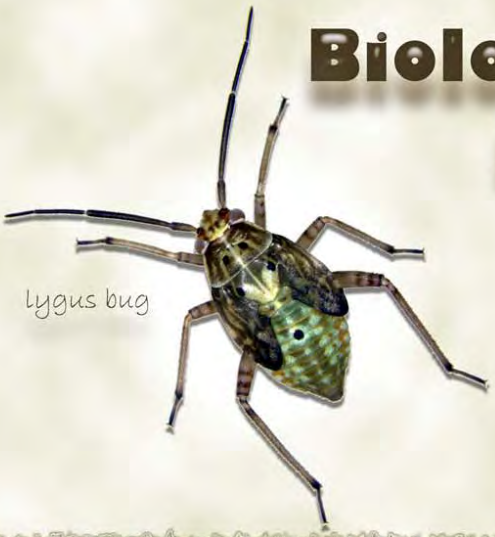
Biological Control Program



lygus bug

Peristenus

California Department of Food and Agriculture

2001

BIOLOGICAL CONTROL PROGRAM

2001 SUMMARY

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PLANT HEALTH AND PEST PREVENTION SERVICES
INTEGRATED PEST CONTROL BRANCH

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Cover developed by Deborah Mayhew. Lygus bug, its parasite, and three commercial crops, cotton, safflower and alfalfa, that are impacted by lygus.

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Preface

M. J. Pitcairn

There have been some changes in the Biological Control Program. Bob Roberson retired. He headed the Integrated Pest Control Branch, of which our Program is a component. His position was filled by the promotion of Nate Dechoretz, our former Program Supervisor. Nate's position was filled by the promotion of Larry Bezark, former Program Manager of the Biological Control Program and I was promoted to fill Larry's position. It has taken time for Nate, Larry, and I to adjust to our new duties but we hope to make the transition as easy as possible for everyone. The changes for the Biological Control Program do not stop there, however. We had the retirement of Joe Ball, an entomologist working with the Insect Group. Joe was an extremely skilled scientist with much expertise in the parasitic hymenoptera that attack aphids, whiteflies, pysllids, and scales. Many of our insect biocontrol projects are directed at these pests and his expertise will be sorely missed. We also hired two new scientists: Syed Khasimuddin, an entomologist who will participate in the research supporting the introductions of parasites against the olive fly, the red gum lerp psyllid, and lygus bug; and Pat Akers, an entomologist who will work on biological control of Russian thistle and several aquatic weeds. We are thrilled to welcome Syed and Pat to the Biological Control Program. Last, Bill Roltsch was transferred from Imperial County to Sacramento. Bill will assume the lead role on the red gum lerp psyllid project and maintain his lead role on the pink hibiscus mealybug project in Imperial County.

The Biological Control Program has had a productive year in 2001 as evidenced by this report. The Program played a major role in establishing two facilities for rearing new biocontrol agents of the glassy-winged sharpshooter, one in Bakersfield and another in Riverside. Staffed by CDFA and USDA personnel, they are now rearing the glassy-winged sharpshooter and several recently imported mymarids, in addition to making field releases of these exotic parasites. Significant progress has been made in the production of parasites for the lygus bug and red gum lerp psyllid. We anticipate that the increase in production will result in more releases of parasites against these two insect pests. Also, there was work on new cotton aphid bioagents. Among the weed projects, significant impacts were observed for the squarrose knapweed and an increase in attack was observed for spotted and diffuse knapweed. A new rust disease against yellow starthistle is pending and may be approved in 2002.

Importation and Establishment of *Lygus hesperus* Nymphal Parasites

C. H. Pickett, K. Godfrey, D. A. Mayhew, K. Casanave, D. Coutinot[1], L. Ertle[2] and K. A.Hoelmer[1]

We report on an ongoing effort to establish nymphal parasitoids that attack the western tarnished plant bug, *Lygus hesperus* (Hemiptera: Miridae). *Lygus hesperus* is a serious pest of cotton in California, strawberries and many seed crops including alfalfa. Parasitoids have been imported and released in central California since 1998 for their permanent establishment. Our first field releases were made into a ½ ac plot of alfalfa at our insect rearing facility in Sacramento August 1998. Alfalfa makes a good nursery crop for our releases since it can be grown for several years without replanting. *Lygus hesperus* nymphs exposed to *Peristenus stygicus* (ex. Southern France; Braconidae: Hymenoptera) were released into this plot. During summers 1999, 2000, and 2001 a combination of both parasitized nymphs and adult *P. stygicus* and *P. digoneutis* were released at four to seven locations in central California and details are reported in respective annual reports. In 2001, we made releases into seven locations consisting of both commercial alfalfa fields and managed plots at experiment stations. We added three new commercial release sites and dropped others with consistently low lygus populations.

CABI Bioscience and the USDA-ARS European Biological Control Laboratory collected parasitoids in Europe from 1997 to 2001. Parasitoids were collected from alfalfa fields infested with *Lygus rugulipennis*. *Peristenus stygicus* was collected from Spain, Italy, and France and a related species, *Peristenus digoneutis* was collected from Italy and Spain (Table 1). A major effort on CDFA's part resulted in an increase in lygus laboratory production over the last four years, which, in turn, supported an increased number of lygus parasitoids for field release. In addition CDFA contracted with Agriculture Canada to produce *Peristenus digoneutis*.

