Bachelor's Button Yellow Starthistle Spotted Knapweed

Biological Control Program

California Department of Food and Agriculture

2000

BIOLOGICAL CONTROL PROGRAM

2000 SUMMARY

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PLANT HEALTH AND PEST PREVENTION SERVICES
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Preface

D. M. Woods

The Biological Control Program of the California Department of Food and Agriculture is an implementation-oriented program within the Integrated Pest Control Branch of the Plant Health and Pest Prevention Services. The 'Program', supervised by a Senior Supervising Scientist, is comprised of nine scientists and three technical support staff. Additional technical support comes from several part time (usually student) employees. Biological control is usually a long-term solution to pest problems, thus the majority of the reports presented here are updates of ongoing projects. Most of the data presented here is of a preliminary nature and will be formally presented when projects are completed. These reports provide a format to express preliminary data as part of the larger scientific discourse.

The program has been facing new tasks, directions and challenges this year that will continue in the upcoming year. Recent retirements within the Plant Health and Pest Prevention Services portion of the Department along with unforeseen biological developments such as the expansion of the range of the glassy-winged sharpshooter (GWSS) have created significant administrative changes for our program. While the general structure of the program remains unchanged, some specific responsibilities and program directions have shifted. Of foremost significance has been the impact the sharpshooter has had. The dramatic impact of its vectoring of Pierces disease, and the degree of concern for control options led to the addition of a new full time staff scientist, Dr. David Morgan. Along with an additional shift in responsibilities for Dr. Pickett to the Pierces Disease Control Program, we begin a huge new project and direction. One report presented in this summary outlines the ground work for this hopefully fruitful project.

Promotions and retirements have also had their impact. Our former Supervising Scientist, Larry Bezark, has accepted a promotion within the branch and one of his first duties will be to hire his successor. The retirement of Dr. Joe Ball early in 2001 leaves a major void and his expertise will be sorely missed. He will remain with the program on a part-time status for a transitional period as Dr. Roltsch relocates to Sacramento and assumes many of these responsibilities. Project responsibilities shifts associated with these personnel changes will continue for some time. A final impact for the insect group has been the initiation of several new projects, such as cereal leaf beetle, which are still in the planning stages.

Biological Control of the Glassy-winged Sharpshooter

D. J. W. Morgan, C. H. Pickett and L. G. Bezark

In the past year, a new program has been initiated in the California Department of Food and Agriculture: the Pierce's Disease Control Program. One of the strategies being investigated to reduce transmission of Pierce's disease is control of the most important vector, the glassy-winged sharpshooter, *Homalodisca coagulata* (GWSS). The Biological Control Program has begun investigating the potential of natural enemies for controlling the glassy-winged sharpshooter.

For the initial stage of the GWSS biological control program, we began investigating natural enemies already present in California, and their impact on established GWSS populations. This initial phase of natural enemy evaluations is focusing on the egg stage of GWSS. In conjunction with the Department of Entomology, UC Riverside, we monitored the fate of GWSS eggs in Temecula organic citrus over the past year. The most important mortality factor for GWSS eggs was the native egg parasitoid*, Gonatocerus ashmeadi*.

Egg parasitoids and other mortality factors acting on the egg stage achieved good control from June onwards (Figure 1). However, low parasitism rates early in the season resulted in large GWSS populations later in the year.

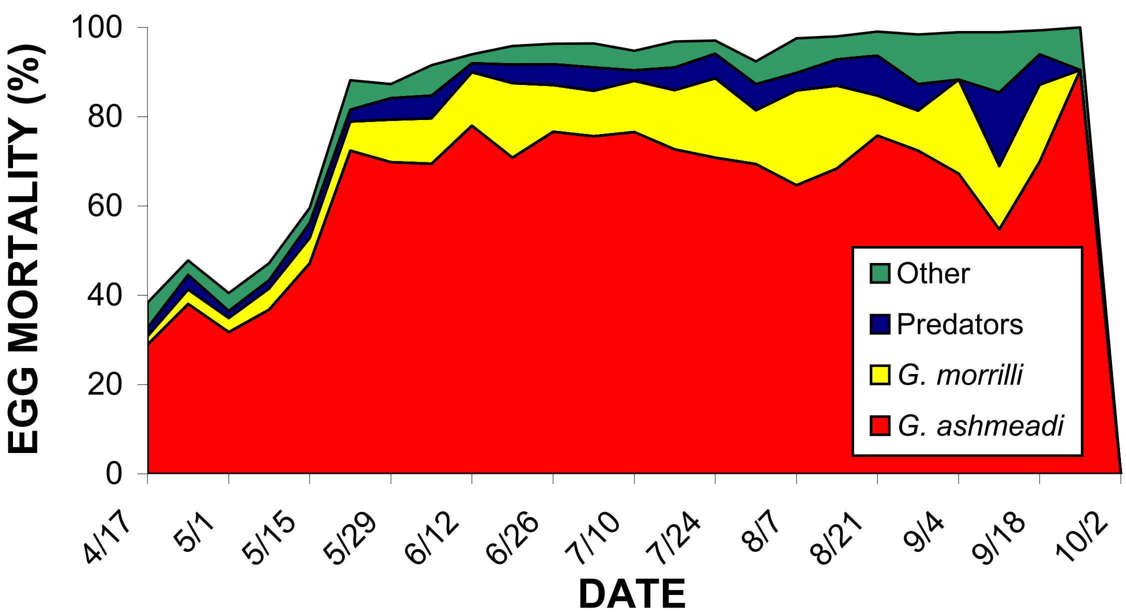

Figure 1: Mortality factors acting on GWSS eggs in organic citrus in Temecula during 2000. Both *G. morrilli*, and *G. ashmeadi* are native egg parasitoids.

The second phase of the program was to initiate searches for natural enemies of GWSS eggs in its area of origin. Over the past year, trips have been made to Texas, Louisiana, Florida, and Mexico (Nuevo Leon, Tamaulipas, San Luis Potosi, and Veracruz states). In addition, egg masses were sent from USDA-ARS in Weslaco, Texas, throughout the year. All collected material was delivered to the quarantine facilities at UC Riverside where UC and CDFA staff identified, reared, and evaluated natural enemies. Permits were obtained to initiate preliminary releases of parasitoids. In 2000, one species, *G. triguttatus*, was released in California (Table 1).

Table 1: Releases of *G. triguttatus* made in California during 2000.

Release site	Date of first release	Number of releases	Wasps per release	Release interval
Riverside, Riverside Co.	24 August	3	180	2 weeks
Temecula, Riverside Co.	24 August	3	100	2 weeks
Fillmore, Ventura Co.	14 September	3	100	2 weeks
Bakersfield, Kern Co.	14 September	3	100	2 weeks

The third phase of the program was to evaluate the recovered natural enemies. Five species of GWSS egg parasitoids were recovered from exploration. Of these, one species died without issue (*Gonatocerus atriclavus*). A further species died after only one generation (*Zagella* sp., undescribed). Of the remaining species, only one was exotic to California, *Gonatocerus triguttatus*, the others being *G. ashmeadi* and *G. morrilli*. All three mymarids were maintained for three generations and evaluated. All species readily parasitized GWSS eggs laid in a range of host plants. Female insects live approximately two weeks at 26°C and parasitize between 30 and 100 eggs.

Finally, we have begun to develop mass rearing strategies for host plants, hosts, and the parasitoids. The greatest difficulty has been to develop a method for producing a reliable and consistent supply of GWSS eggs for parasitism. A range of host plants and hosts were evaluated, and at present, a mixture of potato, *Euonymus*, and *Chrysanthemum* are used to optimize egg production. Leaves containing GWSS egg masses are exposed to wasps for 24 hours, then removed and incubated for seven to ten days at 28°C, 75% RH, 14:10 L:D. Wasps emerge and are either released or diverted to continuing the colony.

A commercial insectary, Foothill Agricultural Research in Corona, has been sub-contracted to supply eggs for natural enemy production and are doing so with great efficiency (currently in excess of 1,000 eggs per day). Ultimately, CDFA facilities in Riverside and Bakersfield will be involved in GWSS egg production. Until these facilities are fully operational, efforts are being concentrated on optimizing parasitoid production from the eggs produced by Foothill Agricultural Research.

In the year 2001, the GWSS biological control program will continue its searches for new natural enemies and intensify releases of beneficial insects, both in terms of the number of release sites, and the number of insects released at each site. Of highest priority is to bring CDFA facilities in Riverside and Bakersfield into full production of beneficial insects.

Insectary Production of the Red Gum Lerp Psyllid and its Parasitoid.

J. C. Ball, J. Brown and D. L. Dahlsten[1]

The red gum lerp psyllid (RGLP), *Glycaspis brimblecombei* Moore (Homoptera: Psylloidea), was first reported in California in June 1998 in the City of El Monte, Los Angeles County. Native to Australia, this psyllid heavily infests red gum eucalyptus (*Eucalyptus camaldulensis)* in California, but has also been found attacking sugar gum, blue gum, *Eucalyptus rudis* and three other species. Red gum lerp psyllid has spread throughout the state at an alarming rate and has rapidly become one of the most serious pests attacking eucalyptus in California.

In 1999, Dr. Donald L. Dahlsten obtained several parasites attacking RGLP in Australia. These were held in quarantine until host testing was performed by the University of California to determine the safety and specificity of the parasites. One species, *Psyllaephagus bliteus* (Hymenoptera: Encyrtidae), was successfully reared, determined to be a host specific primary parasite, and released from quarantine.

The CDFA-Biological Control Program obtained seedling eucalyptus and began germinating eucalyptus seeds in 1999 for insectary production of the psyllid and its parasite. Psyllid colonies were established, CDFA received *P. bliteus* from the University of California in June 2000, and began parasite production. Approximately 660 parasites have since been provided to the University for release in their research plots, but that represents a very small fraction of the numbers required. Some unexpected problems surfaced in the insectary production of RGLP and its parasite. In addition to the normal problem of maintaining "clean" host plants free of disease, pests, and chemical residues, we experienced problems with high RGLP adult mortality in the cages and an apparently low parasite reproductive rate. We needed to understand more about the biology of RGLP and *P. bliteus,* including reproductive rates of the psyllid and its host plant acceptance and, for the parasite, host stage preference, mating requirements (sex ratio problems), and reproductive rates. Several preliminary tests were performed to gain information on some of these attributes.

Two *E. camaldulensis* seedlings, approximately 12" high, were placed in an acrylic sleeve cage (18" cube) on October 19, 2000. Field-collected adult RGLP were released on the plants (150 on October 19 and 180 on October 20) where they remained throughout the study. Sex ratio was not determined. On November 9, six female and one male *P. bliteus* were added to the cage. The average greenhouse temperature up to that time was 70.3°F (56.0°F – 85.1°F). The parasites remained until November 28 when four females were removed. The other two females and one male were not found.

A sample of eight leaves was collected on November 15 (six days after the parasites had been introduced) to obtain a gross estimate of RGLP reproduction, development and parasite production (Table 1). Average greenhouse temperature during the six-day parasite exposure was 63°F (46.8°F – 76.6°F). Almost twice as many RGLP eggs were laid on leaves of the apical half of the plant than on basal leaves (1.9:1), and there were slightly more eggs laid on the ventral leaf surface (1.3:1). Other than several nymphs having necrotic spots, presumably from oviposition probes and host feeding, none of the nymphs showed evidence of having been parasitized, even though all stages were available. On the eight leaves, 217 nymphs of all stages were present. At the temperatures within the greenhouse (mean = 68.6°F), RGLP was able to develop from egg to 5th instar in 28 days and possibly through to adult, as the "unhatched" eggs may represent a second generation (unfortunately, 5th instar exuviae were not recorded).

Table 1. Red gum lerp psyllid reproduction, development and parasitism on 8-leaf sample of red gum eucalyptus. Sample collected November 15, 2000.

Eggs		Nymphs				Lerps + Nymph status			
hatched	unhatched	1st-2nd	3rd	4th	5th	vacant	live	dead	mummy
438	141	14	40	110	53	122	151+	19	0

A second sample of four leaves was collected from the two seedlings on December 6. Counts were confined to the number of lerps, 5th instar nymphs and mummies (Table 2). (Live earlier instar nymphs were not observed in the sample and many of the dead nymphs were partially out of their exuviae apparently dying between moults.) The average greenhouse temperature between November 15 and December 6 was 57.5°F (41°F – 71.8°F).

Table 2. Red gum lerp psyllid reproduction, development and parasitism on 4-leaf sample of red gum eucalyptus. Sample collected December 6, 2000.

Total lerps	5th instar exuviae	Live 5th instars	Parasite mummies
489	101	7	2

Two parasite mummies were present in the second sample, and parasite adults began emerging on Dec. 11. Although the rate of parasitism was low under the described conditions, *Ps. bliteus* was able to complete development in 32 days.

In the coming year, we hope to be able to examine RGLP and *P. bliteus* reproduction and development under more controlled conditions. This is important in obtaining predictable insectary production.

Acknowledgements: We wish to thank John Andrews for providing *P. bliteus* to start our colonies in Sacramento and for his invaluable advice on rearing. We also thank Bill Copper who got us on the right track in field collecting psyllid adults.

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Initial Survey of Parasites of the Citrus Peelminer in Tulare County

K. Godfrey and D. A. Mayhew

The citrus peelminer (*Marmara* new species; Lepidoptera: Gracillariidae) is a native insect of the southwestern United States. It can be found from the Coachella Valley to coastal southern California and into the southern San Joaquin Valley. It is thought that this insect normally attacks willow, but has changed its host plant range to include oleander and citrus where it mines the peels and young stems. In the Coachella Valley, a native parasite (*Cirrospilus coachellae*) provides excellent control of citrus peelminer. During 1999 and 2000, the density of citrus peelminer increased dramatically in the southern San Joaquin Valley, and many growers had 50 – 100% damage to their fruit. As a result of this outbreak, a qualitative survey was initiated to determine if there were native parasites present on citrus peelminer in the southern San Joaquin Valley. In particular, the survey was looking for the native parasite, *Cirrospilus coachellae*.

Collections of citrus peelminers were made from three citrus groves in Tulare County. On September 28, peelminer mines were collected from two grapefruit groves located near Lindsay. On October 5, another collection of peelminer mines was made in a pummelo grove near Porterville. The fruit with the mines were held in the laboratory to allow the emergence of parasites and peelminer adults. All parasites and peelminer adults that were recovered were sent to John Heraty (Dept. of Entomology, University of California, Riverside) for identification.

The initial collections yielded six parasites and one adult peelminer. The parasites belonged to the genus *Pnigalio* (Hymenoptera: Eulophidae) and were all from one grapefruit grove near Lindsay. This genus of parasite is a common gracillariid parasite, but this is the first recorded association of the parasite with citrus peelminer. As the native parasite populations are discovered and studied, decisions on future biocontrol efforts can be addressed. Therefore, the survey of parasites will continue during 2001.

Additional Attempts to Establish a Parasitoid of Olive Fruit Fly in California

C. Pickett, J.C. Ball, R. Messing[1] and D. Asakawa[2]

The olive fruit fly (OLFF), *Bactrocerus oleae* (Gmelin), a major pest of olives in Mediterranean countries and most other areas where present, was found for the first time in California (and the US) in October 1998. At the time of this writing, OLFF has been found as far north as Corning and is extremely abundant in many areas of the state. An olive fruit fly parasite, *Psyttalia concolor* (Szepligeti) was released at sites in Los Angeles and Santa Barbara in September 1999 (see previous annual report). *Psyttalia concolor* was not recovered in post-release samples and additional release attempts, reported here, were made during 2000 in Riverside and Santa Barbara.

All parasite releases in Riverside were made at one location (Jurupa Cultural Center). A total of 300 were released on November 14, 2000, on December 16, and 500 on January 16, 2001. The site had olive trees bordering a driveway along with and a small olive orchard. *Psyttalia concolor* was randomly released amongst the trees. Fruit ranged from green to over-ripe with a high rate of olive fruit fly infestation. Fruit samples were collected from 10, three, and six trees respectively on the three release dates and placed in rearing cartons. The number of fruit collected per tree was variable. Parasites were released in some of the cartons to see if the parasites would attack OLFF when confined. Figure 1 contrasts the emergence of flies from cartons with and without introduced parasites and shows the impact of *P. concolor* on successful emergence of flies. Approximately 80% fewer flies emerged from fruit exposed to the parasites (based on flies per fruit). Two live and 42 dead adult *P. concolor* were recovered two months later from the container receiving 14 adult parasites on January 16. The length of time between exposure of fly infested fruit to live wasps and sampling two months later (March 14, 2001) suggests these wasps are progeny of the original parasites released. The number of recovered adult parasites shows that successful oviposition and adult development of *P. concolor* on olive fruit fly took place.

On November 30, 2000, 1,200 *P. concolor* were released, in approximately equal numbers, on 12 trees at seven properties in Santa Barbara. *Psyttalia concolor* had been released on five of the properties in 1999. Generally, there were several trees at a location, and olive trees were dispersed throughout the area. Table I. shows fruit characteristics and OLFF infestations on the trees at the time of release.

Table 1. Fruit characteristics and OLFF infestation at time of *P. concolor* release.

No. fruit	Weight	% Stung	% Ripeness	OLFF	*Psyttalia*	Other parasites
1085	95.2 oz	75.4	30 - 98	230	0	6

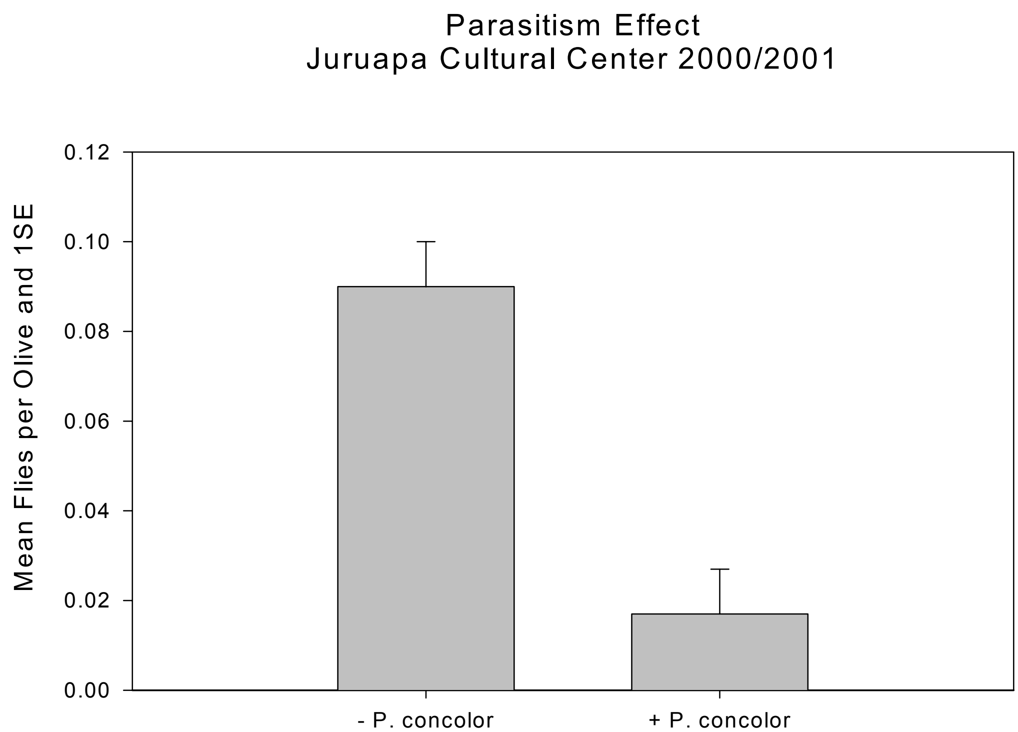

Figure 1. Impact of parasites on infested fruit collected at Jurupa Cultural Center and held in paper cans. Winter 2000-2001.

Psyttalia concolor was not recovered in the pre-release sample in Santa Barbara, implying that it had not established from the 1999 release. However, two "native" parasites [*Eurytoma incerta* (Eurytomidae) and *Pteromalus* sp. (Pteromalidae)] were found. These and another parasite, *Brasema* sp. (Eupelmidae) had previously been found in OLFF infested olives in Fallbrook (D. Penrose, CDFA, PD/EP) and in Los Angeles and Santa Barbara (V. Yokoyama, USDA, ARS).

Although *Psyttalia* has not been recovered from post-release samples taken in Riverside, the caged oviposition study showed that OLFF was an acceptable host for this parasite. Post-release samples have not been taken in Santa Barbara.

Acknowledgements: We wish to thank Steve Heydon (University of California, Davis) for providing identifications of the "native" parasites, Mary Burns (Jurupa Cultural Center), for arranging the release site, Dick Penrose (CDFA – PD/EP, Sacramento) and Victoria Yokoyama (USDA-ARS, Horticulture Crops Research Lab, Fresno) for providing the specimens.

[1] University of Hawaii
[2] CDFA, PD/EP, Goleta, CA

Importation and Establishment of Nymphal Parasites of *Lygus hesperus*

C. H. Pickett, J. C. Ball, D. A. Mayhew, K. Casanave, U. Kuhlmann[1], D. Coutinot[2], L. Ertle[3], and K. A.Hoelmer[2]

Our first releases of exotic parasites for the biological control of *Lygus hesperus* Knight (Hemiptera: Miridae) in California were in September of 1998. The summers of 1999 and 2000 marked the first two years for large numbers of lygus parasite releases. The parasites, *Peristenus digoneutis* Loan and *P. stygicus* Loan (Hymenoptera: Braconidae) were collected in France, Italy, and Spain by CABI Bioscience and the USDA. We were able to increase lygus production in laboratory culture over the last two years, resulting in an increased number of lygus parasites for field release (Table 1). Approximately 1100 parasites were released in fall of 1998, then 6000 and 15,000 during summers of 1999 and 2000, respectively. By maintaining year-round production of parasites in Sacramento, we were able to increase the overall yearly production as well as begin field releases earlier in the year. The earlier in the summer that releases are made, the greater the number of parasite generations produced, and hence, a higher probability for permanent colonization.

We made our first overwintering recovery of *Peristenus stygicus* at CDFA's Sacramento release site. These parasites persisted from releases made in 1999 to May 2000, when the first pre-release samples for 2000 were taken. The proportion of lygus parasitized at this site increased from 3% (n=30) in May (not shown) to 10% (n=40) last August (Table 2). We recovered parasites in 2000 from three of five sites where repeated releases were made beginning in late spring/early summer. The two sites where no parasites were recovered had low (~0.5 nymphs per sweep) lygus populations all summer. In our last sample taken at the Kearney Agricultural Center in 2000, six of 25 lygus nymphs or 24% were parasitized by *P. stygicus*. The high lygus population during the summer (four to eight per sweep) as well as the alfalfa-cutting regime at this site undoubtedly played a role in their quick colonization.

