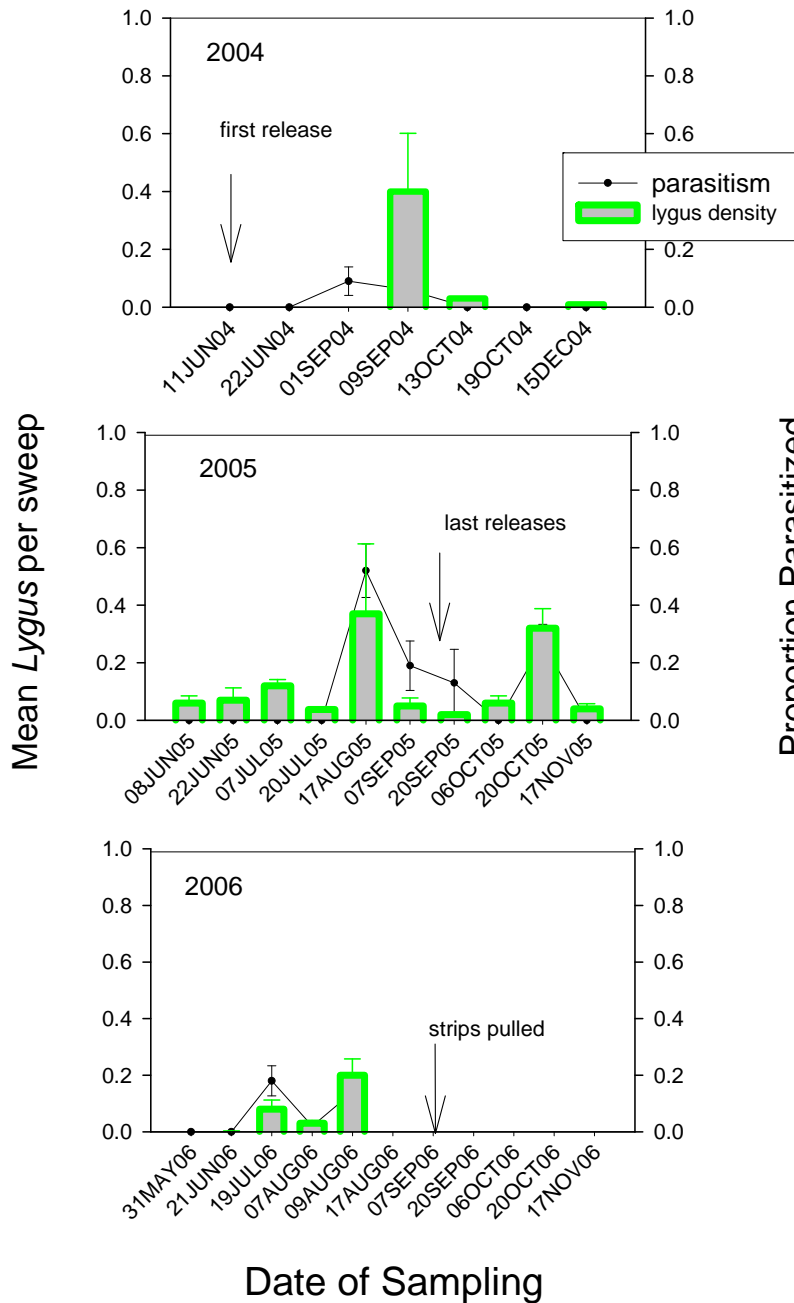


Fig. 2. Parasitism and density of *Lygus* in alfalfa strips. Eagle Tree Site.

Identification of recovered parasitoids was initially based on rearing them to adults, the only stage at which species identification can be made through traditional morphological characteristics. However, beginning in 2005, a technique based on the DNA of the insect was developed (Erlandson) that could identify parasitoid larvae to species. A novel multiplex PCR system showed that all the parasitoids recovered from *Lygus hesperus* were *P. relictus*. Though *P. digoneutis* has not shown up in the DNA samples, it may be present but undetectable through current sampling methods. The PCR results also showed that during spring months *P. relictus* attacked 70% of *C. norvegicus* and 30% *L. hesperus*, and 100% *L. hesperus* during the summer

months. *C. norvegicus* is univoltine and does not occur in sampled vegetation during summer months. Although the native *P. nr. pallipes* attacked 73% and 27 % of *C. norvegicus* and *Lygus shulli*, respectively, in the spring, it was never recovered during the remainder of the year (all in diapause). *Lygus shulli* appears to be univoltine since it was found present primarily in the spring

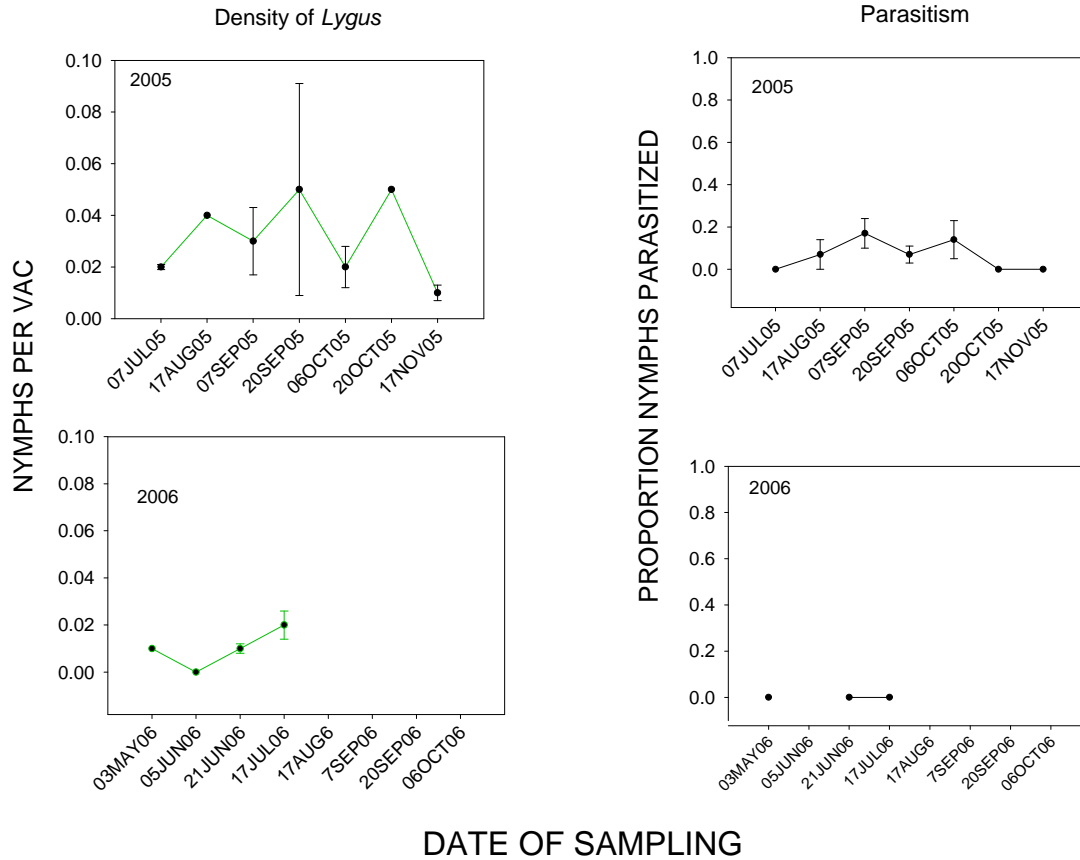


Fig. 3. Density and parasitism of *Lygus* in strawberries near alfalfa release strips. Eagle Tree site. and early summer.

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## Field Releases of the Olive Fruit Fly Parasitoid, *Psytalia lounsburyi*

C. H. Pickett, L. Yang, Karen Sime<sup>1</sup>, Kent Daane<sup>1</sup>, and Alan Kirk<sup>2</sup>

A field release permit for *Psytalia lounsburyi* (Braconidae) was granted late 2005. The first releases of *Psytalia lounsburyi* were made in October 2005 at two locations in northern California. A total of 72 females were received from Kenya, cleared through the USDA-ARS European Biological Control Laboratory, and released at one location in Butte County on October 15<sup>th</sup>. Nine were released into a cage and the rest into the canopy. Another 30 were cleared through quarantine and released into the canopy at one location in Alameda County. No recoveries were made that fall from either site.

Beginning in August 2006, *P. lounsburyi* were released at the same Butte County location, an olive orchard at the UC Davis Student Experiment Farm, and in orchards in Napa and Sonoma County. A total of 500 to 700 females produced between CDFAs Sacramento facility and at University of California, Berkeley were released directly into trees at each of these sites. In addition, small numbers were released into caged branches on trees in the same orchards (Table 1). Within four weeks, small numbers of adult parasitoids were recovered from olives collected inside cages. Recovered adults in this small sample size have been predominately female. No parasitoids have been recovered to date from olives collected outside of cages at each of these locations.

Table 1. Releases of *P. lounsburyi* into small cages enclosing olive branch terminals.

Site Name	Date Released	# Females released	# Olives collected	# Parasites recovered (F:M)	# Puparia Collected
UC Davis <sup>1</sup>	18 Aug 2006	43	30	0:0	21
UC Davis <sup>1</sup>	25 Aug 2006	15	10	2:0	14
Butte Co. <sup>1</sup>	29 Aug 2006	36	31	0:0	29
Butte Co. <sup>1</sup>	26 Sept 2006	94	45	0:1	25
Butte Co. <sup>1</sup>	6 Oct 2006	54	28	1:1	61
Napa Co. <sup>1</sup>	17 Nov. 2006	65	49	5:3	84
Sonoma Co. <sup>1</sup>	14 Dec 2006	40	100	3:1	0 <sup>2</sup>

<sup>1</sup>Parasitoids from South Africa

<sup>2</sup>Parasitoids emerged from puparia which remained inside the fruit

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## Foreign Exploration for Parasitoids of the Olive Fruit Fly

C. H. Pickett, A. Kirk<sup>1</sup>, and B. V. David<sup>2</sup>

A collection trip was made to southern and northern India in October 2006. This country has never been explored for natural enemies of the olive fruit fly. Valuable contacts were made within the government and university, people who will assist in future collections and shipments.

In southern India, two wild olive tree species were found in natural forests. They were closely related to the trees from which previous collections have been made, *Olea europea* subsp. *cuspidata*. Two species were located at about 4000-5000 ft in the western Ghat mountain range. *Olea dioica* was found near Top Slip and both *O. dioica* and *O. panniculata* were found in a forest near the town of Munnar. Although no fruit was collected during this trip from *Olea dioica* and *O. paniculata*, fruit was collected in January 2007 by cooperators and shipped to the European Biological Control Laboratory in France.

