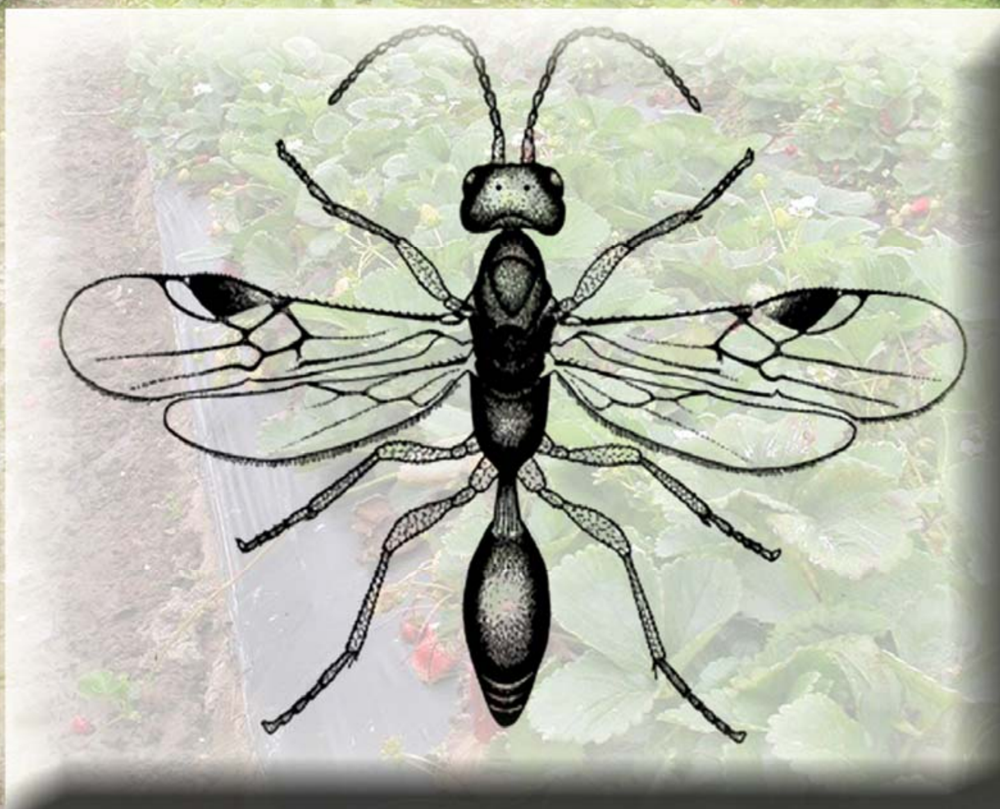
Biological Control Program Annual Report

2007



California Department of Food & Agriculture

BIOLOGICAL CONTROL PROGRAM

2007 SUMMARY

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CALIFORNIA DEPARTMENT OF FOOD AND AGRICULTURE
PLANT HEALTH AND PEST PREVENTION SERVICES
INTEGRATED PEST CONTROL BRANCH

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

Cover developed by Baldo Villegas and John P. Mattia (Orange, CT). The line drawing of the parasitic wasp, *Peristenus relictus* (Ruhte), formerly *Peristenus stygicus* Loan (Hymenoptera: Braconidae), a natural enemy of lygus bugs (Hemiptera: Miridae) was supplied by Dr. Robert Allen Van Steenwyk. The drawing was used in Dr. Van Steenwyk's PhD Dissertation. "Biology, introduction and evaluation of *Peristenus stygicus* Loan, a newly imported braconid parasite of lygus bugs". The strawberry field where the wasps are being released is located in Santa Cruz County, CA and the picture was taken by Dr. Charles Pickett.

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Preface

Michael J. Pitcairn

The concept of Sustainable Agriculture is rapidly spreading throughout California. Sustainable agriculture combines three main concerns: environmental health, economic profitability, and social and economic equity. The goal is meet the needs of the present without sacrificing the needs of the future. It is an ambitious grassroots movement and, despite the diversity of people and perspectives in the agricultural community, many have come to realize that the future survival of agriculture in California may depend on this new philosophy. The Biological Control Program has been working on sustainable solutions for several serious insect pest and weed problems. Our mission is to develop and implement biological controls for invasive insect pests and noxious weeds. When successful, biological controls can provide long-term control of a target pest species with low levels of material inputs, lowering production costs and reducing the pesticide load into the environment.

Good progress is being made in our efforts to introduce biological control agents for the olive fruit fly. At least six parasites have been shipped to the quarantine laboratory at the University of California, Berkeley and, following host testing and other pre-release studies, three have been permitted for release. These are now in mass production and releases are just now starting. All three have proven very difficult to rear so progress on getting them established has been slower than expected. At least three other species are being examined in quarantine for their efficacy and safety. We hope to complete their testing in the next two years.

A new parasite of the lygus bug has been established among the commercial strawberry-growing areas of Monterey and Santa Cruz counties. Lygus bug populations have dropped significantly in both organic and conventional strawberry fields that have been under observation. This drop coincides with a steady increase in parasitism by the new parasite. These are very encouraging results for this early effort in this region.

The giant whitefly had invaded San Diego County in the early 1990s. Control of this pest was achieved following introduction of several parasite species by the Biological Control Program and by the University of California, Riverside. Recently, this whitefly was found in Santa Clara and San Luis Obispo counties. Collections of whiteflies at these infestations found low numbers of the dominant parasite. This suggests that the parasite moved along with its host whitefly and will begin to increase in abundance and quickly spread throughout the new infestations.

We've begun a new release effort against leafy spurge, a serious noxious weed in northern California. In this first year, we released over 58,000 flea beetles on infestations in Siskiyou County. We will be monitoring the release sites for establishment, impact, and spread of this new biological control insect.

These are but a few of the highlights presented in this year's report. I hope you enjoy our 2007 report.

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Insect Projects

Weed Projects

Foreign Exploration for Parasitoids of the Olive Fruit Fly and their Release in California

Charlie H. Pickett, Kent Daane[1], Alan Kirk[2], Karen R. Sime[1], Xingeng Wang[3], John Andrews[1], Lue Yang, and Marshall Johnson[3]

Olive fruit fly is the worst pest of olives to invade California. A single fly maggot feeding in the fruit renders it unmarketable. Through funding provided by the United States Department of Agriculture, Animal and Plant Health Inspection Service, Plant Pest Quarantine (USDA-APHIS-PPQ) Western Region, the Biological Control Program formed a research team consisting of scientists from the USDA Agricultural Research Service (ARS) European Biological Control Laboratory, University of California, and Texas A&M University to develop a biological control program against the olive fruit fly.

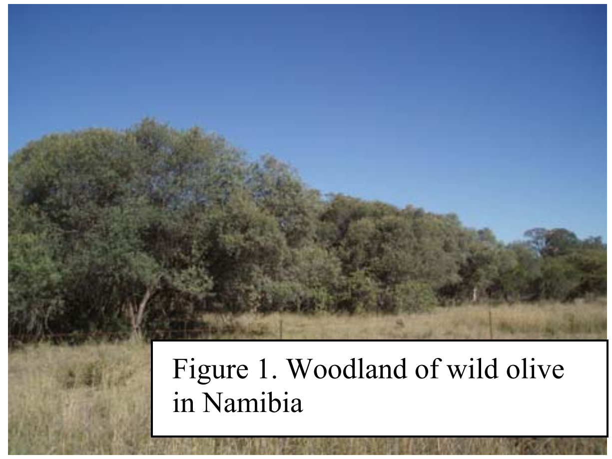

Figure 1. Woodland of wild olive in Namibia

California Department of Food and Agriculture and USDA-ARS scientists have pursued foreign exploration for effective natural enemies of the olive fruit fly in its area of origin and areas of traditional production, including South Africa, Kenya, Namibia, China, and India. In 2007, scientists returned to Namibia and explored areas of this country not covered in the past. A woodland dominated by wild olive trees was discovered and proved rich in a diversity of olive fruit fly parasitoids (Figure 1), including a new population of *Psyttalia concolor*. Previous collections of olive fruit fly parasitoids from other locations in east and south Africa have been dominated by *Psytallia lounsburyi*. In contrast, the collection made in 2006 from this woodland was dominated by *P*. nr. *concolor* (Table 1). The previous collection from Namibia in 2004, though made in the same region, came from urban planted trees and these too, were dominated by *P*. nr. *concolor*. Typically members of the *P. concolor* complex have made up a very low percentage of parasitoids collected in south and east Africa. The dominance of *P*. nr. *concolor* from these collection sites in Namibia on two separate collecting trips suggests it has a much higher degree of specificity for olive fruit fly than other populations or strains collected in Africa.

Table 1. Parasitoids emerging from wild olives (*O. europaea* subspp. *cuspidata*) collected in Namibia.

	Percent of Adults Collected	
	2004 N= 176 adults emerged from fruit	2007 N = 430 adults emerged from fruit
P. lounsburyi	0	23
P. nr. concolor	45	57
Utetes africanus	12	5
Bracon spp.	28	15

The 2007 releases of *Psytallia lounsburyi* began in October. A total of 6,311 *P. lounsburyi* adult females were released in Butte, Napa, Solano, Sonoma and Yolo counties in fall 2007, over four times as many as last year. A second, new strain of *P.* nr. *concolor* collected from Namibia in 2006 was also released**.** A total of 1,275 *P.* nr. *concolor* (Namibia) were released. Adult parasitoids were released directly into the tree with some released into field cages (Figure 2) to facilitate monitoring. Recoveries of small numbers of adult *P. lounsburyi* have been made from olives in the field cages indicating that the parasitoid survived and developed under California field conditions. Results for *P.* nr. *concolor* are pending.

Figure 2. Cage for releasing parasitoids**.**

Table 1. Numbers of adult, female *Psyttalia* spp. released in 2007.

County, Site name	*P. lounsburyi*	*P.* nr. *concolor*
Butte, Leuders	264	318
Fresno, UC Kearney AC	1,100	0
Napa, Spring Mountain	1,383	371
Solano, Wolfskill Field Stn	936	0
Sonoma , Stone Edge	299	0
Sonoma, Hanzell Winery	798	50
Tulare, Lindcove Field Stn.	300	0
Yolo, UC Davis	1,231	536
Total	6,311	1,275

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Aprostocetus vaquitarum, An Egg Predator of Diaprepes Root Weevil

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

Diaprepes root weevil, *Diaprepes abbreviatus*, is a polyphagous weevil with a broad host range and is considered a major insect pest of citrus, woody ornamental plantings, and ornamental plant nurseries. The incompletely described host range may also include avocados, stone fruit, and grapes. Recently it was found infesting parts of Orange, Los Angeles, and San Diego counties (2005 and 2006). In 2006, the California Department of Food and Agriculture (CDFA) began an eradication program in the known infested areas of these three counties. Insecticides are a core component of the eradication program; however, the more effective insecticides have limited registration labels and cannot be used in some of the treatment areas. Therefore, research on biological control was initiated in 2007 to determine if egg predators or parasitoids could be used as a part of the eradication program.

Previous research in Florida identified at least three species of small wasps that readily attack Diaprepes eggs, and one of these wasps, *Aprostocetus vaquitarum*, is maintained in a laboratory colony at the University of Florida, Tropical Research and Education Center. Thus, it was considered for importation to California for further research and potentially field release. This wasp is actually a predator, rather than a parasitoid. The female wasp places her eggs within the Diaprepes egg mass. The eggs hatch, and the wasp larvae begin to feed externally on the Diaprepes eggs within the mass. Each wasp larva requires more than one Diaprepes egg to complete development. The wasp larva eventually pupates within the Diaprepes egg mass. In December 2006, a test shipment of a small number of *A. vaquitarum* was made to the University of California – Riverside Quarantine Facility under a permit held by the CDFA to determine if the wasp could survive shipping from Florida to California. The wasps in this shipment had a 76% survival rate.

Since the wasp could survive shipment, studies on the ability of *A. vaquitarum* to reduce densities of Diaprepes eggs and adapt to the climatic conditions in southern California began in early October 2007. Shipments of the predator were made to the University of California-Riverside Quarantine Facility in early October. The adult wasps from these shipments were used in two studies. The first study, conducted in the quarantine facility, was to determine if the wasp would accept eggs from the California population of Diaprepes weevils. The second study was conducted in the quarantine area of San Diego County to determine if the wasp would adapt to the climatic conditions in southern California.

To determine if the wasp would accept California Diaprepes eggs, 10 mated female wasps were individually confined in vials with eggs laid by field-collected weevils. After five days, the female wasps were given fresh egg masses in which to oviposit. All exposed egg masses were held in the quarantine facility until emergence of the wasp or Diaprepes larvae. Wasps were produced from the California Diaprepes egg masses. This suggests that the Florida wasps would accept California Diaprepes egg masses in the field.