[1]CABI Bioscience, Delemont, Switzerland
[2]USDA-ARS European Biological Control Laboratory, Montfierrier, France
[3]USDA-ARS Newark, Delaware

Table 1 Releases of new parasites, summer 2000

Location	Species and population released	# Parasites released	
		adults	nymphs
Sacramento (CDFA)	*Peristenus stygicus*, France	2105	2100
UC Davis	*Peristenus stygicus*, Italy	3051	3000
Yolo Co. (Fongs)	*Peristenus stygicus*, Spain	3057	2400
UC Kearney, Parlier	*Peristenus stygicus*, Italy	2063	1500
UC/USDA, Shafter	*Peristenus stygicus*, France	2119	1500
UC/USDA, Shafter	*Peristenus stygicus* Umbria, Italy	1213	0
UC/USDA, Shafter	*Peristenus digoneutis*, Umbria, Italy	418	0
Merced Co., Gurr Rd.	*Peristenus stygicus*, Spain	441	0
Kern Co., Sanders	*Peristenus stygicus* Spain	576	300
Total		15,043	10,800

Table 2 Recoveries of released parasitoids

Location	Last collection date	% Parasitism (n)	Cocoon development	
			# nymphs collected	# cocoons developed
Sacramento	25 Aug.	10 (40)	100	12
UC Davis	31 Aug.	0 (40)	102	0
Yolo Co. (Fongs)	31 Aug.	0 (32)	--	--
UC Kearney	16 Aug.	24 (25)	--	0
UC/USDA, Shafter	16 Aug.	2 (50)	--	0

Search for Native Parasitoids of *Lygus hesperus* in Idaho and Nevada

J. C. Ball, C. H. Pickett, C. R. Baird[1], and J. Knight[2]

The Biological Control Program has been attempting to establish nymphal parasitoids of the western tarnished plant bug, *Lygus hesperus* Knight (Hemiptera: Miridae), in California since 1998. The European species *Peristenus digoneutis* Loan and *Peristenus stygicus* Loan (Hymenoptera: Braconidae), as reported elsewhere, were released in California in 1998, 1999, and 2000 with as yet, uncertain results. In 1997, an undescribed, native species of *Peristenus* was found attacking lygus nymphs in Idaho. This species, *Peristenus howardi* Shaw, had multiple generations a year and could produce high rates of parasitism in alfalfa. Because of these properties, an attempt was made to introduce this species into California.

Between July 17 and 19, 2000 collections of *L. hesperus* nymphs were made in alfalfa fields near Boise, Idaho and Lovelock, Nevada. The nymphs were provided green beans and caged over a vermiculite substrate for cocooning of any emerging parasite larvae. The number of alfalfa fields sampled, lygus nymphs collected, and parasite cocoons recovered are displayed below.

Location	Fields sampled	Lygus nymphs	Cocoons
Idaho	3	1,673	164
Nevada	5	1,267	0

No parasites were found in the limited sampling in Nevada, but parasitized nymphs were collected in all three fields sampled in Idaho. Although we obtained cocoons in our samples, no parasite adults had emerged by April 2001, possibly due to handling errors. Dissections in August revealed some cocoons with dead larvae and other cocoons contained adults, but were also partially filled with fluid. The vermiculite may have been too moist, drowning the parasites. In addition, some lygus nymphs were found infected with the entomophagous fungus, *Beauveria bassiana*, which may also have infected *Peristenus*.

Acknowledgements: We wish to thank Michael McGuire, USDA, ARS Shafter Research and Extension Center, for identifying *Beauveria bassiana*.

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Recoveries of Exotic Parasites Released for the Control of Silverleaf Whitefly in the San Joaquin Valley

C. H. Pickett, J. Brown, D. Overholt[1], and B. Abel[2]

Large-scale releases were made between 1997 and 2000 of *Eretmocerus emiratus* Zolnerowich and Rose (M95104), *Eretmocerus* nr. *emiratus* (M96076, Ethiopia), *Eretmocerus. mundus* Mercet (M92014), *Eretmocerus hayati* Zolnerowich and Rose, and *Encarsia sophia* (Girault and Dodd; = *transvena*) (M95107) into four citrus groves in the San Joaquin Valley. Methods for release are described in the last two years' annual reports. The last exotic species was released summer/fall 2000. A total of 124,000 *Encarsia sophia* originally collected from Multan, Pakistan, were released twice monthly into citrus from August to October at a commercial farm near Maricopa in Kern County.

Post-release monitoring of silverleaf whitefly parasites is being conducted at all four original release sites, one each in Fresno and Tulare counties, and two in Kern County. These sites were sampled once each in January and February and twice monthly from August to October. Weedy hosts of silverleaf whitefly, as well as citrus and cotton were sampled. Weed hosts included common mallow, *Malva neglecta* Wallr., sowthistle, *Sonchus*, and spotted spurge, *Euphorbia maculata* L. Fresh weed samples from the field were shipped, using overnight mail to the CDFA Biological Control facility in Sacramento. Sentinel plants were placed in paper cans, allowing for parasite emergence over four to six weeks. Emerged adult *Eretmocerus* parasites were counted. Males were used to discriminate between natives and exotics. Subsamples of females were used for species identification. In addition, sentinel plants were placed in the southern end of the San Joaquin Valley along north-south and east-west transects at 30 to 40 locations. Four to six week old cotton plants were inoculated with silverleaf whitefly nymphs and placed in protected, shaded locations in a mix of locations, e.g. private homes, near cotton fields, and citrus. After one week of exposure, plants were brought indoors, and incubated at constant temperatures of ca. 25° C. Emerged silverleaf whitefly and male *Eretmocerus* parasites were counted with the aid of a dissecting microscope.

In 2000, the four original release sites were sampled in winter, summer, and fall. Of the 25 sampling events (date and site), parasites were recovered seven times on weedy plants. Exotic *Eretmocerus* made up 44% of males recovered. A total of ten female *Eretmocerus* were identified to species. Eight of these were *Ert. mundus* and two *Ert*. nr. *emiratus*. No *Encarsia sophia* was recovered. The most common *Encarsia* recovered was *En. Coquilletti*, native to California. Many of the spurge samples had a mix of silverleaf whitefly and banded wing whitefly, a likely host for this species of *Encarsia*. To our surprise, no species of parasites were recovered from weed samples taken from the Maricopa, Kern County site during summer, where in the past, most exotic parasites had been found. High numbers of silverleaf whitefly were recovered from some of these samples (>20 adults per gm dry wt. plant collection), suggesting that insecticides or some other environmental perturbation affected their populations.

No exotic males were captured by the sentinel plants in 2000, while in 1999 one third of the plants captured exotics. One of six recovered females, however, was identified as *Eretmocerus mundus* (we counted only males); the remainder were native *Eretmocerus*. In 1999 80% of the captured *Eretmocerus* were exotic, however this was during the same period when *Eretmocerus mundus* was being released in large numbers. The poor recovery may be explained by the low number of whiteflies successfully developing to adults on these plants. In 1999, we averaged 34 emerged adults per plant while in 2000, we averaged four.

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Pink Hibiscus Mealybug Biological Control in Imperial Valley

W. J. Roltsch, D. E. Meyerdirk[1] and R. Warkentin[1]

The pink hibiscus mealybug (PHM), *Maconellicoccus hirsutus* (Green), is native within the boundaries of South Asia or Australia. It was recently introduced into the western hemisphere; first in Hawaii in 1984, then the Caribbean islands of Grenada and Carriacou in 1994. Its range soon included over 25 Caribbean Islands. Biological control efforts were implemented in the Caribbean by USDA-APHIS in cooperation with each island country and CABI (Center for Agric. & Bioscience) International. USDA-APHIS has referred to their early program involvement as the "Off shore project", representing a proactive approach to impending pest invasions into North America.

The PHM was first detected in Imperial Valley, California during August 1999, following the submission of a mealybug specimen to the Imperial County Entomologist by a homeowner. This represented the first North American record for the PHM. Population densities of PHM on mulberry, silk oak, hibiscus and natal plum were determined to be high in several communities in southern Imperial Valley. Homeowners in Calexico claimed to have experienced the same mealybug problem in their mulberry trees as early as the summer of 1997. In response to the Imperial Valley infestation, two parasitoid species, *Anagyrus kamali* Moursi and *Gyranusoidea indica* Shafee, Alam & Agarwal, were released at ten infested sites in September of 1999. Approximately 3,000 parasitoids of each species from USDA insectaries were released. These initial releases were followed by additional shipments of parasitoids that were used for release and also the setup of a PHM parasitoid rearing facility in Imperial Valley. The rearing facility, under the direction of the California Department of Food and Agriculture in cooperation with USDA and the Imperial County Agricultural Commissioner's Office began producing and releasing large numbers of parasitoids by late June of 2000 (see associated report).

PHM population densities were monitored quarterly on infested mulberry trees at three parasitoid release sites from September 1999 to October 2000. Three additional sites were incorporated into the study on January 2000, although PHM populations were so low during the winter that samples were not obtained at all sites until April. Samples consisted of the terminal and adjacent five full leaves on eight branches per tree. All egg masses, second and third instars, and adult males and females were counted. The percent parasitization at each site was determined by encapsulating approximately 100 individual mealybugs ranging from late 2nd to third instars and adult females. The majority of samples held for determining parasitism consisted of third instars and early adult female PHM. PHM mummies were collected and held for parasitoid emergence to primarily document hyperparasitism. All specimens were held for 30 days in the laboratory and examined for parasitoid emergence. PHM populations and percent parasitization were similarly evaluated on carob trees and two hibiscus shrubs at three sites.

Population densities on infested mulberry trees averaged over 200 mealybugs per terminal in September of 1999 (Fig. 1). By January of 2000, all leaves had fallen from the trees and only traces of mealybugs were observed during the cooler temperatures of winter, although all life stages were observed. After leaf emergence in April, only 0.3 mealybugs per terminal were observed and most of the PHM eggs that over-wintered had hatched. By June, PHM populations had increased to 22 mealybugs/terminal and by August, densities held at 19 mealybugs per terminal. In September of 2000, one full year after the single release of *Anagyrus* *kamali* and *Gyranusoidea indica*, the PHM population density at the monitored sites was 4% of that found in September of 1999, averaging 9.5 mealybugs/terminal. Parasitism data showed that from the single release of parasitoids in September of 1999, *A. kamali* rapidly colonized PHM infested sites, and was capable of successful overwintering during sporadic cold periods with daily low temperatures ranging from 32 to 39 degrees F for a total of 37 days. During the summer months, *A. kamali* tolerated daily high temperatures above 110^{o} F for 21 separate days (Table 1). Average percent parasitization ranged from 0% in September 1999 (prior to parasitoid release) to 31.7% by September 2000 and 70% in October 2000. In contrast, *G. indica* did not demonstrate a similar degree of rapid establishment. However, data from November of 2000 to March 2001 demonstrated that *G. indica* was common during that time period.

Three sites consisting of carob trees were selected for long term monitoring studies, beginning in June 2000. The initial PHM population densities on carob were high (approx. 100/terminal sample unit) at each of the three sample sites. Parasitoids became established in these carob trees from neighboring releases before our monitoring studies in carob trees were initiated in June. Densities of PHM consistently declined and remained low during the remainder of the year, whereas percent parasitism increased greatly throughout the summer. Although changing host plant quality and weather related factors may have played a role in the PHM's 93% decline from June to September, we feel that parasitism by *A. kamali* played an important if not central role. Percent parasitization ranged from 1.8 % in June 2000 (i.e., pre-release parasitism) to 30.5% by September and 77% in October of 2000.

Hibiscus plants are not common in the PHM infested region of Imperial Valley, although, infested plants at two sites were found and selected for sampling. One site (La Brucherie, El Centro) was heavily overrun with ants that interfered with early PHM suppression by the parasitoids. From late September 1999 to June 2000, PHM populations increased from 71 to nearly 500 mealybugs/terminal. From early July 2000 onward, the homeowner took effective measures to control the ant population. The percent parasitization increased from 0% in September 1999 to 22.8% in August 2000, and over 65% by September and October 2000. By September 2000, the PHM population density decreased to 81 mealybugs/terminal. The second site (Cole Rd., Calexico) showed a PHM population density decrease of 94% from September 1999 to August 2000. The PHM and parasitoid relationships at this second site may have been impacted by poor plant health and a competing mealybug species (solenopsis mealybug, *Phenacoccus solenopsis*).

The impact of native (to Imperial Valley, California) hyperparasitoid species is being monitored. A hyperparasitic species (*Marietta* sp.) was first collected in July 2000. At that time, its occurrence was rare. Upon dissection, we confirmed that the primary parasitoid, *A. kamali*, was under attack by *Marietta* sp. It was common through the remaining months of 2000, as represented by the percent of PHM mummies from which the hyperparasitoid emerged [(mean %, # of sample sites): early August 11%, 5; late August 51%, 6, September 10%, 6; October 38%, 9].

In summary, the two biological control agents released against the PHM have become widely established throughout infested areas of Imperial Valley, and at least one species has had considerable impact to date.

Fig. 1

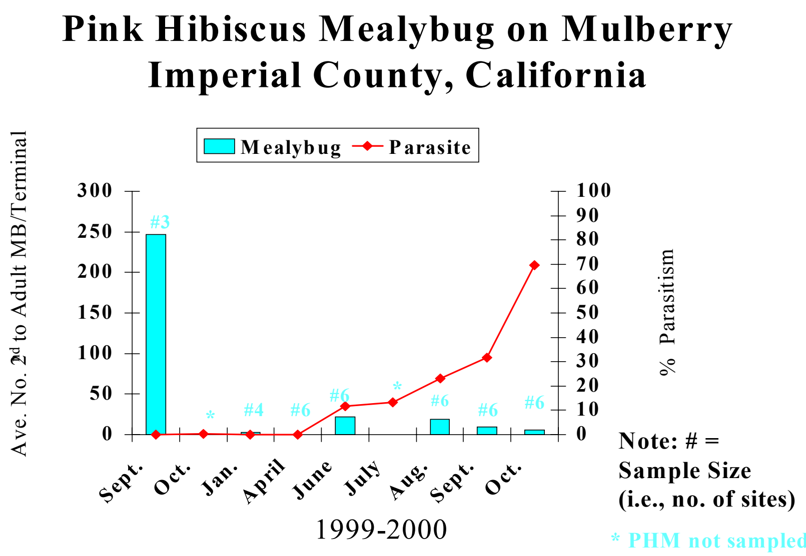

Table 1. Select temperature data summary, Sept 1999-Sept. 2000, Imperial Valley, CA (Meloland)

Temperature (F Degrees)	Number of days (Max. or Min. Temp.)
32	2
33-39	35
100-104	76
105-109	46
110+	21

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Insectary Production of Pink Hibiscus Mealybug Parasitoids

W. J. Roltsch

An insectary was established during the spring of 2000 to produce pink hibiscus mealybug (PHM), *Maconellicoccus hirsutus* (Green), parasitoids for distribution to known infested sites in Imperial Valley, California. Two species in the family Encyrtidae are being reared. These include, *Anagyrus kamali* Moursi and *Gyranusoidea indica* Shafee, Alam & Agarwal The culture of *Anagyrus kamali* is a composite of populations originally collected in China (near Hong Kong) and Hawaii. The *Gyranusoidea indica* culture is a composite of three populations originally collected in Egypt, Pakistan and eastern Australia (near Brisbane). Startup material for each culture was provided by USDA-APHIS. The rearing protocol was adapted from that described in the USDA Biological Control of Pink Hibiscus Mealybug Project Manual. In addition, extensive consultation took place with Dr. Dale Meyerdirk and Mr. Richard Warkentin, USDA-APHIS, NBCI. The rearing facility consists of two, 55' trailers that were rented and located at the Imperial County Annex. Each trailer has a 10x11' room at each end of the trailer, and a center area with a bathroom (10x32' combined). All entry points into the trailers and individual rooms have double door access provided by screened enclosures to prevent the entry of contaminant species.

The PHM culture is maintained in one trailer on young mature Japanese pumpkins (*Cucurbita moschata*, cv. chirimen). The culture is reared in one end-room for eight to 12 weeks, before being moved to the recently cleaned room at the opposite end of the trailer. PHM production is carried out within 48x57x31" (WxHxD) plywood cabinets consisting of a floor space and two shelves. The inside of each cabinet is painted with flat black latex. The front and rear of the cabinets are ventilated with a fine mesh cloth, capable of limiting the escape of crawlers. The PHM rearing environment is intended to be 28^0 C and 50% RH without lighting. Our environment ranged from 25 to 30^o C, and 35 to 65% RH. Developmental time from newly hatched crawlers to egg laying adult females was approximately 30 to 35 days.

A continual supply of Japanese pumpkins is made possible by coordinating field plantings at several locations in the State. Two plantings were implemented in Imperial Valley (January and February plantings produced pumpkins from late April to mid-June), Sacramento (April planting, producing late June to November) and UC Riverside experiment station (July planting, producing October to November). Pumpkins have been successfully held in cold storage (12^o C, 50 to 60% RH) for over 60 days. Many pumpkins continue to be usable up to 90 days, however, they are considerably less attractive to the mealybug crawlers. Prior to placement in cold storage, each pumpkin is washed in a very mild dish soap solution (1drop/gal.) and rinsed in water. The availability of Japanese pumpkins resulted in stable, large-scale production of PHM by late May, and parasitoids by late June 2000. During peak production, 40 to 50 pumpkins were inoculated each week to maintain PHM production and to provide immature PHM on pumpkins to each of two parasitoid cultures. Sprouted potatoes are used for culture maintenance during late spring, prior to the first harvest of pumpkins each year.

Each parasitoid species is reared in separate rooms within the second trailer. Each room has a double door screened entrance. The target temperature and humidity is similar to that in the PHM culture rooms. Overhead lighting consists of eight, cool-white 40-watt fluorescent bulbs. Four to six pumpkins, heavily infested with PHM nymphs, are placed in each rearing cage. Late third instar PHM's are predominantly used to rear *A. kamali*, while *G. indica* is presented with predominantly early third instars. In each case, one pumpkin containing earlier PHM life stages is placed in each cage to supply host material at a later time, because both parasitoid species commonly live for over eight days.

By mid-December 2000, over 200,000 parasitoids of each species were produced in culture (Table 1). Over 160,000 *A. kamali* and 230,000 *G. indica* have been released on several hundred properties within PHM-infested communities (Table 2). The release program is greatly facilitated by an on-going survey program that provides information on infested locations. As a result, releases have been made daily throughout the region with little additional time spent identifying appropriate release sites. In most instances, each release consists of approximately 400 parasitoids of each species at each site. With the exception of several instances in which production cages were male biased, the sex ratio was typically very close to 1:1.

Table 1. El Centro insectary production of pink hibiscus mealybug parasitoids

Month 2000	*Anagyrus kamali*	*Gyranusoidea indica*	Total Production
June	8,600	22,600	31,200
July	25,050	65,050	90,100
Aug	37,850	65,650	103,500
Sept	54,200	50,850	105,050
Oct	57,800	40,300	98,100
Nov	22,000	21,000	43,000
Dec 1-15	11,000	7,700	18,700
Total	216,500	273,150	489,650

Table 2. Parasitoid releases in Imperial Valley (up to 22 December 2000)

City	No. of Release Sites	*Anagyrus kamali*	*Gyranusoidea india*
Calexico	252	89,950	142,300
El Centro*	74	37,400	43,800
Heber	40	16,800	18,200
Holtville	1	2,000	2,200
Seeley**	11	12,200	16,100
Imperial	13	9,200	9,300
Total	391	167,550	231,900

* Includes Camacho's Place Restaurant
** Includes El Centro Naval Air Facility

Further Studies on Vine Mealybug and its Parasitoids on Grapes in the Coachella Valley

K. Godfrey, J. C. Ball, K. Daane[1], and D. Gonzalez[2]

The vine mealybug [*Planococcus* ficus (Signoret) (Homoptera: Pseudococcidae)], is a serious pest of table and raisin grapes in the Coachella and San Joaquin Valleys. Its feeding activity causes direct damage to the clusters. Since 1994, our program has been involved in studies on the biology and dynamics of this species. In 2000, three methods were used to evaluate the impact of two parasites (*Leptomastidea abnormis* and *Anagyrus pseudococci)* on the vine mealybug (VMB) and to evaluate changes in VMB age structures through space and time.

The studies were conducted from February through November in one block of a certified organic vineyard near Thermal (Riverside County). The two parasites (*L. abnormis* and *A. pseudococci*), had been released in/or adjacent to this block in previous years. In February, 270 vines with active VMB infestations were selected and assigned randomly into groups for each of three methods. The methods included vine wrapping (200 vines), field counts (50 vines), and trapping (20 vines).

The vine wrapping method was used to investigate parasitism of VMB. The 200 vines were assigned at random to one of ten subgroups. Each subgroup, (20 vines) was used on a different starting date. On each start date, the loose bark was removed from an area of the trunk and cordon on each vine. A band of bubble wrap (7.62 cm) was wrapped around the exposed section of vine, then left undisturbed to allow parasitization. After four weeks, the bubble wrap was removed, returned to the laboratory, and the developmental stage of VMB, their numbers, and the number of parasitized VMB (mummies) recorded. The VMB and mummies were then held for parasite emergence and identification.

The field count method was used to investigate changes in the VMB population age structure on different parts of the vine through time. At the beginning of the study, each vine within the 50-vine group was assigned at random to one of ten subgroups to be used on one sample date. At four week intervals, all vines in one subgroup were examined for VMB on the trunk, cordon, and roots. The VMB and mummies were collected, returned to the laboratory for counting, and held for parasite emergence and identification. The number and developmental stage of VMB, the number of mummies, and parasite species found at the various locations on the vine were recorded.

The trapping method was used to investigate VMB movement through space and time, and to determine the general pattern of activity of VMB males and parasites. The same 20 vines were sampled from February through November. In February, the loose bark was removed from portions of the trunk and a cordon of all 20 vines. The exposed areas were then wrapped with duct tape and a single width (1.91cm) of double-sided sticky tape placed on top of the duct tape. There was one tape placed on the trunk, and one on a cordon. A 7.6 cm x 12.7 cm yellow sticky card was hung in the canopy of each vine. The sticky tapes and cards were replaced every four weeks and returned to the laboratory to record the number of each stage of VMB and the number of each parasite species.

With the vine wrapping method, all life stages of VMB were found under the wraps. The density of VMB on the trunk peaked in May with a second, smaller peak in late August. On the cordon, the peak density occurred in April through May with a second, smaller peak in late

August. The reason for the smaller peak in August is not clear, but may be an artifact of sample size. Parasites were found in VMB that used the wraps as refugia. Adult *A. pseudococci* and *L. abnormis* were recovered from mummies in the March through June and November sampling dates.

The seasonal population dynamics of VMB revealed using the field count method was similar to that seen with the vine wrapping method. There was a peak in density in April and May, with a second peak in August. For the August peak, three out of the five vines had very large densities of VMB, and two vines had no VMB. This further demonstrates the clumped nature of VMB distribution within a vineyard and, consequently, the limitations of data from only a few samples. In the root samples, VMB was found sporadically. In May and August when substantial numbers of VMB were recovered on roots, most were found on one or two heavily infested vines. In general, VMB does inhabit the roots of grapes, but the extent of its habitation is influenced by many factors such as ant activity, soil texture, temperature, and irrigation regimes. Parasite mummies were recovered from February through October in the field count study, though not from the root samples. The parasite *A. pseudococci* was recovered a few months prior to the recovery of *L. abnormis*.

The seasonal dynamics of the VMB populations on the trunks and cordons of the 20 vines using the trapping method differed slightly from that revealed using the other methods. On the trunk, densities of small and medium VMB peaked in June, and decreased dramatically in July. Very few large VMB were collected on the tapes. On the cordon sticky tape traps, the peak density of small VMB (crawlers and early 2^{nd} instar) occurred in May, and medium (2^{nd} and early 3^{rd} instar) VMB peaked in June. For the small VMB, this peak is one month earlier than that seen on the trunk. Combining the data from this sampling method with that from the others, there appears to be a small resident population on the cordons in early spring that is reinforced by movement of other VMB from the trunk. All life stages of VMB on the cordon traps decreased in July. There was no increase in VMB density in August as seen in the other sampling methods. Yellow card catches of adult parasites was consistent with the other data. The parasite *A. pseudococci* was captured in low numbers in March and April and peaked in June. There was a slight increase in density in October and November. For the other parasite, *L. abnormis*, numbers did not begin increasing until May with a peak occurring in July. As with *A. pseudococci*, there was a slight increase in density in October and November.