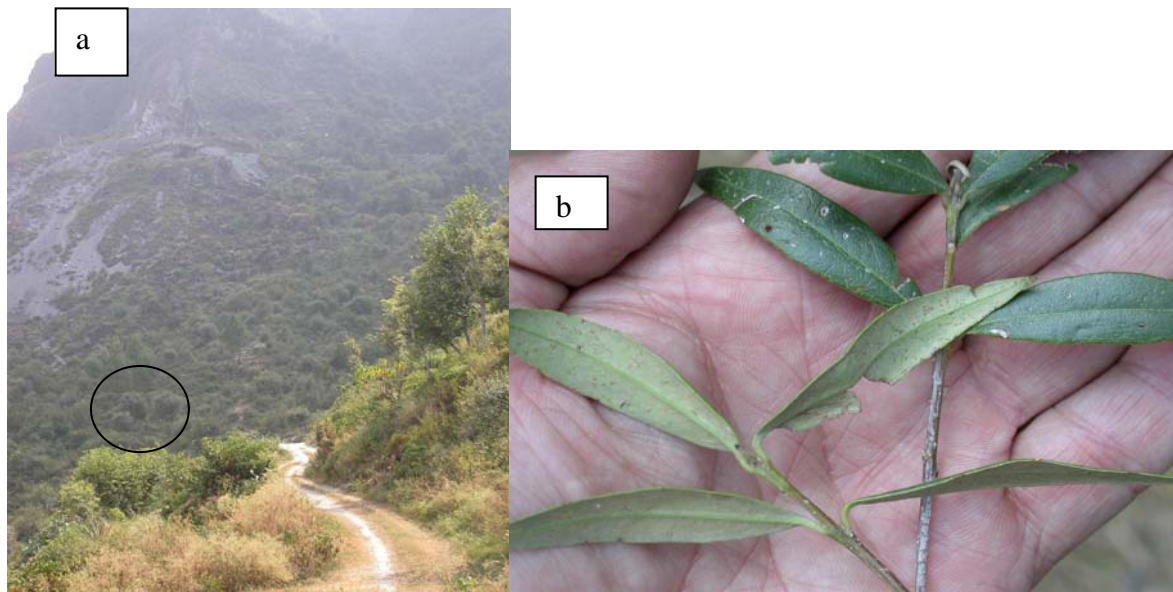


Fig. 1 a & b. First wild olive trees (in circle) seen near city of Mussoorie, in northern India.

Valuable contacts were also made in northern India at the India Agricultural Research Institute in New Delhi and the Forestry Research Institute at Dhra-Dun. The wild olive tree *O. europea* subsp. *cuspidata* was found along two major rivers in the Himalayan foothills near Dehra-Dun, Mussoorie and Shimla (Fig.1 a & b). An extensive grove of wild trees was found near Kempti Falls not far from the Jamuna River. A small cluster of trees on a very remote precipitous road were also found on the Sutlej River near Shimla. All trees lacked blossoms and fruit, however. From conversations with other collectors, it seems weather was a factor in the complete lack of fruit set in these foothill regions. Cooperators, however, will collect from these locations in fall 2007, as well as in Pakistan. Both areas should produce *Psytalia ponerophaga*, a parasitoid species that is not yet in culture, for which permits were recently obtained.





Fig. 2. Wild olive trees, circled, on hillside near Muli, Sichuan Province, China.

An effort was made by Chinese cooperators to collect fruit from wild olive trees located in the fall of 2005 by Pickett and Kirk while exploring for olive fruit fly parasitoids in central China (Fig. 2). Although some fruit was found, no flies or parasitoids were collected from Muli (Sichuan) or Jianshu (Yunnan).

Other material shipped to the UC Berkeley quarantine facility for this project include: *Psytalia lounsburyi* (both South African and Kenyan Stock); *Utetes africanus*, *Psytalia* sp. nr. *humilis* from South Africa, and *P. concolor* from Kenya. *Fopius arisanus* was shipped from Hawaiian cultures to UC Berkeley where it was used in host testing studies.

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## Foreign Exploration for Natural Enemies of the Avocado Lace Bug

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The avocado lace bug (AvLB), *Pseudacysta perseae* (Heidemann), was first found in southern California attacking the foliage of avocado trees during the fall of 2004 (Fig 1). Leaf feeding damage including leaf drop has been extensive at some locations, however, to date the pest has been limited to residential locations in the southern portion of San Diego County. A cooperative effort toward controlling this pest was initiated between CDFA; the University of California, Riverside; the California Avocado Commission; and San Diego County. The use of classical biological control is being pursued to improve management of this pest on a sustained basis.

The AvLB is believed to be native to Mexico (DNA investigations are currently underway at UC Riverside). Foreign exploration was conducted in the state of Veracruz, Mexico during four days in the last week of March 2006 (Figs. 2 & 3). This followed several weeks of travel by Hoddle and Phillips who had visited a number of Caribbean island countries. Small towns and remote avocado groves in the region were visited to collect specimens that were subsequently mailed to the University of California-Riverside quarantine facility. On separate days, we drove 50-75 miles northwest, west and southwest of the coastal city of Veracruz (Fig. 2). We were mostly interested in finding egg parasitoids of the pest, and several thousand eggs were collected and shipped to the quarantine facility.

During the first week of April, the first author traveled to the state of Yucatan to continue collecting. Personnel of the Mexican agricultural agency SAGARPA (Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación) provided assistance in



Fig. 1 AvLB adults with eggs. Eggs covered with resin-like excrement product. Inset - eggs and young nymphs



Fig. 2. Collection areas in 2006 trip to Mexico (black ovals). Proposed collection areas for future exploration (blue ovals).



Fig. 3. Collection site in the highlands, northwest of Veracruz. Avocado trees dispersed throughout a coffee grove.



Fig. 4. Avocado trees in a varietal selection plot near Merida.

collecting over a two-day period. Arrangements for exploration near Merida were made by Hugo Arrendondo-Bernal (Centro Nacional de Referencia de Control Biológica, Colima, México). Collections of AvLB eggs were made at a farm cooperative near the community of Oxkutcab and at a private experiment station near Hunucama (Fig. 4). We also made AvLB egg collections from trees of homeowners within the city of Merida. These collections were sent to the University of California-Riverside quarantine facility for potential parasitoid recovery. In addition, adult specimens of the AvLB were collected and placed in alcohol for DNA analysis by the second author and his colleagues. Collections of adult AvLB are being made in order to utilize DNA analysis to characterize geographical populations of the pest and identify the origin of the population that invaded California.

During our travels in these two regions of Mexico, we noted that AvLB population densities were typically low; however, sizeable populations did occasionally occur. Both in Veracruz and the Yucatan, the populations were in a seasonal decline. It appeared that peak activity had occurred in February. From the material sent to quarantine, no parasitoids were recovered. Two areas of Mexico are of interest for future exploration. The state of Michoacán (west of Mexico City) and a region north of Mexico City, extending nearly to Monterey, are areas where avocado is considered to be native and where they are grown commercially.

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## Host Testing of Olive Fruit Fly Parasitoids

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We are conducting non-target studies to determine the potential host range of each olive fruit fly (*Bactrocera oleae* (Gmelin) (Diptera: Tephritidae) parasitoid species. This is required for submitting requests for permits to USDA-APHIS to rear and release new candidate parasitoid species. Native and exotic tephritid species chosen for these studies represent flies with a range of larval niches including fruit feeders, seed-head feeders, and a gall maker. The non-target species tested in 2006 were *Rhagoletis indifferens* (western cherry fruit fly, a fruit feeder), *Chaetorellia succinea* (yellow starthistle fly, a seed-head feeder) and *Parafreutreta regalis* (Cape ivy fly, a gall maker). Priority was given to testing *C. succinea* and *P. regalis*, which are beneficial species for weed biological control in California.

In fall 2006, we conducted host specificity tests for the parasitoids *Fopius arisanus*, and *Utetes africanus*. For *F. arisanus*, two way choice tests were conducted in small (25 × 25 × 25 cm<sup>3</sup>) cages, comparing responses to OLF-infested olives vs. either *C. succinea* or *P. regalis*. The



Fig. 1. Y-tube olfactometer.

results provide strong evidence that *F. arisanus* will not attack either non-target species, a result that is not surprising as it is consistent with the work of R. Messing and others showing that this parasitoid will not attack hosts that feed in flower buds or galls. The number of replicates in *F. arisanus* testing was eight for Cape ivy and 10 for yellow starthistle ( $P < 0.01$  (unpaired t-test) for both, measuring both number of probes and number of contacts with olives vs. non-target material).

For *U. africanus*, 15 replicates were completed with yellow starthistle ( $P < 0.02$ , binomial probability distribution), 15 with cherry (ns), and nine with Cape ivy ( $P < 0.2$ ). It appears to specialize on fruit-feeding tephritids. For *U. africanus*, cage studies have proved an unsatisfactory method of evaluating behavior as this species shows very little activity in cages. In 2006, therefore, we tested *U. africanus* in a Y-tube olfactometer, in two way choice tests

comparing its responses to olive fly larvae infesting olives vs. *C. succinea*, *P. regalis* (Cape ivy fly), and *R. indifferens* infesting seed heads, galls, and cherry fruit respectively (Fig. 1). The results clearly indicate that *U. africanus* is not attracted to *C. succinea*/thistle heads, but is attracted to *R. indifferens*/cherries at the same rate as infested olives. Results for Cape ivy galls suggest that this host is not attractive, but are based on an insufficient number of replicates and are not conclusive. Poor colony performance prevented us from completing this experiment this year. In combination with evidence from the field, which indicates that *U. africanus* specializes on OLF, the olfactometry data may prove sufficient to apply for a field release permit of this species.

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## Movement of Citrus Leafminer into the San Joaquin Valley

K. Godfrey and E. Grafton-Cardwell<sup>1</sup>

The citrus leafminer (*Phyllocnistis citrella*) is a serious pest of citrus and can facilitate citrus bacterial canker. Citrus leafminer's geographic distribution is expanding from southern California desert valleys and can now be found in southern coastal counties and the southern portion of the San Joaquin Valley. Its phenology throughout California is not well understood. In the desert valleys, citrus leafminer is active primarily in late summer and fall, and active at low levels in early winter. During the remainder of the year, no activity was detected in citrus (J. Heraty, University of California-Riverside, unpublished data). In San Diego County (Escondido area), citrus leafminer is present only from June through about the end of October, although a few moths can be found as early as March, depending upon weather conditions. In the coastal counties and San Joaquin Valley, very little is known about the phenology of citrus leafminer.

In the desert valleys, nine species of parasitoids attack citrus leafminer. The predominant species are *Closterocerus utahensis* and *Cirrospilus coachellae* (family Eulophidae). Both of these species are generalist parasitoids of leafminer species and typically attack gracillariid leafminers. Larval parasitism in the desert valleys ranged from less than 10% to as high as 70% (J. Heraty, University of California-Riverside, unpublished data). In San Diego and Ventura Counties, three parasitoids, *Closterocerus utahensis*, *Pnigalio* spp., and *Chrysocharis* spp., have been identified attacking citrus leafminer (P. Mauk, UCCE – Southern Region, unpublished data). The parasitoids attacking citrus leafminer in the San Joaquin Valley are not known. Therefore, a study was initiated to investigate the phenology and species composition of parasitoids attacking citrus leafminer in the southern San Joaquin Valley.