To determine if *A. vaquitarum* could adapt to California climatic conditions, field releases were initiated in known infested urban properties that would not be sprayed as a part of the eradication program in San Diego, Rancho Santa Fe, Carlsbad, and Oceanside. An additional site, a lemon grove in Encinitas, that is used for other research activities could not be used for these releases because of damage suffered as a result of Santa Ana winds and the wild fires that impacted the area in early October. Adult wasps were transported from the University of California – Riverside Quarantine Facility to San Diego County from October 18 – November 1, 2007. Table 1 summarizes the number of adult wasps released at each site. The release sites will be monitored throughout 2008 to determine the success of the releases. Field cage studies are also planned for the Encinitas site in 2008.

Table 1. The date of release, the number of *A. vaquitarum* adults released, and the location of releases in San Diego County in 2007.

Date of Release	Number Released	Location
October 18	264 adults	San Diego
October 18	292 adults	Rancho Santa Fe
November 1	348 adults	Carlsbad
November 1	304 adults	Oceanside

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Pink Hibiscus Mealybug Parasitoid Insectary Production

William J. Roltsch, Ernesto Leonhardt and Frank Raya

In August 1999, the pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green), was found for the first time in North America in the communities of Calexico and El Centro in Imperial County, California, and in the northern portion of the city of Mexicali, Mexico. Shortly thereafter, California Department of Food and Agriculture scientists, in cooperation with USDA-APHIS and the Imperial County Agricultural Commissioner, established an insectary in El Centro, California to rear parasitoids of the pink hibiscus mealybug. From this insectary, three species of parasitoids from Egypt, Pakistan and areas of southeastern Asia were reared and released throughout the infested area of Imperial County during the next five years (1999-2005).

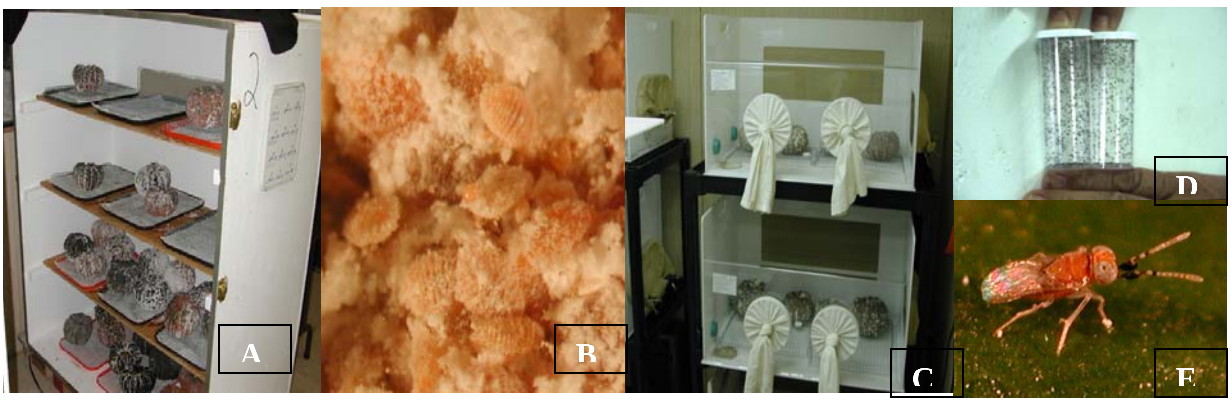

Figure 1. Left to right: a) Production of pink hibiscus mealybugs on squash. b) Mealybug life stages. c) Parasite rearing cages. d & e) Adult parasites prepared for release.

Eight years after the insectary production was initiated, the pink hibiscus mealybug population in Imperial Valley continues to be less than one percent of its former abundance. Past parasitoid recovery studies have provided strong evidence that two species, *Anagyrus kamali* and *Gyranusoidea indica*, have provided a permanent means of biological control of pink hibiscus mealybug populations without a need for any chemical control applications. Without this protection, many species of ornamental trees and shrubs would have been destroyed in these communities and the spread of the pink hibiscus mealybug to other regions of the state would have rapidly occurred, likely affecting ornamentals as well as several commercial crops.

In more recent years, the pink hibiscus mealybug has been found in Florida and Louisiana. As a result, USDA-APHIS contracted again in 2007 with the Department's Biological Control Program to continue production of the parasites at facilities in El Centro, California, for shipment to Louisiana (Table 1). Both *A. kamali* and *G. indica* were reared. Each colony consisted of a combination of strains. The colony of *A. kamali* consisted of populations from Hawaii, China, and Taiwan, while the colony of *G. indica* consisted of populations from Egypt and Australia. In 2007, the Department produced and shipped 105,400 parasites to Louisiana for field release. In addition, 43,425 parasites were released in Imperial Valley, because production exceeded the amount requested by Louisiana. This also represented an opportunity to release *A. kamali* with genetic background from Taiwan. The strain from Taiwan was obtained late during the time that biological control was being implemented in Imperial Valley and therefore a relatively limited number of them had been released.

Table 1. Shipments of pink hibiscus mealybug parasites from California to other states in 2007

Date Sent	Recipient	*A. kamali*– strains: Hawaii/China/ Taiwan	*G. indica*- strain Egypt/Australia
January	Louisiana	3,600	4,400
February	Louisiana		9,400
June	Imperial Valley		3,000
July	Imperial Valley		4,000
August	Imperial Valley		21,925
September	Louisiana	48,000	40,000
September	Imperial Valley	4,000	2,000
October	Imperial Valley	6,000	2,500
Total		61,600	87,225

Figure. 2. Mealybug rearing cabinet with isolation boxes and new squash to be infested with mealybug crawlers. Boxes sealed with weather stripping. Holes on box lids and sides covered with fine mesh silk screen.

Unfortunately, *G. indica* contaminated the host pink hibiscus mealybug colony that had been housed in a trailer separate from where the parasitoids were reared and a decontamination protocol had to be implemented. To decontaminate the mealybug culture, we prepared tightly sealed plastic boxes with top and side "windows" covered with a very fine mesh silk screen fabric. The mesh size was only large enough for first instar mealybugs to crawl through. As a result, each box having fine screen windows contained the parasitoids that emerged from late instar mealybugs on pumpkins, as well as parasitized yet active second instar through adult mealybugs. The crawler stage (i.e., first instar), which is not parasitized by *G. indica*, were free to move through the screen and infest pumpkins outside each box. These pumpkins were used to initiate a new parasitoid-free generation of mealybugs. This approach took two to three generations to eliminate *G. indica* from the parent mealybug culture.

Monitoring for Scuttle Flies for Biological Control of Red Imported Fire Ants

Kris Godfrey, David Morgan[1], and Anne-Marie Callcott[2]

Red imported fire ant (*Solenopsis invicta*) is an A-rated pest in California, and is currently a focus of an eradication program. The insecticide applications in the eradication program are expensive, and in some areas, may raise environmental and health issues. In the southeastern United States, biological control using two species of scuttle flies, *Pseudacteon tricupis* and *Pseudacteon curvatus*, has reduced the activity and abundance of the red imported fire ant. In 2005 and 2006, one species of this fly, *P. tricuspis*, was released at four sites in the city of Lake Elsinore in Riverside County. In June 2005, 1,093 flies were released in northern Lake Elsinore, and 1,373 flies were released in southern Lake Elsinore. In April 2006, 1,550 flies were released in central Lake Elsinore and 1,360 flies were released in eastern Lake Elsinore.

To determine the success of these releases, monitoring of the release sites has been conducted since 2005. Progeny of the flies released were found at one release site one month after release in 2006. No flies were found at the 2005 release sites in 2006. The lack of recoveries of the flies one year after release is not to surprising because in other parts of the United States, it has taken up to three years to get recovery of the flies. In 2007, monitoring of the release sites was conducted in May and November. Visual surveys of the release sites were conducted in May. These surveys are done by disturbing of the mound (tapping on the top) and crushing some ants to attract the flies. No flies were seen in these surveys. In November, a trap provided by USDA, APHIS was used to survey for the scuttle flies. The traps were placed at the four release sites for 24-hours on November 6 and November 14. No flies were caught on the traps during these surveys. Monitoring will continue next year. In addition, if permit issues can be resolved, the second species of scuttle fly, *P. curvatus*, may be released in 2008 in Lake Elsinore.

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Distribution of *Bemisia* Parasitoids in Central California

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

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

Two sampling protocols were conducted for several years in late summer to early fall, one to measure the spread of *E. mundus*, and the other to determine in-field host specificity. The first is based on whitefly infested cotton leaves and was conducted by the Pink Bollworm Program (CDFA) as part of a larger effort to monitor insect pests in cotton. It was described in the 2004 annual report. The second sampling effort focused on identification of parasitoids and host whiteflies. Parasitoids were reared from isolated whitefly hosts to insure identification of host species, and these methods are also described in the same report. Collections of cotton leaves came from Kern, Tulare, Kings, and Fresno counties. Additional samples for the parasitoid-host survey work came from nightshade (*Solanum* sp.), spurge (*Euphorbia* sp.), holly hock (*Alcea rosea*), purple potato vine, *Solanum rantonnetii*, wild lettuce (*Lactuca* sp.), mulberry (*Morus* sp.), and sunflower (*Helianthus*). Two other species of whitefly were recovered from these plants, the banded wing whitefly, *Trialeurodes abutilonea* and the mulberry whitefly, *Tetraleurodes mori*.

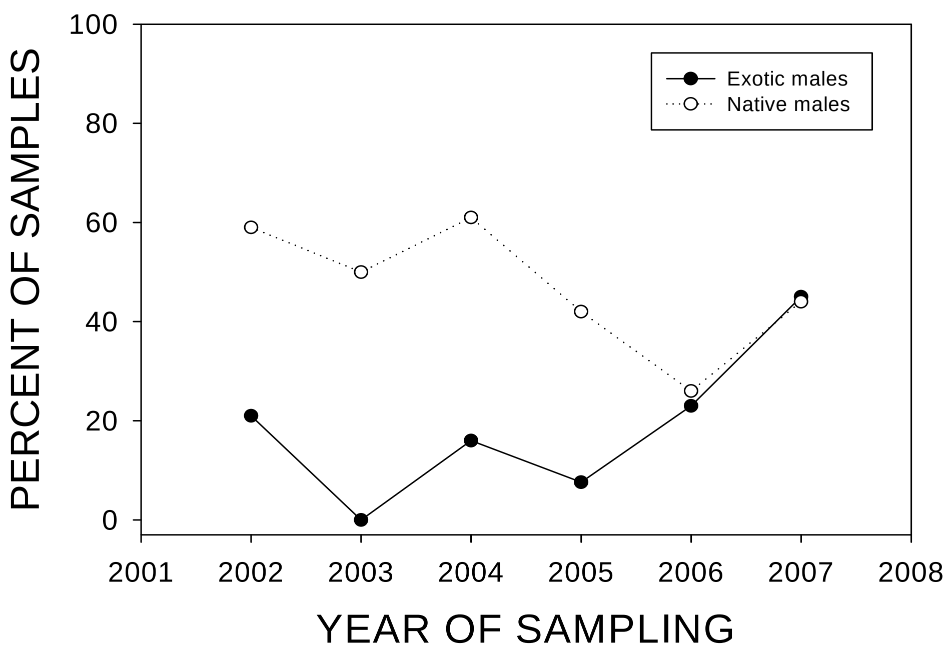

Figure 1. Percent of samples with Eretmocerus. San Joaquin Valley, California

Eretmocerus mundus is spreading throughout the cotton growing acreage in central California (Figure 1, Table 1). An increasing percentage of cotton leaf samples were recorded positive for the presence of *E. mundus* from 2002 to 2007. Most of this increase has taken place in the last two years. The percentage of samples with *E. mundus* is approaching amounts that have been recorded for the native *Eretmocerus*. Native *Eretmocerus* numbers have fluctuated between 26 and 61%, and appear to be decreasing while the exotic numbers increase. Initially *E. mundus* were found more in areas where releases were made (Kern and Tulare counties and the very eastern end of Fresno County). The distribution of collected parasitoids has more recently spread out over the four counties being sampled (Table 1). Kings county, once with virturally no records of *E. mundus*, had 55% of its samples positive for *E. mundus* in 2007. Fresno is the last county to show any substantial gains in *E. mundus*. The cotton growing area in this county is farthest from any of the original main release sites. The drop in samples with *E. mundus* from Kern County may have to do with decreasing cotton acreage.

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

Year of collection	Percent of samples with *E. mundus*, by county (number of samples)			
	Kern	Tulare	Kings	Fresno
2002	33.3 (12)	30.0 (10)	0 (1)	0 (6)
2003	0 (7)	0 (5)	0 (1)	0 (1)
2004	50.0 (6)	5.0 (20)	0 (3)	8.3 (12)
2005	33.3 (6)	0 (14)	0 (1)	0 (1)
2006	27.0 (52)	22.0 (45)	50.0 (10)	14.3 (16)
2007	16.0 (19)	63.6 (22)	55.5 (27)	20.0 (5)

The introduced *E. mundus* showed a very high preference for the exotic target pest, *Bemisia tabaci*. With only one exception, introduced *E. mundus* was only recovered from this whitefly. Native *Eretmocerus* spp. were associated with the native banded wing whitefly, *T. abutilonea*, and mulberry whitefly *Tetraleurodes mori*. The native *Encarsia*, including *E. pergandiella*, *E. meritoria*, and *E. coquilletti*, were found on *Bemisia* and banded wing whitefly.