The results of this study show that there was a peak in VMB density in mid to late spring followed by a dramatic decline in mid summer. The data were consistent with the idea that there was a resident population of VMB on all parts of the vine throughout the year. The dramatic increases in density in the spring were most likely the result of increased reproduction and movement of the VMB throughout the vine. The role of the roots on VMB seasonal dynamics requires further study because many factors may impact that role. The two parasites, *A. pseudococci* and *L. abnormis,* appeared to have established resident populations within this vineyard. The densities of both parasites responded to changes in VMB density. In our studies, *A. pseudococci* became active earlier in the season than *L. abnormis*.

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Establishment of Introduced Parasitoids of the Silverleaf Whitefly in Imperial Valley, CA

W. J. Roltsch

In an intensive, multi-institutional effort lasting over six years, numerous species and strains of aphelinid wasps in the genera *Eretmocerus* and *Encarsia* were studied and released in Imperial Valley and elsewhere to combat the silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring. Data collected in recent years indicates that the most promising *Eretmocerus* for the Imperial Valley desert region includes *E. emiratus* Zolnerowich & Rose, *E.* nr. *emiratus* from Ethiopia and *E. mundus* Mercet. *Encarsia sophia* (=*Encarsia transvena*) from Maltan, Pakistan appears promising as well. Identification to species is accomplished using recently published keys and by DNA analysis (RAPD-PCR) by the USDA-APHIS, Mission Biological Control Center, Mission, Texas. This report is a summary of silverleaf whitefly (SLWF) parasitoid population development specifically in long-term refuge nursery plots maintained by CDFA from 1994 to 2000, and more generally throughout the valley system via the survey of numerous cotton fields in Imperial Valley.

From 1994 through 1997, exotic parasitoids were released into long-term refuge field plots on multiple occasions each year. Plots (1/2 to 1 acre) were located at the Imperial Valley Research Center near Brawley, California, and at an organic farm at the south end of the county. During the warm season, plots consisted of okra and basil. During the cool season, cole crops (esp. collard) and sunflower were present. Kenaf, roselle and eggplant were also periodically present (1994-1996) along with adjacent plantings of cotton and spring cantaloupe. Leaf samples were taken approximately six times during each year to determine parasitoid population increase and persistence. Neither *Ert. tejanus* or *E. staufer*i (i.e., *Eretmocerus* spp. from Texas) have been recovered following their release. During 1995, *E. melanoscutus* was released in large numbers, but recoveries of this parasitoid were rare (Fig. 1a). Releases of *E. mundus, E. hayati* and *E. emiratus* began in April of 1996. Numbers of exotic parasitoids compared to natives were high during early summer; however, the proportion of the sample consisting of exotic species dropped markedly by late July, indicating poor performance (population increase and persistence) during this very warm summer period (Fig. 1b). During 1997, *E. emiratus* and *E.* nr. *emiratus* were released. The relative performance of exotics was considerably better than in 1996 (Fig. 1c). The proportion of exotic *Eretmocerus* relative to native *Eretmocerus eremicus* declined once again during late summer, however, not to the same extent. From 1998 until today, no additional releases have been made into these refugia. This made possible the assessment of populations released in previous years at these sites, in terms of their ability to overwinter and compete with native species of silverleaf whitefly parasitoids. Overwintering on cole crops was confirmed albeit in low numbers. During the summer of 1998 and 1999, *Eretmocerus* densities soared on okra, basil and adjacent cotton (Figs. 1d and e). By late August, there was a greater proportion of exotic *Eretmocerus* (upwards of 80% on okra and cotton) than native *Eretmocerus*. The order of dominance of exotic *Eretmocerus* species was *E.* nr. *emiratus*, *E. emiratus* and *E. mundus*. *Encarsia sophia* reached high densities during the summer and fall of 1998 and 1999 in several of the refuge field plots as well. Results during 2000 were similar to those found in 1999 (Fig. 1f). The lack of early spring samples was a result of low whitefly populations on collard and a delay in establishing spring planted crops. The status of exotic species composition for 2000 is pending.

Surveying cotton fields from 1998 to 2000 provided further evidence regarding the extent of exotic parasitoid establishment. Leaf samples were obtained from three edges of conventionally managed cotton fields in Imperial Valley during each year from late August to early October. Exotic *Eretmocerus* were detected in 9 of the 23 fields (i.e., 39%) sampled in the fall of 1998, and 22 of 23 fields (i.e., 96%) sampled in 2000 (Table 1). In fields where exotic *Eretmocerus* were detected, 6% of the *Eretmocerus* were exotics in 1998 and 26% were exotic in 2000. Similarly, an increase in *Encarsia sophia* was noted from 1998 to 1999. *Encarsia sophia* was detected in only one of 23 cotton fields (i.e., 4%) in 1998. However, *E. sophia* was detected in 27 of 42 cotton fields (64%) in 1999, and 24 of 29 (82%) fields in 2000.

Native *E. eremicus* is a common parasitoid of SLWF in Imperial Valley on most host plant species throughout much of the year. Given this, it is reasonable that by expressing exotic *Eretmocerus* as a percentage of total *Eretmocerus,* a useful (although simplistic) means of assessing the establishment of exotic species in this genus is provided. In summary, up to three exotic species of *Eretmocerus* are well established in Imperial Valley. By comparing them to native *Eretmocerus eremicus*, the relative yearlong abundance of exotic parasitoids increased consistently over the past three years. Likewise, the establishment of *Encarsia sophia* (a fourth exotic parasitoid species) appears to be well underway.

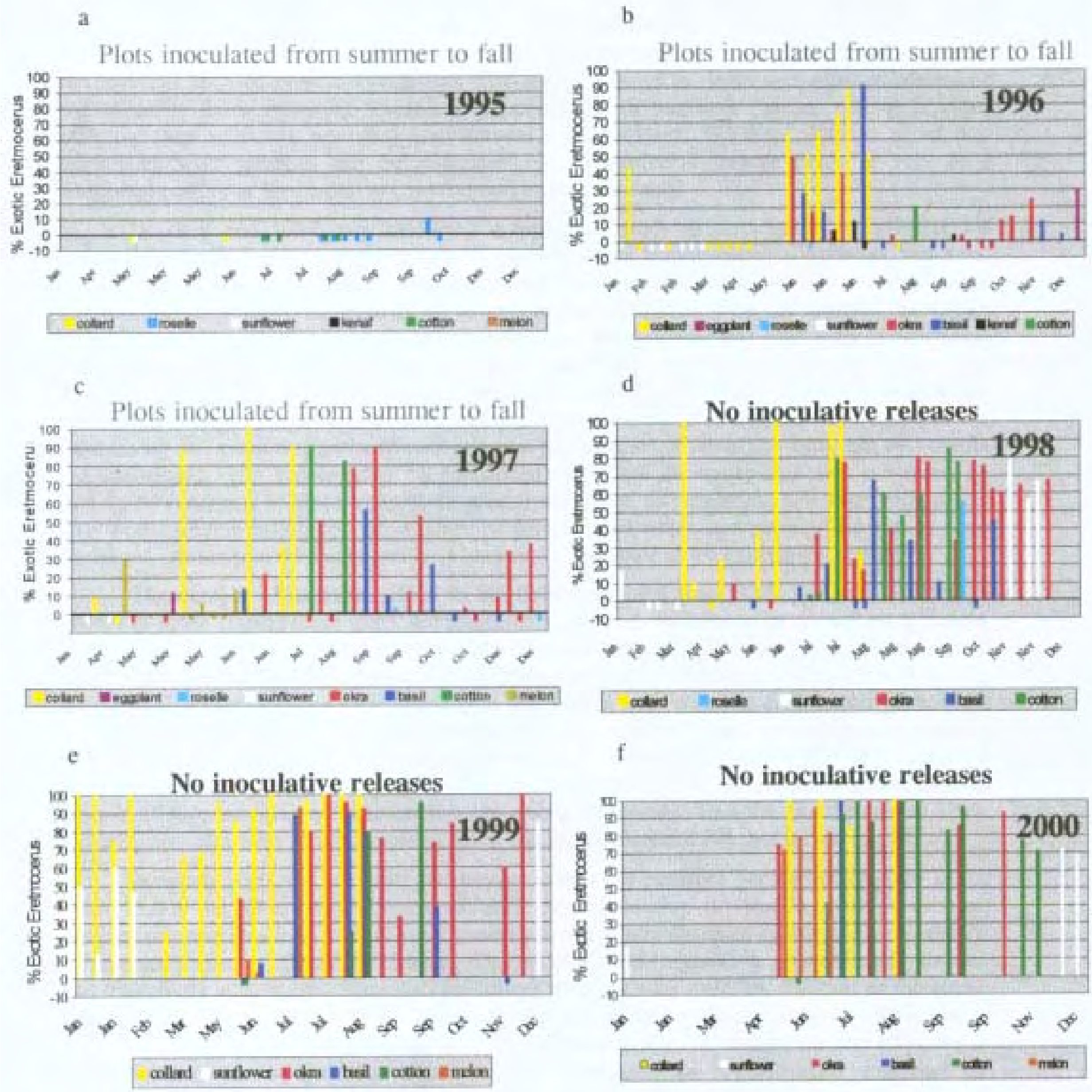
Table 2. Cotton field survey of exotic *Eretmocerus* in Imperial Valley

	Fall 1998	Fall 1999	Fall 2000
Fields sampled	23	42	24
Fields with exotics	43%(10)	74%(31)	96%(23)
Mean exotics in all fields	2%	21%	46%
Mean exotics in fields where detected	4%	28%	48%

Table 3. Cotton field survey of *Encarsia sophia (= E. transvena)* in Imperial Valley

	Fall 1998	Fall 1999	Fall 2000
Fields sampled	23	42	32
Fields with exotics	4%(1)	64%(27)	75%(24)

Fig. 1. Exotic *Eretmocerus* as a Percentage of All *Eretmocerus* Collected From 3-4 Refuge Field Plots From 1995 – 2000 in Imperial Valley, CA



NOTE: X-axis is presented in unequal monthly time intervals.
Bars less than zero represent zero percent; i.e., no exotic *Eretmocerus* in sample.

Augmentative Biological Control of Silverleaf Whitefly Using Transplants

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We report on a novel approach to enhancing early season field populations of *Eretmocerus* sp. using cantaloupe transplants. Cantaloupe seedlings prior to placement in fields were inoculated with silverleaf whitefly and then exposed to a highly specific whitefly parasite, *Eretmocerus* sp., recently imported from Ethiopia. We want to determine whether control of whiteflies in fields receiving transplants inoculated with parasites (banker plants) is more effective than in fields receiving conventional hand releases. We also want to show that transplants with parasites can be integrated into imidacloprid treated fields at very little additional cost, or at least equal to conventional insecticide costs.

In 1998 and 1999, we conducted a replicated study at an organic farm where we compared the effect of banker plants against plots receiving hand-releases of parasites, and a no-release control. We completed our third and final field season spring 2000. We set up paired comparisons in seven commercial farms of cantaloupe in Imperial Valley to measure the impact of banker plants on parasite and whitefly populations: three organic and four using conventional practices, i.e. imidaclopird for whitefly control. At each farm, two, 1-acre plots were delimited in a much larger field (20 acres or more), spaced at least 1 acre apart. In one of these plots, cantaloupe transplants inoculated with *Eretmocerus nr. emiratus* (Ethiopia) were added to the field. Every 10th plant was replaced with our own banker plant of about the same age, two-month old transplants. The survivorship of parasitized whiteflies on transplants was measured by staking 40 randomly selected transplants at each study site, and sampling and examining plants five weeks after planting for the number of parasite exit holes and pupae. The values were compared against control transplants held back and maintained at the experiment station.

The number of parasites added to the fields increased from 9000 per acre in 1998, to 24,000 in 1999. During these years, we measured significant differences on several sample dates in whitefly nymphal populations between the different treatment plots (see Pickett et al. annual report 1998 and 1999). Both years, the whitefly numbers were lowest in the plots receiving parasites. However, no consistent, significant differences were detected across both years when comparing insect population means between the two release strategies: hand versus banker plants. Parasitism means were consistently higher in the transplant plots in 1999, but about the same as in hand release plots in 1998. Plots were smaller (1/3 acre) and much closer to each other in 1998, promoting cross contamination among treatments. This was not the case in 1999. Whitefly numbers were generally lowest in the transplant plots in 1998, but mixed in 1999.

In spring 2000, we increased the average number of parasites per transplant up to 25, or about 25,000 per acre: range 5,000 to 45,000. No relationship is visually apparent between numbers released in a field and seasonal parasitism or whitefly densities (Table 1, Figs. 1 and 2). A 'repeated measures ANOVA', blocking for type of grower (organic vs. conventional), showed that transplants significantly increased parasitism in study sites over the duration of the study, April through June 2000 ($p = 0.03$). Parasitism was higher in organic fields (seasonal average = 51%) than conventional ones (seasonal average = 10%; Fig. 3). The average number of parasites per transplant was numerically higher on the transplants in organic sites compared to conventional plots after five weeks, but was not significantly different (32 vs. 21 per plant; $p>0.05$). These data suggest that the imidcloprid treatment in conventional fields retarded the population growth of released parasites. Other factors such as poor transplant survival, may also have contributed to poorer parasitism levels in conventional fields.

A repeated measures ANOVA showed that transplants, on average, had no impact on whitefly populations. Plots receiving transplants, whether organic or conventional, had the same silverleaf whitefly population density from April through June as the paired controls (α=0.05). However, some study sites showed a consistent suppression of whiteflies in treated plots over control plots (Fig. 1 and 2, e.g. Bornt's organic field #2 and Strahm's conventional field, # 6). Although one of the Bornt plots achieved very high levels of parasitism (#1), the releases had no net affect on the silverleaf whitefly densities. This occurred most likely because the study site was within 200 m of a hedge planted specifically for promoting the overwintering survivorship of whitefly parasites. Results from Heger's field are more difficult to explain, since the transplant treated plot had consistently high levels of parasitism, yet no measurable impact on the whitefly population. Silverleaf whitefly are known for their ability to migrate long distances. The imidacloprid treatment in the two conventional fields, Abbatti 30 and 182, probably had a greater impact on the whitefly population than did the banker plants. We did not monitor exactly how and when applications of this systemic were applied. Timing and rates of application could easily have caused between-site differences in our results.

The high levels of parasitism achieved in this study, plus suppression of whitefly numbers in replicated studies in 1998 and 1999, and in our paired-plot work of 2000 (two of seven fields), one organic and one conventional, show promise for this method. However, more work needs to be done to make this strategy a more predictable method. One area for research is the type of plants used. A tougher plant, capable of supporting more parasitized whiteflies deserves greater attention. Controlling a highly mobile pest such as silverleaf whitefly may be beyond the ability of augmentative biological control, at least in vegetables that have a very short growing season. Using transplants for augmenting natural enemies in other settings, such as greenhouses, perennial crops, or against pests with less propensity for rapid growth and dispersal would have greater potential.

Table 1. SLWF and *Eretmocerus* density on banker plants five weeks post planting.

Field Name	Production method	Number	Mean *Eretmocerus*	Mean SLWF
Bornt's near house	organic	1	20.3	1.5
Bornt's away from house	organic	2	45.3	1.5
Abbatti 30	conventional	3	5.02	0.48
Abbatti 182	conventional	4	40.6	1.96
Heger	organic	5	32.1	1.55
Strahm	conventional	6	7.1	2.42
Black Dog	conventional	7	29.8	1.56

Fig. 1. Organic plots receiving banker plants. Imperial Valley, spring 2000.

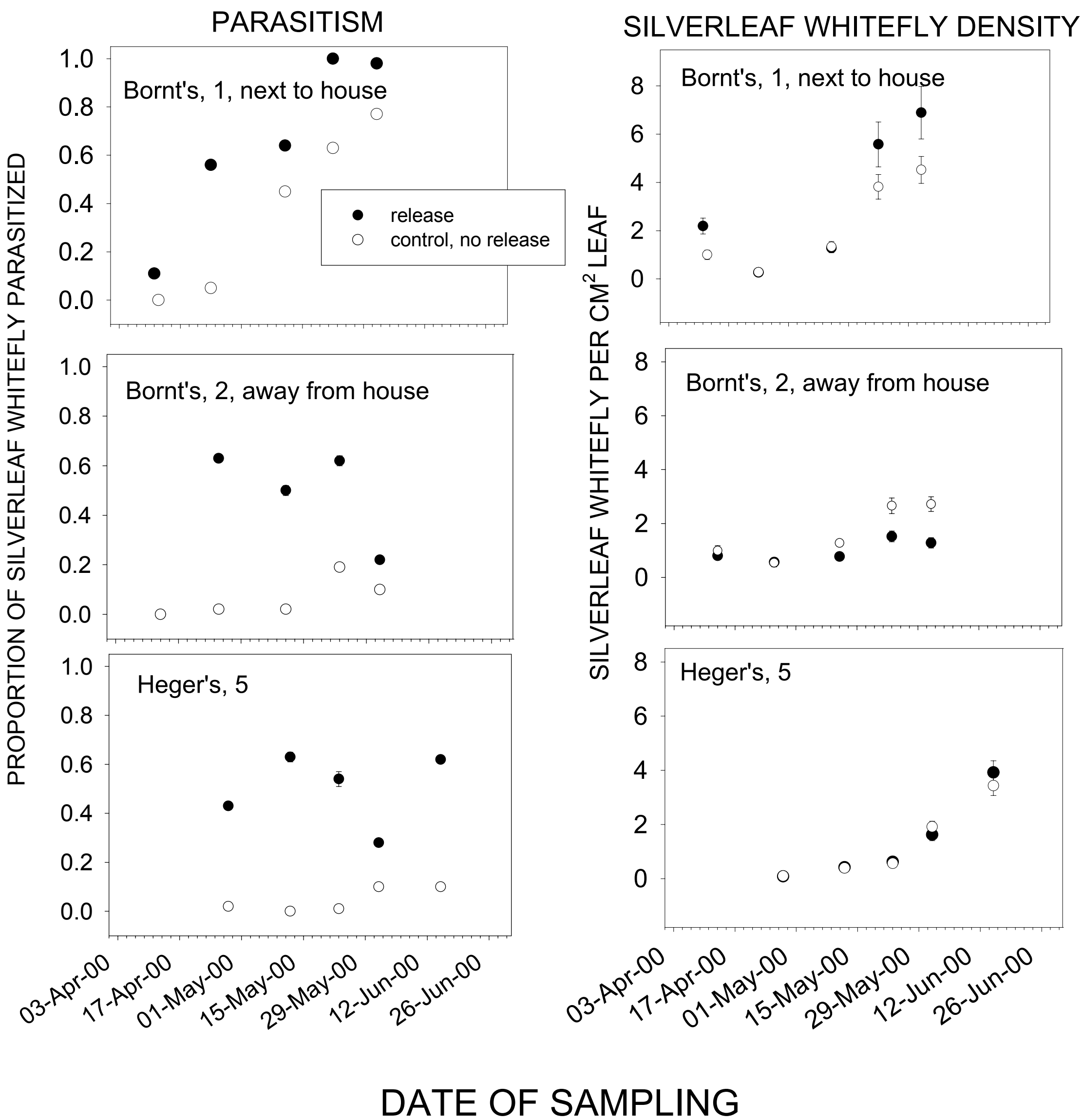


Fig. 2. Conventional plots: treated with both banker plants and imidacloprid.

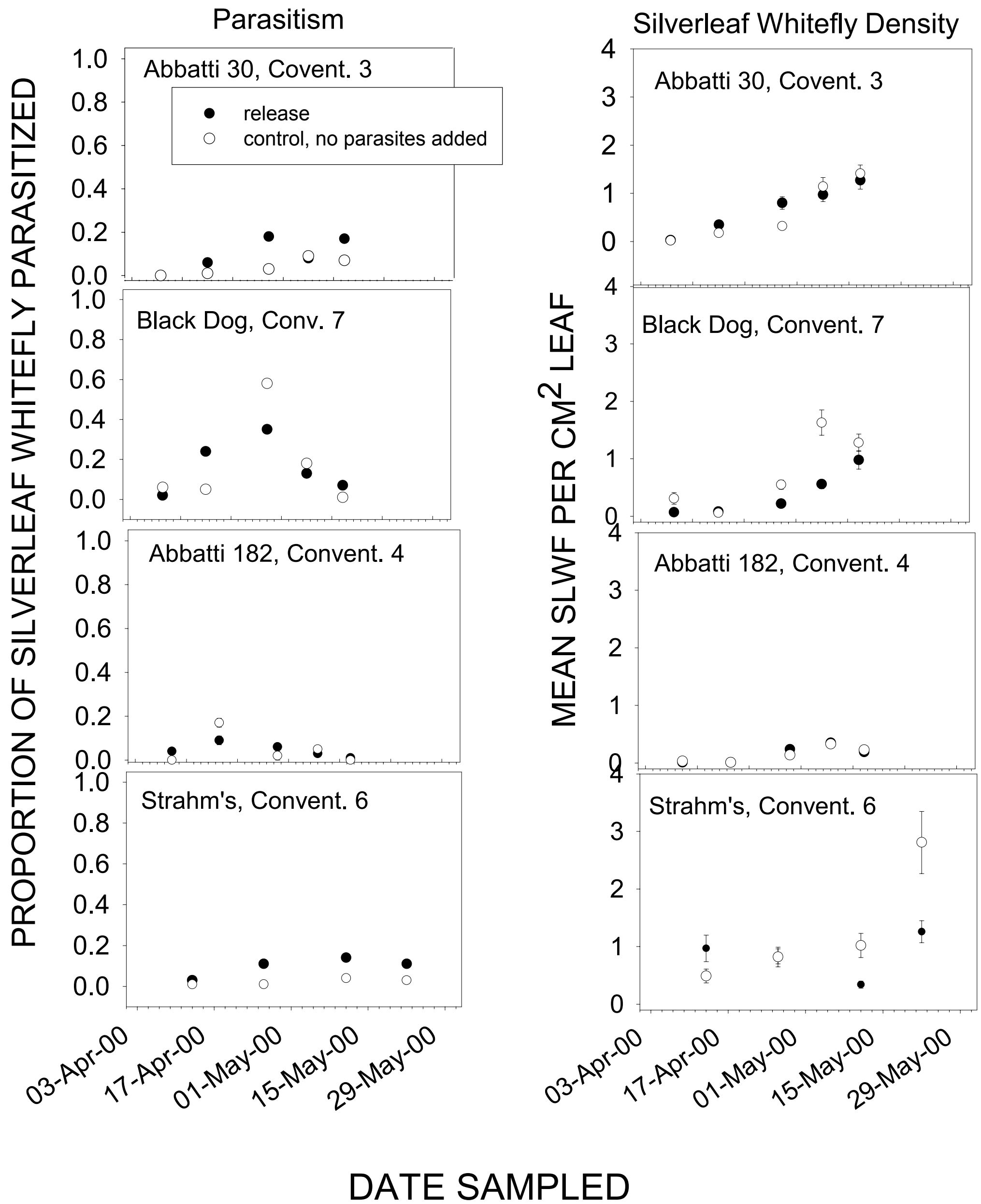
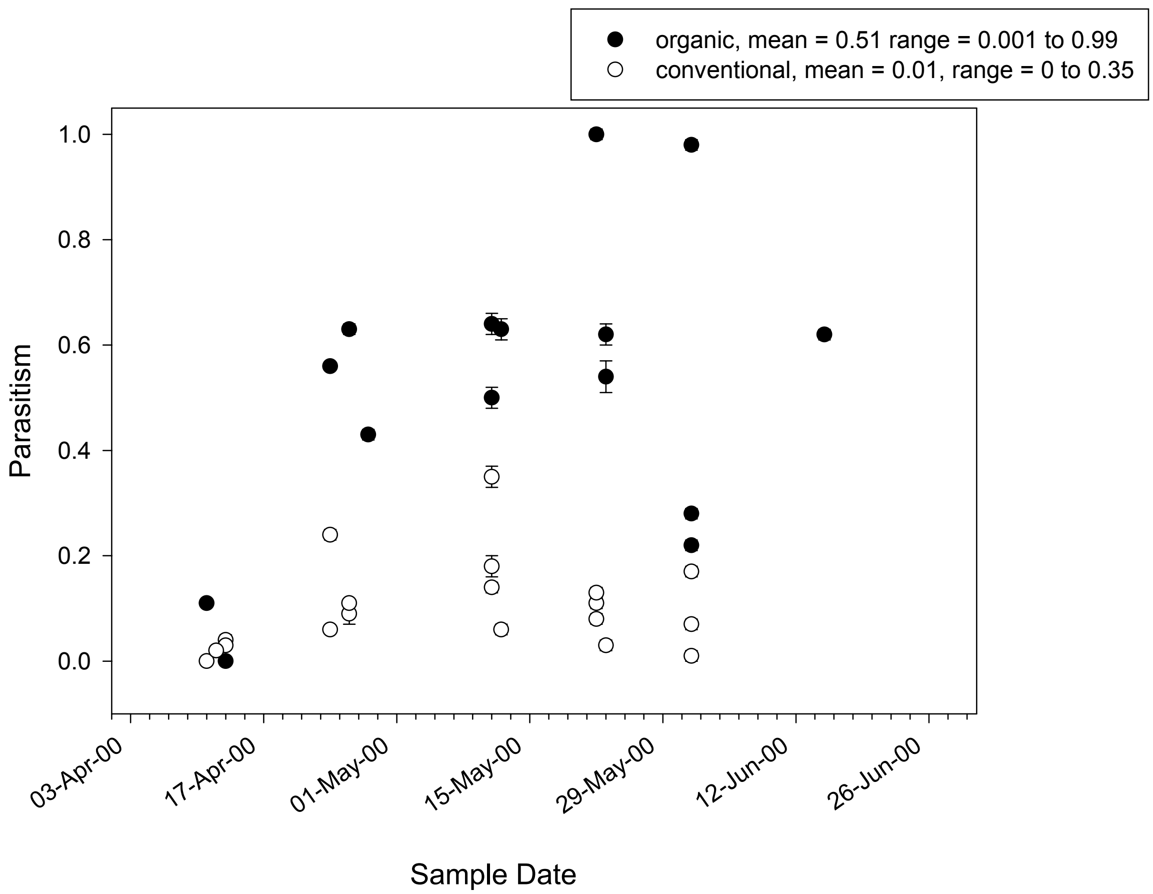


Fig. 3. Transplant Treated Plots,
Spring 2000. Parasitism in organic vs. conventional plots.