The studies were conducted in four geographic areas of Tulare County, located near or in the cities of Porterville, Strathmore, Ivanhoe, and Visalia. A single pheromone-baited trap was placed in a tree at the edge of a citrus grove or in a dooryard at each site beginning 19 April 2006. At the Porterville site, additional traps were placed north and west of the original site on 15 August. At approximately monthly intervals, each trap and lure were replaced. The number of moths found in each trap was recorded. At all sites, the trees surrounding the tree with the trap (or the tree containing the trap for the dooryard tree in Visalia) were inspected for the presence of citrus leafminer larvae. For the sites near Porterville, Strathmore, and Ivanhoe, five terminals on 10 trees were examined for the presence of larvae.

Adult moths first appeared in the trap located near the original Porterville site on 16 May (Porterville Main; Table 1). The traps located near Strathmore, Ivanhoe, and the additional traps located near Porterville began catching moths after 15 August (Table 1). The peak trap catches occurred in traps placed in the field on 29 September (Table 1). By mid-October, trap numbers were declining possibly due to colder temperatures. The differences in trap catches among the different locations were most likely due to the quality or growth characteristics of the citrus variety in which the traps were placed. For example, the trap near Strathmore was placed in a grove of early-maturing navels that have a more lush growth habit than the later-maturing navels found in the other groves that were trapped.

Table 1. The number of citrus leafminer moths captured in pheromone-baited traps during each sampling period at four geographic areas in Tulare County in 2006.

Date of	Location of Traps					
Trap	Porterville					
Placement	North	Main	West	Strathmore	Ivanhoe	Visalia <sup>a</sup>
19 April	-	0	-	0	0	0
16 May	-	1	-	0	0	0
19 June	-	1	-	0	0	0
11 July	-	0	-	0	0	0
15 August	2	1	0	2	1	0
29 September	5	1	5	18	8	0
18 October	3	1	5	3	1	0
29 November	0	0	0	0	0	
28 December	0	0	0	0	0	

<sup>a</sup>Trap removed on 29 November

The trap placed in the dooryard in Visalia caught no moths during the trapping period. This trap was placed in this tree because a large retailer about two blocks from this site had CLM-infested nursery stock found in its garden center on January 31, 2006. This tree was the closest lemon tree to the large retailer.

In the survey of foliage in the groves, one mined leaf with a CLM larva inside was found on 18 October in the grove near Strathmore (Fig. 1). No other mined leaves with larvae were found in the surveys.



Fig. 1. Citrus leafminer larvae mining the underside of a young citrus leaf.

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## Biological Control of the Vine Mealybug in Central Valley Vineyards

K. Godfrey and K. Daane<sup>1</sup>

The vine mealybug, *Planococcus ficus*, is a serious pest of vineyards throughout much of the grape-growing regions of California. This insect causes direct damage to the berries, causes decline in the vines and may vector some leaf-roll viruses. Current management programs rely heavily on insecticides, and more sustainable management programs are needed. Biological control will be one component of the sustainable management program for vine mealybug. A cooperative project with the University of California-Berkeley was established in 2005 in an attempt to increase the contribution of biological control to the management of vine mealybug populations. In the present study, vineyards in the Central Valley were established as parasite release sites, and a colony of one of the exotic parasites, *Anagyrus pseudococci* (Sicilian strain), was established at the CDFA Biological Control Facility in Sacramento. The parasite colony was established in late fall of 2006, too late for releases of the parasites in 2006. The objectives for 2006 were to identify two vineyards for future parasitoid releases and characterize vine mealybug densities and resident *A. pseudococci* activity prior to the release of the Sicilian strain.

Two vineyards located in San Joaquin County were identified as parasite release sites for the Sicilian strain of *A. pseudococci*. The owners of the vineyards agreed to not spray insecticides on a small portion of each vineyard, so the area could serve as a parasite nursery. Prior to the release of any parasites, the vine mealybug and native parasite populations in both vineyards were characterized. In late summer, 100 vines in each vineyard were rated as to vine mealybug density using the following scale: 0 = no vine mealybug present; 1 = a few vine mealybugs present; 2 = large number of vine mealybugs present. In addition, any clusters found on these vines were rated for vine mealybug damage using the following scale: 0 = no damage visible; 1 = honey dew and a few vine mealybugs present; 2 = honey dew and more than 10 vine mealybugs present; and 3 = cluster unmarketable due to honey dew and vine mealybugs. Collections were also made of mealybugs on the bark, leaves, and if available, clusters. These mealybugs were placed individually in gelatin capsules and held in the laboratory for parasite emergence. Three traps containing vine mealybug pheromone were also placed in each vineyard. These traps were replaced on monthly intervals and the number of male vine mealybugs within each trap counted.

The two vineyards differed somewhat in their vine mealybug densities (Fig. 1). The vineyard in Galt had fewer vines with large densities of vine mealybug than the vineyard in Walnut Grove. Argentine ants tended the vine mealybugs in the Walnut Grove vineyard, while native fire ants tended those in the Galt vineyard. The vineyard in Walnut Grove had been harvested prior to mealybug sampling, so no clusters sampling was done at this vineyard. In the Galt vineyard, 82% of the clusters sampled had no damage, 9% had a honey dew and a few mealybugs present, 8% had honey dew and more than 10 mealybugs, and only 1% were unmarketable. Native *A. pseudococci* were reared from collections of mealybugs made from leaf samples in the Walnut Grove vineyard. The parasitism rate was very low (3.31%). No parasites were recovered from samples from the Galt vineyard. Trap catches of males were very large from September through November and declined dramatically in December with the onset of colder temperatures.

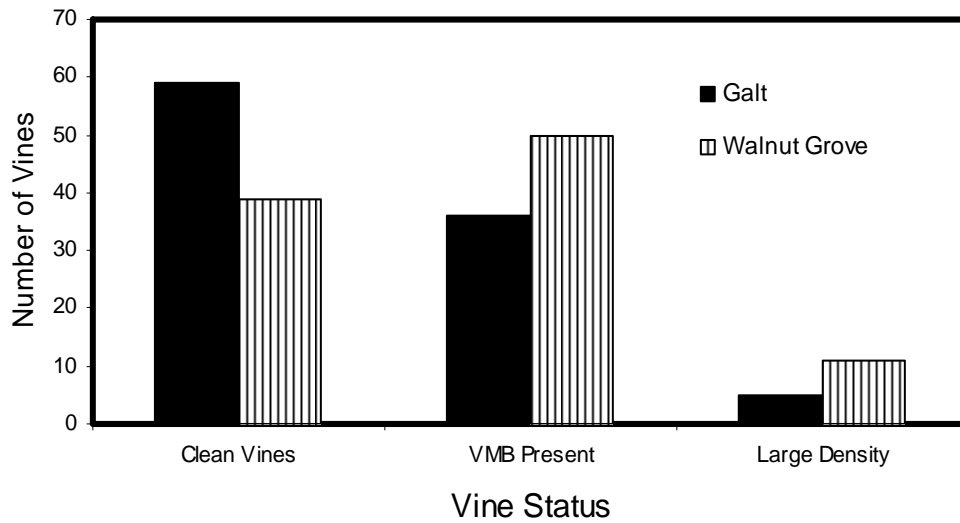


Fig. 1. The number of vines in each vine status category for two vineyards in San Joaquin County in the fall of 2006.

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## Avocado Lace Bug Geographic and Seasonal Population Patterns in San Diego County

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In the 1990's, outbreaks of avocado lace bug (AvLB), *Pseudacysta perseae*, occurred in Florida, and were believed to represent a secondary pest outbreak resulting from death of its natural enemies by pesticides applied against other avocado pests at the time. In recent years, the AvLB has expanded its range into the Caribbean, resulting in devastating leaf damage and production loss in avocado groves. Feeding damage typically results in the formation of large necrotic areas on the foliage (Fig. 1).

Found in San Diego County in late summer of 2004, AvLB has occurred solely in urban landscapes and has not been detected in commercial groves. High population densities have caused significant leaf damage and leaf drop at a number of residential locations. A cooperative project to manage this pest has been initiated between CDFA; the University of California, Riverside; the California Avocado Commission; and San Diego County.

A survey throughout San Diego County in late spring of 2006 indicated that AvLB was limited to areas south of Interstate 8 (Fig. 2, only sites with avocado are represented). An additional survey was conducted in the fall of 2006, including

78 residential sites, each with avocado trees, located north of Interstate 8. In this survey, AvLB was present at several new locations, illustrating a limited expansion of its range in the county. In addition to a site near Lake Murray (right blue cross) where it was found to occur in 2004, the new area of infestation included two new sites located in the vicinity of La Jolla (Fig. 2).



Fig. 1. Adult avocado lace bugs, eggs, nymphs, and leaf feeding damage.

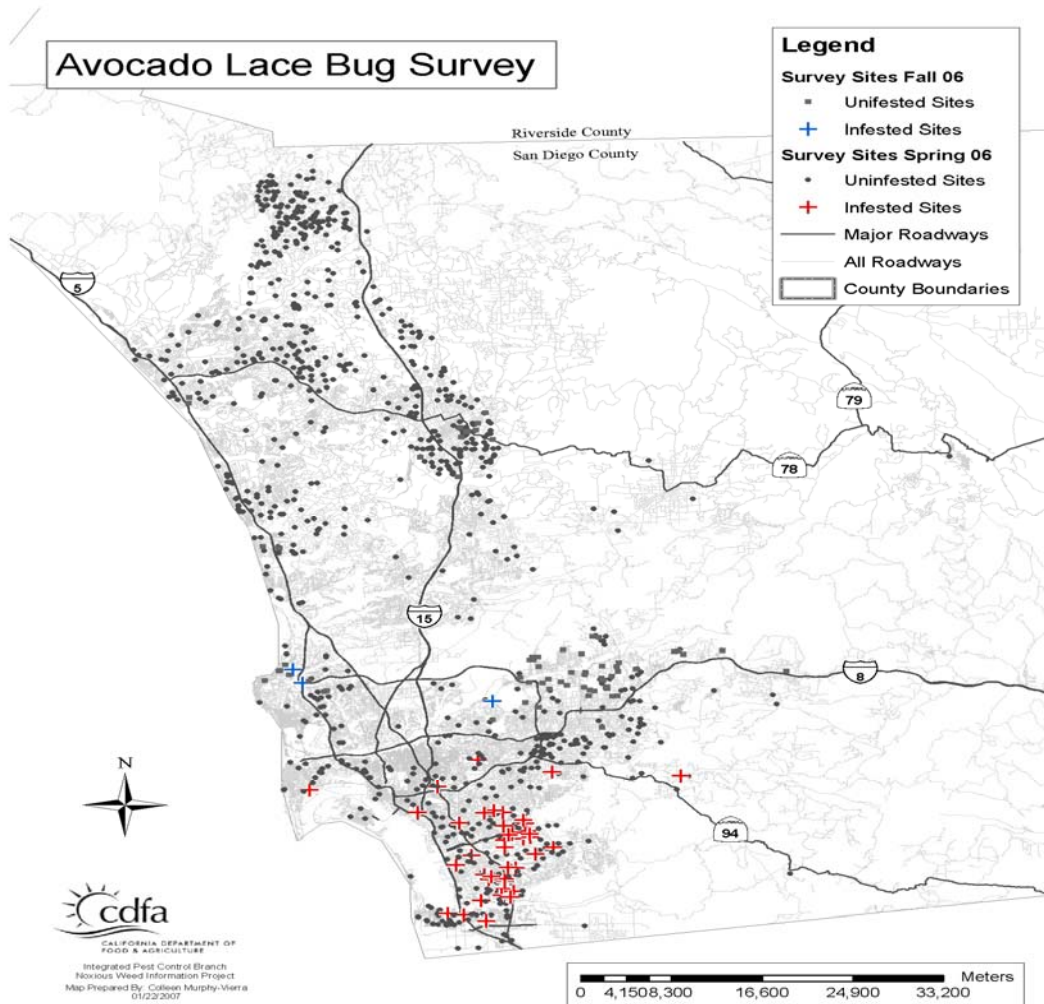


Fig. 2. Results from 2006 spring and fall survey in San Diego.