Table 2. Host associations, on weedy plants and cotton, 2002 - 2007 combined data.

	Bemisia tabaci	*Trialeurodes abutilonea*	*Tetraleurodes mori*
Exotic *E. mundus*	247	1	0
Native *Eretmocerus* spp.	106	27	20
Native *Encarsia* spp.	12	14	0

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

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

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

The most serious insect pest of strawberries grown in the Monterey Bay region of California has been *Lygus hesperus* Knight (Hemiptera: Miridae). Until recently *Lygus* has been managed through applications of broad spectrum insecticides. Cultural and biological alternatives have not been considered useful. Importation of the nymphal parasitoid, *Peristenus digoneutis* Loan (Hymenoptera: Braconidae), into eastern United States during the 1980's, and more recently, the establishment of *P. digoneutis* and *P. relictus* in northern California has created interest in colonizing these parasitoids in the Monterey Bay region. *Peristentus* spp. were released from 2002 to 2005 into four release sites, two of wild vegetation and two of organically produced strawberries.

Peristenus relictus has been recovered four years after it was last released at the original release site of wild vegetation bordering conventionally grown strawberries (Figure 1). Up to 60% parasitism of *Lygus* collected from strawberries adjacent to the wild vegetation has been recorded from this location despite frequent applications of pesticides in the strawberries. Most likely the bordering vegetation serves as a refuge from which the parasitoids can reproduce and re-enter the strawberry fields.

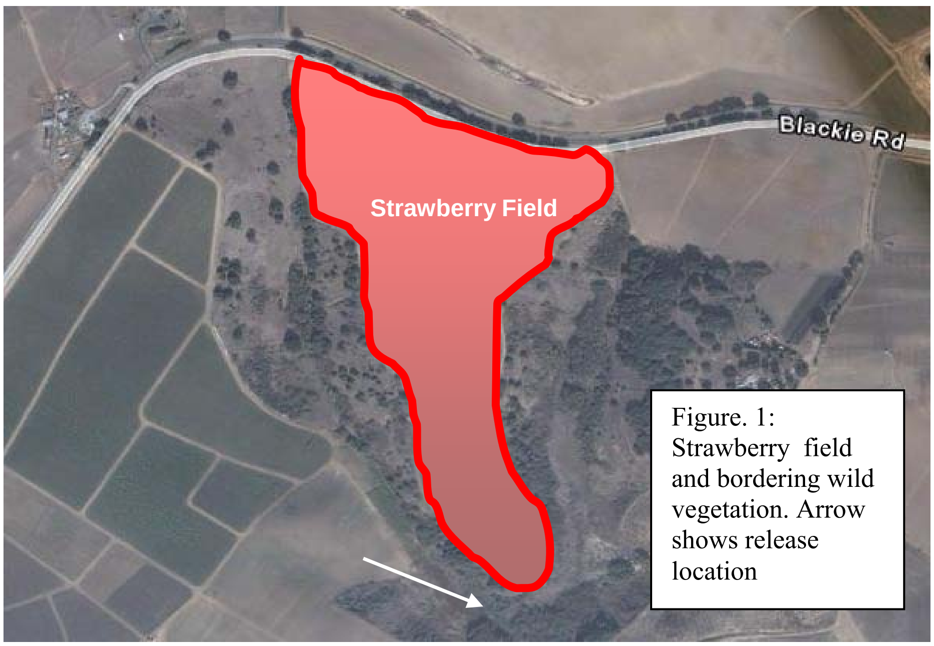

Figure. 1: Strawberry field and bordering wild vegetation. Arrow shows release location

Parasitoids were also released into two organic strawberry farms that lacked the same scale of bordering vegetation as shown in Figure 1. Rather, *Peristenus relictus* was released into single row wide strips of alfalfa serving as a trap crop for *Lygus*. The alfalfa strips were interspersed every 34 rows and were routinely vacuumed with a tractor mounted vacuum device. Parasitoids have been recovered from these fields one and two years after last released. *Peristenus relictus* rapidly spread from one farm and colonized trap crops of alfalfa over 2 km away in a newly planted field of strawberries grown by the same owner with the same management practices. Parasitism of *Lygus* reached 44% (n = 91 dissections) in the trap crop of alfalfa and 36% in the strawberries (n = 51 dissections). *Peristenus digoneutis* has yet to be recovered, post-release, at any sites.

The numbers of *Lygus* in strawberries at the first organic farm receiving parasitoids has dropped significantly over the last five years, from a seasonal high of 0.1 to about 0.01 per suction in 2007 (using a modified reversed Stihl leaf blower). It is likely that the vacuuming of trap crops has played some role in this reduction. However the continual drop over this period of time, along with the strong correlation in numbers of *Lygus* and parasitism (2006 report), suggests that the establishment of *P. relictus* has also played a role. Furthermore, evidence from our initial release site in northern California indicates that a regional impact may not be fully realized until several years after establishment. The maximum seasonal density of *Lygus* at the Sacramento alfalfa site has dropped from a seasonal high of 20 per sweep in 2003 to 1 per sweep last summer (2007) (Figure 2). Finally, *Peristenus relictus* was collected for the first time over 4.5 km distant from the initial release site of alfalfa in Sacramento, spring 2007, indicating that the population is gradually spreading through the region.

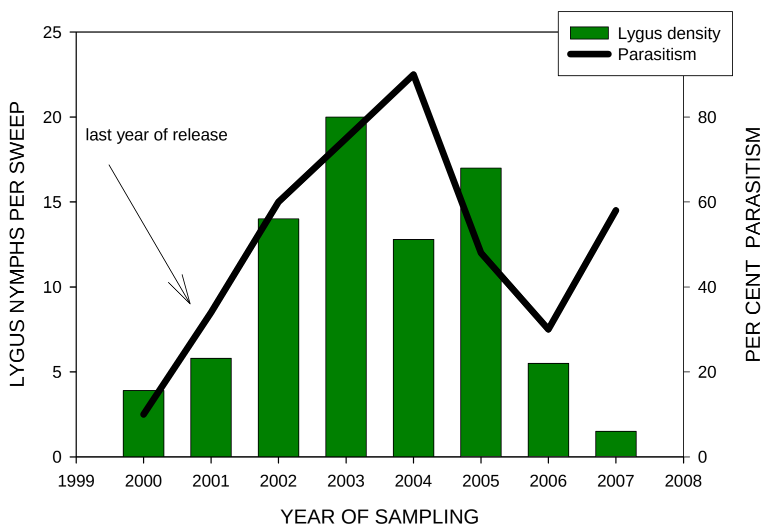

Figure 2. Seven Year Population Trend in Alfalfa Sacramento, California. Annual maximum values.

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Establishment of a Laboratory Culture of the Omnivorous Leafroller to Mass-produce Parasitoids with a Potential Use Against the Light Brown Apple Moth

Syed Khasimuddin

The Light Brown Apple Moth (LBAM) which has been recorded from several counties in the northern and central coast of California presents a significant threat to California's agriculture. In addition to efforts to eradicate this pest, other approaches are being considered including a biological control strategy targeting the egg, larval, and pupal stages of the pest.

The LBAM has a complex of egg, larval, larval-pupal parasitoids in its place of origin (Australia). A fairly good number of native leafroller species have been recorded in California. One possible tactic for California is to make use of the already existing parasitoid species in the state. Efforts are being focused on five Tortricidae that have attained pest status on various field and orchard crops. These species are:

1. *Platynota stultana*, the omnivorous leafroller (OLR)
2. *Argyrotaenia franciscana*, the orange tortrix (OT)
3. *Archips argyrospila,* the fruittree leafroller (FTLR)
4. *Argyrotaenia velutiana*, the redbanded leafroller (RBLR)
5. *Choristoneura rosaceana*, the obliquebanded leafroller (OBLR)

Here, we report on efforts to initiate laboratory cultures of some of the leafroller species. The goals of having laboratory cultures are: 1) to mass produce field-collected parasitoids on various leafroller species; 2) to expose the immature stages in the field to naturally occurring parasitoids, then brought back to the laboratory for parasitoid recovery and rearing; and 3) provide non-target host material for pre-release testing of LBAM parasitoids in quarantine.

The most readily available species during October of 2007 was the omnivorous leafroller (OLR), *Platynota stultana*. A laboratory culture of OLR was started with eight pupae of OLR received on October 9, 2007 in individual 1 oz. clear plastic cups. The larvae were provided by Dr. Lucia Varela of U.C. Cooperative Extension, from a culture being maintained by her. Moths that emerged from these pupae were put together for mating and oviposition in clear plastic tubes. Hatching larvae were carefully transferred by a camel hair brush onto an artificial diet (Standard Oriental Fruit Moth diet – Citrus supplied by "Bio Serv" – Product # F9649B) in 1 oz. clear plastic cups, at the rate of one or two larvae per cup. A total of 28 cups were set up. These cups were held in the laboratory with 22^{o} C $\pm$ 2^{o} C temperature and ambient humidity ranging between 43% and 46%, and ambient daylength. After 21 days, all cups were transferred to an environmental chamber maintained at 26^{o}C, 55% R.H , and a 14/10 light/dark cycle. The first adults started emerging 30 days from the time the larvae hatched. Emerging adults were put together in plastic cups supplied with a 10% honey solution. Oviposition took place on the walls of the cups and eggs were harvested by cutting out parts of the cups with egg masses.

The next (2nd) generation larvae were bulk-reared on the same artificial diet in square, clear plastic "lunch boxes" with airtight lids (28.5 oz. capacity). From the initial eight pupae, a total of 22 next generation adults were realized. This generation took a total of 30 days from egg hatch to adult emergence. The next generation (3rd) produced 76 adult moths and the time from egg-hatch to first adult emergence was 32 days in the environmental chamber.

Citrus Leafminer Phenology in Tulare, Kern, and San Diego Counties

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

The citrus leafminer (*Phyllocnistis citrella*) is a serious pest of citrus and can facilitate citrus bacterial canker when the disease is present. Citrus leafminer is continuing to move throughout California and can be found in the desert valleys of southern California, along the southern coast, and is now gradually moving northward through the San Joaquin Valley. The phenology of citrus leafminer and its potential parasitoids throughout the range of citrus in California is not well understood. In parts of southern California, citrus leafminer has been established for a number of years, and native gracillariid parasitoids have been attacking the leafminer larvae for some time. Very little is known about the native gracillariid parasitoids that may attack citrus leafminer larvae in the San Joaquin Valley. Therefore, this study was initiated in 2006 to investigate the phenology of citrus leafminer along the extreme southern coast of California and the southern end of the San Joaquin Valley, and to begin to survey the parasitoids attacking the leafminer in the San Joaquin Valley.

The phenology studies were conducted at five locations in San Diego County, three locations in Tulare County, and three locations in Kern County. Pheromone-baited traps that attracted male moths were used at all locations. The starting date of the trapping and the number of traps placed varied with location. In San Diego County, two traps were placed at opposite ends of each site on March 8 and were replaced at approximately monthly intervals through December 31, 2007. One trap was placed at each Tulare County location on January 27 and in Kern County on August 27. Traps were replaced at approximately monthly intervals until December 19, 2007. An additional trap was placed at the opposite end of the block from the original trap at Tulare site 1 on June 20, 2007.

The survey of native parasitoids was conducted at two locations in Tulare County beginning on April 17 and three locations in Kern County beginning September 12. The survey consisted of examining five terminal branches on 10 randomly selected trees at each site. The first 10 – 15 leaves on the tip of each terminal branch were inspected for the presence of leaf mines. Leaves with active mines were collected, returned to the laboratory, and held for the emergence of any parasitoids.

The phenology of citrus leafminer varied with location in the state. In San Diego County, male moths were caught in traps beginning in early March and continued to increase in number throughout the summer and into the fall (Figures 1 and 2). For the Escondido traps (east and west), a decline in trap catches occurred in October due to the very close proximity of the traps to areas that burned in the regional wild fire (Figure 1). In the southern San Joaquin Valley, more moths were captured in traps in Kern County than in Tulare County (Figures 3 and 4). For the San Joaquin Valley, there appears to be a small flight of moths in late February through March, with an increase in moth density occurring from August through the end of October (Figures 3 and 4).

Surveys of native parasitoids in Tulare and Kern counties recovered no parasitoids or evidence of parasitoids. The amount of leaf mining found at all survey sites was very low, although it did increase in November and December in Kern County (Table 1). The lack of parasitism and the low densities of citrus leafminer larvae in the San Joaquin Valley suggest that citrus leafminer has not been present long enough or in large enough numbers to attract the native gracillariid parasitoids that are in adjacent habitats.

These results suggest that the seasonal phenology of citrus leafminer varies with the location in the state. Some of the phenological differences may be due to weather factors such as the southern coastal areas being slightly warmer or having temperature conditions more favorable for citrus leafminer throughout the year than the San Joaquin Valley. The question still remains as to where citrus leafminer can find refuge in times when no flush growth is present in the citrus groves.