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Florida Collecting Trip for *Delphastus* spp.

C. H. Pickett and K. A. Hoelmer[1]

The correct identification of reared natural enemies is a constant problem for commercial insectaries. Recently, confusion about the identity of a whitefly predator has affected both homeowners and researchers. The native coccinellid beetle, *Delphastus* sp., is a valuable predator of whiteflies and is cultured and sold commercially worldwide as a whitefly-specific predator for greenhouse production and homeowners. Attempts have also been made to establish it in new regions to assist in permanent reductions in whitefly populations. The initial observations of this beetle as a predator of *Bemisia* were made in central Florida in the late 1980's by L. S. Osborne (University of Florida), and a culture established from this population was used to conduct the first research documenting the efficacy of the beetles. Specimens from this population were initially identified as *D. pusillus,* native to eastern North America. This research culture was also the source of all known distributions to other researchers and to commercial insectaries throughout North America and internationally during the early 1990's.

Growing interest in the use of *Delphastus* as a whitefly predator led to a taxonomic revision of the genus by the USDA coccinellid specialist Robert Gordon. This work in turn, raised questions about the actual identity and historical origin of the species originally studied and cultured commercially. Recently collected specimens from Florida include both *D. catalinae* (native to coastal California, Central and South America and the Caribbean), and *D. pusillus*. *Delphastus* recently purchased from private insectaries were inadvertently identified as *Delphastus pusillus* rather than *D. catalinae*. Obscure records indicate that, earlier in the century, there were releases of *D. catalinae* obtained from California into Florida, but these were not thought to have persisted. Because of these developments we thought that it would be useful to re-examine the current distribution of naturally-occurring *Delphastus* populations in Florida to support future use and artificial spread of the beetle by commercial insectaries. One of us (CHP) traveled to Florida in October 2000. The *Delphastus* collection at the Florida Department of Agriculture and Consumer Services was examined and a collecting trip conducted.

The earliest collection of *Delphastus* at the Florida Department of Agriculture's collection in Gainesville was in 1918, and the most recent in 1988 by Ru Nguyen. Twenty-six collections were examined (Table 1). One taxonomic separation was made, based on the presence or absence of punctuations on the pro-sternum of adults. All the examined specimens were previously labeled as *D. pusillus*, and came from drawers labeled as such. Until recently it was the only species present in Florida, according to Robert Gordon's latest taxonomic revision. Two collections that were examined came from a drawer marked as "unidentified" specimens. Based on the punctation character alone, all specimens collected prior to 1960 were identified as *D. pusillus*. The first *D. catalinae* showing up in this collection came from Tampa, Florida, in May 1960. From 1960 to 1988 there were 19 additional entries (i.e, unique collections that could have more than one pointed specimen). Of these, five were *D. catalinae*, and mis-identified as *D. pusillus*. One of the unidentified collections was a mix of both *D. pusillus* and *D. catalinae*. Both were collected from the same site (citrus) on the same day, the host being *Dialeurodes citri*.

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Table 1. Florida Dept. Agriculture Collection of Delphastus. All specimens came from trays marked as *Delphastus pusillus* or from unidentified specimens

Date of Collection	County in Florida	City	Host plant	Host whitefly	Identification: Current	Identification: by CHP	Determined or collected	# Examined/# in collection
Sept. 14, 1918	Manatee				pusillus	pusillus	Matchlor	3/18
Oct. 24, 1921		Oakland			pusillus	pusillus		4/8
Aug. 7, 1953	Dade	Miami			pusillus	pusillus	Link	1
Oct. 16, 1953	Alachua	Gainesville	Gardenia		pusillus	pusillus	Frost	5/8
Jun. 5, 1954	Dade		*Callicaspa americana*		pusillus	pusillus	Merril	1
Jan. 26, 1955	Sarasota	F. W. Mead Stn.	Lychee		pusillus	pusillus		1
Aug. 2, 1955	Santa Rosa		*Prunus carolina*		pusillus	pusillus	F. W. Mead	1
Jan., 12, 1957	Dade	Miami	Trema		pusillus	pusillus	Weems	1
May 10, 1960		Tampa			pusillus	catalinae	C. W. Hale	1
May 27, 1964	Alachua	Gainesville			pusillus	pusillus	R. White	1
Oct. 1, 1964		Apopka	Citrus		pusillus	pusillus		1
Sept. 18, 1967		Edgewater			??	??		1
May 2, 1974	Alachua	Gainesville		whitefly	pusillus	pusillus		1
Sept. 20, 1974	Alachua	Gainesville		Citrus whitefly	pusillus	catalinae	R. Gordon	1
July 10, 1975	Alachua	Gainesville?	Citrus?	*Dialeurodes citri*	pusillus	pusillus		3
July 10, 1975	Alachua	Gainesville?	Citrus?	*Dialeurodes citri*	pusillus	catalinae		2
Feb. 9, 1976	Dade	Miami			pusillus	pusillus		2
Feb 24, 1976	Broward				pusillus	pusillus		2
Jun 22, 1977	Broward	Ft. Lauderdale	Citrus		pusillus	pusillus	R. Schimmel	1
April 7, 1978	Highland	Archibold Biol. Stn.			pusillus	pusillus	H. W. Weems Jr. & L.L. Lampert Jr.	2
Aug. 29, 1979	Broward	Ft. Lauderdale			pusillus	pusillus	R. Gordon	1
Sept. 10, 1982	Broward	Hollywood	Guava		pusillus	catalinae	B. Harrell	1
Dec. 16, 1982	Dade	Homestead	Black olive		pusillus	catalinae	R. Gordon	1
Aug. 30, 1987	Alachua	Gainesville		*Dialeurodes*	pusillus	pusillus	F. Bennett	1
April 15, 1988	Manatee	Ellenton	Citrus	Citrus black fly	pusillus	pusillus	R. Nguyen	2
July 24, 1988	Manatee	Palmetto	Citrus	*Aleurocanthus woglumi*	pusillus	catalinae	F. Bennett	2
Oct. 7, 1988	Pinellas	Clearwater	Carica		pusillus	pusillus		3

Based on these findings, we propose that *D. catalinae* was introduced purposely or by accident sometime during the first half of the last century. Today, Florida has two species of *Delphastus*. The collections that Dr. Lance Osborne of the University of Florida made around 1990 and shipped to California could have had both species present. He made his collection from one site in Apopka, Florida, from *Bemisia* infested papaya. The CDFA Biological Control Program was rearing *Delphastus* at that time which originated from Dr. Osborn's shipments. Releases of *Delphastus* were made into Imperial County and the San Joaquin Valley using this culture that was thought to be *D. pusillus*. Voucher specimens from the same culture were later identified as *D. catalinae*, yet this species was never recovered in the field. Dr. Robert Gordon has identified specimens coming back from the San Joaquin Valley recoveries as mainly *D. dejavu*, but were identified as *D. pusillus*. The latter species are supposed to be east coast in origin. We hypothesize releases of both species were made back in 1993/1994, but only the *pusillus* type survived

Five locations were sampled from Gainesville south to Ft. Pierce. Dr. Ru Nguyen of the Florida Department of Agriculture contacted state workers in Orlando, Bradenton/Palmetto, and Ft. Pierce to assist. *Delphastus* was not recovered at any locations. The closest relative found was *Nephaspis* feeding on papaya whitefly at the Epcot Center. Dr. Osborn identified these beetles. The other locations where sampling took place are shown in Table 2. *Delphastus* needs high numbers of whiteflies to develop into large populations. None were found on this trip.

Table 2. Sampling locations for Delphastus

Location	Date	Plant	Whitefly	Beetles?
Orlando, Univ. Central Fl. arboretum	Sept. 31	Magnolia	Wooly whitefly	Coccinellidae
Orlando, Univ. Central Fl. arboretum	Oct. 5	Magnolia	Wooly whitefly	Coccinellidae
Apopka, Magnolia Pk	Sept. 31	Citrus	Wooly whitefly	
Apopka, Epcot Ctr	Oct. 2	Papaya	Papaya whitefly	>20 Nephaspis
Gainesville, UF	Oct. 1	Citrus,	Wooly whitefly	
Gainesville, Steck's house	Oct. 1	Magnolia	Wooly whitefly	
Bradenton/Palmetto	Oct. 3	Sea grape	Wooly whitefly	1 Nephaspis
Bradenton/Palmetto	Oct 3	Citrus	Wooly whitefly	
Vero Beach (N. of Ft. Pierce)	Oct. 3	Sea grape	Wooly whitefly	
Ft. Pierce	Oct. 3	Hibiscus	Giant whitefly	

We hope to make the same trip again in mid summer to increase the chances of finding whitefly hosts that support these beetles. The current distribution of *Delphastus* would show how *Delphastus catalinae* has spread throughout Florida and whether is has displaced *D. pusillus* in any areas.

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Establishment of Nursery Sites for Two Introduced Parasites of the Cotton Aphid

K. Godfrey, M. McGuire[1], D. Ballard[1], D. A. Mayhew, and K. Casanave

The cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae), can attain pest status in a variety of crops in the San Joaquin Valley. Management of these populations must be done with care so as to avoid problems of insecticide resistance and/or cotton aphid population resurgence. Biological control may be one tactic that could be used to manage aphid populations within an area of spatially or temporally adjacent crops or habitats.

In an attempt to increase the amount of biological control on cotton aphid populations, a cooperative project among USDA-Agricultural Research Service, CDFA-Biological Control Program, UC Cooperative Extension, and the University of Arkansas was initiated in 1996 and continues currently. The long-term objective of this project was to construct a natural enemy complex using natural enemies not currently found in California to complement the existing natural enemy complex of the cotton aphid. After four years of research, two parasite species, *Aphelinus* near *paramali* and *Aphelinus gossypii* Timberlake (Hymenoptera: Aphelinidae), were identified as the first components of the introduced natural enemy complex. Therefore, distribution of these two parasite species throughout the San Joaquin Valley began. In addition, studies on other natural enemies that may be candidates for inclusion in the introduced natural enemy complex will continue as natural enemies become available.

The distribution of the two introduced parasites, *Aph.* near *paramali* (ANP) and *Aph. gossypii* (AG), began with the establishment of ten parasite nurseries; two nurseries each in Merced and Madera Counties, and six nurseries in Kern County. Most of the nursery sites have a variety of habitats that are favorable for cotton aphid throughout the year. Beginning in early July, each site was visited and the aphid population sampled by examining 40 to 80 plants within the site for the presence of aphids. Once cotton aphids were found, parasite releases began and continued until the cotton was harvested. Weekly sampling to determine if the parasites were using the cotton aphids that were present began approximately two weeks after the first release. Any aphids or mummies recovered from the sampling were returned to the laboratory and held for parasite emergence. Once the cotton was harvested, other plants that could support cotton aphid were sampled at approximately monthly intervals.

From the releases and sampling, black mummies, indicative of the introduced parasites, were recovered from all nursery sites. In addition, ANP and AG were recovered from nine out of the ten nursery sites. In the nursery sites producing adult parasites, more ANP were recovered than AG. At the one nursery site in Kern County where black mummies only were recovered, hyperparasites emerged from the mummies. The differences in production of parasites at the nursery sites may have been due to the suitability of habitats surrounding the nurseries for the development of cotton aphid populations. This study will continue for a second year.

Research supported with a grant from the California Cotton Pest Control Board
[1]USDA, ARS, Shafter Research and Extension Center, Shafter, California

Evaluation of Habitats for Introduced Parasites of the Cotton Aphid in the San Joaquin Valley: A Two-Year Study

K. Godfrey, M. McGuire[1], D. Ballard[1], K. Casanave, and D. A. Mayhew

The cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae), is a ubiquitous pest in the San Joaquin Valley and can take advantage of the mosaic of habitats throughout the Valley because of its broad host range. Introductions of new natural enemies that should complement the existing natural enemy complex are currently underway. Although the target crop for these releases is cotton, it would prove beneficial if the introduced natural enemies would move to habitats that are spatially and temporally adjacent to cotton along with the cotton aphid. The ability of two introduced parasites, *Aphelinus* near *paramali* and *Aph. gossypii* Timberlake (Hymenoptera: Aphelinidae), to attack cotton aphid in a variety of habitats and move with cotton aphid was investigated. The habitats included crops such as cotton, citrus, melons, nectarines and almonds, as well as non-crop plants that grew at the edges of fields.

The ability of *Aph.* near *paramali* (ANP) and *Aph. gossypii* (AG) to move with the cotton aphid through space and time was investigated at four geographically separated sites in Kern County. Each site had at least four habitats that could support cotton aphid at some time during the year, one of which was cotton. Releases of both parasites were made at each site. Since both parasites are exotic to California, all recovered individuals came from our releases. To monitor the movement of the parasites and cotton aphids, yellow sticky cards were placed around the appropriate habitat (i.e., the habitat most likely to have cotton aphid) and changed weekly throughout the year. Directional pan trapping was also conducted, but only near cotton and at times of the year when flights of cotton aphid alates were most likely. Collections of aphids from host plants were also made when aphids could be found. These aphids were held in the laboratory under the appropriate environmental conditions to allow the development of any parasites. Adult parasites recovered were identified and the number recorded.

Episodes of movement of alate cotton aphids appeared to be similar throughout the trapping area in both years. Winter and early spring movement of the alate aphids appeared to be related to searching for suitable host plants within a habitat. As these host plants declined in suitability, movement out of the habitat began in late spring and early summer. In both years, there was an easily detectable flight of cotton aphid near cotton in mid to late July. The flight in 2000 was approximately one week later in July than the flight that occurred in 1999. The slight difference in timing was probably related to weather factors that influenced the rate of decline in suitability of the host plants used before cotton was invaded. The flights of alate cotton aphid throughout the rest of the summer and early fall were probably a combination of movement into the cotton field and movement out of the cotton field as the densities of non-alate cotton aphid increased within the field. Detectable flights of alate cotton aphids also occurred at cotton harvest and stalk destruction in all fields. The directional pan trap data supported the data from the yellow sticky cards. In addition, there was a trend of capturing more alate cotton aphids in the northern and western quadrants. This suggests that there was a general movement by the aphids from the north and west to the south and east. Much more extensive trapping would have to be conducted to determine if this is a real trend or an artifact of this data set. In addition, both introduced parasites, ANP and AG, were recovered from the yellow sticky cards and from the aphid collections made in the various habitats. The recovery of the parasites demonstrated that the introduced parasites would move short distances to find host aphids and could successfully reproduce in a variety of habitats that can support cotton aphid in the San Joaquin Valley.

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[1]USDA, ARS, Shafter Research and Extension Center, Shafter, California

Evaluation of Survival of Introduced Aphid Parasites in Mature Citrus Groves: A Two-Year Study

K. Godfrey, E. Grafton-Cardwell[1], L. Whitendale[2], D. Mayhew, and K. Casanave

The cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae), impacts citrus through its ability to vector citrus tristeza virus. A reduction in the density of cotton aphid will reduce the threat of citrus tristeza virus spread. Biological control is one management tactic that can be used to achieve this reduction in cotton aphid densities. Two introduced parasites, *Aphelinus* near *paramali* and *Aphelinus gossypii* Timberlake (Hymenoptera: Aphelinidae), have been shown to reduce cotton aphid densities in other habitats such as cotton, young citrus, and winter non-crop areas (see annual reports 1998 and 1999). In this study, the ability of these two parasite species to establish in a mature citrus grove was investigated.

The survival of *Aph.* near *paramali* and *Aph. gossypii* was investigated in a grove of Valencia oranges at the Lindcove Research and Extension Center from April 19, 1999, to October 26, 2000. Releases of the parasites were made by placing potted hibiscus plants containing cotton aphid and mummies of each of the parasites underneath the canopy of ten mature orange trees. These plants were left undisturbed for three weeks. After that time, the plants were replaced with new aphid and parasite-infested plants. The plants that had been in the field for three weeks were returned to the laboratory and examined for evidence of parasite reproduction.

Recoveries of both species of parasites were made from the hibiscus during the first year of the study, but only *Aph.* near *paramali* adults were recovered in the second year. However, numerous black mummies, indicative of the two introduced parasites, were found throughout the entire sampling period. The majority of these black mummies were found on the hibiscus. This is not too surprising considering the largest density of cotton aphids within the grove was on the hibiscus. The parasites may have begun to establish within the grove, but with the low density of cotton aphid normally within the grove, they will be extremely difficult to detect.

Lindcove Research and Extension Center provided the Valencia orange grove.
[1]University of California – Riverside, Kearney Agricultural Center, Parlier, California
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Long-Term Evaluation of the Ash Whitefly Parasitoid, *Encarsia inaron*

C. H. Pickett and R. Wall

The ash whitefly, *Siphoninus phillyreae* (Haliday) (Homoptera: Aleyrodidae), invaded southern California in 1988. Populations rapidly spread throughout the state, infesting ornamental street trees commonly planted by city governments. Clouds of adult whiteflies in urban centers were common. An imported wasp, *Encarsia inaron* (Walker) (Hymenoptera: Aphelinidae), reduced their populations to levels difficult to detect. Despite its dramatic success, there is some question as to the stability and long-term behavior of the host-parasitoid relationship. We designed a study to characterize the long-term population dynamics of ash whitefly and *E. inaron* among several geographic and climatic areas of central and northern California. Original release trees in Contra Costa, El Dorado, Madera, Sacramento, Shasta, San Mateo, and Yolo Counties have been monitored since 1993 for ash whitefly abundance and percentage parasitism. There are four release trees per county, yielding 24 trees consisting of 14 ash, 5 pomegranate, and five ornamental pear trees. Sampling has been conducted once each year at all release sites (single trees). Sites were visited once over a two-week period beginning in mid-July. We chose this time since it corresponded with the peak number of ash whitefly recorded in our earlier, statewide study. The abundance of whiteflies was estimated from 30 leaves selected arbitrarily within arms reach from the lower canopy of each tree. These were examined under a microscope and all ash whitefly eggs, immatures and pupae were counted. The impact of the parasitoid was estimated by dissecting 30 ash whitefly immatures from each tree. Ten to 15 leaves with ash whitefly 4th instar nymphs or pupae were collected from each tree and returned to the lab for dissection. No more than three nymphs or pupae were removed from a single leaf for the total of 30 immatures selected for dissection.

Figure 1 shows the ash whitefly abundance and parasitism levels over the last 10 years. Data from the first three years, 1990 – 1992, were collected as part of a statewide parasitoid release/establishment effort (Pickett et al. 1996) and thereafter as part of a long-term study. Populations of ash whitefly peaked in 1991 at 13 whiteflies (eggs and nymphs) per cm^2. Since 1993 the population has not exceeded 0.26 individuals per cm^2 leaf, 50 times below the highest value recorded in the absence of *E. inaron*. Seasonally averaged parasitism peaked in 1992, just two years after most parasites were released. Parasitism during the single yearly sample since that time has varied from 8.6% to 58.4%. Within year parasitism values often increased to 100% (Table 1). The whitefly specific predator, *Clitostethus arcuatus* (Rossi) (Coleoptera: Coccinellidae) was also observed in trees. It too was released as part of a statewide effort to control ash whitefly. This beetle could have contributed to whitefly mortality. It has been recovered each year since we began sampling in 1994, but has been sporadic in its presence on sample trees varying from 25 to 50% of those sampled. The results from earlier field studies (Gould et al. 1992, Pickett et al. 1996), the persistent populations of *E. inaron,* significant correlation between host and parasitoid populations ($r = 0.25$, $p = 0.003$; Fig. 1), and results from exclusion cages reported in last year's annual report show this parasitoid is primarily responsible for maintaining low population densities of ash whitefly since its introduction.

Fig. 1. Long term Impact of Encarsia inaron on ash whitefly.

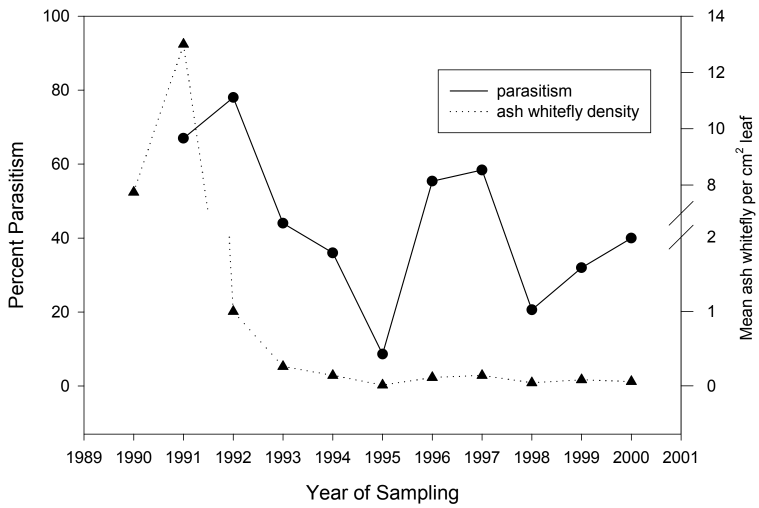

Table 1. Ash whitefly abundance on leaves, parasitism by *Encarsia inaron,* and percentage of sampled trees with *Clitostethus arcuatus* present in California.