Since July of 2005, repeated monthly sampling of populations at approximately six residential sites indicated that on average, population levels were greater in 2006 than in 2005 (Fig. 3). However, increases in density did not occur at all sample sites. Whereas densities at two sites in 2006 were very high, densities at several other sites decreased markedly. These findings are exemplified by high standard error values in the data (Fig. 3). Additionally, densities in 2006 were somewhat inflated compared to those in 2005 because a site with moderate densities was lost due to a homeowner insecticide spray application, and was replaced by a site with high densities.

Because classical biological control using egg parasitoids is being pursued, we have been particularly interested in egg survivorship under field and laboratory conditions. Presently, AvLB in California does not appear to be attacked by egg parasitoids, nor has any other life stage been found to be parasitized. To determine egg fate under field conditions, unhatched egg masses were identified at several sites. Eggs were counted using a 10X hand lens, the egg mass was encircled with the ink from a black marking pen, and each leaf was flagged using orange ribbon. After approximately 30 days, leaves were returned to the lab where the number of eggs was

counted, recording the number of hatched and unhatched eggs. This was done successfully in July and September of 2006 at two sites each month. Eggs at a third site could not be assessed because mold grew over the eggs by the time they were collected, preventing the determination of egg fate. Egg hatch in July and September was found to be more than 70% at these field sites (Fig. 4). The impact of mold is unknown. In contrast to these field observations, egg hatch among eggs held under laboratory conditions (i.e., eggs on leaf sections and severed leaves) has been dramatically lower, even when leaf portions are held at 60% RH. Currently, methods are being developed to improve egg hatch under lab conditions in order to provide greater flexibility in rearing egg parasitoids in the event that promising egg parasitoids are found during foreign exploration.

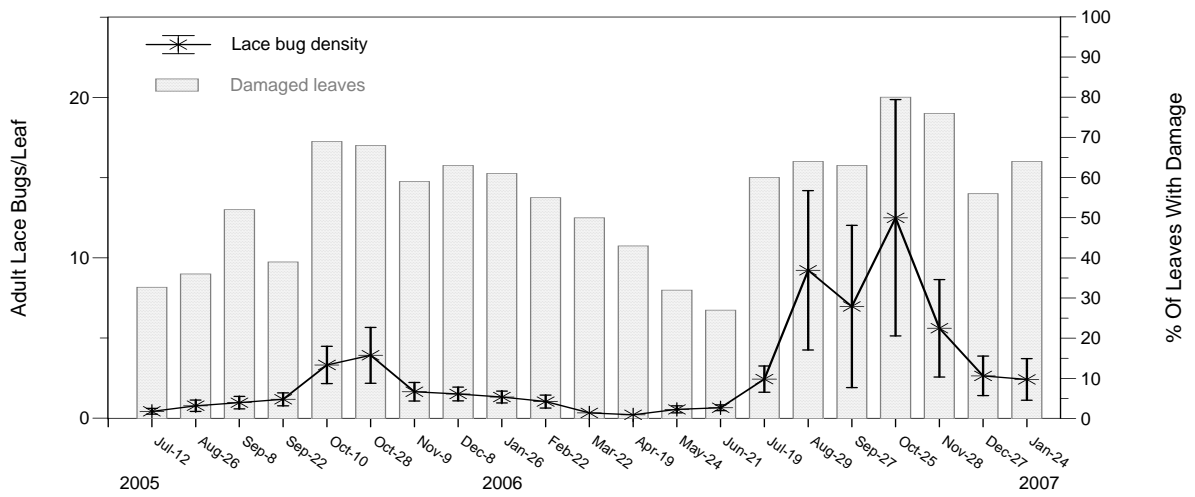


Fig. 3. Adult lace bug densities at sample sites in San Diego County and associated leaf damage.

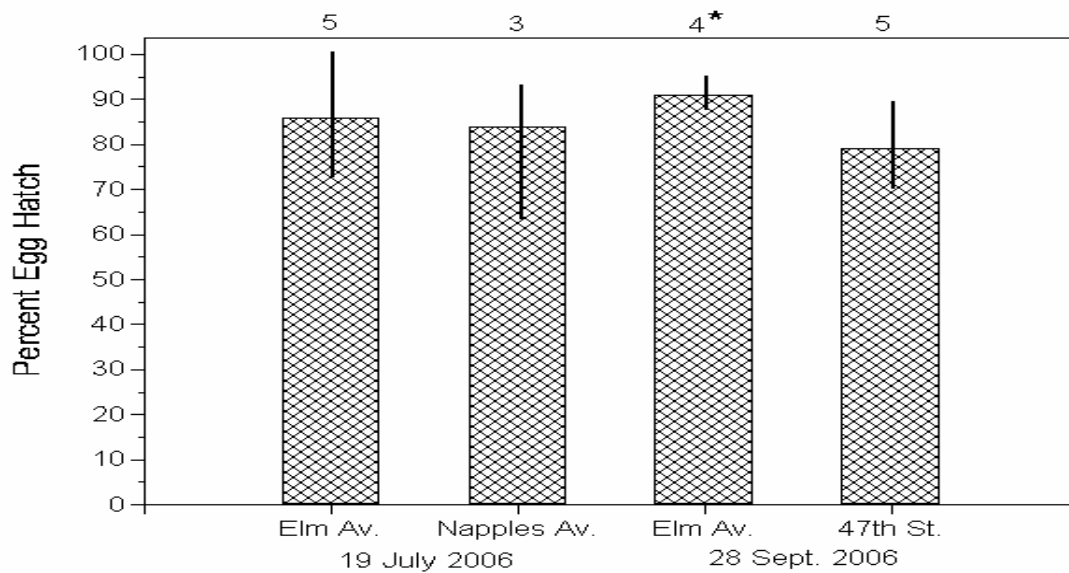


Fig. 4. Mean egg hatch recorded under field conditions during July and September 2006 at two locations each month. Vertical lines represent the range of percent hatch recorded among egg masses. Number of egg masses is noted above bars. The asterisk denotes that percent hatch (5%) for one egg mass (a fifth egg mass) was removed as an outlier.

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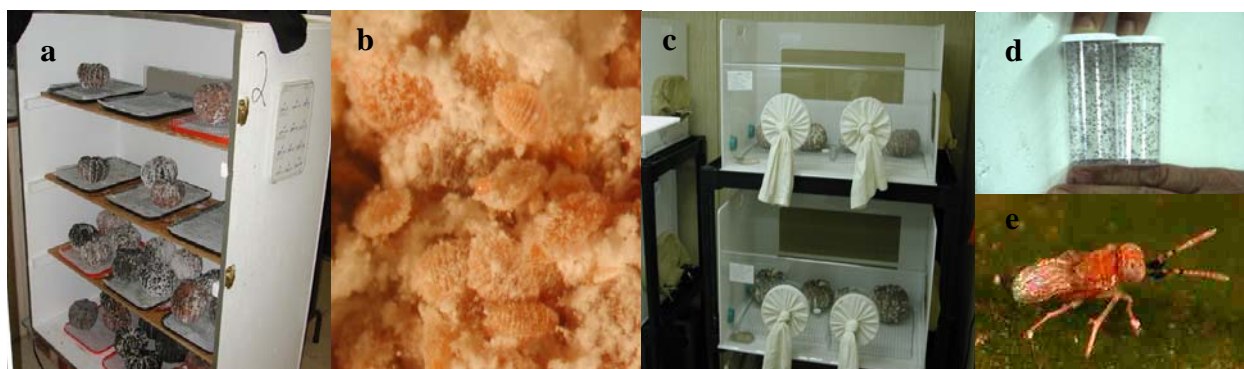
## Update of the Pink Hibiscus Mealybug in Imperial Valley and Parasitoid Insectary Production

W. J. Roltsch, J. Zuniga and R. A. Aguilar

In August 1999, the pink hibiscus mealybug, PHM, *Maconellicoccus hirsutus* (Green), was found in North America for the first time in the Imperial Co. communities of Calexico and El Centro, California, and in the northern portion of the neighboring city of Mexicali, Mexico. Shortly thereafter, an insectary was setup in El Centro, CA, in cooperation with USDA-APHIS and Imperial County Agricultural Commissioner's Office. From this insectary, three species of parasitoids from Egypt, Pakistan, Australia and areas of Asia were reared, and released throughout the infested area of Imperial Co. during the next five years.



Fig. 1. Two trailers setup as insectaries for rearing the pink hibiscus mealybug and its parasitoids in El Centro, CA.



Figs. 2 a-e. a) Production of mealybugs on infested squash. b) Mealybug life stages. c) Parasitoid rearing cages. d) Adult parasites prepared for release. e) Adult *Anagyrus kamali*.

Studies on the impact of the parasitoid complex from 2000 to 2004 showed that two parasite species (*Anagyrus kamali* and *Gyranusoidea indica*) have become permanently established in the region and provide over 99% control of the PHM. Without this protection, many species of ornamental trees and shrubs would have been destroyed in these communities and the spread of PHM to other regions of the state would have rapidly occurred, likely impacting several commercial crops. A check on the population density in late August of 2006 showed that population densities of PHM continues to exist at a very low level. Of 64 terminals from mulberry and carob at eight separate locations, PHM was detected on two, with an average



of .3-second instar to adult mealybug per terminal. There continues to be no reports of the pest within California outside of Imperial County.

In recent years the PHM has been found in Florida (2004) and Louisiana (2006). As a result, USDA has contracted with the CDFA Biological Control Program to continue production of the parasites for shipment to these states (Table 1).

Table 1. Number of parasitoids shipped to Florida and Louisiana in 2006.