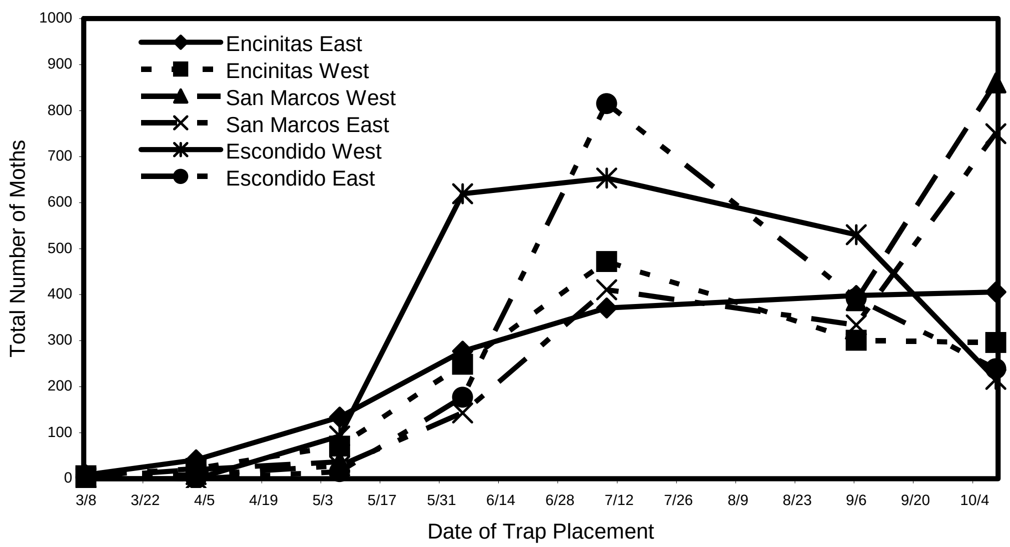

Figure 1. The total number of male citrus leafminer moths caught in pheromone traps placed on the west side of San Diego County in 2007.

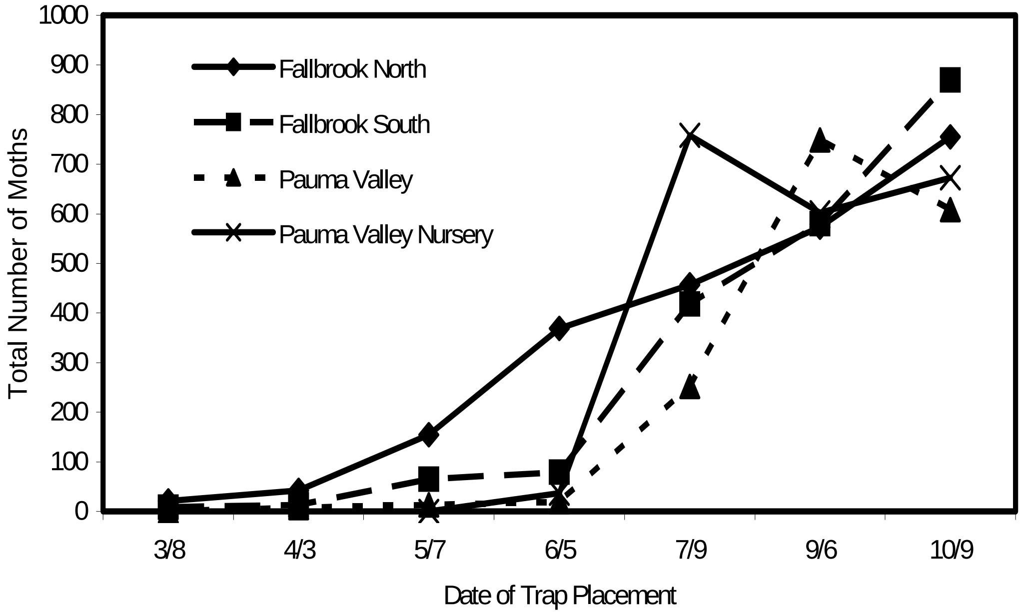

Figure 2. The total number of male citrus leafminer moths caught in pheromone traps placed on the east side of San Diego County in 2007.

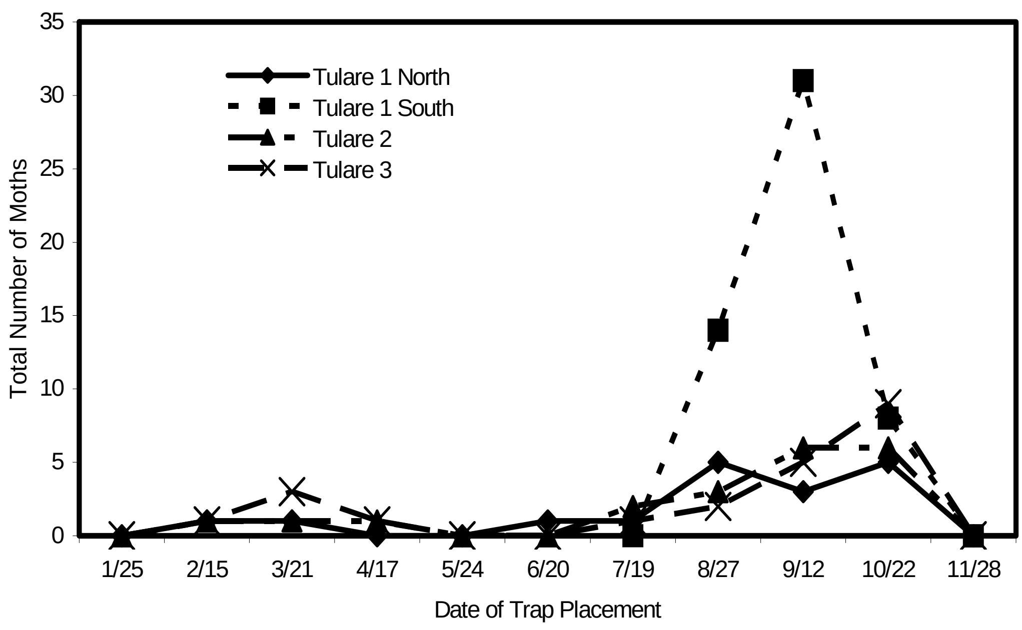

Figure 3. The total number of male citrus leafminer moths caught in pheromone traps placed in Tulare County in 2007. The Tulare 1 South site was added in June.

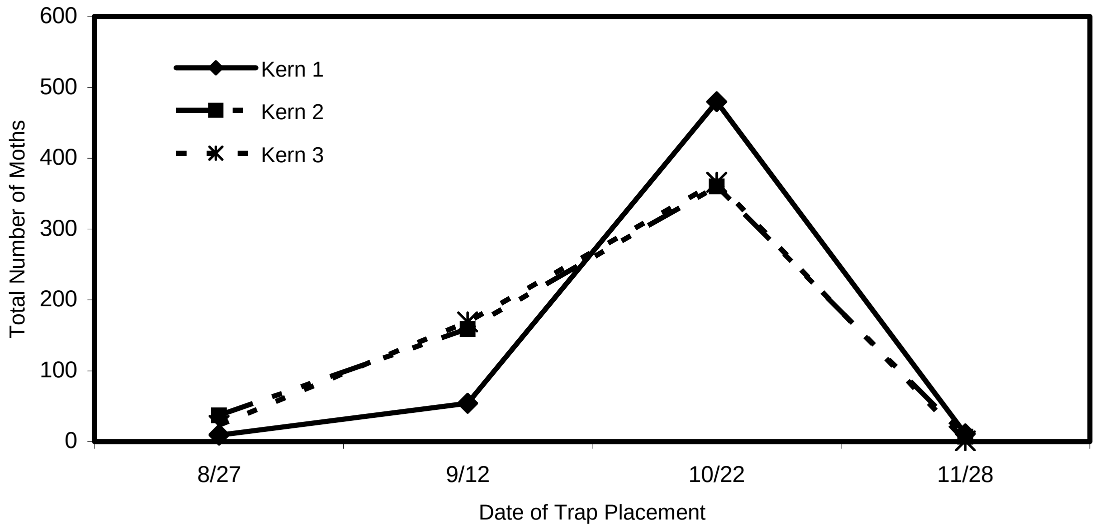

Figure 4. The total number of male citrus leafminer moths caught in pheromone traps placed in Kern County in 2007.

Table 1. The number of mined leaves in 50 branch terminal samples, emerged mines, larvae, and pupae found in Tulare and Kern Counties in 2007. Results given for sample dates and sites with positive finds.

Survey Date	Site	Number of Leaves Mined	Emerged Mines	Larvae	Pupae
July 19	Tulare 1	1	0	1 Dead	0
Sept. 12	Kern 1	1	2	0	0
	Kern 3	1	1	0	0
Oct. 22	Tulare 1	1	0	1 Large	0
	Kern 3	1	1	0	0
	Kern 2	2	0	3 Large	1
	Kern 3	7	1	3 Medium, 5 Large	1
Dec. 19	Kern 1	4	2	2 Large	0
	Kern 2	13	6	3 Large	4

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Biological Control of the Vine Mealybug in Central Valley Vineyards

Kris Godfrey, Kathleen Casanave, Ruby Shin, Monica Cooper[1], and Kent Daane[1]

The vine mealybug, *Planococcus ficus*, is a serious pest of vineyards throughout much of the grape-growing regions of California. This insect causes direct damage to the berries, decline in the vines and may vector some leafroll viruses. Current management programs rely heavily on insecticides, and more sustainable management programs are needed. Biological control will be one component of the sustainable management program for vine mealybug (VMB). A cooperative project with the University of California-Berkeley (UCB) was established in 2005 in an attempt to increase the contribution of biological control to the management of VMB populations. In late 2006, four vineyards were selected in San Joaquin and Sacramento Counties as parasitoid release sites. As a part of the cooperative project, CDFA sampled and made releases at two of the vineyards (Galt and Walnut Grove), while UCB sampled and made releases at the other two vineyards in 2007. A colony of one of the exotic parasitoids, *Anagyrus pseudococci* (Sicilian strain), was established at the CDFA Biological Control Facility in Sacramento. The objectives of this project for 2007 were to characterize VMB populations within the vineyards, make releases of *A. pseudococci* (Sicilian strain), and monitor parasitism within the vineyards. The results from the sampling by CDFA are contained in this report.

A core area (the area of the vineyard with the highest densities of VMB) and a transect area (area with a lower VMB density that surrounded the core) were established at each vineyard. These areas for the Galt vineyard were much smaller than that for the Walnut Grove vineyard, due to differences in VMB population densities between the two sites. The two vineyards were sampled monthly from May 15 – October 18, 2007. Parasitoid releases were made in the core area of each vineyard by releasing adult *A. pseudococci* females while walking along a row. Parasitoids were released from June 29 – October 18 in the Galt vineyard, and from April 26 – October 15, 2007, in the Walnut Grove vineyard. In August, cooperators at UCB began supplying additional parasitoids to CDFA for release. In total, 883 parasitoids (425 from CDFA and 458 from UCB) were released at the Galt vineyard, and 1,335 parasitoids (1,029 from CDFA and 306 from UCB) were released at the Walnut Grove vineyard. On each sample date, the trunks, cordons, canes, leaves, and clusters on randomly selected vines in the core area (10 vines for the Galt vineyard and 20 vines for the Walnut Grove vineyard) and transect area (10 vines in both vineyards) were visually examined for three minutes. During this time, the number and life stage of all VMB, and the number and condition of parasitized mealybugs (i.e., mummies) were recorded. Any VMB or mummy (parasitized mealybug) found was collected, returned to the laboratory, and held for parasitoid emergence.

The density of VMB increased through the season at both vineyards, peaking in August with most of the mealybugs found within the core area (Tables 1 and 2). As expected, there was a resident population of VMB in protected locations on the vine (e.g., under the bark) throughout the season. The density of VMB in exposed locations (e.g., leaves, clusters) increased in mid-summer with the expansion of the leaf canopy and development of clusters on the vines (Tables 1 and 2).

Anagyrus pseudococci was recovered from VMB collected in both vineyards (Table 3). The parasitism rates were low throughout the season. In the Walnut Grove vineyard, several parasitoids were recovered in the transect area, suggesting the movement of the parasitoids out of the release area.

Table 1. The mean number (std. error) of the nymphs, mummies, and adults, found in protected and exposed locations in the core and transect areas at the Galt vineyard in 2007.

Date Sampling area	Protected Nymphs	Protected Mummies	Adults	Exposed Nymphs	Exposed Mummies
May 15					
Core	0	0	0.2 (0.2)	0	0
Transect	0	0	0	0	0
June 13					
Core	1.3 (1.2)	0.5 (0.5)	0	0	0
Transect	0	0	0.1 (0.1)	0	0
August 2					
Core	0	0	0.8 (0.7)	1.1 (0.7)	0
Transect	0	0	0.1 (0.1)	0	0
August 23					
Core	1.2 (0.99)	0	0	0.4 (0.4)	0
Transect	0	0	0.1 (0.1)	0	0
September 20					
Core	0	0.2 (0.2)	0.1 (0.1)	0	0
Transect	0	0	0	0	0
October 18					
Core	0	0	0	0	0
Transect	0.2 (0.2)	0	0.1 (0.1)	0.1 (0.1)	0

Table 2. The mean number (std. error) of the nymphs, mummies, and adults found in protected and exposed locations in the core and transect areas at the Walnut Grove vineyard in 2007.