Year	Mean AWF/CM^2 (range), N	%Parasitism (range), N	% sites with *C. arcuatus* (N)
1990 [a]	7.74 (0 – 24), 54	----	---
1991 [a]	13.06 (0 – 84), 243	67.0 (not available), 77	---
1992 [a]	1.04 (0 – 24), 300	78.0 (not available), 53	---
1993	0.26 (0 – 1.85), 25	44.5 (0 – 96.7), 21	---
1994	0.14 (0 – 0.98), 25	36.0 (0 – 84.2), 23	37.5 (25)
1995	0.01 (0 – 0.10), 24	8.6 (0 – 55.0), 15	25.0 (24)
1996	0.11 (0 – 1.10), 28	55.4 (0 – 100.0), 25	37.5 (28)
1997	0.14 (0 – 0.73), 28	58.4 (0 – 100.0), 25	44.7 (28)
1998	0.04 (0 – 0.33), 28	20.6 (0 – 75.0), 21	25.0 (28)
1999	0.08 (0 – 0.36), 28	32.4 (0 – 82.0), 7	30.0 (28)
2000	0.056 (0 – 0.44), 14	39.8 (0 – 82.0), 15	14.3 (14)

[a]From Pickett et al. 1996

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Insect Natural Enemies Mass Reared for Research and Colonization Projects

K. Casanave, J. Brown, D. Mayhew, L. Brace, L. Lee, E. Stallions, and L. Braddock

Each year, one or more insect natural enemies are mass reared for a variety of projects conducted by the Biological Control Program or other state and federal agencies. These research or colonization projects may not be reported elsewhere in our annual summary. Below we list these projects, the agency primarily involved in the work, and a description of the project goals. This past year, we reared natural enemies for control of cotton aphid, *Aphis gossypii,* silverleaf whitefly, *Bemisia argentifolii*, and the western tarnished plant bug, *Lygus hesperus*. The USDA-ARS and USDA-APHIS provided material for starting cultures. The DNA "banding patterns" reported below for whitefly parasites are from a PCR fingerprinting technique developed by the USDA-APHIS, PPQ, Plant Protection Center at Mission, Texas. The patterns are considered unique to strains or species of parasites that have not been described or identified using traditional morphological techniques.

2000 Releases of Natural Enemies by CDFA's Biological Control Program

Natural Enemy	Host	Source Population	Agency Receiving Shipments	Project Description	Stage Delivered	Total Delivered
Psyllaephagus sp.	Red gum lerp psyllid	Australia	UC Berkeley	Red gum lerp psyllid	adults	500??
Eretmocerus M96076 (Ethiopia)	silverleaf whitefly	ERET-13(DNA banding pattern)		Greenhouse, control	pupae	70,000
Encarsia sophia (=*transvena*; Pakistan)	silverleaf whitefly		CDFA, Shafter	Nursery sites	Pupae	124,000
			CDFA, Brawley	Nursery sites	Pupae	60,000
Aphelinus nr paramali	cotton aphid		USDA-ARS, Shafter/Kern	Nursery Sites	Adults & pupae	8,950
			CDFA/Madera & Merced	Nursery Sites	Adults & pupae	6,400
			UC Lindcove Field Stn.	Citrus, open release	pupae	4,250
			USDA-ARS, Shafter/CDFA	Movement of Parasites, Kern Co.	Adults & pupae	9,450
Aphelinus gossypii	cotton aphid		USDA-ARS, Shafter/Kern	Nursery Sites	Adults & pupae	28,420
			CDFA/Merced & Madera .	Nursery Sites	Adults & pupae	9,220
			UC Lindcove Field Stn.	Citrus, open release	pupae	3,400
			USDA-ARS, Shafter/CDFA	Movement of Parasites, Kern Co.	Adults & pupae	16,730
Peristenus stygicus	Lygus	France(Herault, Lattes)	CDFA, Sacramento, North B St. facility	Cage, open release into alfalfa	Larvae/adults	2105/2100
			UC/USDA Shafter Field Station	Open release, alfalfa	Larvae/adults	2119/1500
		Italy (Umbria)	UC/USDA Shafter Field Station	Open release, alfalfa	Larvae/adults	1213/0
		Italy (San Donna de Piave)	Yolo Co. (UCDavis)	Cage, open release into alfalfa	Larvae/adults	3051/3000
			UC Kearney Ag Stn.	Open release, alfalfa	Larvae/adults	2063/1500
		Spain(Catalognia, Navata; nr. Figueras)	Yolo Co. (Fongs nr. Woodland)	Open release into alfalfa	Larvae/adults	3057/2400
		Spain (Catalognia, Navata; nr. Figueras)	Kern Co. (Sanders nr. Maricopa)	Into alfalfa	Larvae/adults	576/300
		Spain (Catalognia, Navata; nr. Figueras)	Merced Co. (Gurr Rd.)	Into alfalfa	Larvae/adults	1410/3450
Peristenus digoneutis	Lygus	Italy (San Donna de Piave)	UC/USDA Shafter Field Station	Open release, alfalfa	Larvae/adults	418/0
Gonatocerus triguttatus	glassy-winged sharpshooter	Mexico(Tamaulipas & Nuevo Leon)	UC Riverside	Nursery site, open release	Adults	300
		Mexico(Tamaulipas & Nuevo Leon)	Fillmore, Ventura Co.	Nursery site, open release	Adults	300
		Mexico(Tamaulipas & Nuevo Leon)	General Beal Rd., Kern County	Nursery site, open release	Adults	300
		Mexico(Tamaulipas & Nuevo Leon)	Temecula	Nursery site, open release	Adults	300

Update on New Russian Thistle Natural Enemies for the Western United States

M. J. Pitcairn, R. Sobhian[1], M. Cristofaro[2], J. Kashefi[3], G. Campobasso[4], L. Smith[5], and B. Bruckart[6]

A renewed effort is underway to achieve biological control of Russian thistle, *Salsola tragus* L. (=*Salsola kali spp. tragus*), in the western United States. A team of USDA-ARS scientists and cooperators located in Eurasia has been pursuing this effort, and during 1998 through 2000, several new natural enemies were discovered attacking Russian thistle in Europe and Asia. The information reported here summarizes the status of the research effort for each natural enemy to date.

***Lixus salsolae* (Coleoptera: Curculionidae)** R. Sobhian discovered this weevil attacking *S. tragus* in several locations in eastern Europe and Asia. The insect causes substantial damage to stems of infested plants that can result in mortality of the host. Examination of sugar beet fields near infested plants in Turkey failed to find this weevil, suggesting that it may be specific to *Salsola*. However, in a preliminary host specificity test carried out in Turkey, oviposition and slight feeding was observed on sugar beet, but the larvae did not develop to adults. Unfortunately, a similar weevil was found on sugar beet plants in a commercial beet field in China. The difference in behavior between these two populations suggested that they may be different species. Enzo Colonnelli, a curculionid taxonomist in Italy, examined adult *L. salsolae* from Turkey and China, but he was unable to find morphological differences between these populations. The use of molecular taxonomy may be necessary to clarify this problem. Still, because of the high amount of damage on *Salsola* caused by this weevil, additional host testing of the weevils from Turkey will be pursued.

***Aceria salsolae* (Acari: Eriophyidae)** This mite deforms the growing tips and leaves of the plant, stopping growth of infested branches. Preliminary host tests by R. Sobhian and J. Kashfi show that this mite can attack both biotypes A and B from California and to be highly host specific. Lincoln Smith, USDA-ARS, will perform final host specificity testing in quarantine facilities in Albany, California. R. Sobhian will collect and ship infested plant material to Albany. The mite population from Turkey will be used for the final tests, because the mite was more common in Turkey and the effect on plants was more serious than the population in Greece.

***Piesma salsolae* (Hemiptera: Piesmatidae)** This plant bug occurs widely throughout Europe and Asia. It damages the plants by killing the growing tips of infested branches and sucking the green paranchymal tissue. Preliminary host specificity testing of this insect by R. Sobhian has begun. Unfortunately, the results indicate that, under no-choice conditions, this insect completes development on sugar beets and several other related species. A decision on whether to proceed further with this insect will be made once currents tests are completed.

***Baris soricinae* (Coleoptera: Curculionidae)** G. Campobasso discovered this weevil attacking *S. tragus* plants in Sicily. While not common, it does cause a considerable damage to the roots of infested plants. Preliminary host testing of this agent will begin in 2001 at the European Biological Control Laboratory substation in Rome, Italy.

***Ritocnema* n. sp. (Coleoptera: Chrysomelidae)** This flea beetle was recently discovered by M. Cristofaro and G. Campobasso in Russia. Adult specimens sent to the US National Museum in Washington, D.C., were identified as a new species. Little is known of its biology or life history. For 2001, efforts will be made to collect field material to provide adults for laboratory studies. If enough adults are available, they will be exposed to California biotypes A and B to determine if this flea beetle will attack these plants. M. Cristofaro, in cooperation with Marcel Volkovitsh from the Institute of Zoology, St. Petersburg, Russia, will perform this work.

***Gymnacella canella* (Lepidoptera: Pyralidae)** Larvae of this moth feed on young shoots and developing seeds of infested plants, moving from seed to seed up and down a branch. In 2000, no-choice tests were performed by R. Sobhian by transferring emerging 1st instar larvae to sugar beets and other related host plants. Most larvae died but a few survived to pupae on some plants other than *S. tragus*. In 2001, choice oviposition tests will be performed with adult moths.

***Uromyces salsolae* (Fungi)** This rust disease is being studied by Bill Bruckart, USDA-ARS, Frederick, MD. Preliminary host screening shows that the rust will infect Type A plants from California, but not Type B. Further host screening will occur in 2001.

***Desertovellum stackelbergi* (Diptera: Cecidomyiidae)** R. Sobhian discovered this small fly galling the stems of *S. kali* in Uzbekistan. Preliminary host specificity testing was performed in 1999 and 2000, in cooperation with A. Khamraev in Uzbekistan. Results show that the fly has a strong preference for Type A plants (100% attack), but will attack Type B plants (15% attack). In 2001, host testing of this species will be performed at the EBCL laboratories in France.

***Chromoderus declivis* (Coleoptera: Curculionidae)** Larvae of this weevil were discovered attacking the roots of *S. tragus* in Russia. This species feeds internally and causes a swelling or gall in the root. For 2001, efforts will be made to collect field material for rearing of adults to learn more of its biology and host preference. M. Cristofaro and Marcel Volkovitsh from the Institute of Zoology, St. Petersburg, Russia, will perform this work.

Cecidomyiid sp. (Diptera: Cecidomyiidae) M. Cristofaro discovered a small fly galling *Salsola. tragus* plants in southern Russia near Kazakstan. This species has not been identified and little is known of its biology. For 2001, efforts will be made to collect field material for rearing of adults for taxonomic identification. M. Cristofaro and Marcel Volkovitsh from the Institute of Zoology, St. Petersburg, Russia, will perform this work.

Curculionid sp. (Coleoptera: Curculionidae) Larvae of an unknown species of curculionid were discovered feeding externally on the roots of *S. tragus* in Russia. For 2001, efforts will be made to collect field material for rearing of adults for taxonomic identification. M. Cristofaro and Marcel Volkovitsh from the Institute of Zoology, St. Petersburg, Russia, will perform this work.

A stem-boring moth (Lepidoptera) Larvae of an unknown species of lepidoptera were discovered feeding inside the stems of *S. tragus* in Italy. For 2001, M. Cristofaro will collect field material for rearing of adults for taxonomic identification.

***Sitona* sp. (Coleoptera: Curculionidae)** Larvae of a *Sitona* sp. were discovered feeding on *Salsola tragus* in Italy. For 2001, efforts will be made to collect field material for rearing of adults for taxonomic identification. M. Cristofaro will perform this work.

***Cassida* sp. (Coleoptera: Chrysomelidae)** Larvae of a *Cassida* sp. were discovered feeding on the leaves of *S. tragus* in several locations. This group of herbivourous beetles is not considered to be very host specific so this species was assigned a low priority at this time.

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[3]USDA-ARS, European Biological Control Laboratory, Thessaloniki, Greece
[4]USDA-ARS, European Biological Control Laboratory, Rome, Italy
[5]USDA-ARS, Western Regional Research Center, Albany, CA
[6]USDA-ARS, Foreign Disease-Weed Science Research Unit, Frederick, MD

Update on New Natural Enemies for Yellow Starthistle in the Western United States

M. Pitcairn, M. Cristofaro[1], W. Bruckart[2], G. Campobasso[3], J. Kashefi[4],
L. Smith[5], and N. Carruthers[6]

A renewed effort is underway to achieve biological control of yellow starthistle, *Centaurea solstitialis* L., in the western United States. A team of USDA-ARS scientists and cooperators located overseas has discovered several new natural enemies attacking yellow starthistle in Europe and Asia. The information reported here summarizes the status of the research effort for each natural enemy to date.

***Ceratapion bassicorne* (Coleoptera: Apionidae)** This apionid weevil is a common root feeder on *C. solstitialis* throughout Europe and Asia. Through the work of Joe Balciunas and Boris Korotyaev, the taxonomy of this species appears to have been worked out. There appears to be at least two species of *Ceratapion* attacking *C. solstitialis*: *C. bassicorne* and *C. orientale*. *Ceratapion bassicorne* appears to be specific to yellow starthistle; *C. orientale* is thought to be polyphagous. M. Cristofaro found *C. bassicorne* in 17 localities throughout Europe and Asia, five localities with high numbers. M. Cristofaro will perform open field tests to examine the host specificity and attack rate of this and other natural enemies of yellow starthistle (listed below) endemic to the locality of the study. This would be accomplished by setting up a garden of several plant species then sampling plants during the growing season to determine attack by the natural enemies. If all details and arrangements can be made, gardens would be located at one site in Italy (near Rome), in Turkey, and in Russia (near Krasnodor). A similar garden will be set up by J. Kashefi in Greece to examine infection by *Puccinia jaceae* var. *solstitialis* (see below). Plants in this garden will also be examined for attack by *C. bassicorne*.

Obtaining high numbers of *C. bassicorne* adults for quarantine evaluation has been difficult. A more efficient method to field-collect adult *C. bassicorne* will be developed in 2001 using methods similar to those used to collect *Apthona* flea beetles on leafy spurge. If large numbers of adult *C. bassicorne* can be collected, they will be shipped to Rome, Italy, or Albany, California, for no-choice host testing.

***Puccinea jaceae* var. *solstitialis* (Fungi)** This rust disease has been extensively studied by Bill Bruckart, USDA-ARS, Frederick, Maryland. A petition for its release in the United States has been submitted to APHIS and is currently being reviewed by Technical Advisory Group, (TAG). Initial feedback from TAG suggests a favorable review and only minimal additional host testing may be required. Several sites in California have been identified for initial release of the rust. Its colonization and spread will be monitored by CDFA and USDA-ARS. Post-release impact studies will also be performed.

In 2001, a study will be performed near Thessaloniki, Greece, to test inoculation methods under field conditions. Plants of local *C. solstitialis*, *C. solstitialis* from California, and a commercial variety of safflower will be interplanted in an outdoor garden by J. Kashefi. Plants will be monitored for infection by the rust throughout the growing season.

***Psilloides* nr. *chalcomera* (Coleoptera: Chrysomelidae)** This newly discovered flea beetle is known from only one site near Krasnodar, Russia. For 2001, this beetle will be tested against *C.*

solstitialis from the United States. Also, the biology of this species needs to be researched, especially when it emerges from diapause, what stage of the plant is attacked, number of generations, etc. M. Christofaro and M. Volkovitsh, Institute of Zoology, St. Petersburg, Russia, will perform these studies. Fieldwork in 2001 will consist of monitoring indigenous and California *C. solstitialis* plants for attack and damage at known recovery site and examination of related endemic plants for attack as a preliminary assessment of host specificity.

A blister mite (Acari: Eriophyiidae) This newly discovered mite is known from only one site near Goreme in Turkey. It has not been identified nor has its biology been examined. Jeff Littlefield, Montana State University, has agreed to examine this species as he has extensive experience with blister mites on other *Centaurea* species. M. Cristofaro will work with Sibel Uygur of the Chukurova University, Adana, Turkey, to collect and ship infested material to the quarantine facility in Bozeman, Montana.

***Cyphocleonus morbillosus* (Coleoptera: Curculionidae)** This species of *Cyphocleonus* was first discovered in the early 1960's by Lloyd Andres, USDA-ARS, Albany. Since then, few individuals have been recovered. G. Campobasso, USDA-ARS-EBCL Rome, reported that high numbers of weevils are not consistent from year to year and, currently, few weevils are available for host testing. Nada Carruthers, USDA-APHIS, Albany, is currently working on an artificial diet for *Cyphocleonus achates*, a natural enemy of spotted knapweed, *Centaurea maculosa*. It is hoped that individuals of *C. morbilosus* attacking *C. solstitialis* encountered in 2001 will be shipped to the USDA-ARS quarantine in Albany for use in her studies.

A mycoplasma-like organism Yellow starthistle plants were found infected with a mycoplasma-like organism (MLO) in Italy and Greece. Recent examination by a pathologist in Italy suggests that it is different from other known MLOs. In 2001, Tim Widmer, USDA-ARS-EBCL, Montpellier, France, will attempt to transfer it into California *C. solstitialis*, indigenous *C. solstitialis* and several non-target plants including safflower and artichoke to determine pathology and to measure changes in titer (to see if it multiplies in the host). If the agent appears to be highly specific, subsequent investigations to identify actual and potential vectors could be initiated.

***Phytoecia humeralis* (Coleoptera: Cerambycidae)** Larvae of this longhorn beetle were discovered infesting *C. solstitialis* plants in Turkey; however, little is known of its biology. For 2001, efforts will be made to collect field material for rearing and biological studies. Massimo Cristofaro and Sibel Uygur, Chukurova University, Adana, Turkey, will perform this work.

***Lixus scolopax* (Coleoptera: Curculionidae)** Larvae of this weevil was discovered in the stems of flowering plants of *C. solstitialis* in Turkey and Greece. For 2001, efforts will be made to collect field material for rearing of adults to learn more of its biology and host specificity. Massimo Cristofaro and Sibel Uygur, Chukurova University, Adana, will perform this work.

***Lixus filiformis* (Coleoptera: Curculionidae)** Larvae of this weevil was discovered attacking the stem and root crown of *C. solstitialis* plants in Turkey and Greece. For 2001, efforts will be made to collect field material for rearing of adults to learn more of its biology and host specificity. M. Cristofaro and Sibel Uygur, Chukurova University, Adana, will perform this work.

A stem-boring moth (Lepidoptera) Larvae of an unknown species of lepidoptera was discovered attacking the stem of *C. solstitialis* in Russia. For 2001, efforts will be made to collect field material for rearing of adults for taxonomic identification. M. Cristofaro and Marcel Volkovitsh from the Institute of Zoology, St. Petersburg, Russia, will perform this work.

A tip-boring fly (Diptera) Larvae of an unknown species of diptera was discovered attacking the tips of stems of *C. solstitialis* in Russia. For 2001, efforts will be made to collect field material for rearing of adults for taxonomic identification. M. Cristofaro and Marcel Volkovitsh from the Institute of Zoology, St. Petersburg, Russia, will perform this work.

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[1]Biotechnology and Biological Control Agency, Rome, Italy
[2]USDA-ARS, Foreign Disease-Weed Science Research Unit, Frederick, MD
[3]USDA-ARS, European Biological Control Laboratory, Rome, Italy
[4]USDA-ARS, European Biological Control Laboratory, Thessaloniki, Greece
[5]USDA-ARS, Western Regional Research Center, Albany, CA
[6]USDA-APHIS, Western Regional Research Center, Albany, CA

Releases of the Flower Weevil, *Larinus curtus*, for the Biological Control of Yellow Starthistle in California in 2000

B. Villegas

The flower weevil, *Larinus curtus* (Hochhut) (Coleoptera: Curculionidae) was introduced from Greece into California, Idaho, Oregon, and Washington in 1992-1994 by the USDA-ARS, for the biological control of yellow starthistle, *Centaurea solstitialis* L. (Asteraceae). This insect is called the flower weevil because the adult oviposits inside the open flowerheads. The larvae feed directly on the seeds and a single larva can consume most of the seeds in an infested seedhead.

There are currently two sites where the flower weevil is broadly established. One is in the Sutter Buttes of Sutter County and the other near Ione in Amador County. Because weevils collected from the Sutter County site have been shown to harbor the gut protozoan, *Nosema* sp., no weevils have been moved from that site. On the other hand, weevils collected in June and July 1999, from the Ione area were free of the gut protozoan.

Two workshops occurred at the Ione site during June 2000. The workshops drew 11 biologists from Calaveras, Contra Costa, Marin, Placer, Sacramento, Solano, Sonoma and Yolo Counties. Additional flower weevils collected by the Biological Control Program were distributed to counties participating in the hairy weevil workshops in Shasta County. A third collection of *L. curtus* took place for research releases of the flower weevil at Fort Hunter Liggett, Monterey County. Four releases totaling 800 weevils were made there. Surveys for establishment at all sites will be made during 2001 by county and CDFA Biological Control personnel.

Table 1: Releases of the flower weevil, *Larinus curtus* in California in 2000

County	Release sites	Releases
Calaveras	4	1,103
Contra Costa	5	2,500
Glenn	1	200
Marin	2	450
Monterey	4	800
Nevada	1	200
Placer	1	200
Sacramento	4	1,350
Sierra	1	200
Solano	2	400
Sonoma	4	1,400
Tehama	2	400
Trinity	1	300
Yolo	2	500
Yuba	1	200
15 Counties total	35	10,203

Releases of the Hairy Weevil, *Eustenopus villosus,* in California During 2000

B. Villegas

The hairy weevil, *Eustenopus villosus* (Boheman) (Coleoptera: Curculionidae), was introduced from Greece in 1990 for the biological control of yellow starthistle, *Centaurea solstitialis* L. (Asteraceae). The USDA-ARS, in cooperation with the Biological Control Program and the County Agricultural Commissioners, established the first colonies of the hairy weevil in Nevada, El Dorado, Mendocino, Napa and Shasta Counties in 1990-1991. Since 1992, the weevil has been moved to other sites in California. A large number of weevils have also been sent to cooperating agencies in Idaho, Nevada, and Arizona.

The hairy weevil has one generation per year. The adults overwinter in the duff and litter at the base of previous-year stands of yellow starthistle and emerge to feed on young, closed buds as temperatures warm up in May and June. Unlike other yellow starthistle natural enemies, this damage can be very extensive and readily noticeable. The weevils mate on top of the buds and the females lay individual eggs inside feeding holes on the sides of mature buds from late May through July depending in the area in California. After oviposition, the feeding hole is filled with a distinctive black plug that protects the egg from dehydration and potential predators. The larvae feed on receptacle tissue and on developing seeds inside the seedheads. A single larva is capable of consuming most of the seeds in an infested seedhead.

The hairy weevil is one of the more effective natural enemies introduced for the biological control of yellow starthistle. In comparison to the bud weevil, *Bangasternus orientalis*, and the flower weevil, *Larinus curtus*, the hairy weevil is a relatively poor flyer and does not seem to disperse very far from release areas. Workshops are needed to help in the distribution of the weevil throughout California's yellow starthistle infestations, especially at the edge of the infestations.