<b>Date Sent</b>	<b>Recipient</b>	<b><i>A.kamali</i>- strain: Hawaii/China</b>	<b><i>A. kamali</i>- strain: Taiwan</b>	<b><i>G. indica</i>- strain Egypt/Australia</b>
January 2006	Florida		3,000	46,400
February	Florida	4,800	7,000	8,000
August	Florida	5,00	3,500	
September	Florida	4,400	16,800	12,500
October	Florida	2,000	2,800	1,800
October	Louisiana	6,750	8,800	11,800
November	Louisiana		4,500	2,000
December	Louisiana	1,800	3,300	7,300
<b>Totals</b>		<b>20,250</b>	<b>49,700</b>	<b>89,800</b>

In 2006 a total of 159,750 parasitoids were produced and shipped to Florida and Louisiana. Production numbers were considerably lower than during previous years for two reasons. First, the Japanese pumpkin crop that we grow in Imperial Valley for mealybug production was almost entirely destroyed by early, exceptionally high temperatures of over 48° C. These squash are relied upon as host material for producing mealybugs in large numbers for parasitoid production. Second, the mealybug host population became contaminated with *G. indica*, further diminishing our ability to produce parasitoids. These two events created a major season-long challenge for the production of parasitoids.

## Releases of Two *Galerucella* Leaf Beetles for the Biological Control of Purple Loosestrife in Butte and Fresno Counties

B. Villegas and D. Kratville<sup>1</sup>

The two leaf-feeding beetles, *Galerucella californiensis* L. and *G. pusilla* (Dufft.) (Coleoptera: Chrysomelidae), were released at two purple loosestrife infestations during 2006. The leaf beetles were collected from the Big Lake cove site bordering Ahjumawi Lava Springs State Park near McArthur, California (Figs. 1-3). Collections at this site had been difficult in the past as the site is a swamp and the borders of the lake are difficult to penetrate due to the thick vegetation. During the 2005, season several collection methods were tried without much success due to the shallow water in many areas infested with loosestrife and the absence of a solid lake bottom. In 2006, an airboat was used to penetrate the shallow areas of Big Lake, Horr Pond, and adjacent rivers in order to survey the loosestrife infestations as well as gain access to safe sites with good leaf beetle populations.



Figs 1-4. 1) Air boat used for making surveys and collections along shallow riparian areas. 2) Baldo Villegas making collections from the bow of the air boat. 3) Baldo Villegas making collections in solid ground areas along the cove at Big Lake. 4) Release site along the Kings River site in Fresno County (Picture taken by the CDFA Purple Loosestrife Control Program).

Approximately 8000 leaf beetles were collected on August 23, 2006. About 95% of the beetles collected appeared to be *Galerucella californiensis*. Of these, about 5000 beetles were released at a site on Wyman Ravine near Lone Pine Road in Palermo, Butte County, CA. The

other 3000 beetles were released on August 24, 2006 at a loosestrife infestation located in a creek that empties into the Kings River in Sanger, Fresno County, CA (Fig. 4).

The two release sites were chosen because they are not subject to flooding and cattle grazing as have been the problems in other release sites in California. It is hoped that the beetles will become established in these two sites and are able to control the loosestrife.

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<sup>1</sup>California Department of Food and Agriculture, Purple Loosestrife Control Program, Sacramento, CA.

## Releases of the Chevronated Waterhyacinth Weevil for the Biological Control of Waterhyacinth in Coastal Santa Cruz County, California

B. Villegas and J. Brown

The chevronated waterhyacinth weevil, *Neochetina bruchi* Hustache (Coleoptera: Curculionidae), was released in the San Joaquin-Sacramento Delta region in the 1980's for the biological control of waterhyacinth. The weevil has become well established throughout the Delta region and is achieving some level of biological control. The weevil (Figs. 1 & 2) damages waterhyacinth by feeding directly on the foliage. Its larvae cause extensive damage on the leaf blades and petioles and the plant stems resulting in dead or weakened plants.

On September 12, 2006, approximately 400 weevils were collected from the Whiskey Slough area located west of Stockton, California. The weevils were collected individually from waterhyacinth plants showing the distinctive feeding damage by the adult weevils (Figs. 1-3). The weevils were released on September 13, 2006 at a new waterhyacinth infestation that was recently found taking over Freedom Lake in Watsonville, CA (Fig. 4). The weevils were released at this site in the hopes that the weevils would become established on the waterhyacinth plants and eventually control the infestation.



Figs. 1-4. 1) *Neochetina bruchi* adult and on leaf surface. 2) Feeding scars made by adult *Neochetina* weevils and indicative of their presence. 3) UC Davis student collecting weevils at Whiskey Slough, San Joaquin County. 4) Santa Cruz County biologist releasing the *Neochetina* weevils at the edge of Freedom Reservoir in Watsonville, CA.



## Establishment of the Crown Weevil, *Phrydiuchus tau*, for the Biological Control of Mediterranean Sage in Northeastern California

B. Villegas, C. Gibbs<sup>1</sup> and K. Haas<sup>2</sup>

Mediterranean sage, *Salvia aethiopsis* L. (Lamiaceae), is strongly aromatic and distasteful to cattle, horses, and wildlife. It is also a highly invasive weed that has become well established in open rangeland, pastures, roadsides, and abandoned fields in Modoc and Lassen Counties in northeastern California. In other areas of the state, the weed has been eradicated or it occurs at extremely low levels. Mediterranean sage is native to the Mediterranean area of Europe and Northern Africa and into western Asia. This biennial plant produces rosettes the first year and during the second year, the plants bolt producing a flowering stock with numerous white flowers. The plants then dry up and break off at the soil level and travel across the roads and open lands like small tumbleweeds spreading seed.

*Phrydiuchus tau* Warner (Coleoptera: Curculionidae) is one of two weevils introduced in 1971 from Europe for the control of Mediterranean sage. The adult weevils impact the plants by feeding directly on the leaves (Figs. 1 & 4) and by the larvae burrowing and girdling the crown of the plants. Severe adult feeding oftentimes results in stressed and weakened plants that die without bolting and producing seed.



Figs. 1-4 (left to right): 1) *Phrydiuchus tau* weevil on a Mediterranean sage leaf; 2) Collection container with weevils ready to be released; 3) Carolyn Gibbs releasing weevils at a Lassen County site; 4) Mediterranean sage plant showing leaf feeding damage by the weevils indicative of their presence.

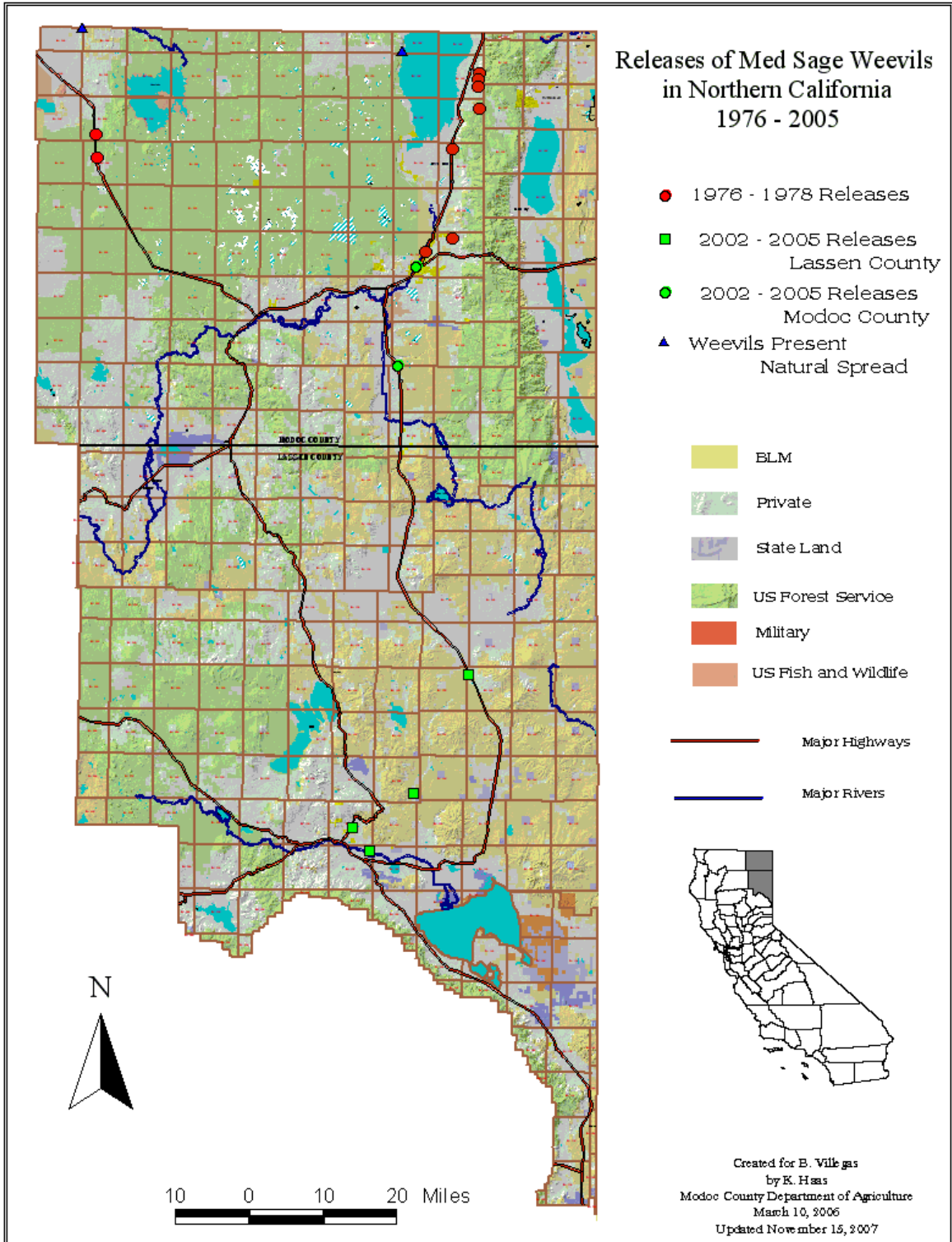


Figure 5: Map of Modoc and Lassen Counties showing release sites and survey points for the Mediterranean sage weevil.

Between 1976-1980, a total of 13 releases totaling 2600 weevils were made at 10 sites in Modoc County in an attempt to establish the weevil (Fig. 5). Unfortunately, no organized surveys were made at the time to determine if the effort was successful. A second effort to start a biological control program was made between November 2002 and June 2005 with the weevil collections made in southern Oregon near Summer Lake and Lake Abert. This effort resulted in 1500 weevils released at two sites in Modoc County and 3781 weevils released at four sites in Lassen County (Figs. 2, 3 & 5).

Surveys for establishment at previous release sites were done during 2005 and 2006. All release sites were surveyed at least once. An attempt to find and survey the 1976-1978 release sites was made twice. In the overall survey effort, the weevils were found at three sites that indicate long distance movement from previous release sites by the weevils in northern Modoc County. One such place was a site in the west side of Goose Lake and a second site located at the Oregon-California State line, where no releases took place. The third site was north of Alturas near the junction of Highways 395 and 299, near a 2005 release site. In most of the sites where releases were made in 1976-1978, the Mediterranean sage was at low population levels or almost absent. Weevils were found on plants located in undisturbed areas and areas of low human interference. On disturbed areas and areas along major highways and roads, the plants were devoid of weevils or feeding damage. This could be attributed to Modoc County Department of Agriculture's Weed Control Unit's aggressive weed control program which sprays any targeted weed growing within the roads' shoulders.