Date Sampling area	Protected Nymphs	Protected Mummies	Adults	Exposed Nymphs	Exposed Mummies
May 15					
Core	2.4 (1.5)	0	0.35 (0.2)	0	0
Transect	0	0	0	0	0
June 13					
Core	12.7 (5.4)	0	2.2 (1.3)	0.15 (0.11)	0
Transect	0	0	0	0	0
August 1					
Core	3.7 (1.5)	0.05 (0.05)	1.5 (0.6)	33.25 (11.6)	3.3 (3.1)
Transect	0	0	0	0	0
August 21					
Core	1.9 (0.7)	0.3 (0.2)	3.1 (1.2)	41.5 (11.5)	0.4 (0.3)
Transect	0	0	0.05 (0.05)	0	0
September 19					
Core	3.9 (1.3)	0	1.7 (1.3)	15.2 (5.1)	0.4 (0.2)
Transect	0	0.1 (0.1)	0.2 (0.2)	0	0.2 (0.1)
October 24					
Core	6.9 (2.1)	0.5 (0.2)	1.0 (0.4)	20.8 (6.8)	0.3 (0.1)
Transect	1.2 (1.2)	0	0.1 (0.1)	0.1 (0.1)	0

Table 3. The number of *Anagyrus pseudococci* reared from VMB and the percent parasitism found in the Galt and Walnut Grove vineyards in 2007.

Date of Sample	Vineyard	No. of Parasitoids/ No. of VMB	Percent Parasitism
May 15	Galt	0/2	0
	Walnut Grove	0/14	0
June 13	Galt	0/16	0
	Walnut Grove	7/26	26.9
August 1 and 2	Galt	1/70	1.4
	Walnut Grove	1/570	0.18
September 19	Walnut Grove	14/58	24.1
October 18 and 24	Galt	6/51	11.8
	Walnut Grove	11/75	14.7

The studies to characterize VMB and parasitoid populations and releases of *A. pseudococci* will continue in 2008. Rearing methods for the parasitoid at CDFA have greatly improved and it is expected that larger numbers of parasitoids can be produced and released in 2008.

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Avocado Lace Bug Seasonal Population Patterns in San Diego County

William J. Roltsch, Eduardo C. Humeres[1], Mark S. Hoddle[1], Joseph G. Morse[1], David Kellum[2]

In the 1990's, outbreaks of avocado lace bug (AvLB), *Pseudacysta persiae*, occurred in Florida, primarily as secondary pest outbreaks resulting from death of its natural enemies by pesticides applied against other avocado pests. In recent years, the AvLB has expanded it range into the Caribbean, resulting in devastating leaf damage and production loss in avocado groves. This lace bug is now also known from French Guyana in South America, having most likely invaded from the Caribbean. Feeding damage by nymphs and adults typically results in the formation of large necrotic areas on the foliage (Figure 1). Unless leaves are very heavily damaged, damaged leaves typically remain on trees throughout the season with leaf drop coinciding with that of the annual cycle of healthy leaves.

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

Figure 1. Avocado lace bug adults, leaf damage and field sampling.

Since July of 2005, repeated monthly sampling of populations was performed at six residential sites. Sampling consists of visually examining 25 leaves in the lower canopy of selected trees at each site, aided by the use of a headset magnifier (Figure 1). On average, population levels were greater in 2006 than during 2005 and 2007 (Figure 2). However, increases in density did not occur at all sample sites, e.g. densities at two sites in 2006 were very high, but densities at several other sites decreased markedly. These findings are highlighted by high standard error values in the data (Figure 2). Similar findings occurred in 2007, where the three highest population density sites in 2006 declined markedly in 2007.

In the fall of 2006, two additional sites were discovered in the coastal community of La Jolla. These sites contained younger infestations than the original set of sample sites. Data from these sites, first sampled in February of 2007, were not combined with data from the other sample sites. We are sampling to see if densities at these sites follow a pattern similar to population trends at other locations where population densities escalated and declined. In this case we expect that populations that are high in 2007 will subside in 2008. Visual leaf inspection over the past three years has provided no insight into the role that natural enemies may play in population fluctuations. Occasionally we find a green lacewing larva feeding on the AvLB. Perhaps nocturnal predators play a role and tree variety may also have considerable impact on lace bug numbers. At sites where multiple tree species are present, the Hass-like avocado varieties rarely support high lace bugs densities. Interestingly, in the Dominican Republic, massive outbreaks of AvLB in commercial Hass avocados has been observed and the dominant predator working pest populations was a predatory thrips, *Franklinothrips vespiformis*, which has congeneric representation in California.

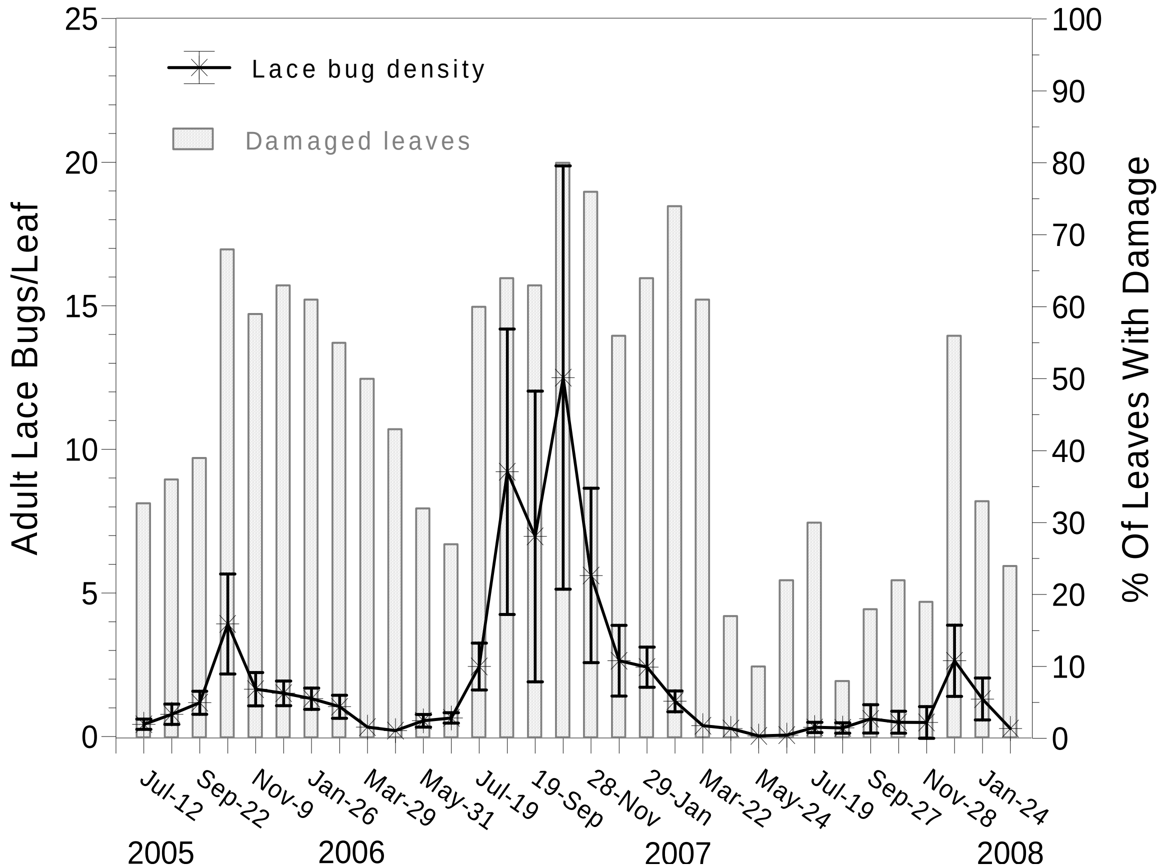

Figure. 2. Adult lace bug densities at sample sites in San Diego County and associated leaf damage.

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Fortuitous Establishment of Giant Whitefly Parasitoids in Northern California

Charlie. H. Pickett, Stan Maggi[1], Marc Lea[2], and Ciprian Simon

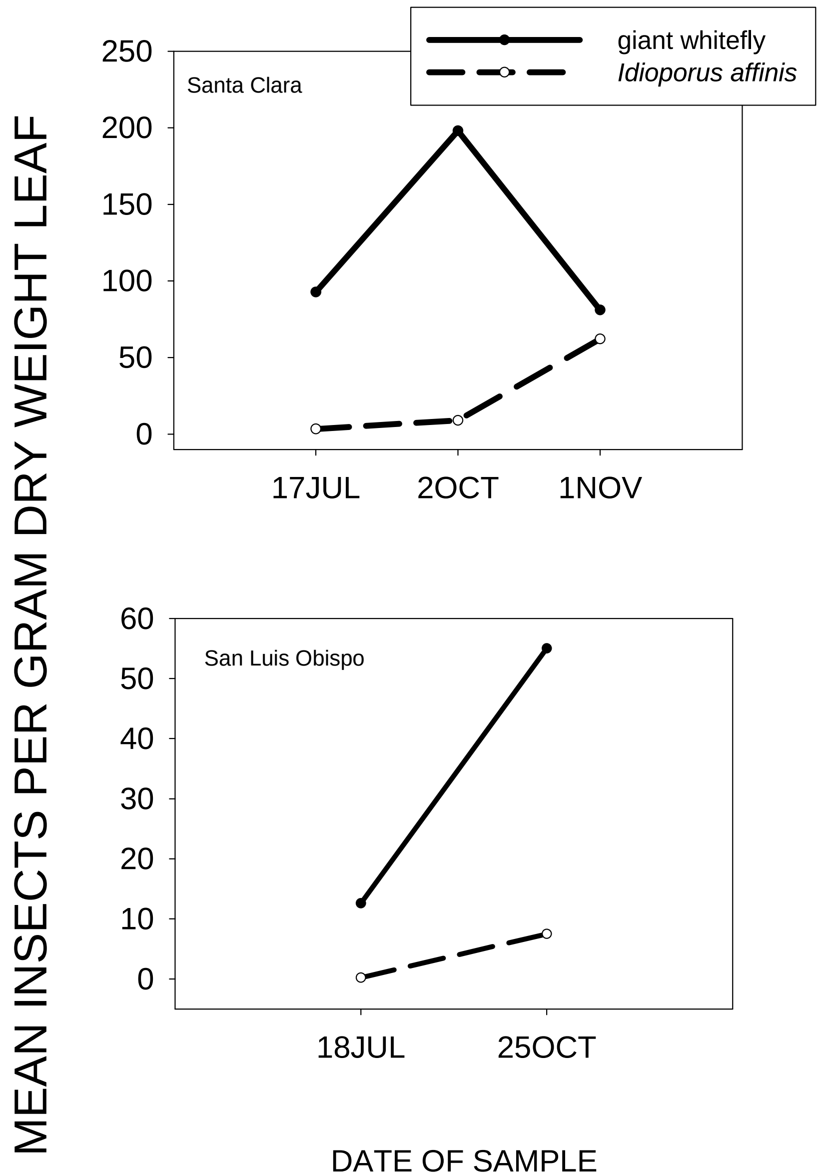
The giant whitefly, *Aleurodicus dugesii* Cockerell, was first discovered in San Diego October 15, 1992. It has been previously reported from north central Mexico (Zacateca) and the southern tip of Baja California (Ray Gill, pers. comm.). The giant whitefly has also recently invaded and established populations in eastern Texas, Florida, Louisiana, and Hawaii. The original finds in southern California were mainly from hibiscus but the whitefly spread to a broad range of subtropical perennial trees and shrubs in southern California, including *Xylosma*, palm, apricot, bird-of-paradise, and morning glory. It also attacks avocado and citrus, two important agricultural crops in San Diego County. This whitefly poses a serious health problem to the general public and a potential economic problem to California agriculture. The nymphal stages produce long waxy hairs up to six inches long that can break off and float in the air, carrying honeydew with it. Leaves with high densities of giant whitefly are flocked with this white material (Figure 1). Sooty mold eventually builds up on leaves reducing their photosynthetic capability.

Figure. 1. Giant whitefly on *Xylosma*, Santa Clara.

Three parastoid species were released in southern California during the mid-1990's for control of giant whitefly. *Entedononecremnus krauteri* was imported into San Diego in 1995 (by Pickett and Rose), while *Idioporus affinis* and *Encarsiella noyseii* were imported in 1996 by the University of California, Riverside (Hoddle and Headrick). Today in southern California, *I. affinis* is the dominant parasitoid found attacking giant whitefly.