Two field workshops for the mass collection of the hairy weevil were held in Lindsay, (Tulare County) and Redding (Shasta County) during June 2000. Hairy weevils were also collected at two workshops near Ione (Amador County), for the flower weevil, *L. curtus*. Table 1 summarizes the releases made by participating counties. Sixteen counties participated in the four workshops. From Tulare County, approximately 2,600 weevils were collected and released at four sites as part of the Yellow Starthistle Control Demonstration implemented at the Fort Hunter Liggett Military Base in Monterey County.

Table 2 summarizes the within-county releases made by counties, which have well-established populations of the hairy weevil. In 2000, nine counties made over 100 within-county releases totaling approximately 21,980 weevils. Three counties, Shasta, Trinity, and Tulare Counties did most of these releases.

Table 1. Releases of the hairy weevil, *Eustenopus villosus* to establish county nursery sites in 2000

County	Sites	Releases	Source nursery
Amador	1	250	Tulare: Lindsay
Calaveras	4	463	Amador: Ione
Contra Costa	2	1,000	Amador: Ione
Humboldt	3	600	Shasta: Redding
Kings	2	500	Tulare: Lindsay
Marin	2	50	Amador: Ione
Monterey	4	2,600	Tulare: Lindsay
Placer	1	250	Amador: Ione
Plumas	4	1,340	Shasta: Redding
Sacramento	1	300	Amador: Ione
Santa Barbara	3	985	Tulare: Lindsay
Sierra	1	150	Amador: Ione
Solano	1	200	Amador: Ione
Stanislaus	4	2,800	Tulare: Lindsay
Tehama	5	1,000	Shasta: Redding
Trinity	1	205	Shasta: Redding
Ventura	2	630	Tulare: Lindsay
17 Counties	41	13,323	

Table 2. Within county redistribution releases of the hairy weevil, *Eustenopus villosus* in 2000

County	Sites	Releases	Source nursery
Glenn	7	1,400	Clarks Valley
Lassen	1	370	Honey Lake
Monterey	8	2,000	Argyle Rd
Napa	2	460	Angwin
Shasta	23	4,600	Redding
Tehama	8	1,600	Paynes Cr
Trinity	30	6,000	Weaverville
Tulare	23	4,600	Lindsay
Yuba	5	950	BAFB
9 Counties	107	21,980	

Biological Control of Bull Thistle in California

B. Villegas and E. Coombs[1]

Bull thistle, *Cirsium vulgare* (Savi) (Asteraceae), is a biennial weed native to Europe, western Asia, and North Africa. It was introduced into the western United States about 1900. In California it is widespread, usually associated with a high degree of disturbance, such as overgrazed rangeland and pastures as well as woodland clearings. The numerous spines on leaves, stems, and seedheads make the plants a feeding hazard to livestock and a problem in recreational areas.

The bull thistle gall fly, *Urophora stylata* (Fabricius) (Diptera: Tephritidae), is being released in California for the biological control of bull thistle. It was first released in Canada in 1973, and then into the United States in 1983. *U. stylata* is a host specific biological control agent of bull thistle in its native range as well as in the United States. It has one generation per year. The adult flies emerge from overwintering seedhead galls from late May through early July depending on the weather and microclimate of the area. The adults are thought to be long lived, living perhaps as long as several weeks. The flies lay their eggs in developing flower buds and the eggs hatch after about one week. After hatching, the larvae migrate to the receptacle where they begin feeding. In the process of feeding, the larvae induce gall tissue formation and a chamber forms around each larva feeding on the receptacle. Eventually a multi-chambered gall forms on the receptacle with as many as 20 larvae inside of it. The galls are thought to be energy sinks for the plants and seedheads infested with the gall flies produce less seeds.

In California, the seedhead gall fly was released at several sites in 1993-1995 by the USDA-ARS and the CDFA Biological Control Program, but the flies did not appear to establish at the time. A second release effort was initiated in 1997 and continued through 2000. All the releases in California from 1993-2000 were made with flies emerging from infested bull thistle seedheads collected in northwestern Oregon. The releases were made through direct releases of the adult flies or by setting out bags containing 150-200 infested seedheads near bull thistle infestations. Starting in 1998, most releases of the flies were made by the bagged seedhead method since it appears to be just as effective as the releases of the adult flies and it takes less time to do. The bagged seedhead releases were made by including 150-200 infested bull thistle seedheads inside empty #10 orange bags or bags made of netting material. The bags containing the infested seedheads were hung near bull thistle infestations and the flies were allowed to emerge from the seedheads. Table 1 lists all the releases that took place during 2000. It is estimated that about 9,023 flies were released at 17 sites in Glenn, Humboldt, Marin, Plumas, San Luis Obispo, Shasta, and Sonoma Counties. Only one release of adult flies was made in 2000.

A follow-up survey of most release sites for colonization/establishment was made by examining heads for galls during the fall and winter months. The presence of galls in a seedhead can be determined in the field nondestructively by squeezing the seedhead. Uninfested heads are soft and depress with gentle pressure, while infested heads have a hardened gall that is detectable under firm pressure. Excellent establishment of the gall fly occurred at all coastal release sites made 1993-1995. The only sites that did not establish were two sites located in Mendocino County near Ukiah and one site south of Redding in Shasta County. The same trend is noted for the releases made during 1997-2000. Coastal sites are ideal for establishment of the fly. It is difficult to evaluate establishment of the fly in interior sites in California due to variable populations of bull thistle and continued disturbance by livestock and fire. No establishment surveys were made in mountainous sites in northern California as the weather patterns made surveys of the areas impossible. Table 2 shows estimates of the infestation rate by the gall fly at several coastal sites in San Luis Obispo, Marin and Humboldt Counties and at one interior site in Tulare County.

Table 1: Releases of *Urophora stylata,* flies for the biological control of bull thistle during 2000

County	Nearest City	Location	*Flies Released*
Glenn	Nat Forest	Buck Point, Hull Road	600
Glenn	Nat Forest	Hokey Pokey Ridge, Road 24N02	600
Glenn	Willows	Rd 49 1/2	600
Humboldt	Arcata	James Rd	300
Humboldt	Arcata	Hwy 101 & 299	300
Humboldt	Eureka	Jackson Rd	300*
Humboldt	Pepperwood	Pepperwood	173
Humboldt	Pepperwood	Pepperwood	150
Marin	Tomales	Caselli Ranch	600
Marin	Tomales	Fallen Rd nr Hwy 1	600
Plumas	Beskeen	Hwy 70 @ Golden Eagle	600
Plumas	Loyalton	York Ranch	1200
San Luis Obispo	Los Osos	Hwy 1	600
Shasta	Anderson	Hill St & Hwy 273, 1 block N of Jct.	600
Shasta	McArthur	Dee Knox Rd & Jim Day Rd, 0.5mi E Jct.	600
Sonoma	Valley Ford	Valley Ford	600
Sonoma	Valley Ford	Valley Ford	600
TOTAL FLIES:			9023

*Direct release of adult flies

Table 2: Infestation of bull thistle seedheads by *Urophora stylata,* in Marin, Humboldt, San Luis Obispo and Tulare Counties – winter 2000

Location County: Site	Year of Release	Sampling	# Plants	# Seedheads	No. and (%) Infestated
Marin: Walker Cr.	1995, 97	1999	15	218	153 (70.2%)
		2000	10	360	164 (45.6%)
Marin: BMC	1995, 97	1999	15	436	58 (13.3%)
		2000	22	215	23 (10.7%)
Marin: Hwy 1 MP46	1998	1999	15	391	20 (5.1%)
		2000	15	250	10 (4.0%)
Marin: Alexander Rd	1998	1999	15	259	134 (51.7%)
		2000	15	206	31 (15.1%)
Marin: Jensen Ranch	1998	2000	15	273	86 (31.6%)
Marin: Caselli Ranch	2000	2000	10	765	152 (19.8%)
Marin: Fallen Rd	2000	2000	10	591	94 (16.0%)
San Luis Obispo: Montana de Oro	1999	2000	10	398	47 (11.8%)
San Luis Obispo: Madonna Rd	1995	1999	20	284	133 (47%)
.		2000	11	426	226 (53.1%)
San Luis Obispo: San Simeon Park	1995	2000	10	410	103 (25.1%)
Tulare: Globe Dr.	1998	2000	11	270	3 (1.1%)

[1]Oregon Department of Agriculture, Salem, Oregon

Releases of Biological Control Agents for the Biological Control of Purple Loosestrife in California

B. Villegas and E. Coombs[1]

Purple loosestrife, *Lythrum salicariae* L. (Lythraceae), is an exotic, invasive weed of wetlands, riparian areas and irrigation canals in many parts of the United States and Canada. It is native to Europe, Asia, and northern Africa and was introduced into the United States prior to 1800 because of its herbal and ornamental qualities. Once established, the plant produces millions of small seeds that can be spread by wind, wildlife, and water currents. In California, purple loosestrife has been reported in a number of locations throughout the state. Small infestations are under control, but well established infestations remain in Butte, Kern, Shasta and Siskiyou Counties.

In 1996, the Biological Control Program initiated biological control releases at the largest infestations of purple loosestrife in California. To date, four biological control agents have been released in the state: *Hylobius transversovittatus* Goeze (Coleoptera: Curculionidae), a root boring weevil: *Nanophyes marmoratus* (Goeze) (Coleoptera: Curculionidae), a flower-bud weevil; and two leaf-feeding beetles, *Galerucella calmariensis* L., and *G. pusilla* (Dufft.) (Coleoptera: Chrysomelidae).

The root-boring weevil, *H. transversovittatus* was the first agent released in California. The early releases involved the placement of eggs in loosestrife stems at two sites in Butte and Shasta Counties. Subsequent releases involved the releases of adult weevils in the same areas (Table 1). The main damage from this agent is caused by the larva, which can extensively damage the roots of purple loosestrife. To date there have been no recoveries of the weevils at any of the sites. In other states and countries where the weevil has been released, it plays an important role in the decline of purple loosestrife because of its extensive damage to the roots.

The flower-bud weevil, *N. marmoratus,* was released at two sites in Butte and Shasta Counties in 1997. The weevil was also released in very small numbers in conjunction with field releases of the leaf beetles in 1998-2000. Adults and the larvae cause flower-bud abortion, thus reducing the seed output by the plants. Table 2 summarizes the releases that have been made in California to date. The weevil was recovered in small numbers from one site in Butte County during 1999-2000.

The recent (since 1998) focus of the releases on purple loosestrife has been on two species of leaf-feeding beetles, *G. calmariensis* and *G. pusilla.* These beetles damage the plant by both adult and larval feeding on the foliage and the new tips. In many areas of Canada and the United States where these beetles have been released, the damage to the foliage has been spectacular especially three to six years after the initial release of the beetles. There are also documented cases where the beetles are having an impact in reducing infestations of purple loosestrife. The releases that were made in California from 1998-2000 are summarized in Table 3. The beetles are established at most locations, especially where spring generation releases were made. No recoveries occurred where only the summer generation of the beetles was released. To date, most release sites have been surveyed for establishment, but populations remain weak at all areas surveyed. It is hoped that if larger numbers of the leaf beetles are released, establishment and population build-up can be achieved faster than we have seen in the past two years.

Table 1. Releases of the root-boring weevil, *Hylobius transversovittatus* Goeze for the biological control of purple loosestrife, 1996-2000

County	City	Sites	Date	Generation	Releases
Butte	Oroville	1	June 1996	Spring	323 eggs
Butte	Oroville	1	July 1997	Summer	20 adults
Butte	Oroville	1	April 2000	Spring	100 adults
Shasta	Fall River Mills	1	June 1996	Spring	511 eggs
Shasta	Fall River Mills	1	April 2000	Spring	100 adults

Table 2. Releases of the flower-bud weevil, *Nanophyes marmoratus* (Goeze) for the biological control of purple loosestrife, 1997-1998

County	City	Sites	Date	Generation	Releases
Butte	Oroville	1	July 1997	Spring	160 adults
Butte	Oroville	2	July 1998	Summer	20 adults
Shasta	Fall River Mills	1	July 1997	Summer	150 adults

Table. 3: Releases of *Galerucella calmariensis* and *G. pusilla* in California for the biological control of purple loosestrife, 1998-2000

County	City	Sites	Date	Generation	Releases
Butte	Oroville	3	May 1998	Spring	600
Butte	Oroville	4	July 1998	Summer	2,000
Butte	Oroville	3	May 2000	Spring	1400
Kern	Onyx	4	July 1999	Summer	2,800
Kern	Onyx	1	May 2000	Spring	600
Nevada	Bear River	2	May 1998	Spring	400
Nevada	Bear River	3	July 1998	Summer	1,500
San Joaquin	Lodi	1	July 1998	Summer	1,000
Shasta	Fall River Mills	3	May 1998	Spring	600
Shasta	Fall River Mills	2	July 1999	Summer	1200
Shasta	Fall River Mills	2	May 2000	Spring	1,200
Siskiyou	Tulelake	2	May 1998	Spring	400
Siskiyou	Tulelake	1	July 1998	Summer	1,000

Monitoring Purple Loosestrife in California Following Release of Biological Control Agents

D. B. Joley, D. M. Woods, and V. Popescu

Purple loosestrife is an exotic invasive, semi-aquatic weed in many North American states and provinces. Several biological control agents have been released and are being monitored for impact, especially in areas most directly impacted by loosestrife. California has relatively little purple loosestrife due to continued exclusion and chemical control efforts. There are, however, a few areas of large densities where biocontrol has recently been attempted. We are participating in a continent-wide monitoring effort utilizing standardized approaches to monitoring the impact of purple loosestrife biocontrol agents.

Monitoring sites were selected in Shasta and Butte Counties in 1998, and five permanent $1m^2$ quadrats marked at the corners with wood stakes. The site in Shasta County is located on the bank of a stream associated with a natural spring, thus purple loosestrife is not often flooded. The Butte County site is associated with the Oroville Dam afterbay and is often flooded until mid summer. Standard protocol calls for a minimum of two visits per site (both spring and fall) to record data associated with plants and released insects. Counts were taken in spring (June) and autumn (September) in Shasta County, but only in autumn in Butte County due to high water during spring.

Single releases of approximately 200 adult *Galerucella* (both *G. calmariensis* and *pusilla)* were made during 1998 at the Little Tule River monitoring site in Shasta County, and 500 adults at the Oroville Dam afterbay monitoring site in Butte County. Another 600 beetles were released at the Butte County site in 2000. Although three years of data collection is usually too short to indicate any permanent trends, it appears that the leaf beetles failed to establish at both sites. Adults and eggs were visible after release in 1998 and in 1999, but none were observed in 2000.

Some variables show continued declines over the years, such as 'heights of 5 tallest plants' and number of inflorescences/quadrat, but they are not likely to be linked to biocontrol agents as we have seen little typical damage on the plants to date. Plant parameters in Shasta County showed various levels of variability between seasons and among years (Table 1), but showed little variability among years in Butte County (Table 2).

Table 1. Purple loosestrife plant parameters measured at the Little Tule River site in Shasta County, where biocontrol agents were released in 1998.

	1998		1999		2000	
	Spring	Fall	Spring	Fall	Spring	Fall
Cover (%)	67.5	67.5	42.5	67.5	62.5	47.5
Stems/M^2	99.2	73.4	64.4	75.2	73.4	49.6
Ave. Height of 5 Tallest Plants (cm)	68.6	126.2	40.2	87.2	64	79.6
No. Inflorescences of 5 Tallest Plants	--	8	--	5	--	5
Ave. No. of Inflorescences/Quadrat	--	24	--	9	--	7

Table 2. Purple loosestrife plant parameters measured at the Oroville Dam afterbay site in Butte County, where biocontrol agents were released in 1998.

	1998		1999		2000	
	Spring	Fall	Spring	Fall	Spring	Fall
Cover (%)	--	--	--	--	--	--
Stems/M^2	--	39.9	--	33.8	--	36.4
Ave. Height of 5 Tallest Plants (cm)	--	165.8	--	160.8	--	169
No. Inflorescences of 5 Tallest Plants	--	--	--	--	--	--
Ave. No. of Inflorescences/Quadrat	--	25	--	19	--	29

After comparing our results with those in other states where biocontrol agents have established, we have decided to release much larger numbers of the leaf beetles at suitable sites for multiple years, and plan to continue annual measures of plant and insect parameters as well. Evaluation of our data and comparison to that collected elsewhere will aid in evaluating methods for this as well as future weed biocontrol projects. The Butte County site will be chemically treated beginning in 2001; therefore, it will be abandoned. The current Shasta County site will be expanded to 10 quadrats in 2001. The second set of five quadrats will likely be established along the edge of the Little Tule River where the roots of purple loosestrife are often underwater. In addition, a second site may be established in a different location in Shasta County.

Fate of Seedlings of Musk Thistle Infected with Rust, *Puccinia carduorum*

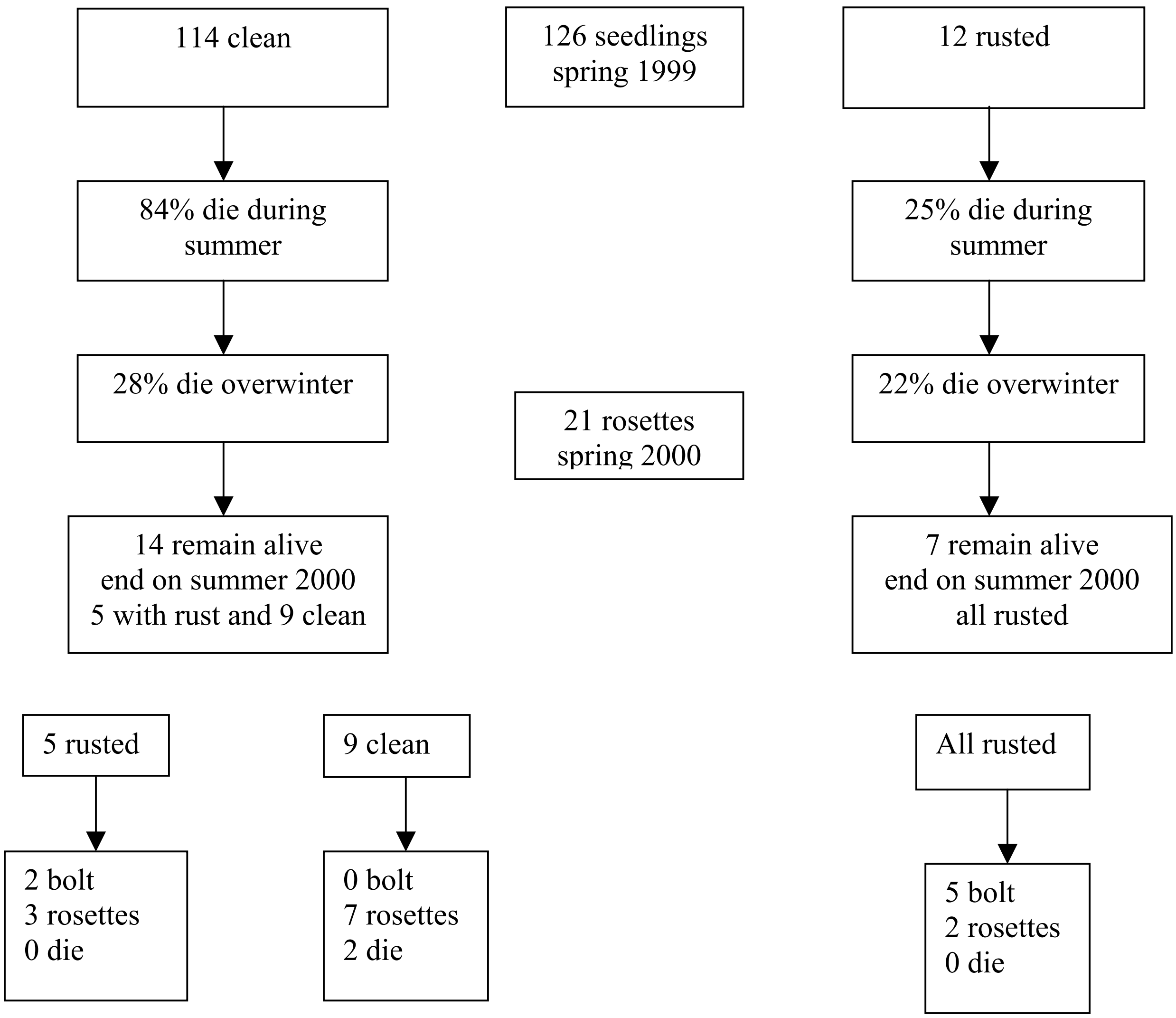
D. M. Woods, D. B. Joley and V. Popescu

The musk thistle rust, *Puccinia carduorum* Jacky, originally collected in Turkey in 1978, was introduced into the eastern United States as a potential biological control agent for musk thistle, *Carduus nutans* L. (Asteraceae). The rust was field released in Montgomery County, Virginia, from 1987-90, and has subsequently spread across the United States. On September 22, 1998, we detected rusted musk thistle plants on the shoulder of Mt. Shasta in northern California.

We initiated evaluation studies of the rust at the Mt. Shasta infestation on May 26, 1999. Our goal was to measure impact of the rust on plant survival and seed production, especially on plants infected as seedlings or young rosettes. We established two transects through a musk thistle population, selecting and flagging all plants in five one square meter permanent plots spaced one meter apart. Seedlings were evaluated for the presence of rust pustules. Plants were again evaluated on September 28 to compare the change over the season for both the rusted and non-rusted plants. Any surviving plants were again evaluated in the spring and fall of 2000. Musk thistle is considered a biennial species; so most plants were expected to bolt during this second year. A schematic of the progression of the plants through the two years is shown in Figure 1.

A large proportion of the seedlings produced each year died prior to bolting. This mortality was not evenly distributed among rusted and clean seedlings. Over the 15-month duration of this study, 90% of the initially clean seedlings died without bolting. During this same time, only 42% of the initially rusted seedlings died. Also, while less than 2% of the initially clean seedlings bolted during the monitoring, over 41% of the initially rusted seedlings had bolted during the 15 months. While it appears that the presence of the rust may be associated with a higher probability to survive and to bolt, additional research is needed to clarify this connection. It is possible that the rusted seedlings were slightly older or larger, thus more likely to sustain a disease load and survive. Interestingly, approximately 10% of the original musk thistle seedlings remained as rosettes in September 2000, 15 months after we first found them.

Figure 1. Progression of musk thistle from seedling stage through two years at Mt. Shasta California.



Establishment of Root Attacking Biological Control Agents on Spotted Knapweed

D. M. Woods, D. B. Joley and V. Popescu

Spotted knapweed, *Centaurea maculosa* Lamarck (Asteraceae), is consistently detected at several locations in California. All but one of these locations are slated for eradication using chemical and/or physical means. The central portion of the remaining location, a large infestation flanking a portion of the Pit River in Shasta County, is being addressed with exotic biological control agents, while chemical/mechanical methods are employed at the periphery. Several natural enemies have been imported into California in an effort to establish enduring biological control of spotted knapweed. Most of the insects attack seedheads but two, the yellow-winged knapweed root moth, *Agapeta zoegana* Linnaeus (Lepidoptera: Cochylidae) and the knapweed root weevil, *Cyphocleonus achates* (Fahraeus) (Coleoptera: Curculionidae), were imported for their ability to attack root tissue.

Adult *A. zoegana* were obtained from Jim Story (Montana State University), and released in 1993, 1994 and 1995. The root weevil, *C. achates,* was released in 1993, 1994, 1995, and 1997. Initial efforts to confirm establishment were largely nonquantitative, based on visual searches. Subsequent monitoring, focusing on changes in populations of the knapweed root feeding insects, was initiated in 1997, by monitoring populations in plant roots collected in late summer or early fall. A minimum of 100 plants was uprooted at each of two locations about ½ mile apart within the knapweed infestation. Roots diameters were measured, and the roots scraped and split to determine the attack by the root feeding insects; either observing damage consistent with attack by each insect, or detecting the presence of the biological control agents within the roots.