The same conclusions can be arrived at with the 2002-2005 release sites. At the Bureau of Land Management (BLM) sites located near the Belfast Tablelands and Ravendale, the weevils established very well. At the other two sites located east of Susanville on Road A27 and at the Old Susanville Landfill, the weevils did not establish well. We will be looking into the reasons why the weevils failed to show a good establishment.

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## Long-term Population Changes in Yellow Starthistle at Hairy Weevil Release Sites

D. M. Woods, M. J. Pitcairn, and V. Popescu

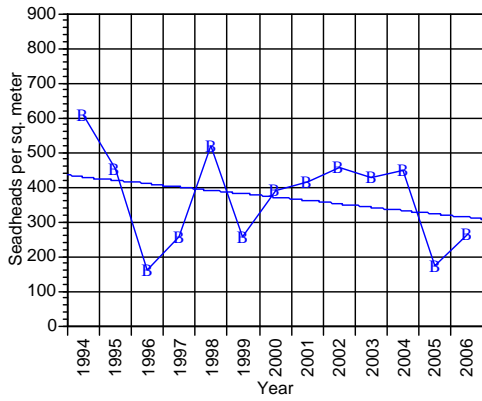
Classical biological control of weeds is an inherently long-term process. Estimates of 10 to 20 years are not uncommon to achieve success. Yellow starthistle is expected to be a long-term project because of the extremely large number of seeds produced by healthy plants and the highly plastic nature of plant adaptation to environmental stresses. In spite of notable indications of success in some sites in California, not all locations in the state seem to be experiencing population level impacts that can be associated with biological control agent development. The hairy weevil, *Eustenopus villosus*, is considered one of the more successful biological control insects on yellow starthistle. Seed destruction as well as bud kill seems to be high at many sites due to the weevil. This report includes a preliminary analysis of the impact of the hairy weevil acting principally on its own.

Two sites were selected for the earliest releases of the hairy weevil in California. The Dog Bar site in Nevada County was the first site in the state to receive releases of the weevils (1990). The Mead site in Napa was the third site, receiving weevils in 1991. In 1994, we established monitoring transects at each site. Each fall, the total number of yellow starthistle plants was counted in 40 20x20cm squares. The total number of seedheads was also counted. The results are shown in Fig. 1.

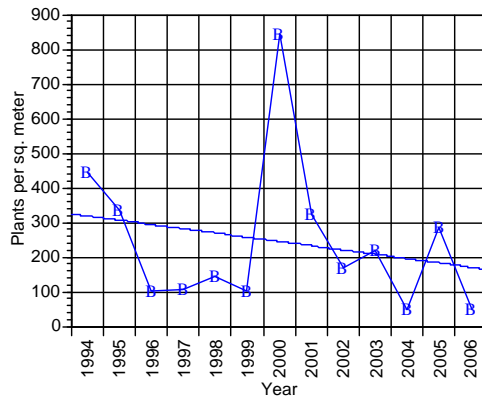
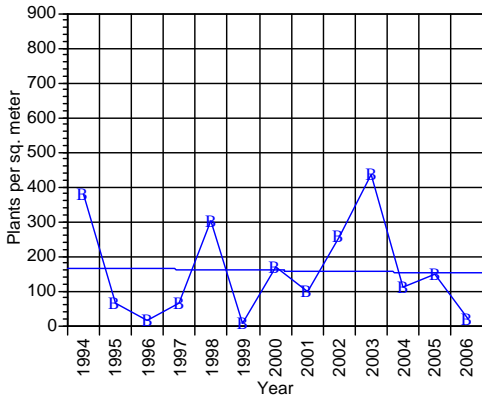
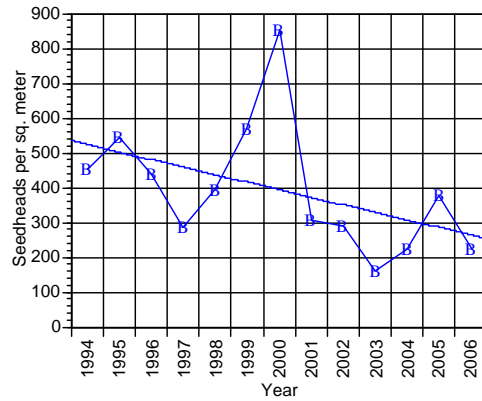
Both sites showed a decline in plant number during the term of the study but neither site demonstrated a dramatic decline. Declines were more significant at the Napa site, but the variation was very high year to year. Both sites had substantial declines in seedhead density over the project life but again variation was high year to year. Both sites are primarily infested with the hairy weevil, with year-to-year variation due primarily to seasonal environmental differences. Attack at the Dog Bar site varied between 35-80% of the seedheads, while Napa varied from 60-85% attack. The lone exception was at Napa where only 12% of the heads were attacked by the weevil in 2000. A wet winter followed by a moderate spring allowed an uncharacteristic percentage of plants to survive, far too many for the number of emerging weevils to attack. Both sites do have a limited population of other biological control agents that migrated from other starthistle infestations. The total attack rate for the other agents never exceeded 10% in any single year.

Fig. 1. Repeated measure of yellow starthistle plants at two sites in California. The mean number of seedheads per square meter is shown in the upper graphs, the mean number of plants is in the lower two graphs. Data for Dog Bar is on the left and Napa on the right. Linear regression lines are included on each plot.

**Dog Bar Site (Nevada County)**



**Mead Site (Napa County)**



## Seedling Emergence Trends in Spotted Knapweed

D. M. Woods and V. Popescu

Seven biological control agents have been established at the spotted knapweed biological control site on the banks of the Pit River in Northern California. One of the insects, *Larinus minutus*, is by a wide margin, the most successful biological control agent at this site. Attack rates are routinely high with over 70% of the seedheads attacked in any single year. Additional root attacking insects including *Cyphocleonus achates*, may contribute to a reduced lifespan for this perennial weed. Presumably, the extensive seed destruction will over time translate into reduced seedling emergence and the root agents will impact emerged plants. We established a permanent transect at this site with five one-square meter plots setup to monitor seedling emergence, rosette establishment and eventually plant flowering. Each spring beginning in 1999, seedlings emerging in each plot were counted at peak emergence. Mortality was ascertained during the year by recounting each plot. During the fall, surviving plants were individually marked with a miniature numbered flag. Each subsequent spring and fall, flagged plants were monitored and re-flagged. The data are tabulated for each years emerging cohort in Table 1.

Table 1. Survival of spotted knapweed seedlings emerging in plots in Shasta County.

Year of Emergence	Spring Seedlings	One Year Old	Two Year Old	Three Year Old	Four Year Old	Five Year Old
1999	97	3	0			
2000	776	7	0			
2001	1272	4	1	1	1	1
2002	7347	25	12	10	5	
2003	247	0				
2004	923	0				
2005	3669	63				
2006	55					

There was a very large year-year variation in the number of seedlings emerging in the plots. Seedling emergence is primarily controlled by three variables; seed input from the previous year, environmental effects on germination, and the residual seed bank. The successful establishment and population development of the seedhead insects has led to a drastic reduction in seed production at the site. Preliminary estimates indicate that for the entire location, 4,129 seeds fell per square meter. Declining plant numbers, seedhead numbers and seed production per seedhead resulted in 1,568 per square meter in 2000, and only 1,224 in 2002. The monitored plots had a higher seedhead density than the site as a whole so seed rain in these plots was likely higher. None-the-less, a trend of

declining seed numbers seems to exist across the area. Sufficient seed still seems available to produce a viable knapweed population each year. Environmental constraints seem the dominate factor in seedling emergence. During the years of this study, significant variation has occurred in the amount and distribution pattern of winter rainfall. Also winter temperature effects may be relevant. The extremely high mortality from seedlings to rosettes seems the controlling factor at this site. Only four plants have matured to seed production during the course of this study (the fifth is remaining as a rosette).

## Early Season Impact of the Rust Fungus, *Puccinia jaceae* var. *solstitialis*, on Yellow Starthistle, Leaf Maintenance

D. M. Woods and V. Popescu

Yellow starthistle, *Centaurea solstitialis*, has proven to be a highly invasive plant in California and the western United States. It is able to function over a fairly wide geographic area and has covered large acreages. Biological control is considered an essential component of long-term management for such widely distributed weeds and an active program exists for yellow starthistle. Some biological control agents, particularly plant pathogens, have the ability to negatively impact plant fitness by reducing photosynthetic area. The rust fungus, *Puccinia jaceae* var. *solstitialis*, was the first plant pathogen approved for release as a biological control under the modern permit and review system. Decades of research and work went into the preparation of host specificity documentation insuring a safe release of the pathogen. Impact studies were necessarily limited during the pre-release evaluations due to the difficulty of performing those studies in a quarantine environment. Impact studies have had to be postponed until the pathogen was permitted, released and established in sufficient numbers to establish substantial field plots.

We established a field plot in Sacramento to evaluate various aspects of the rust's impact on yellow starthistle. Yellow starthistle seeds were sown on wet blotter paper for three to five days then transplanted to four-inch plastic pots in a commercial soil mix. Potted plants were grown on benches in a greenhouse for five to seven weeks. Plants were then transplanted into a field plot and allowed to adjust for another 10 days. Subplots were inoculated with a spore suspension of various amounts of rust spores in 100 mls. water and three drops Tween 20 (polyoxyethylene sorbitan monolaurate) as a wetting agent. Three replicates of 10 plants each, were inoculated with spore suspensions of 0, 25, 50, 100, or 200 mg *P. jaceae* urediniospores in 100 mls of water. Inoculated plants were covered with a short plastic tent overnight to maintain an ideal environment for spore germination and penetration.

Mature pustules began to appear at 10 days, and we began monitoring leaf development and rust development on all plants. Leaf evaluations were made every seven days for 12 weeks. A portion of the results are shown in Fig. 1 and Fig. 2. Successful infection of plants with the rust caused increased leaf mortality in all cases for the first 10 weeks of monitoring. By that time, a substantial portion of the non-inoculated plants became naturally infected with the rust. The higher levels of inoculum initially caused higher pustule density on the leaves, which results in a generally shorter leaf lifespan. The greatest effect was five weeks into the monitoring, when the most diseased plants were losing as much as five times as many leaves as the non-inoculated plants. Per plant leaf loss then evened out between the treatments such that inoculated and non-inoculated plants were losing leaves at the same rate. Most plants were however becoming universally infected at a lower level than the actual experimental inoculation. The early season leaf loss was not compensated for by the end of the experiment. The initial setback has a distinct and permanent effect.