The giant whitefly has recently been found in high numbers in Santa Clara and San Luis Obispo counties. Having been contacted by these counties, the Biological Control Program of CDFA conducted a pre-release survey in these counties with the intention of eventually moving parasitoids from southern California to central (San Luis Obispo) and northern (Santa Clara) California. Several private properties in each county were visited in mid summer and collections of leaves from heavily infested *Xylosma* were made. Samples were placed in paper cans, transported to the Program's laboratory in Sacramento and allowed to incubate in a controlled environment chamber for two months. *Idoporus affinis* was discovered in both counties, but in very low numbers (Figure 2). A second parasitoid, *Encarsia*, most likely *hispida,* was also recovered from collections. Additional samples were taken later in the year to monitor changes in insect populations. Rapid increases in the number of *I. affinis* found in these latter collections indicate this parasitoid is permanently established and quickly spreading in both counties, eliminating any need to move this parasitoid from southern California. Counties will continue to be monitored for giant whitefly and its parasitoids this coming year.

Figure. 2. Densities of giant whitefly and its parasite on *Xylosma* leaves collected in Santa Clara and San Luis Obispo counties summer 2007.



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[1]Santa Clara Agricultural Commissioner's Office, San Jose, California
[2]San Luis Obispo County Department of Agriculture, San Luis Obispo, California

Change in Populations of Weevils Attacking Squarrose Knapweed

Dale M. Woods and Viola Popescu

Biological control of squarrose knapweed appears to be one of the most promising weed biological control programs in California. Three insects, *Bangasternus fausti*, *Larinus minutus*, and *Sphenoptera jugoslavica*, have been successfully introduced and established throughout most of the range of squarrose knapweed in the state. A fourth biological control agent, the knapweed seed fly, *Urophora quadrifasciata*, migrated to the state unaided and also established throughout the range of squarrose knapweed. Of the four exotic biological control insects, the two weevils, *B. fausti* and *L. minutus*, have increased in population density the fastest and seem to be the most effective biological controls. Populations of both weevils have developed rapidly when introduced in new areas of California such that, in a few years time, over 90% of the seedheads are attacked each year by the weevils. Both species deposit their eggs in or around the seedhead, then the larvae develop within seedheads, and consume the developing seeds within the seedhead. Squarrose knapweed seedheads are not large enough to support more than one insect, thus competition exists for the food resource. As the populations of the weevils have increased and greater competition exists, we have detected shifts in the relative density of the two weevil species. We have repeatedly sampled from several sites over multiple years and evaluated attack rates by each insect. Results from six of those sites, all in northeastern California, are shown in Figure 1.

The first releases of the weevils in the area occurred in 1998 at the B. fausti site and 100 meters away at the L. minutus site. Extremely rapid increases of both species occurred at both of these sites and all other locations where we have released them. A total of 300 *B. fausti* adult weevils were released at the B. fausti site and a much larger number (2,000) adult *L. minutus* weevils were released nearby at the L. minutus site. Populations of *L. minutus* exploded within two years at the L. minutus release site increasing to over 80% attack rate on the seedheads, and had also spread across the road to the B. fausti site building to equally high population levels. Populations of *B. fausti* steadily increased at the B. fausti release site, increasing more slowly than *L. minutus* but from a lower starting point. After a one-year delay for migration, populations of *B. fausti* began to increase at the L. minutus site also, again slowly but steadily. In both cases, when the populations of *B. fausti* increased enough to attack more than 10% of the seedheads at the site (year three or four), success of *L. minutus* was affected. *L. minutus* populations decreased at the same rate that the *B. fausti* populations increased such that within six years of release, *B. fausti* had replaced *L. minutus* as the primary agent at both sites.

The weevils were released at the Foothill site as a mixture of both species. Again, *L. minutus* developed to high populations faster but was replaced by *B. fausti* at the six year mark. *B. fausti* has continued to attack an increasingly larger portion of the seedheads since that time. The Kane site also received a mixed weevil release but at this site, the weevils seem to be co-existing so far. This release was different from most of the earlier releases in the state in that while others were direct releases from collections made in Oregon, and had been collected from diffuse and spotted knapweed, the Kane site releases came directly from squarrose knapweed in California and may have been adapted to mixed populations. The Peterson site also received mixed releases (from Oregon) but the numbers of *L. minutus* released were 20 fold the numbers of *B. fausti*. After a substantial lag period, populations of *B. fausti* appear to be replacing *L. minutus* at this site just as was found at the L. minutus site.

The Wild rice site was initially intended to be a *B. fausti*-only site as it is several miles from the other release sites and would provide a unique contrast. Small numbers of *L. minutus* have however, arrived at the site. Of particular interest at this site is the continued development of *L. minutus* populations, in spite of a substantial predominance of *B. fausti*. This site is our principle opportunity to evaluate the ability of *L. minutus* to survive as a secondary biological control agent.

It appears from most of our sites that *B. fausti* will replace *L. minutus* as the primary seedhead biological control agent in squarrose knapweed in California. Adults of *B. fausti* emerge earlier than *L. minutus* and thus emerging larvae have a head start within the seedhead. It is possible that, over an extended period of time, a balance will be established with perhaps *L. minutus* attacking over 30% of the seedheads, and 60% going to *B. fausti,* as is currently the case in several of our sites.

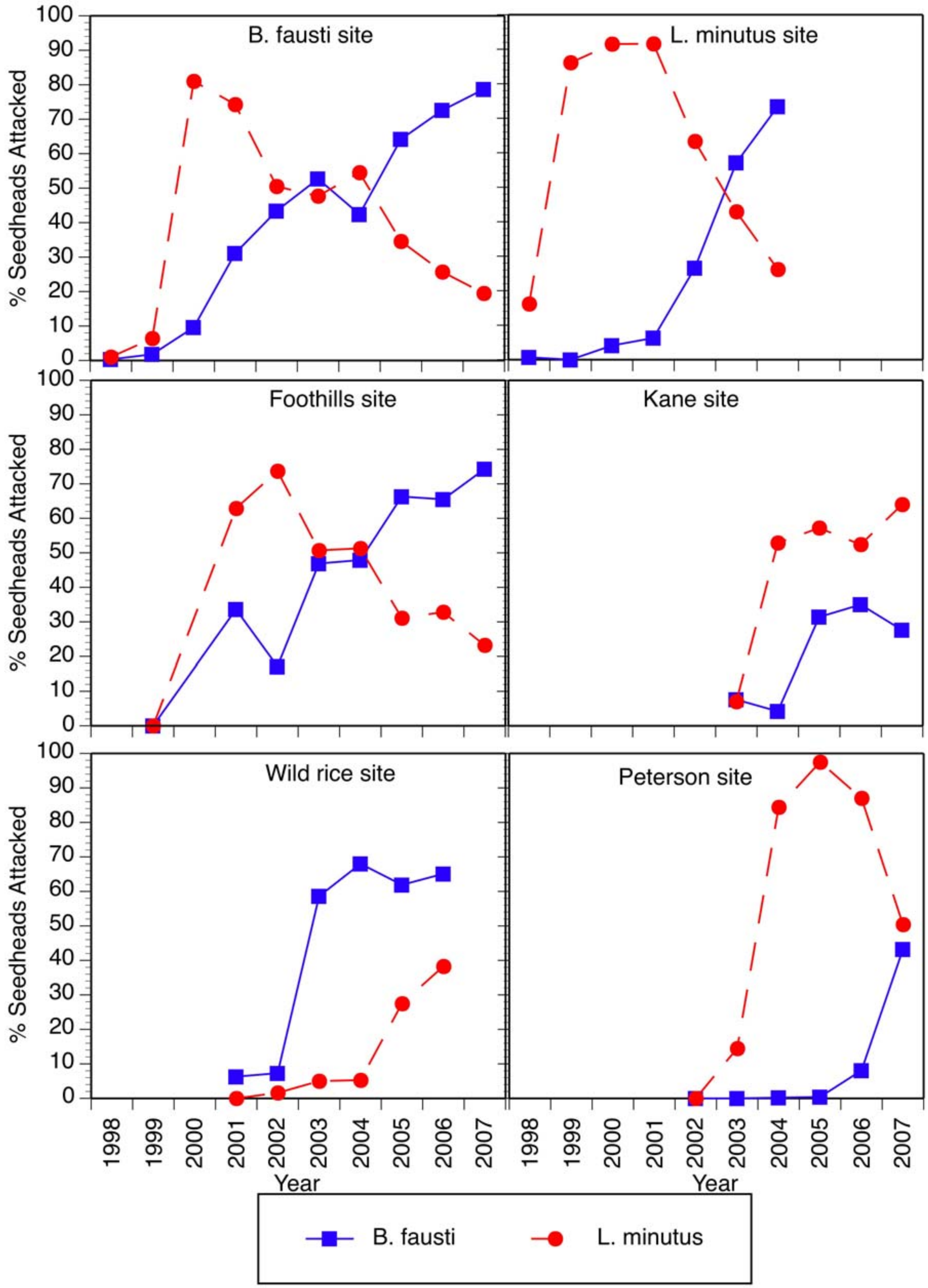


Figure 1: Percentage of squarrose knapweed seedheads attacked by *Bangasternus fausti* or *Larinus minutus* at six sites in California.

Persistence of the Yellow Starthistle Rust Following Field Release

Dale M. Woods, Alison Fisher[1], Baldo Villegas and Viola Popescu

The rust fungus, *Puccinia jaceae* var. *solstitialis,* is the only disease approved as a biological control for yellow starthistle, *Centaurea solstitialis*. The rust was first released in the United States in 2003 at two sites in California: a field site in Napa County and in our greenhouse in Sacramento. Extensive efforts were made to mass produce the rust in the greenhouse and then make it available for field release through assistance by the local Agriculture Commissioners. Each release plot (one square meter) of yellow starthistle was spray inoculated with urediniospores of the fungus, then covered overnight with a plastic tent to provide artificial dew which aids infection. A limited number of releases were made in 2004, and then larger numbers were made in 2005 and 2006 as more rust inoculum became available. For most releases, we and/or staff from the Agriculture Commisioners' offices made repeated field monitoring visits to check on the status of the rust the year it was released and again in subsequent years. During 2007, we made a final follow-up effort to check on all 2004-2006 release sites in the state. By checking on all sites we hoped to create a 'snapshot' picture of the status of the rust in the state and evaluate its ability to persist in-field over a several year period. This report is a preliminary examination of the results obtained from three years of monitoring the rust release sites.

Statewide, between 2004 and 2006, we made 176 releases of the rust. These were spread over 41 counties encompassing the range of yellow starthistle in the state. By surveying these sites in 2007, the sites provided a diverse history. That is, sites had been in existence for one, two or three years and been monitored repeatedly over that time. Eleven sites were not visited in 2007 due to time constraints and/or the remoteness of the sites. Of these 11 sites, only one had any indication of rust in previous visits. Fifteen sites had been destroyed at some point after release of the fungus. The factors in destruction included: herbicide use (intentional and accidental), flooding, wildfires (Figure 1), repeated mowing, and construction projects.

Figure 1. Wildfire destroyed a yellow starthistle rust site in Yolo County.

The rust successfully infected yellow starthistle at most sites as a result of the spray inoculation (Table 1). In fact, although sites were located in different areas of the state, roughly 90% of the sites inoculated in 2004 and 2005 had pustules (visible infection), one to three months after inoculation. This confirmed some of our earlier work indicating that there does not appear to be any variation in susceptibility in yellow starthistle populations throughout the state, and that our field inoculation techniques are effective. Releases in the first two years were limited to locations and to time frames expected to be more conducive to the rust based on greenhouse data. For the 2006 releases, cooperators were allowed to attempt releases at less than ideal times (early summer vs spring) as well as less than ideal locations such as dry hill sides and urban areas. Consequently, the first year failure rate in 2006 was much higher, likely due to environmental limitations on infection.

The percentage of sites in which rust reappeared a full year after release was fairly consistent over all years at roughly 20%. This number is substantially lower than we had hoped for but there was no data to provide us insight about what to expect. Unfortunately, survival through an additional year has also proved difficult. The rust showed up in only 9.5% of the sites at the two year interval. Overall, based on these observations the rust has not demonstrated a strong record of persistence in the field in California. We are evaluating environmental parameters to better understand limiting factors on the success of the rust. Additional releases will be made in 2008 to test modifications of the release strategy in order to increase year to year survival.

Table 1. Summary of field survey of rust release sites for three years after release

Year released	Number of release sites	% of sites with rust at the time monitored			
		1-3 months	12-14 months	24-26 months	36-38 months
2004	29	93%	21%	10%	3%
2005	76	87%	21%	9%	
2006	71	58%	18%		
Total	176	76%	20%	9.5%	3%

[1] USDA-ARS, Albany, California

Seedhead Insects in Meadow Knapweed

Dale M. Woods, Baldo Villegas, Mary Huerter[1], and Viola Popescu

Meadow knapweed, *Centaurea* x *pretensis* (Figure 1), is a fertile hybrid between black knapweed (*C. nigra*) and brown knapweed (*C. jaceae*). All three of these knapweeds are not native to North America and are increasingly being recognized to be invasive pests. Of the three knapweeds, meadow knapweed is the most problematic in California. It is also a pest weed in the Pacific Northwest where populations are much higher and continue to increase. Local efforts to control or eradicate are being attempted in California in order to avoid development of a larger invasive weed problem. Biological control may be of value in the largest stands but has not been evaluated for usefulness. We attempted to establish biological control insects from other knapweeds (spotted and diffuse knapweed) on meadow knapweed stands in California.