At least some level of reproduction of *A. zoegana* has occurred at our release site, as a single adult was found in 1996, one year after the last release. Larvae have also been found in roots of spotted knapweed as recently as 1997. Unfortunately, neither adults nor larvae have been found since 1997. Damage suggestive of attack by *A. zoegana* continues to be found as recently as this year, but the low numbers (Table 1) and equivocal nature of the damage makes pronouncements of establishment questionable. Researchers and managers in other states have had good success establishing this insect on spotted knapweed. Recent research suggests that small releases have a poor establishment record in spotted knapweed. This may well be the case in California as the combined total released adults are less than 600 moths. Consequently, one or more large releases of *A. zoegana* may be warranted.

Table 1. Proportion of knapweed roots with damage consistent with feeding by *Agapeta zoegana* larvae at two locations along the Pit River.

	Year			
	1997	1998	1999	2000
Plot				
Large roots >1cm diam	48%	0	0	6%
Small roots <1cm diam	4%	4%	0	0
Hill				
Large roots >1cm diam	10%	8%	0	5%
Small roots <1cm diam	0	0	0	3%

Adults of the knapweed root weevil, *C. achates,* are somewhat difficult to detect in the field, but the larval damage is distinctive. The weevil is, however, clearly established, as adults have been found throughout the summer in each of the last three years. Attack rates of the larvae have continued to rise, particularly on large diameter roots (Table 2). With a quarter to half of the larger roots attacked, we may soon reach infestation levels sufficient to demonstrate impact.

Table 2. Proportion of knapweed roots with damage consistent with feeding by *Cyphocleonus achates* larvae at two locations along the Pit River.

	Year			
	1997	1998	1999	2000
Plot				
Large roots >1cm diam	4%	17%	13%	61%
Small roots <1cm diam	0	7%	10%	5%
Hill				
Large roots >1cm diam	8%	10%	7%	24%
Small roots <1cm diam	0	0	0	5.9%

Population Fluctuations of Diffuse Knapweed and Two of its Natural Enemies, *Bangasternus fausti* and *Larinus minutus*, in Trinity County

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

Diffuse knapweed, *Centaurea diffusa* Lamarck (Asteraceae), occurs in California as single plants or in small patches, and is under eradication in most areas of the state except in Trinity County. The Biological Control Program has had an ongoing project to release available biological control agents on this weed in Trinity County since 1976. Six biological control agents are currently established on diffuse knapweed in Trinity County, but the focus of this report is limited to results obtained with the two weevils, *Bangasternus fausti* (Reitter) (Coleoptera: Curculionidae) and *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae).

Seedhead samples were collected annually, 1998-2000, from four sites along Miller Road where *B. fausti* and *L. minutus* had been released in previous years to compare population buildup and fluctuation of these weevils. The infestation rate was estimated by harvesting all or parts of 10 plants in mid-autumn, removing and combining all seedheads, then dissecting a sub-sample of heads (minimum 100) from each site for insects, empty pupal cells, or other evidence of larval damage under a microscope. Although there was similarity in damage caused by the two weevils, damage could usually be ascribed to a single species, so percent infestation is reported for each weevil (Table 1).

Table 1. Percent infestation of seedheads by the weevils, *Bangasternus fausti* (released in 1994 and 1995) and *Larinus minutus* (released in 1995 and 1996), at various sites along Miller Road.

	B. fausti			*L. minutus*		
Locations along Miller Road	1998	1999	2000	1998	1999	2000
Bottom	11	16	34	49	47	38
Lower	27	26	56	37	48	28
Mid (Primary monitoring site)	74	49	54	4	19	30
Upper	0	5	13	32	48	69

It appears that neither weevil has established dominance on diffuse knapweed in Trinity County, with the numbers of each weevil infesting flower heads shifting to accommodate microhabitat requirements. Nevertheless, in 1999 and 2000, there were numerous seedheads with *B. fausti* eggs that contained adults or other life stages of *L. minutus*, indicating that some degree of competition exists between them. Both weevils showed different infestation rates among sites, potentially a function of when the other weevil moved into the site. Unfortunately, it will not be possible to determine the maximum potential infestation rate for each weevil because both weevils have spread throughout the knapweed infestation and now co-occur. At the bottom site, *L. minutus* infested about 50% of the seedheads in 1998, remaining about the same in 1999, but decreased in 2000 when *B. fausti* doubled to 34%. At the upper site, *L. minutus* increased in 2000, to 69%, the highest level observed. Since *B. fausti* did not move to the upper site until 1999, their numbers were still low in 2000, and therefore, appear not to have significantly interfered with *L. minutus*. At the primary monitoring site where *B. fausti* was first released, the highest infestation rate occurred in 1998 (four years after release), but declined when populations of *L. minutus* increased.

Densities of reproductive stage diffuse knapweed plants and infestation rates of *B. fausti* and *L. minutus* in seedheads were evaluated from 1995 through 2000, at the primary monitoring site. Density estimates were made by counting plants within a 0.25-m^2 frame placed at 30 contiguous locations along two permanent, parallel transects. Plant density and combined infestation rate of both weevils are shown in Table 2.

Table 2. Combined percent infestation of seedheads by the weevils, *Bangasternus fausti* and *Larinus minutus*, and density of reproductive diffuse knapweed plants at the primary monitoring site.

Year	Weevil Infested Heads (%)	Plants/m^2 (No.)
1995	8	30
1996	23	39
1997	43	63
1998	78	75
1999	69	45
2000	85	43

Both the percent of heads infested by the weevils and the knapweed plant density increased significantly at this site through 1998. Both declined in 1999, but percent infestation then increased while plant density remained relatively stable in 2000. The decline in infestation rate in 1999, following the previous dramatic rise, may have been due to the cooler than normal early- to mid-summer temperatures. Conditions in 2000 were generally warmer and drier than in 1999, thus improving synchronization between flower development and weevil oviposition.

It is possible that *B. fausti* and *L. minutus* may complement each other in Trinity County by collectively maintaining a higher infestation rate than either weevil could alone. Further study will be done as the weevil populations expand and increase mutual contact to determine if infestation levels remain high or if interference creates a barrier to additional increases. Finally, if reduction in diffuse knapweed abundance is to be achieved with these two weevils at this site, it will apparently take several more years.

Impact of Biological Control Agents on Squarrose Knapweed

D. M. Woods and V. Popescu

Knapweeds have long been a target for biological control in the western U.S. but the effort has been largely limited to spotted and diffuse knapweeds. We are evaluating the potential of two species of weevils, *Bangasternus fausti* (Reitter) (Coleoptera: Curculionidae), and *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae), both imported as biological control insects for spotted and diffuse knapweed, to attack squarrose knapweed.

A single release of 2,000 adult lesser knapweed flower weevils, *L. minutus,* and 200 adult broad-nosed seedhead weevils, *B. fausti,* was made in 1998 on a large stand of squarrose knapweed near Pittville in Lassen County. The weevils were released separately 40 meters apart, within the infestation. Establishment of the weevils, and impact on seed production were monitored by collecting ten mature squarrose plants at both release sites at the end of each growing season during 1998, 1999, and 2000. Seedheads were dissected to determine insect attack and the number of viable seed. Seedheads were also collected at two more distant sites during 2000 in order to evaluate spread of the insects.

Larinus minutus rapidly colonized the *L. minutus* release site. Within two years, 86% of the seedheads were attacked by *L. minutus* (Table 1). *Larinus minutus* also appears to be spreading outward from the original release site. Infestation levels at release site 2 (the *B. fausti* site) have also risen dramatically (Table 2), increasing to nearly 80% by 2000. The most dramatic increase occurred this year at the *B. fausti* site, as attack increased from seven to 80%. Although capable of flying, the weevils remain relatively sedentary on the plant, resulting in a rapid local buildup prior to extensive outward spread. Only moderate numbers of seedheads were attacked by *L. minutus* 300 meters east, and very few were attacked one kilometer east (Table 3).

Table 1. Infestation rate of three biological control agents on squarrose knapweed at release site 1, (*L. minutus* site) near Pittville California. Number indicates the percentage of seedheads attacked.

	1998	1999	2000
Larinus minutus	16.3%	86.5%	91.3%
Bangasternus fausti	0.6%	0	4.1%
U. quadrifasciata	4.6%	2.4%	0.6%
Total infested	21.6%	88.5%	96.0%

The broad-nosed seedhead weevil, *B. fausti,* also established well from the 1998 release. Only 200 adults were available for release, and the population growth has been much less dramatic than for *L. minutus* perhaps due to the lower release numbers. The overall trend of growth, however, was very good at the release site (Table 2). This weevil also seems to be spreading (Table 1 and 3).

The ‘UV seedhead fly’, *Urophora quadrifasciata*, (Meigen) (Diptera: Tephritidae), was likely present in the area several years prior to our release of the weevils. It is a strong flier and may have flown from established populations in Oregon. The fairly uniform numbers at the two monitoring locations over the three years suggests that the UV fly is not increasing in density and will have limited impact as it fails to establish substantial population levels. It may, in fact, actually be declining in abundance as both weevils easily chew through the thin gall material housing the fly larvae thereby decreasing larval survivorship.

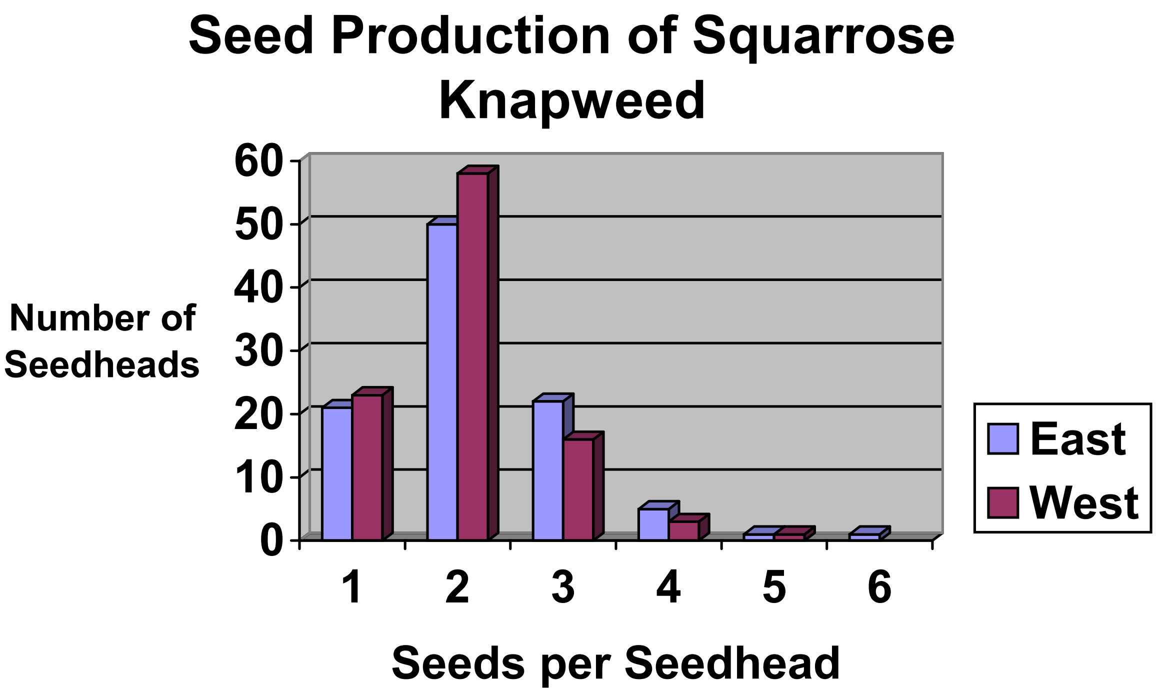
Table 2. Infestation rate of three biological control agents on squarrose knapweed at release site 2, (*B. fausti* site) near Pittville California. Number indicates the percentage of seedheads attacked.

	1998	1999	2000
Larinus minutus	1.0%	6.9%	79.3%
Bangasternus fausti	0.3%	1.8%	9.6%
U. quadrifasciata	1.3%	0.9%	0.2%
Total infested	2.6%	9.7%	89.0%

Table 3. Distribution of biological control agents on squarrose knapweed near Pittville California in 2000. Weevils were released early summer 1998 at the *L. minutus* and *B. fausti* release locations The gall fly immigrated some time prior to 1998. Number indicates the percentage of seedheads attacked.

	L. minutus site	*B. fausti* site	300 meters east	1 km east
L. minutus	91.3%	79.3%	36.8%	0.5%
B. fausti	4.1%	9.6%	0.6%	0
U. quadrifasciata	0.6%	0.2%	0	3.2%
Total infested	96.0%	89.0%	37.2%	3.7%

Squarrose knapweed does not produce many seeds per head, although there are many seedheads per plant. Seed production in uninfested seedheads was measured at the two locations distant from the releases. Uninfested seedheads at these locations had an average of 1.44 seeds per head; and most heads have two seeds (Fig 1). Seed destruction is a meaningful measure of impact for seedhead feeders that establish high populations like those in our study. A total of 2,566 seedheads were dissected in 2000 over the four collection sites. Damage consistent with *Larinus minutus* was found in 1,964 of these seedheads. All of the seed in the infested heads were completely destroyed except for three seedheads, which had one seed each. Overall, we calculated that 76% of the potential seed production for all heads collected at the four locations in 2000 was destroyed by *L. minutus*. Directly around the release location, 91% of the seeds were destroyed. There were only 162 seedheads damaged by *B. fausti* at the four sites, but all seeds were destroyed in all but one head. That head had two mature seeds along with a small larva. It appears that both weevils are well adapted to squarrose knapweed, completing their life cycle, building to high populations, but most importantly, destroying most of the seed. Additional studies will be established to investigate the potential of these insects to impact plant density.



Six Year Population Buildup and Combined Impact of Biological Control Insects on Yellow Starthistle

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

Six exotic insect species have been introduced for biological control of yellow starthistle in the western United States. Five species are established in California; three species, *Bangasternus orientalis* (Capiomont) (Coleoptera: Curculionidae), *Urophora sirunaseva* (Hering) (Diptera: Tephritidae), and *Eustenopus villosus* (Boheman) (Coleoptera: Curculionidae), are widespread. The two other species, *Chaetorellia australis* Hering (Diptera: Tephritidae) and *Larinus curtus* Hochhut (Coleoptera: Curculionidae), are abundant in the Pacific Northwest, but are limited to isolated populations in California. In addition, the seedhead fly, *Chaetorellia succinea* (Costa) (Diptera: Tephritidae), an accidental introduction into western North America, is now widespread throughout California and the Pacific Northwest. All of these insects attack the flower heads of yellow starthistle and destroy developing seeds.

Evaluations of the impact of individual insect species on yellow starthistle seed production in California suggest that no single agent will be the dramatic silver bullet in reducing yellow starthistle abundance. Rather, a combination of the current, and possibly, future natural enemies may be necessary to control this noxious weed. A study was initiated in 1993 to evaluate the population buildup, combined impact, and interaction of all available biological control insects on yellow starthistle. Field sites were established in Yolo, Placer, and Sonoma Counties to represent three different climatic regions where yellow starthistle occurs in abundance. Four insects (*B. orientalis*, *U. sirunaseva*, *E. villosus*, and *L. curtus*) were released at each site in 1993 and 1994, and long-term monitoring of the weed and insect populations was initiated. A fifth insect, *C. succinea*, invaded these sites on its own between 1996-1998. The Yolo County site is open Sacramento Valley rangeland located west of Woodland; the Placer County site is at 1300-ft elevation in the Sierra Nevada foothills east of Auburn; the Sonoma County site is at 1200-ft elevation in the Coast Range foothills southeast of Santa Rosa. Various aspects of the weed-insect interaction are being monitored annually, including canopy cover estimates of yellow starthistle and competing species, yellow starthistle seedling recruitment, adult plant density, seedhead numbers, seed production, and insect infestation rates. Preliminary results from 1995-2000 are presented in Table 1.

Six years after the first releases, we have evidence that attack by these biological control agents has reduced seed production by yellow starthistle at all three sites. The weevil, *Eustenopus villosus*, has become the most abundant insect at all these sites. In addition to seed destruction by larvae, adult *E. villosus* feed on and kill young developing buds. The loss of early buds produces a structural change and results in plants dominated by stems. Instead of flowers born on the tips of stems, new flowers are produced on short stems arising from the leaf axils along the main stems. Population densities of *E. villosus* increased steadily from 1995-1998 but, interestingly, declined in 1999 at all three sites. Little change in attack rate was observed in 2000 suggesting that the population densities of *E. villosus* may be beginning to level off.

The infestation rates of *B. orientalis* and *L. curtus*, have been consistently less than 13% and have not shown a steady upward trend. The infestation rate of the gall fly, *U. sirunaseva*, has been less than 30% but slight, upward trends appear to continue for the Placer and Sonoma County sites. The false peacock fly, *C. succinea*, was first recovered in 1996 at the Yolo County site and in 1998 at the Placer and Sonoma County Sites. While infestations rates have steadily increased each year thru 1999, they declined this year at the Yolo and Sonoma County sites but continued to increase at the Placer County site. This decline may have been due to the unusually cool spring temperatures, which delayed growth and flower production by yellow starthistle until later in summer, but failed to delay spring emergence of adult flies. The result was gravid adult flies with few seed heads ready for oviposition and may have resulted in a lower attack rate.

The Sonoma County site has had the most dramatic changes in both insect populations and yellow starthistle seed production. The rapid increase of *E. villosus* seems associated with a steady decline in the number of flowerheads per plant and the number of seed per head. The percentage of mature heads infested by at least one biological control insect increased from 22% in 1995, to 80% in 1997, and has remained high since then (range 78-83%). In addition, there has been a concurrent decrease in seed production (13,839 to 3,802 seeds per sq. m) and seedling density (897 to 234 seedlings per sq. m). While the decline in attack rate by *E. villosus* in 1999 resulted in a significant increase in seeds per head and total seed production (seed per sq. m), this insect was able to rebound in 2000 and maintain a high level of attack (64%) on a larger crop of seed heads.

The Yolo County site was the first location in California to be confirmed with established populations of all five natural enemies. Significant declines in adult plant density and seed production occurred from 1995-1997. Interestingly, despite the increase in plant density and seed production observed in 1998, seed per head and total seed production (seed per sq. m) declined in 1999 and 2000.

The density of biological control agents at the Placer County site built up quickly, but showed little change from 1995-1997. An increase in insect attack rates was observed in 1998 (83%) and, since then, has been maintained above 70%. The weevil, *E. villosus* is the most abundant insect, infesting 62% of the seedheads in 2000; the other biological control agents occurred at rates one to 13%. There has been little change in plant density and flower production at this site, but seed production was consistently held below 3,500 seeds per sq. m, a decline of 52% from 1995-97 (mean seed production 1995-97 vs. mean 1998-00). We hope *C. succinea* continues to increase at this site and cause additional seed loss.

These observations provide evidence that these natural enemies have reduced yellow starthistle seed production at all three sites. The weevil, *E. villosus,* is clearly the most important insect to date at these sites, increasing to quite high levels. However, plant samples show that activity of this insect is limited to early summer (June-August) and that flowers produced after mid-August are not attacked. It is hoped that the seed head fly, *C. succinea*, which has two or more generations per year, will continue to increase and attack these late-season flowers.

Table 1. Status of yellow starthistle and its natural enemies at three multi-agent research sites. Values in parentheses indicate percent survivorship of seedlings to adult plants.

Placer County

Plant		**95**	**96**	**97**	**98**	**99**	**00**	**01**
Seedlings/square meter		-	651	669	883	666	842	762
Adult plants/square m		332	83(12.7)	108(16.1)	151(17.1)	54(8.1)	109(12.9)	
Heads/ square meter		679	280	438	378	256	355	
Seed/head		8.2	18.0	16.2	6.7	10.6	9.3	
Seeds/square meter		5,568	5,040	7,096	2,533	2,730	3,303	
Insect & release year								
B. orientalis	93	6.7%	0.6%	1.6%	12.0%	9.4%	8.7%	
U. sirunaseva	93	4.7%	5.0%	8.7%	7.4%	6.0%	9.3%	
E. villosus	93	51.6%	50.9%	54.8%	79%	58.4%	61.9%	
L. curtus	94	0	0	0.2%	0%	0%	0.3%	
C. succinea	-	0	0	0	3%	8.0%	13.1%	
Heads w/ 1 or more sp		58%	60%	60%	83%	73.8%	76.3%	

Yolo County

Plant		**95**	**96**	**97**	**98**	**99**	**00**	**01**
Seedlings/square meter		-	1095	1928	1076	642	992	840
Adult plants/square m		975	322(29.4)	180(9.3)	422(39.2)	72(11.2)	285(28.7)	
Heads/ square meter		1181	369	343	830	249	439	
Seed/head		23	26	13	18	17	9	
Seeds/square meter		27,163	9,594	4,459	14,691	4,275	3,951	
Insect & release year								
B. orientalis	91	5%	2%	4%	3%	4%	2%	
U. sirunaseva	93	13%	18%	17%	13%	11%	13%	
E. villosus	93	5%	19%	23%	50%	24%	32%	
L. curtus	94	0	0	0.2%	0%	0%	0%	
C. succinea	96	0	2%	8%	12%	23%	14%	
Heads w/ 1 or more sp		19%	33%	31%	57%	36%	43%	

Sonoma County

Plant		**95**	**96**	**97**	**98**	**99**	**00**	**01**
Seedlings/square meter		-	897	822	624	234	1020	310
Adult plants/square m		241	233(25.9)	222(27.0)	231(37.0)	64(27.4)	435(42.6)	
Heads/ square meter		547	442	508	486	414	622	
Seed/head		25.3	14.9	8.0	7.8	15.1	8.1	
Seeds/square meter		13,839	6,586	4,064	3,802	6,232	5,038	
Insect & release year								
B. orientalis	94	5.4%	9.5%	4.2%	12.4%	12.9%	8.3%	
U. sirunaseva	94	4.8%	16.3%	19.7%	22.7%	22.2%	26.7%	
E. villosus	94	12.9%	37.3%	73.9%	72.7%	65.5%	63.6%	
L. curtus	94	0	0	0.7%	0.5%	0%	0%	
C. succinea	-	0	0	0	1.0%	12.9%	10.7%	
Heads w/1 or more sp		22%	56%	80%	83%	78%	78%	

Integrated Management of Yellow Starthistle at Fort Hunter Liggett

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Yellow starthistle (*Centaurea solstitialis* L.) is a major weed at the Fort Hunter Liggett Military Reservation in Monterey County. Dense stands restrict military training activities, increase fire danger, displace native vegetation and threaten endangered species located on the installation. Surveys showed that yellow starthistle occurs in five different habitats (stream corridors; military use; oak woodlands; purple amole; and vernal pools). Each of these habitats requires control methods that are specific to that habitat. A cooperative project between the California Department of Food and Agriculture and the University of California, Davis, was initiated in 1999 to demonstrate large-scale integrated control of yellow starthistle, and a multi-year management plan was developed for each of the five habitats.

The management goals for Fort Hunter Liggett are to substantially reduce the abundance of yellow starthistle, enhance the utility of the lands for military training, prevent invasion by other noxious weed species, and enhance the growth of native vegetation. Management goals for each habitat depend on their projected use following control (i.e., military training access and use, forage for livestock and wildlife, native vegetation, or protected rare or endangered plant or animal species).