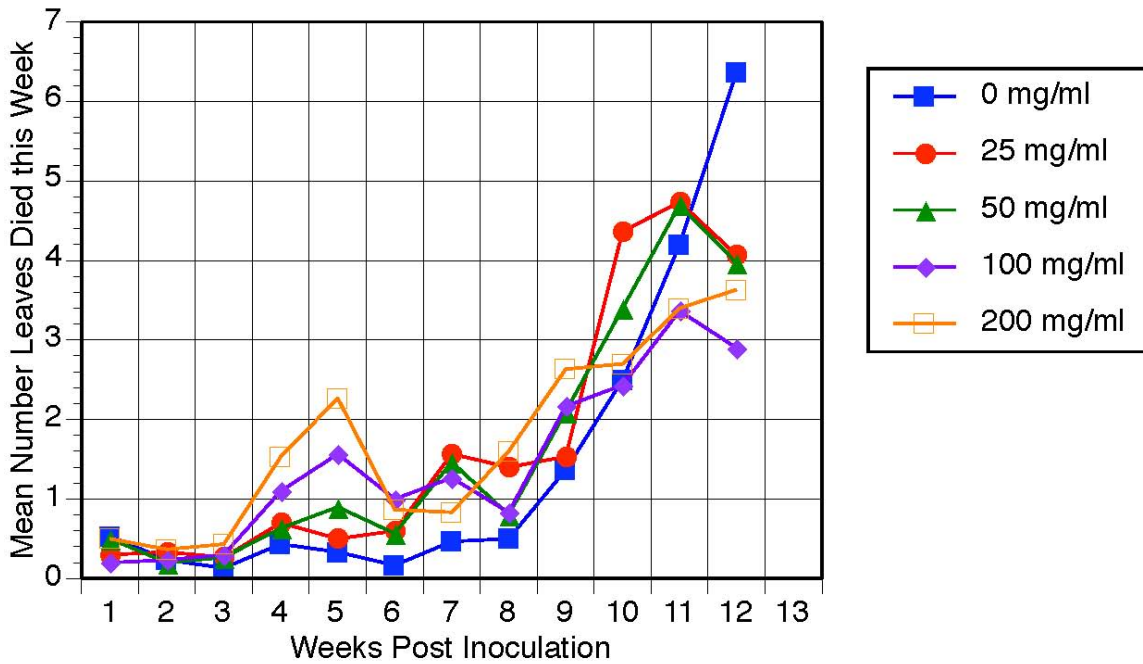


Fig. 1. Impact of various inoculation concentrations of *Puccinia jaceae* on yellow starthistle leaf mortality. Data are the per plant mean of dead leaves during the previous week for 30 plants at each spore concentration.

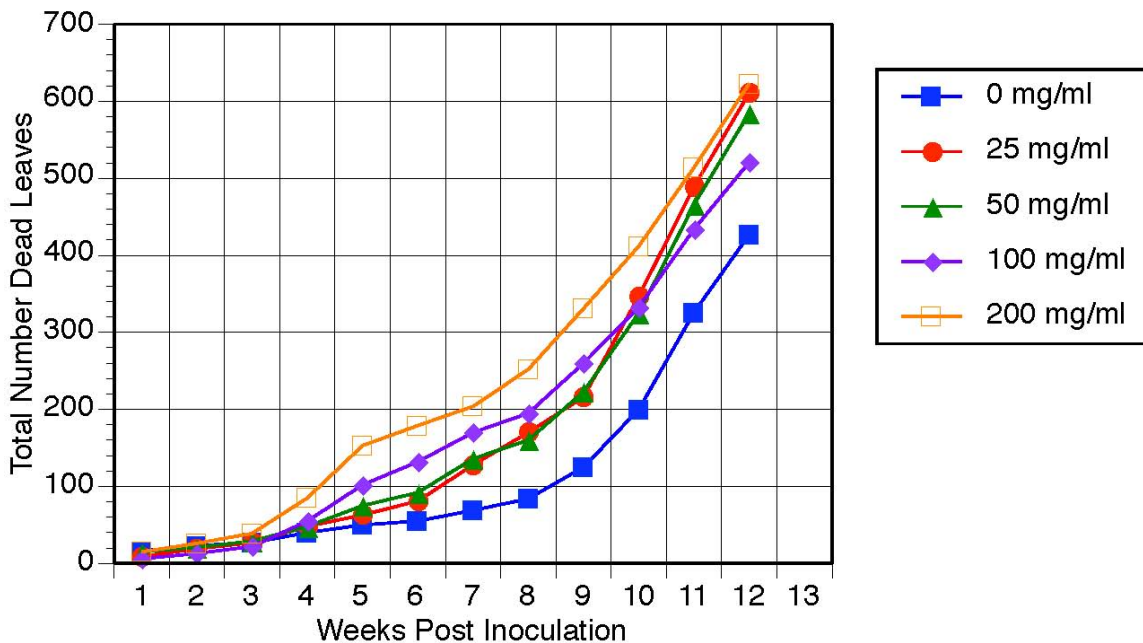


Fig. 2. Impact of various inoculation concentrations of *Puccinia jaceae* on yellow starthistle leaf mortality. Data are the accumulated sum of dead leaves for 30 plants at each spore concentration.

## A Third Season of Distribution of the Yellow Starthistle Rust Fungus

D. M. Woods B. Villegas and V. Popescu

The rust fungus, *Puccinia jaceae* var. *solstitialis*, was approved for release as a biological control of yellow starthistle, *Centaurea solstitialis*, in California in 2003. Since that time extensive efforts have been made to distribute the rust around the state into as many environmental situations as possible. The California Department of Food and Agriculture in cooperation with the California Agricultural Commissioners and Sealers Association have created an extensive network to facilitate that large-scale distribution. This report outlines the most recent distribution effort in California, an effort that resulted in rust spores being released in 71 locations over 32 counties during 2006.

Greenhouse production of the rust occurred at our Meadowview facility and the spores were stored prior to release. County agriculture departments were given the opportunity to participate in the release program. Counties with substantial yellow starthistle infestations were provided enough urediniospores for one to four releases. The California Department of Food and Agriculture also provided equipment for effective releases. Because this was the third year of releases, field locations that might be severely problematic but not ideal for establishment were considered. Sites in active cattle ranches, near traditional crop agriculture and similar sites were inoculated. The field survey results are not complete but we expect to evaluate all sites in 2007.

The 2006 releases came on the heels of successful release programs in 2004 and 2005. Field studies in 2004 had demonstrated that greater success might be achieved with a smaller amount of rust released at each site than was widely used in 2004. Consequently, we reduced the amount of rust applied at each location in 2005 and 2006. County agricultural biologists were provided with a 100 mg sample of rust spores (200 mg in 2004) and a 'dew tent' made of PVC pipe covered with black plastic sheeting. Biologists could then return to their sites and perform the inoculation, covering the plot overnight with the dew tent to maintain heat and moisture. Each inoculation was designed to be one square meter in size and remained covered overnight with the dew tent. Biologists removed the dew tents the morning following inoculation, then continued to monitor the sites regularly for the first appearance of disease symptoms (two to four weeks) as well as natural spread from the inoculated plants. Infection results are not yet tabulated, but preliminary indications are that the infection rate is somewhat reduced from previous years.



## Status of the Two *Chaetorellia* Seedhead Flies Introduced into California for the Biological Control of Yellow Starthistle

B. Villegas

A survey was carried out during the 2004 and 2005 seasons in California to determine the status of the peacock fly, *Chaetorellia australis* Hering, and *C. succinea* (Costa), the false peacock fly, on yellow starthistle, *Centaurea solstitialis* and on bachelor button, *Centaurea cyanus*. Introductions and releases of *C. australis* occurred between 1988-1994 (Turner, et al., 1996). The first releases were made with flies emerging from seedheads of bachelor button. These seedheads were collected in the Oreokasto area of Greece and sent to the Albany USDA-ARS quarantine containment facility. There, the adult flies were allowed to emerge from the seedheads and then sent to cooperators in California, Oregon, Washington, and Idaho where the flies were released on to yellow starthistle infestations. In California, site visits were made by both USDA-ARS and CDFA personnel from 1988 through 1993 at all release sites, but no flies were ever recovered at any of the sites. In other states, recoveries of the flies were made at some of the sites where the flies were released. From 1994 to 1996, collections of *Chaetorellia* flies were made from a site in southern Oregon, and released at 26 sites throughout California (Table 1). The sudden recoveries of *Chaetorellia* flies at all release sites as well as at other yellow starthistle monitoring sites in the fall of 1995, led to a suspicion that another fly species could have been accidentally introduced from Greece. However, it wasn't until July 1996 that specimens were identified as *Chaetorellia succinea* (Costa) by fly taxonomists Louis Blanc and Eric Fisher from the California Department of Food and Agriculture in Sacramento. The identity of the flies was later verified by Dr. Ian White at the British Museum of Natural History who is a known authority on the taxonomy of *Chaetorellia* flies. Voucher specimens and records retained at the Albany USDA-ARS quarantine containment facility subsequently showed that a 1991 shipment of yellow starthistle seedheads originating from Oreokasto, Greece, contained the contaminant species, *C. succinea* as well as the peacock fly, *C. australis*. No other shipments apparently had the contaminant fly (Balciunas and Villegas, 1999). To date, *C. succinea* has become established at almost all yellow starthistle infestations in California. This widespread establishment of the false peacock fly led to the need to find out the current status of the intended biological control agent of yellow starthistle, the peacock fly, *C. australis*.

For this survey, two different collection periods were undertaken as bachelor button develops and blooms about four to six weeks before yellow starthistle. All *Chaetorellia* release sites (Table 1) were surveyed in 2005 and 2006 to determine the predominant host plant and the ideal time to collect a mixture of seedheads likely to be infested by the *Chaetorellia* flies. Collection of the bachelor button samples occurred from May through August (Table 2) while those of yellow starthistle occurred from July-August (Table 3). If the site was no longer available due to development, fire, inaccessibility, etc., another site was chosen as close to the original release site as possible. At least 100 seedheads were collected from all sites with an ideal sample size of 300 seedheads as a goal. Collection of bachelor button seedheads was made at all locations where they were seen growing, either as naturalized populations or in private gardens. Whenever possible, a collection of yellow starthistle seedheads was made from all bachelor button collection sites. Because of the difference in flowering periods, a second trip to the location was often necessary. After the seedhead samples were collected, the samples were kept

cool and transported to Sacramento, CA where they were transferred to emergence containers and monitored for about six weeks. During this monitoring period, all emerging insects were separated from the seedheads and placed in separate containers for subsequent recording and tallying. After that time, the containers were not monitored until the winter period when the containers were emptied, and any dead insects remaining in the samples were counted and identified. All seedheads were disposed of as soon as they were counted.

Results of the survey show that *C. australis* is more widely distributed than was once thought. In this survey, it was found at all sites containing naturalized stands of *C. cyanus* (Table 2). At the time when the original releases were made, the only naturalized stands known in California and southern Oregon were located in the mountainous areas of Modoc, Siskiyou, Lassen, Shasta, Plumas, and Mariposa Counties and in Josephine and Jackson Counties in Oregon. This survey found additional stands in Tuolumne, Fresno, and Tulare Counties as well as in nearby Washoe County in the state of Nevada. This survey also found the peacock fly to be present in cultivated stands of bachelor button (e.g., Los Molinos in Tehama County) (Table 2). On yellow starthistle, the peacock fly is most common where bachelor button stands are nearby but hardly ever in more than 10 percent of the seedheads. However, the fly was recovered, at very low levels, from four sites that had no visible bachelor button plants growing nearby.