Figure 1. Flowerhead with *Larinus* adult

In July 2001, 2,500 adult *Larinus* weevils were collected from spotted knapweed near Mount Hood in northern Oregon. The collection was divided in half and released at two sites in Siskiyou County near Mt. Shasta City. We returned to sample the sites in the fall of that year. We found that in addition to our released weevils, a third biological control insect, the UV knapweed seed fly, *Urophora quadrifasciata* had previously migrated to at least one site and was already well established. During this inspection, seedheads were examined and numerous emergence holes were found in the meadow knapweed confirming that the released *Larinus* weevils had completed development over the summer at both sites and emerged as adults (Figure 2). Follow-up evaluations of the emerged adult weevils revealed that the release from Oregon material had been a mixture of two knapweed biological control agents, the lesser knapweed flower weevil, *Larinus minutus*, and a larger species, the blunt knapweed flower weevil, *Larinus obtusus*.

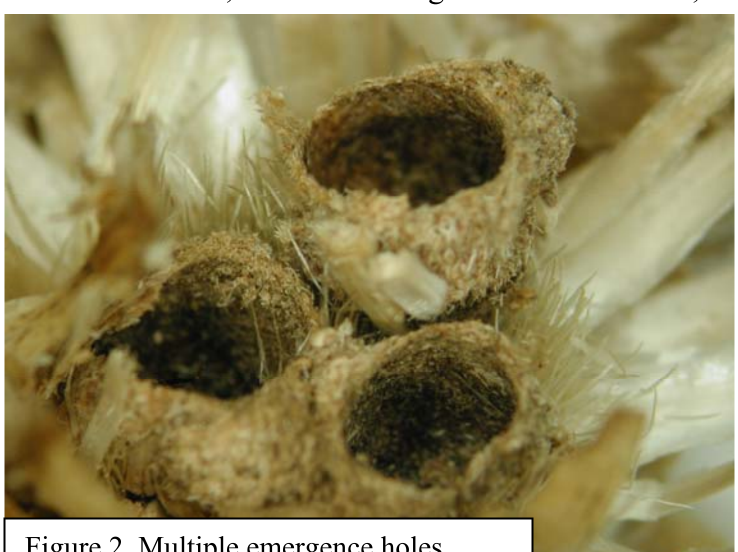


Figure 2. Multiple emergence holes

In 2003, we began field collections of seedheads at the release sites as very little was known about the potential of these weevils to impact seed production of meadow knapweed. Samples were collected from both sites on July 28, 2003 and October 28, 2003. At the July sampling, the heads of the plants were at two developmental stages; flowering, and immediately past flowering with recently spent florets. Relatively few heads were still in the flowering stage, and they had little evidence of attack by seedhead insects. There were a few eggs of *Larinus* spp. that had recently been oviposited adjacent to the florets within these seedheads (Figure 3). Heads in the spent flower stage were far more common and these supported weevil larvae in various amounts (one to four per head, Figure 4). Because meadow knapweed plants do not degrade quickly at the end of summer, we were able to sample in the fall before snowfall. We gathered 10 plants from both sites on October 28, 2003, bulked the heads for each site and dissected 50 random heads from each site (Table 1).

Figure 3. *Larinus* egg on knapweed floret

Meadow knapweed has substantially larger seedheads than most other knapweeds and might be expected to support more biological control insects in each seedhead. It is also likely that a larger number of insects would have to infest each head in order to impact these large seedheads. The gall fly did reach 'in-head' densities as high as 10 at Site #2. However, this was a single seedhead, and the second highest density detected in all samples was only four galls (again a single seedhead). With most seedheads supporting three or fewer galls, we expect little impact from this agent in spite of the history of this agent building to higher 'in-head' levels in other knapweeds. The low attack rate, particularly at Site #1, also does not suggest much promise.

Larinus weevils seldom develop to maturity in groups of two or more per head in other knapweeds but this phenomenon was quite common in meadow knapweed. The high attack rate (76%) and the mean 'in-head' density of 1.55 weevils per attacked seedhead may be enough to impact seed production at a population level, but we did not measure this. Seedheads with a larger diameter were consistently associated with higher numbers of weevils per seedhead. This relationship did not remain true for gall flies in meadow knapweed (Figure 5).

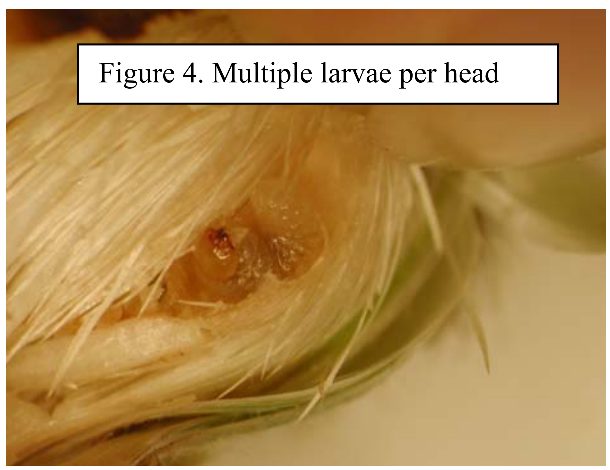
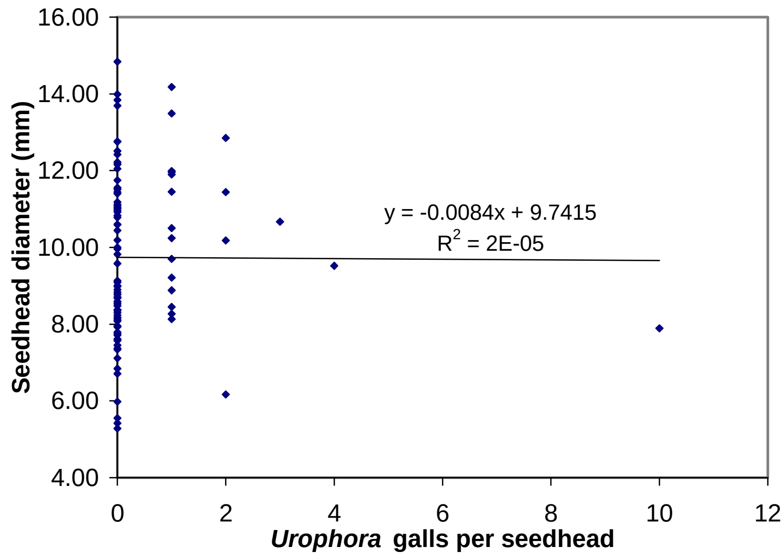


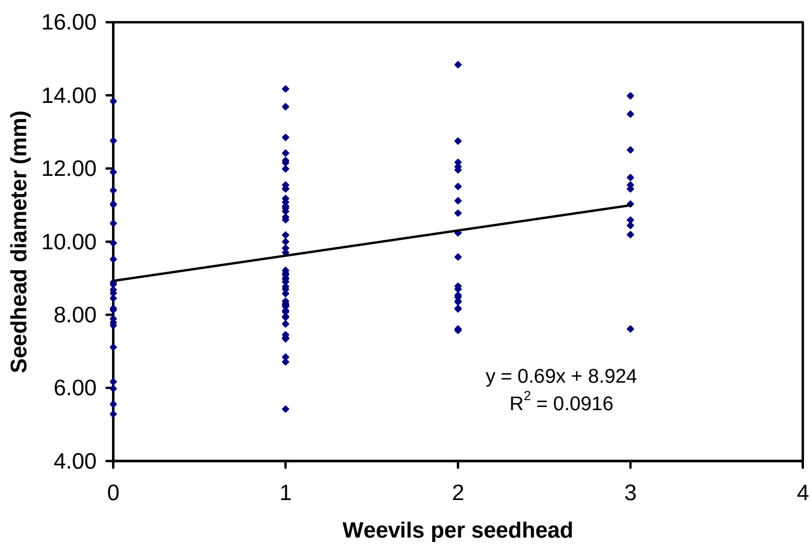
Figure 4. Multiple larvae per head

Table 1. Attack of meadow knapweed by *Larinus* spp and *Urophora quadrifasciata*

	Site 1	Site 2	Total
Larinus spp.			
% seedheads attacked	78%	74%	76%
Mean weevils per attacked seedhead	1.56	1.54	1.55
Maximum weevils per head	3	3	3
Urophora quadrifasciata			
% seedheads attacked	4%	38%	22%
Mean galls per attacked seedhead	1.50	1.89	1.86
Maximum galls per head	2	10	10

Figure 5. Relationship of seedhead diameter to attack by multiple biocontrol individuals





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Puncturevine Biological Control Workshop in Lassen County

Baldo Villegas, Carolyn Gibbs[1], Rob Wilson[2] and Norm Smith[3]

Puncturevine (*Tribulus terrestris* L.) is a widespread weed of European and North African origin. It is a summer annual that thrives in hot and dry areas. It grows low to the ground, extending two to six feet in diameter and plants are commonly found growing close together, forming dense mats along roadsides, irrigation canals, vacant lots, and abandoned agricultural fields (Figure 1A). The plant produces small yellow flowers and prickly spiny fruit which separate at maturity into five hardened segments each containing a seed inside. The hardened segments, oftentimes called goatheads, are armed with very sharp divergent spines (Figure 1B), and can become embedded in bicycle and automobile tires, shoes, and bare animal and human feet.

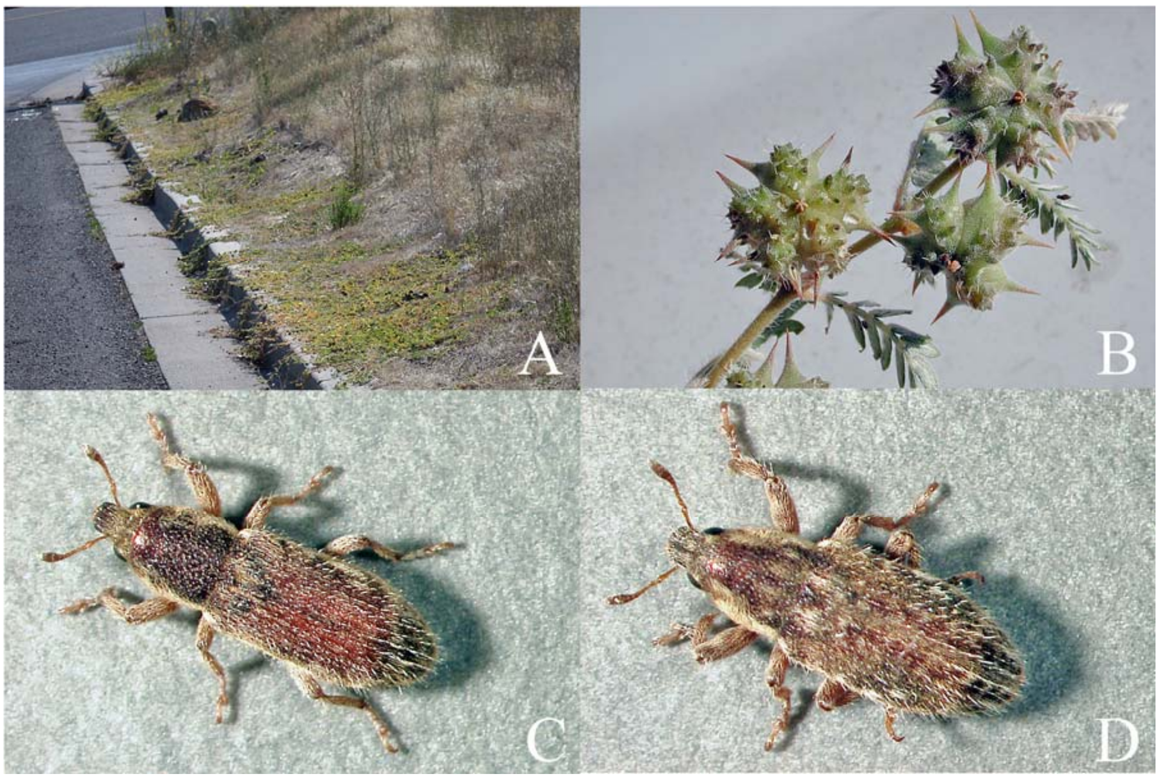

Figure 1: A) Infestation of puncturevine along a sidewalk in northern California; B) Puncturevine seedpods armed with diagnostic divergent spines; C) Stem-boring weevil, *Microlarinus lareynii*; D) Seed-feeding weevil, *Microlarinus lypriformis*.