The plan, in essence, is to reduce yellow starthistle with conventional control methods, such as burning or herbicides, and then use biological control to delay resurgence of the weed, thereby decreasing long-term costs and potential environmental damage. Two weevils, *Eustenopus villosus* and *Larinus curtus*, are being collected elsewhere in California and released at the installation. *Bangasternus orientalis* and *Chaetorellia succinea* are natural enemies of yellow starthistle that have been observed at various locations on the installation, and their numbers will not be augmented. Biological control agent release histories for each site are shown in Table 1. Release sites were located just outside the proposed treatment zones. Presumably, established agents will migrate into the treatment areas following treatment.

In 1999, U.S. Fish and Wildlife Service raised concerns regarding the potential impact that the use of herbicides may have on arroyo toads found in the San Antonio River drainage and halted their use within 2 km of the river and its tributaries. Similar concerns have also been raised regarding other endangered species, purple amole (*Chlorogalum purpureum* Brandegee var. *purpureum*), tiger salamanders, and fairy shrimp. As a result of these concerns, work at the Stream Corridor habitat was discontinued. Work was also discontinued at the Old Oak Woodland and Military Use Training Area habitats, and alternate locations were selected (New Oak Woodland and New Military Use Training Area habitats). Management plans for the purple amole and vernal pool habitats were changed to not include herbicides.

Implementation of the integrated control effort began in 1999. Here we report on the biological control activities. Releases occurred in June and July 1999 and 2000 (Table 1). The seedhead infestation rate for all biological control agents was monitored at release sites by collecting whole plants at the end of the flowering season and examining heads for attack. Results are presented in Table 2.

Activities associated with each demonstration plot

Stream Corridor Site. This site is no longer part of the integrated management study due to concerns by U.S. Fish and Wildlife Service regarding the arroyo toad. Adult *E. villosus* had been released at three locations in this site during 1999, and these were to be monitored in 2000 for establishment. Unfortunately, the site burned in 2000 from sparks blown from an accidental fire across the river. No work is planned for this site in 2001.

Military Use Site (Training Area 15). The specific goal for this site is to remove yellow starthistle from the area. Because this site is subject to heavy troop activity, no efforts to increase plant biodiversity are employed. The site was burned in 1999, then treated with clopyralid in 2000. Plant and community parameters (Table 3) were measured in 2000 and will be measured again in 2001.

Approximately 600 *E. villosus* adults were released east of the treatment area and 400 released west of the treatment area (Table 1) in June 2000. Approximately 200 adult *L. curtus* were released southwest of the site, near a small reservoir. Additional releases of *E. villosus* adults will be made at both release areas in 2001. *E. villosus* was recovered at a very low level (2%) at one of the three release sites, whereas *C. succinea* occurred at moderate levels (21-48%) at all three sites. *B. orientalis* was observed at only one site (9%). *L. curtus* was recovered at the treatment site itself and also at the west *E. villosus* release site, indicating movement >400 meters in less than one year. Monitoring will be continued in 2001.

Oak Woodland Site (Training Area 27). The goal for this site is to increase plant biodiversity and reduce the abundance of yellow starthistle. This site was burned in summer 1999, and treated with clopyralid in 2000. The objective of burning was to open the plant canopy and encourage germination of the yellow starthistle seed bank. Plant and community parameters (Table 3) were measured in 2000 and will be measured again in 2001.

Approximately 1,000 *E. villosus* adults were released at four locations outside the treatment site (Table 1) in June 2000. Approximately 200 adult *L. curtus* were released just south of the treatment site, but was not monitored in 2000. Additional releases of *E. villosus* populations will be made in 2001.

E. villosus was recovered at very low levels at three of the four release sites at the end of the season (Table 2). *B. orientalis* was recovered at very low levels at three of four sites. *C. succinea* was recovered at uniformly moderate levels (38-42%) at all four release sites. *L. curtus* was recovered at the two southern *E. villosus* release sites, indicating probable movement >400 m from the *L. curtus* release site in less than one year. Monitoring will be continued in 2001.

Purple Amole Site. The goal for this site is to protect purple amole plants from the invading yellow starthistle and, possibly, to increase their density. The site was burned in September 2000, to remove the heavy thatch, open the plant canopy, and reduce yellow starthistle seed. It is expected that growth of grasses and purple amole plants will increase in 2001. The site will be examined in June 2001, to determine the need for additional control measures. If yellow starthistle density is high, another burn will be scheduled for the area; if density is low, the plants will be removed by hand labor. All yellow starthistle plant and community parameters (Table 3) were obtained at both the treated and untreated (control) study areas at this site during 2000. These plant parameters will be measured again in 2001.

Approximately 400 adult *E.villosus* were released at the untreated (control) area of the Old Woodland Site west of the Purple Amole Site, and approximately 200 weevils were released on the east side of the proposed treatment area in 1999 (Table 1). Unfortunately, the east release site was burned during the burning of the treatment area in 2000, effectively eliminating this site as a biological control agent nursery. In June 2000, a release of 200 *E. villosus* adults occurred at a location immediately south of the site and across the main road.

Eustenopus villosus was recovered at the West (Old Woodland) and South sites. The recovery at the West site was lower than expected since weevils had been released there for two consecutive years. *Bangasternus orientalis* was recovered at one of the sites. *Chaetorellia succinea* was recovered at moderate levels at both sites. Monitoring of biological control agent populations will continue in 2001.

Vernal Pool Site. The goal for this site is to remove the threat of yellow starthistle from invading and displacing native species in the vernal pool habitat. To date, only releases of biological control agents have occurred at this site. None of the plant and community parameters (Table 3) was measured at this site in 2000.

Initial releases of *E. villosus* occurred near each of the vernal pools [200 adults at Vernal Pools 1 and 2, 400 adults (200 at two locations) at Vernal Pool 3] in 1999 (Table 1). Additional releases occurred on June 21, 2000, at Vernal Pools 1 and 3 (200 each pool).

Eustenopus villosus was recovered at moderate levels at Vernal Pools 1 and 3; no samples were collected from Vernal Pool 2. Recoveries near the Vernal Pools were much higher than at other release sites on the installation. *Bangasternus orientalis* was recovered at a low level at Vernal Pool 1. *C. succinea* was low at Vernal Pool 1, but moderate at Vernal Pool 3. The recovery of an adult *Larinus curtus* in a single head is surprising because the nearest release site is several miles away. Monitoring of biological control agent populations will continue in 2001.

Summary and future releases. Generally, infestation rates of biological control agents were fairly uniform over the installation. Rates of *C. succinea* were generally high, especially where there was little competition from the other agents. *Bangasternus orientalis* and the two recently released weevils, *E. villosus* and *L. curtus*, were generally at low levels. It is anticipated, however, that infestation rates of *E. villosus* and *L. curtus* will increase during the next couple of years.

Releases of *L. curtus* occurred at three locations not associated with the treatment study areas in the northern portion of the military reservation. In 1999, approximately 400 adults were released along Sulphur Springs Road and 400 released in a wooded area along Upper Springs Road. In 2000, an additional 200 adults were released at the Upper Springs Road area and 200 adults were released in a small valley located northeast of Training Area 27. The purpose is to establish nursery colonies from which personnel can move insects throughout the installation in the future. No plant samples were collected at these locations in 2000. All three sites will be visited in June 2001 to determine if *L. curtus* has colonized.

Table 1. Releases of *Eustenopus villosus* (1999 and 2000) and *Larinus curtus* (2000) on yellow starthistle at various sites at Fort Hunter Liggett, Mendocino County.

	E. villosus		**L. curtus**	
Locations of Sites	1999	2000	1999	2000
Stream Corridor Site[1,2]				
South of River	200	0	0	0
North of River	200	0	0	0
Old Military Use Site (Jackhammer)[1]				
West	200	0	0	0
South	200	0	0	0
East	200	0	0	0
New Military Use Site (Training Area 15)				
East	0	600	0	0
West (creek side)	0	040	0	0
Southwest (reservoir)	0	0	0	200
Old Oak Woodland Site				
South (Purple Amole Control)	200	0	0	0
North[2]	200	0	0	0
New Oak Woodland Site (Training Area 27)				
South (ca .25 mi southeast of barn along road)	0	0	0	200
Southwest (south of barn)	0	200	0	0
Southwest (north of barn)	0	200	0	0
Northwest	0	200	0	0
Northeast	0	400	0	0
Purple Amole Site				
East[2]	200	0	0	0
South (Power pole, south of road)	0	200	0	0
Vernal Pool Site				
Vernal Pool #1 (West)	200	200	0	0
Vernal Pool #2 (Mid)	400	0	0	0
Vernal Pool #3 (East)	200	200	0	0

[1]Sites abandoned
[2]Areas burned in 2000

Table 2. Percent of yellow starthistle seedheads infested with four exotic insects at Fort Hunter Liggett, Monterey County. The weevils, *E. villosus* and *L. curtus*, were released intentionally (Table 1), whereas *B. orientalis* and *C. succinea* moved from nearby locations.

	Seedheads Infested (%)			
	E. villosus	*L. curtus*	*B. orientalis*	*C. succinea*
New Military Use Site (Training Area 15)				
East	2	0	9	48
West (creek side)	0	1	0	46
Southwest (reservoir)	0	2	0	21
New Oak Woodland Site (Training Area 27)				
South (ca .25 mi se of barn along road)	ND*	ND	ND	ND
Southwest (south of barn)	<1	<1	4	38
Southwest (north of barn)	4	<1	2	42
Northwest	0	0	<1	39
Northeast	5	0	0	41
Purple Amole Site				
West (Old Woodland Control)	1	<1	0	24
South (Power pole, south of road)	11	<1	4	18
Vernal Pool Site				
Vernal Pool #1 (West)	17	1	4	7
Vernal Pool #2 (Mid)	ND	ND	ND	ND
Vernal Pool #3 (East)	13	0	0	32

*ND - no data

Table 3. Yellow starthistle plant and community parameters measured at the Fort Hunter Liggett yellow starthistle demonstration plots

Parameter	Month of Activity
Seedling Counts	March
Plant Community Cover (all identifiable species)	May
Yellow Starthistle Plant Cover	July
Yellow Starthistle Plant Biomass	July
Yellow Starthistle Plant Density	July
Yellow Starthistle Plant Seedhead Density	July
Yellow Starthistle Density in Soil Seed Bank	October

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Survivorship and Increased Emergence of Scotch Thistle Following Seed Rain and Fire

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

We have been monitoring Scotch thistle, *Onopordum acanthium* L., at several sites in northeastern California in preparation for a potential release of biological control agents. One of the sites is at the Modoc National Wildlife Reserve where we have been developing baseline information on the biology of Scotch thistle since 1996. Unfortunately, the first potential biological control agent (*Lixus* sp.) was eliminated from further consideration because it readily attacked native *Cirsium* spp. during quarantine evaluations. Recently, the USDA-ARS Exotic and Invasive Weeds Research Unit in Albany, California, decided to curtail testing of other potential biological control agents. As a result, the Scotch thistle field studies were scaled down to concentrate on survivorship and growth habits in northeastern California. Plans are to complete current studies and possibly continue monitoring seedling emergence to assess rate of depletion of the seed bank. After the 1997 field season, we decided to avoid jeopardizing control efforts by preventing further seed production at field sites through removing plants at the bolting stage. This provides an opportunity to monitor depletion of the seed bank under natural field conditions.

The Modoc plot is comprised of four transects with 30 contiguous 1 sq. meter quadrats each (total = 120 quadrats). Emerging seedlings were marked with small flags and monitored over several years. In late summer 1999, a control burn of weed vegetation escaped containment and burned most of the study plot. Subsequently, a large number of Scotch thistle seedlings emerged within the burn area, suggesting that fire stimulated germination of the seeds. Here we report on the fate of seedlings in several cohorts followed at this plot

1998 seedling cohorts. Emerging Scotch thistle seedlings were divided into four cohorts totaling 568 seedlings during 1998 (Table 1). Although rainfall was plentiful until mid-June, very few seedlings emerged during the early summer visits of 1998. Presumably, temperatures were too low in May and June for germination to occur. The greatest numbers of seedlings were flagged late in the season, on 19 August and 8 October. A storm in late July provided approximately 30 mm of precipitation and subsequent storms provided rainfall in early and late September. Maximum daily temperatures during the late July and early September storms ranged from the mid-20's to above 30° C. Most of the seedlings occurred in quadrats close to two large plants that dispersed their seeds in 1997, indicating that some of the fresh seeds were nondormant. Of the total flagged seedlings in 1998, none bolted in 1998, 104 bolted in 1999, 121 bolted in 2000, and 14 remained alive in October 2000. The remainder (57%) perished over the three years.

1999 seedling cohorts. A total of 86 Scotch thistle seedlings in three cohorts were flagged during 1999. The weather pattern was generally dry after early March. There was a single-day storm (21mm) on 14 July and scattered precipitation in early and late August, but seedling emergence was poor. Either the moisture level and air temperatures were inadequate to induce germination, or most seeds were dormant. Two of the 86 plants bolted in 2000, and the remainder (95%) died, with all but two dying during 1999. We feel that the much greater mortality of seedlings emerging in 1999 than in 1998 is due to differences in precipitation during early stages of development. Whereas seedlings emerging in 1998 were supported by timely rainfall during their first year, seedlings emerging in 1999 were provided little rainfall at this critical stage.

2000 seedling cohorts. The number of seedlings emerging following the burn was too great to monitor in all quadrats as we had done in previous years. A total of 318 seedlings were flagged on 22 June 2000 (June/00 cohort). None of these plants bolted during the summer, but 266 remained alive on 12 October 2000. In addition to the flagged seedlings, there were 1,530 that were counted in the quadrats, but not marked with a numbered flag on 22 June 2000. Most of the seedlings emerged from a small area, especially in the quadrats close to where seeds were last shed in 1997. Nevertheless, seedlings also emerged in other, distant quadrats, which were also burned between 2 September and 20 October 1999. It is not known whether fire directly stimulated germination or whether removal of cover indirectly allowed seeds to germinate. Either way, fire might be a useful tool in controlling Scotch thistle. By using fire judiciously along with other methods, land managers conceivably could accelerate depletion of the seed bank.

Table 1. Scotch thistle emergence and survivorship during 1998-2000 at the Modoc National Wildlife Reserve Site, Alturas, California

Month/Year of Cohort	Initial # of Plants	# Bolted in 1999	# Bolted in 2000	# Remaining October 2000	#Un-flagged seedlings
June/98 = 10/3/97– 6/23/98	14	8	1	0	0
July/98 = 6/23/98– 7/14/98	9	0	6	0	0
August/98 = 7/14/98– 8/19/98	344	96	119	13	0
October/98 = 8/19/98– 10/8/98	201	0	1	1	0
Total 1998	**568**	**104**	**127**	**14**	**0**
June/99 = 10/8/98– 6/8/99	52	0	0	2	0
July/99 = 6/8/99– 7/1/99	22	0	2	0	0
September/99 = 7/1/99– 9/2/99	12	0	0	0	0
Total 1999	**86**	**0**	**2**	**2**	**0**
June/00 = 10/20/99– 6/22/00	318	--	0	266	1530

Chaetorellia Seedhead Flies and other Seedhead Insects on *Cirsium* Thistles in Close Proximity to *Centaurea* spp.

B. Villegas, F. Hrusa, and J. Balciunas

A survey of native *Cirsium* thistles was initiated in 1998 and continued through 2000 to address the question that two seedhead flies, *Chaetorellia succinea* (Costa) and *Ch. australis* Hering, introduced for the biological control of yellow starthistle, might also attack California native thistles. During this survey, efforts were made to evaluate *Cirsium* thistles naturally occurring within close proximity of known host plants of the *Chaetorellia* seedhead flies. Prior to its accidental introduction in the U.S., little was known about the host range of *Ch. succinea* beyond yellow starthistle. In contrast, the documented hosts of *Ch. australis* in California were known from host testing to include both yellow starthistle and bachelor button, (*Centaurea cyanus* L.). During the survey, botanical specimens were collected from all thistle populations evaluated and deposited with the Herbarium of the California Department of Food and Agriculture's Plant Pest Diagnostics Center. Collection permits for rare or endangered native thistles were obtained from the California Department of Fish and Game.

Seedheads collected from each host location were kept separate in emergence containers in the laboratory. The *Cirsium* thistle samples collected included; opened flower heads, unopened flower buds, along with parts of the peduncles and stems in order to insure a complete collection of the seedhead insects. This is a modification of the seedhead sampling protocol done during 1998, which emphasized mature seedheads between flowering and seed dissemination. This change was needed as some insects oviposit on the seedheads, but the mature larvae migrate some distance into the stems and peduncles. In the laboratory the emergence containers were periodically monitored and any emerged insects were collected, pinned, labeled, recorded, and stored in entomological collection trays for subsequent identification.

To date, a total of 64 samples of 13 native *Cirsium* species and two exotic weedy *Cirsium* species were sampled for attack by the seedheads flies, *Ch. succinea* and *Ch. australis* (Table 1). No *Chaetorellia* seedhead flies of either species were reared from any of the thistles collected. Native phytophagous insects were found in all samples and a complete report of these insects will be reported when this survey is completed in 2002. The only non-native insect reared from a number of thistles in the California thistle collections was *Rhynocyllus conicus* (Frolich), a seedhead weevil introduced into California for the biological control of musk thistle, *Carduus nutans* L., milk thistle, *Silybum marianum* (L.) Gaertn., and Italian thistle, *Carduus pycnocephalus* L. A second non-native weevil, *Larinus planus* (F.), was reared from three species of native thistles (*C. brevistylum, C. remotifolium*, and *C. undulatum*) collected in northcentral Oregon. According to Peter Harris, this weevil is thought to be an accidental introduction. It was first reported in the northeastern United States prior to 1968, and it is considered a good biological control of Canada thistle (*Cirsium arvense* (L.) Scop.). Since its accidental introduction, the weevil has moved naturally or has been released as a biological control agent in parts of Canada and from Wyoming west to Washington and northcentral Oregon.

Table 1: *Cirsium* Thistles sampled during 1998-1999 for *Chaetorellia* seedhead flies

Scientific Name	Common name	N/I*	Year	Samples	County	Emergence Notes
C. andersonii (A. Gray) Petrak	red stemmed thistle	N	1998	3	Nevada and El Dorado	No *Chaetorellia* flies
C. arvense (L.) Scop.	Canada thistle	I	1998	2	Modoc, Plumas	No *Chaetorellia* flies
C. brevistylum Cronq.	clustered thistle	N	1998, 2000	4	Humboldt, Del Norte & two sites in Oregon	No *Chaetorellia* flies
C. canovirens Rydb.	gray-green thistle	N	1998, 1999, 2000	6	Nevada, Alpine and Oregon	No *Chaetorellia* flies
C. crassicaule (Greene) Jepson	slough thistle	N	1998, 2000	2	Kern & Monterey	No *Chaetorellia* flies
C. cymosum (Greene) J. T. Howell	peregrine thistle	N	1998, 1999, 2000	6	Modoc, Siskiyou, Lassen	No *Chaetorellia* flies
C. douglassi DC	swamp thistle	N	1998, 2000	8	Humboldt; Nevada; Trinity	No *Chaetorellia* flies
C. edule Nutt.		N	1998	1	Oregon	No *Chaetorellia* flies
C. loncholepis Petrak	bog thistle (La Graciosa thistle)	N	1999	1	San Luis Obispo	No *Chaetorellia* flies
C. occidentale var. *californicum* (A. Gray) Keil & Turner	sierra thistle	N	1998, 1999, 2000	4	Los Angeles & Santa Barbara	No *Chaetorellia* flies
C. occidentale var. *candidissimum* (Greene) J.F. Macbr.	snowy thistle	N	1998, 1999, 2000	8	Modoc, Mono, Plumas, Shasta, Siskiyou, Trinity, & Alpine	No *Chaetorellia* flies
C. occidentale var. *occidentale* (Nutt.) Jepson	cobwebby thistle	N	1999	1	San Luis Obispo	No *Chaetorellia* flies
C. occidentale var. *venustum* (Greene) Jepson	venus thistle	N	1999	5	Humboldt, Kern, Fresno, Mendocino & San Benito	No *Chaetorellia* flies
C. ochrocentrum A. Gray	yellow-spined thistle	N	1998	2	Modoc & Oregon	No *Chaetorellia* flies
C. remotifolium (Hook.) DC.	Klamath thistle	N	2000	2	Oregon	
C. scariosum Nutt.	elk thistle	N	1998, 1999	2	Plumas	No *Chaetorellia* flies
C. undulatum (Nutt.) Spreng.	wavyleaf thistle	N	1999	2	Oregon	No *Chaetorellia* flies
C. vulgare (Savi) Ten.	bull thistle	I	1998, 1999	6	Humboldt; Siskiyou; San Luis Obispo; Marin; Oregon	No *Chaetorellia* flies

*N = Native thistle; I = Introduced weedy thistle

Attack of Napa Thistle by Yellow Starthistle Biological Control Insects

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Pre-release host specificity studies are designed to insure that the risk of attack on non-target native and economic species by introduced biological control agents is extremely low. However, utilization of closely related exotic species may be expected. Following release, we initiated field studies to confirm the lack of attack to non-target species and determine the extent of utilization (if any) of other exotic *Centaurea* species occurring in California. Here we report utilization of Napa thistle, *Centaurea melitensis* L. (Asteraceae), also known as Tocalote. Although Napa thistle is widely spread in California, the densest stands are located in the lower San Joaquin Valley. The sharp spines, brilliant yellow flowers, and plant shape often lead to its confusion with yellow starthistle, *Centaurea solstitalis*. These weeds are in fact, quite closely related and co-exist in many areas, although Napa thistle has a somewhat more southern distribution range. During 1998, we reported results from a site in Tulare County confirming that *E. villosus* can have a significant effect on seed production of Napa thistle. This report is based on additional sites that were evaluated during 1999.

A minimum of ten mature Napa thistle plants were collected in early June 1999, at several sites by pacing through the infestation and collecting the closest plant to every third step. All seedheads were dissected and examined in the laboratory for evidence of attack by yellow starthistle biological control insects. The green seedhead category represents late season seedheads that were still somewhat immature when the plants were collected in mid June. The results are shown in Table 1.

Plants located at the Hwy 43 site and the Hwy 124 site had very high levels of attack by *Chaetorellia succinea* (Costa) (Diptera: Tephritidae). With nearly 60% of the mature heads attacked by one or more individuals, we believe that it is reasonable to expect that this insect is affecting the overall seed production at these sites. While adult *C. succinea* flies were visible at the Camino del Cielo site, none of the collected heads were infested. Napa thistle flowers earlier than yellow starthistle, so these early flowers are the only host available for early season *Chaetorellia succinea*. The reduced attack rates for the green seedheads are consistent with our expectation that *C. succinea* is moving to yellow starthistle later in the season.

Napa thistle also faces exposure to the other yellow starthistle biological control insects at these and other sites. In particular, the Hwy 124 site has *Larinus curtus, Bangasternus orientalis*, *Urophora sirunaseva* and *Eustenopus villosus* nearby in great abundance and was the only site with damage caused by species other than *C. succinea* this year. One mature and one green head had damage suggestive of *E. villosus*, one seedhead had damage of *L. curtus*, and one had an egg of *B. orientalis*.

Table 1. Attack rates on mature and late season (green) seedheads of Napa thistle by the false peacock fly, *Chaetorellia succinea.* The number in parentheses is the number of heads in each category.

Location	County	Mature seedheads		Green seedheads	
		Clean	*Chaetorellia*	Clean	*Chaetorellia*
Hwy 43	Kern	58%(122)	42%(89)	88%(54)	12%(8)
Hwy 41 west	Kern	96%(91)	4%(4)	100%(15)	0
Hwy 124	Amador	59%(54)	41%(37)	78%(38)	22%(11)
Camino del Cielo	Santa Barbara	100%(92)	0	100%(108)	0