This study found the false peacock fly, *Chaetorellia succinea*, present at all yellow starthistle sampling sites and in all yellow starthistle samples taken. This fly is a strong flier and has been able to move from its initial release sites to other areas where yellow starthistle grows. Bachelor button is a poor host for the false peacock fly. Before this study, there were no records of this fly attacking bachelor button. In this study, 13 flies were recovered from five samples of bachelor button collected from Lassen, Modoc, Plumas and Siskiyou Counties. The samples in these areas were taken from well established naturalized stands of bachelor button.

## References:

- Balciunas, J. and B. Villegas. 1999. Two new seed head flies attack yellow starthistle. California Agriculture, 53(2): 8-11.
- Turner, C.E., G.L. Piper, and E.M. Coombs. 1996. *Chaetorellia australis* (Diptera: Tephritidae) for biological control of yellow starthistle, *Centaurea solstitialis* (Compositae) in the western USA: Establishment and seed destruction. Bulletin of Entomological Research 86:177-182.

Table 1. Listing of all *Chaetorellia* seedhead fly releases made in California 1988-1996.

<u>COUNTY</u>	<u>CITY</u>	<u>LOCATION</u>	<u>YEAR</u>	<u>RELS</u>	<u>REL</u>	<u>HOST</u> <sup>1</sup>	<u>ESTABLISHED?</u> <sup>2</sup>
Amador	Jackson	New York Ranch Rd.,	1995	1	164	CESO	CHSU
Contra Costa	Concord#1	Willow Pass Rd.	1996	1	300	CESO	CHSU
Contra Costa	Concord#2	Lime Ridge Open Space	1996	1	300	CESO	CHSU
Contra Costa	Lafayette	Pleasant Hill Rd	1988	1	200*	CESO	CHSU
El Dorado	Latrobe	Latrobe Rd nr Deer Creek	1996	1	300	CESO	CHSU
Fresno	Fresno	Watts Valley Rd	1996	1	400	CESO	CHSU
Glenn	Willows #1	W Hwy 99 & Norman Rd	1996	1	250	CESO	CHSU
Glenn	Willows #2	W Hwy 99 & Rd 60	1996	1	500	CESO	CHSU
Madera	Chowchilla	Berenda Reservoir	1996	1	200	CESO	CHSU
Marin	Novato	Hwy 37 @ Novato Creek	1996	1	320	CESO	CHSU
Mariposa	El Portal	Crane Creek Rd	1994 & 1995	4	1,151*	CECY CESO	CHSU CHAU
Napa	Mankas Corner	Gordon Valley Rd.	1989	1	120*	CESO	CHSU
Napa	Yountville	Silverado Trail & Cross St.	1996	1	500	CECY** CESO	CHSU CHAU
Nevada	Penn Valley	Hwy 49 & Penn Valley Rd.	1989 & 1990	1	322*	CESO	CHSU
Placer	Loomis	Barton Rd & Nute Rd.	1995	2	460	CESO	CHSU
Plumas	Quincy	Quincy Airport	1989	1	42*	CECY CESO	CHSU CHAU
Sacramento	Rancho Cordova	Hazel Av.	1995	2	972	CESO	CHSU
San Luis Obispo	San Luis Obispo	CA Men's Colony	1995	1	560	CESO	CHSU
Santa Barbara	Santa Barbara #1	E Camino Cielo Rd (PF#2)	1996	1	460	CESO	CHSU
Santa Barbara	Santa Barbara #2	Sta Ynez River Rd. (PF#1)	1996	1	410	CESO	CHSU
Shasta	Anderson	Panorama Point Rd	1996	1	300	CESO	CHSU
Shasta	Fall River Mills	Hwy 299 @ Glenburn Rd.	1991 & 1995	2	494*	CECY CESO	CHSU CHAU
Siskiyou	Yreka	Anderson Grade Rd.	1995	1	500	CECY** CESO	CHSU CHAU
Sutter	Pleasant Grove	Howsley Rd & Hwy 99	1996	1	300	CESO	CHSU
Tulare	Farmersville	Rd 196 & Av 324	1996	1	430	CESO	CHSU
Yolo	Esparto	Rd 19 & Rd 92-C	1996	1	400	CESO	CHSU
<b>20 Counties</b>		<b>26 Sites</b>		<b>32</b>	<b>10,355</b>		

\* Some of the flies originated from host material collected in Greece.

\*\* Bachelor button seeds were purposely planted by the property owners to facilitate establishment of the *Chaetorellia* flies.

<sup>1</sup>CESO = *Centaurea solstitialis*, yellow starthistle; CECY = *Centaurea cyanus*, bachelor button.

<sup>2</sup>CHSU = *Chaetorellia succinea*, the false peacock fly; CHAU = *Chaetorellia australis*, the peacock fly.

Table 2: Bachelor button seedhead samples taken at *Chaetorellia* release sites and at naturalized and cultivated growing areas

COUNTY	CITY	RELEASE SITE?	SAMPLES	HEADS	CHSU FLIES	CHAU FLIES	YST NEARBY?
Fresno	Miramonte		1	270	0	184	Yes
Fresno	Pinehurst		1	332	0	80	Yes
Humboldt	Orleans		1	78	0	0	Yes
Lassen	Nubieber		2	574	1	377	Yes
Lassen	Susanville		4	1441	0	592	Yes
Madera	Hwy 49		1	294	0	191	Yes
Mariposa	El Portal #1	Yes	3	1522	0	535	
Mariposa	Midpines		1	227	0	151	Yes
Modoc	Alturas #1		2	499	0	152	
Modoc	Alturas #2		1	631	0	37	
Modoc	Adin		1	403	0	95	
Modoc	Cedar Pass		1	154	0	5	
Modoc	Fort Bidwell		1	965	3	41	
Modoc	New Pine Cr.		2	364	0	239	
Plumas	Quincy #1	Yes	1	1190	5	675	Yes
Plumas	Quincy #2		2	753	3	517	Yes
Plumas	Lake Almanor		1	259	0	91	Yes
Shasta	Burney		2	594	0	327	Yes
Shasta	Fall River Mills	Yes	2	1520	0	471	Yes
Siskiyou	Callahan		1	261	0	136	Yes
Siskiyou	Etna #1		1	300	1	59	Yes
Siskiyou	Etna #2		1	220	0	150	Yes
Siskiyou	Fort Jones		2	535	0	185	Yes
Siskiyou	McCloud		1	300	0	57	Yes
Siskiyou	Mt Shasta		1	237	0	86	Yes
Tehama	Los Molinos		1	150	0	6	Yes
Tulare	Badger		1	520	0	361	Yes
Tuolumne	Groveland		1	100	0	8	Yes
Washoe, NV	Verdi #1		2	726	0	215	
Washoe, NV	Verdi #2		1	300	0	103	
Josephine, OR	Merlin	Yes	4	2380	0	907	Yes
			<b>47</b>	<b>18099</b>	<b>13</b>	<b>7033</b>	

Table 3: Yellow starthistle seedhead samples taken at *Chaetorellia* release sites and near bachelor button infestations

COUNTY	NEAREST CITY	LOCATION	REL SITE?	SAMPLES	YST HEADS	CHSU FLIES	CHAU FLIES	CECY Nearby?
Amador	Jackson	New York Ranch Rd.,	Yes	1	205	252	0	
Contra Costa	Concord #1	Lime Ridge Open Space	Yes	1	200	118	0	
Contra Costa	Concord #2	Willow Pass Rd	Yes	2	591	161	1	
Contra Costa	Lafayette	Pleasant Hill Rd	Yes	2	636	95	0	
El Dorado	Latrobe	Latrobe Rd	Yes	2	584	244	0	
Fresno	Miramonte	Miramonte	No	1	32	18	0	Yes
Fresno	Pinehurst	Pinehurst	No	1	75	124	2	Yes
Fresno	Watts Valley Rd	Watts Valley Rd.	Yes	2	569	110	0	
Glenn	Willows #1	Hwy 99, near Norman Rd	Yes	2	648	176	2	
Glenn	Willows #2	Hwy 99, near Rd 60	Yes	1	233	5	0	
Madera	Chowchilla	Berenda Reservoir	Yes	2	832	141	0	
Mariposa	Midpines	Hwy 140 @ Midpines	No	1	148	45	36	Yes
Mariposa	El Portal #2	Rancheria Flat Rd	No	1	114	22	0	
Mariposa	El Portal #3	Incline Rd	No	1	251	188	6	
Mariposa	El Portal #4	Hwy 140 @ Cedar Lodge	No	1	241	118	0	Yes
Marin	Novato	Hwy 37 @ Novato Creek	Yes	2	351	125	0	
Napa	Mankas Corner	Gordon Valley Rd	Yes	2	513	32	0	
Napa	Yountville	Silverado Trail	Yes	2	606	422	0	
Nevada	Penn Valley	Hwy 49 & Penn Valley Rd.	Yes	2	663	171	0	
Placer	Loomis	Barton Rd & Nute Rd.	Yes	2	613	154	1	
Plumas	Quincy #1	Quincy Airport	Yes	1	334	478	14	Yes
Sacramento	Rancho Cordova	Hazel Av @ S Folsom Canal	Yes	2	539	290	1	
Santa Barbara	Sta Barbara #1	Sta Ynez River Rd. (PF#1)	Yes	1	468	13	0	
Santa Barbara	Sta Barbara #2	Camino Cielo Rd (PF#2)	Yes	2	629	24	0	
Shasta	Anderson	Panorama Point Rd	Yes	2	746	102	0	
Shasta	Burney	Hwy 299 @ Power lines	No	1	260	5	10	Yes
Shasta	Fall River Mills#1	Glenburn Rd	Yes*	1	401	33	40	Yes
Shasta	McArthur #1	Hwy 299 near Storr Rd	No	1	260	17	2	Yes
Shasta	McArthur #2	Hwy 299 near Cemetery	No	1	259	12	0	Yes
Siskiyou	Callahan	Hwy 3 near Old Hotel	No	1	150	18	8	Yes
Siskiyou	Etna #2	Hwy 3 @ MP 15.27	No	1	403	24	36	Yes
Siskiyou	Fort Jones	Hwy 3 near Moffett Cr	No	1	540	16	3	Yes
Siskiyou	McCloud	Hwy 89 & McCloud Rd	No	1	263	32	18	Yes
Siskiyou	Mt Shasta	North Shore Rd	No	1	325	23	10	Yes
Siskiyou	Yreka	Anderson Grade Rd.	Yes	2	799	36	0	Yes
SLO	SLO	Hwy 1 near CA Men's Prison	Yes	2	642	370	0	
Sutter	Pleasant Grove	Howsley Rd & Hwy 99	Yes	2	491	163	0	
Tulare	Farmersville	Rd 196 & Av 324	Yes	2	695	403	0	
Yolo	Woodland	Rd 19 & Rd 92-C	Yes	2	988	99	0	
Josephine, OR	Merlin	Merlin Road & I-5 Fwy	Yes*	2	1103	12	62	Yes
				60	18400	4891	252	

\**Chaetorellia* flies originating from Greece emerging from seedheads of yellow starthistle were released at this site in 1991.