Puncturevine has become a major weed pest in mountainous areas of California. This is believed to be partially due to the lack of cold hardy forms of the biological control agents. Biological control has a long and successful history on puncturevine in many parts of California. In 2005, the Lassen County Agricultural Commissioner and the Lassen County Agriculture Board requested releases of the two biological control agents, *Microlarinus lareynii* (Jacquelin du Val) [the stem-boring weevil] (Figure 1C) and *Microlarinus lypriformis* (Wollaston) [the seed-feeding weevil] (Fig. 1D). These two insects were mass collected in Tulare County and released at seven high elevation sites above 3500 feet in Lassen and Shasta counties. Some of the sites selected were in urban areas since these sites would be warmer than those in agricultural areas. In the agricultural setting, the release sites contained mixed vegetation near the puncturevines so that the weevils could migrate and be protected from the cold winter temperatures. The sites were surveyed for weevil establishment after the winters of 2005 and 2006. The weevils successfully established at most of the sites suggesting that the weevils are more winter hardy that we thought and that if the release sites are properly selected, the weevils can survive in high elevation areas.

On July 26, 2007, an educational workshop comprising of all aspects of puncturevine control was organized in Susanville, California. Approximately 3700 weevils containing both biological control agents were mass collected in Fresno, California and separated into 18 individual containers for redistribution during the workshop. The 18 recipients were asked to release the weevils onto puncturevine infestations in Lassen, Modoc, and Shasta counties. The weevils were divided in lots ranging from 200-300 weevils each. Surveys for establishment will be done in 2008 to determine the success of this biological control distribution effort.

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Biological Control Releases on Leafy Spurge in Siskiyou County, California

Baldo Villegas, Eric Coombs[1], Gary Brown[2] and Jodi Aceves[3]

Leafy spurge, *Euphorbia esula* L., is a Eurasian weed that is widely distributed in the United States and Canada. The plant is a deep-rooted perennial and can very quickly become widespread and dominate in agricultural lands, meadows, grasslands, and riparian areas. The plant produces latex which can cause dermatitis to humans and grazing animals. Leafy spurge is currently listed as a federal noxious weed and it is under eradication in California.

Leafy spurge has been the target of biological control research in the United States for over 30 years. A complex of insects has been introduced and those that have shown good biological control of leafy spurge in the Great Plains are several species of flea beetles in the genus *Aphthona* (Coleoptera: Chrysomelidae). The larvae of these beetles feed on the roots and root hairs while the adults feed on the leaves and flower bracts (Figures 1A and 2). Six species of *Aphthona* have been released in North America. These beetles are small and some of the species are difficult to identify. *Aphthona lacertosa* (Rosh) and *A. nigriscutis* Foudras are the two most common flea beetles that have impacted leafy spurge where they were released and were identified for use in California. Site selection for these species is very important as sandy soil types are less desirable for the beetle larvae. In addition to the flea beetles, the longhorn beetle, *Oberea erythrocephala* (Schrank) (Coleoptera: Cerambycidae) has been widely utilized in biological control programs. The larvae of this beetle mine leafy spurge stems and keep them from flowering. The adults feed on the foliage causing minor damage (Figure 1B). Fortunately, it attacks leafy spurge over a wider range of conditions independent of soil types.

The largest infestation of leafy spurge in California occurs in the Scott Valley of Siskiyou County. There it is found in irrigated pasturelands, private properties, and along the riparian areas of the Scott and Klamath Rivers and their tributaries. The Siskiyou County Department of Agriculture has been actively treating the more easily accessible leafy spurge infestations for many years. However, many infestations occur along the riparian areas of the two river systems that are inaccessible and difficult to treat. Thus, biological control releases were attempted in 2001. Fifteen releases were made utilizing some 53,000 flea beetles at eight sites along the Scott and Klamath Rivers.

Figure 1: A) *Aphthona lacertosa* flea beetles feeding on leafy spurge foliage, B) *Oberea erythrocephala* longhorn beetles feeding and laying eggs on a stem of leafy spurge.

The beetles were collected in the Medora area of North Dakota and shipped overnight to Siskiyou County where they were released the following day. Approximately 99% of the beetles released were *A. lacertosa*. The two beetles failed to establish at any of the sites. This failure was attributed to the shallow sandy soils where the flea beetles were released.

In 2007 a renewed biological control program was started, but with biological control agents originating from Oregon and making additional releases with the longhorn beetle. Also, more attention was given to the selection of release sites. A large private property along the Scott River north of Fort Jones, California was selected to be used as a nursery site for all of the introduced biological control agents. Additional sites along the two river systems were chosen based on their soil type and their proximity to the rivers. Those sites with loamy soils near the roads were chosen over sandy soils near the rivers.

The first set of releases were started on June 13, 2007 with approximately 8,000 *A. lacertosa* flea beetles and 50 *O. erythrocephala* longhorn beetles originating from the Prineville area of central Oregon. The flea beetles were released at five sites and the longhorn beetles were all released at the Fort Jones nursery site. The releases of the flea beetles ranged from 1,000 to 2,000 per release site. A second set of releases occurred on June 14, 2007 with an additional 25,000 *A. lacertosa* flea beetles and about 100 *Oberea* longhorn beetles released at the Fort Jones site in order to supplement the releases that were made the day before. The second set of biocontrol agents was collected from the Lost River area of southern Oregon near the California-Oregon border (Figure 2). A third set of releases totaling another 25,000 *A. lacertosa* flea beetles and 200 *Oberea* longhorn beetles were made on July 18, 2007. The beetles were also massed collected from the Lost River area of southern Oregon.

All sites will be monitored for establishment for the next two years. Additional releases of both biological control agents are also being planned as suitable sites are found in the infested areas of Siskiyou County.

Figure 2. *Aphthona lacertosa* flea beetles being mass collected in the Lost River area of southern Oregon

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[2]USDA-PPQ, Portland, Oregon
[3]Siskiyou County Department of Agriculture, Yreka, California

Biological Control of Saltcedar in California with the Crête Biotype of the Leaf Feeding Beetle, *Diorhabda elongata*

Baldo Villegas, Ray Carruthers[1] and John Herr[1]

Saltcedar, *Tamarix* spp., is a very serious riparian weed in California as well as other western states. In the West, it infests over a million acres and it is very difficult and expensive to control with conventional methods. A biological control program against *Tamarix ramossissima* was started by the United States Department of Agriculture Agricultural Research Service (USDA-ARS) at Temple, Texas in 1987 and after extensive host specificity testing a leaf beetle, *Diorhabda elongata* Brullé (Coleoptera: Chrysomelidae), from central Asia, was determined to be host specific and safe for introduction into North America. Before the beetles were released in the Southwestern United States, the United States Fish and Wildlife Service (USFWS) raised concerns over the potential impacts on the habit areas being used by the endangered bird, the southwestern subspecies of the willow flycatcher (*Empidonax traillii extimus*). The flycatcher uses saltcedar in parts of Arizona, New Mexico and Nevada for nesting in areas where saltcedar has displaced willows and cottonwoods. Consequently, releases of the beetles were limited to areas away from the reproductive range of the flycatcher. In California, the beetles were released at sites in Inyo, Monterey, and Yolo counties on both *Tamarix ramossissima* and *T. parviflora* using several release methods but no establishment occurred. Later, a better climatically suited biotype of the beetles was introduced from the island of Crête off the coast of Greece. This biotype appears to feed readily on *T. parviflora* which is the predominant species in Northern and Central California. This biotype was also tried at the same sites in California where early releases occurred but has only become well established in the Cache Creek area near Rumsey in Yolo County.

During 2007, the USDA-ARS Invasive Weeds Research Unit located in Albany, California officially turned the redistribution phase of this biological control program to the Biological Control Program for implementation. The final evaluation included some additional host testing experiments using several native species in the genus *Frankenia* which are known to also occur in several areas of California infested with saltcedar. In August, a team of scientists from the California Department of Food and Agriculture, University of California, and native plant societies reviewed the data collected on the possible impact on the *Frankenia* species tested and concluded that the risk was minimal and recommended redistribution of the Crête leaf beetles throughout California.

Figure 1: A) *Diorhabda elongata* saltcedar beetle; B) *D. elongata* larvae devouring saltcedar foliage and flowers; C) *D. elongata* beetles mass collected with a sweep net; D) Cooperator and rancher making a release of the saltcedar beetles.

Due to the lateness of the season, releases of the Crête biotype of saltcedar leaf beetle were made only at four sites infested with *T. parviflora* in northern California on September 5-6, 2007. Approximately 12,000 beetles were released at two sites in the Stony Creek area of Glenn County and about 4,000 beetles were released at two sites in Tehama County (Figure 1). The leaf beetles were collected along Cache Creek near Rumsey, California on September 5, 2007. The collection site as well as the 2007 release sites will be monitored during 2008 to determine the best collection and release methods for establishment.

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

Releases of Biological Control Agents on a Newly Detected Infestation of Squarrose Knapweed in Northeastern California

Baldo Villegas, Judy Perkins[1], and Carri Pirosko[2]

Squarrose knapweed (*Centaurea squarrosa* Willd) is limited in distribution to Lassen, Modoc, Shasta, and Siskiyou counties in northeastern California. A biological control program against this weed was started in this area in 1998. In subsequent years, most of the biological control agents available for both diffuse and spotted knapweeds were mass collected in central and southern Oregon and released at many sites located in these four counties. From all the biological control agent species released on squarrose knapweed, only the rootboring beetle, *Sphenoptera jugoslavica* (Figure 1A), and the seedhead weevils (*Larinus minutus* [Figures 1B] and *Bangasternus fausti* [Figures 1C]) have become widely established and are successfully controlling squarrose knapweed.

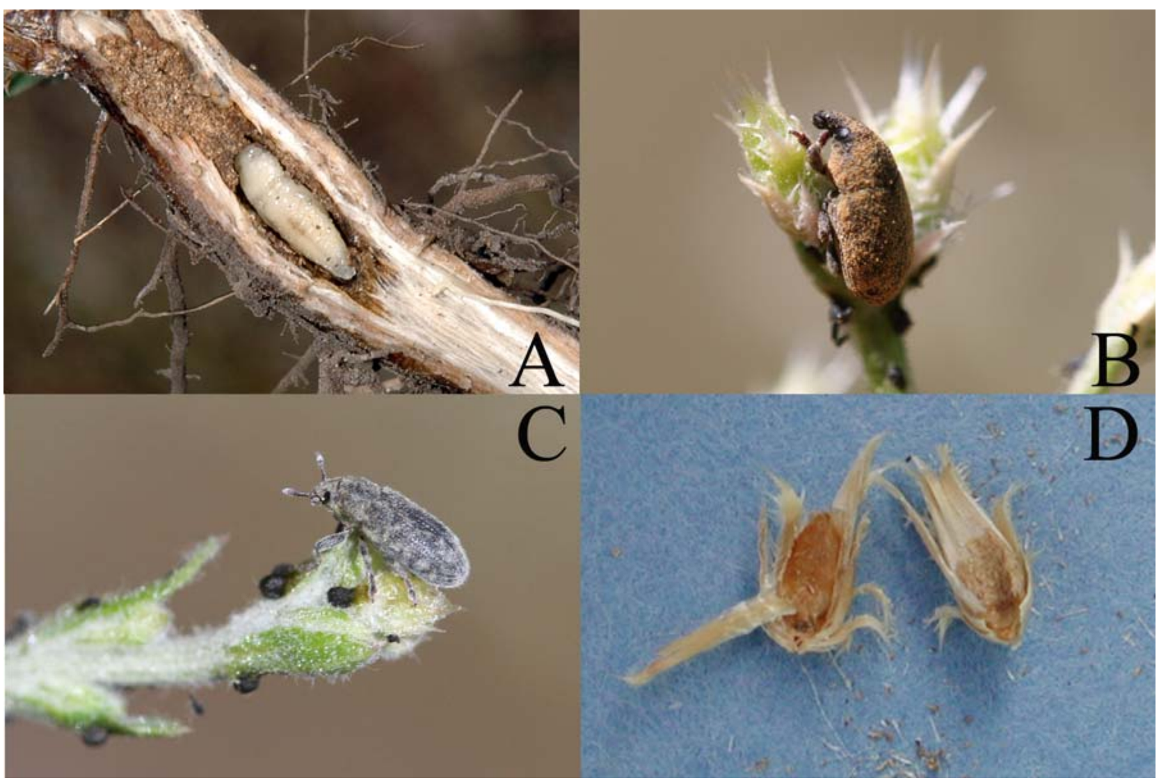

Figure 1: A) Pupa of the rootboring beetle, *Sphenoptera jugoslavica* and damage to the taproot of squarrose knapweed; B) The flower weevil, *Larinus minutus* on new buds of squarrose knapweed; C) The bud weevil, *Bangasternus fausti*, laying eggs on squarrose knapweed buds; D) Seedhead damage by *B. fausti* (left) and *L. minutus* (right);

In 2007 a new infestation of squarrose knapweed near Cassel, California was brought to the attention of the Biological Control Program by botanists with the Lassen National Forest. The area was surveyed on May 22, 2007 and it was found to be far enough from previous release sites that new releases of available biological control agents were justified. On the same day, approximately 180 *B. fausti* and 20 *L. minutus* seedhead weevils were released during the survey. A second release of the weevils occurred on July 17th with approximately 1800 *B. fausti* and 200 *L. minutus* weevils near the initial release site. Along with the seedhead weevils, about 100 *S. jugoslavica* rootboring beetles were released. All the biological control agents were collected from an established nursery site near Pittville, California.

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[2]CDFA, Integrated Pest Control Branch, Burney, California