Approximately 1100 parasites were released in fall of 1998, then 6000, 15,000, and 14,710 during summers of 1999, 2000, and 2001, 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 (Table 2). The earlier in the summer that releases are made, the greater the number of parasitoid generations produced, and hence, higher the probability for permanent colonization.

We made our first overwintering recovery of *Peristenus stygicus* at CDFA's Sacramento release site spring 2000. *Peristenus stygicus* was also recovered in spring 2001, prior to additional releases, at sites in Sacramento and Davis. These parasitoids persisted from releases made in fall 1999 until at least May 2000 (Sacramento), and from fall 2000 until at least spring 2001 (Davis), showing they can reproduce and survive during this period of time. The proportion of lygus parasitized at the CDFA site increased from 3% (n=30) in May 2000 to a yearly maximum of 34 % (n=32) August 2001 (Table 3). We recovered parasitoids this last year, summer 2001 from 6 of 7 sites where we made repeated releases beginning in late spring/early summer. The sites where no parasites were recovered had low (~0.5 nymphs per sweep) lygus populations all summer.

[1]USDA-ARS European Biological Control Laboratory, Montpellier, France
[2]USDA-ARS Newark, Delaware

Table 1. Locations for releases of lygus parasites, 1998 to 2001

Species and population released	Location released	Lat/Long		Year released
Peristenus stygicus, France (Herault, Lattes)	Sacramento, N. B	38º35.607N	121º29.519W	1998, 1999,2000
	UC/USDA Shafter	35º31.952N	119º16.701W	2000
Peristenus stygicus, Italy (San Dona' di Piave)	UC Davis	38º32.403N	121º45.919W	1999, 2000
	KAC	35º31.353N	119º17.274W	2000
Peristenus stygicus, Spain (Catalognia, Navata)	Fong's Farm, Yolo Co.	38º41.145N	121º53.574W	1999, 2000
	Sander Farm, Kern Co.			1999, 2000
	Triple S Farms-Merced	37º08.508N	120º18.604W	2000, 2001
Peristenus stygicus, Italy (Umbria)	UC/USDA Shafter	35º31.952N	119º16.701W	2000, 2001
	Sacramento, N. B	38º35.607N	121º29.519W	2001
	KAC	35º31.353N	119º17.274W	2001
Peristenus stygicus, Spain (Granada)	Triple S Farms-Merced	37º08.508N	120º18.604W	2001
	Madera	36º59.54N	120º20.724W	2001
	Kern2, Poplar Ave	35º33.4N	119º17.64W	2001
Peristenus digoneutis, (San Dona' di Piave)	Sacramento, N. B	38º35.607N	121º29.519W	1999
Peristenus digoneutis, Italy (Umbria)	Sacramento, N. B	38º35.607N	121º29.519W	2001
	UC/USDA Shafter	35º31.952N	119º16.701W	2000, 2001
	Kern2, Poplar Ave.	35º33.4N	119º17.64W	2001
	Madera	36º59.54N	120º20.724W	2001
	Coast, Santa Cruz			2001
Peristenus digoneutis, Spain (Catalognia)	Coast, Santa Cruz			2001

Table 2. Releases of lygus parasites, summer 2001

Location	Species and population released	# adult parasites released	# nymphs exposed to parasitism released
Sacramento (CDFA)	*Peristenus stygicus*, Italy (Umbria)	1520	0
	Peristenus digoneutis, Umbria, Italy	795	0
Merced	*Peristenus stygicus*, Spain (Granada)	600	0
	Peristenus stygicus, Spain (Catalognia)	1724	550
Madera	*Peristenus stygicus*, Spain (Granada)	1050	0
	Peristenus digoneutis, Italy (Umbria)	123	0
UC Kearney, Parlier	*Peristenus stygicus*, Italy (Umbria)	2054	0
	Peristenus digoneutis, Italy (Umbria)	1736	600
UC/USDA, Shafter	*Peristenus stygicus*, Italy (Umbria)	1732	0
	Peristenus digoneutis, Italy (Umbria)	1889	0
Kern2, Poplar Ave.	*Peristenus stygicus*, Spain (Granada)	1974	0
	Peristenus stygicus, Spain (Granada, Catalognia)	655	0
	Peristenus digoneutis, Italy (Umbria)	479	0
Coast, Santa Cruz	*Peristenus stygicus*, Italy (Umbria)	156	0
	Peristenus digoneutis, Spain (Catalognia)	53	300
	Peristenus stygicus, Spain (Catalognia)	1100	0
TOTAL	*Peristenus stygicus*, all populations	11,218	
	Peristenus digoneutis, all populations	3,482	

Table 3. Recoveries of released parasitoids in 2001. Parasitism was determined through dissection of 2nd-4th instar nymph lygus. All samples came from alfalfa plots where releases had been made.

	Percent Parasitized (n) by Location						
Date	Sac. NB	UC Davis	Kearny AgCenter	Madera	Merced	Kern2	UC/USDA Shafter
April 11	-	-	-	-	0 (2)	-	-
April 16	-	-	-	-	-	-	0 (11)
June 20	13 (45)*	4 (50)*	-	-	-	-	-
July 11	-	-	-	-	0 (2)	-	-
July 16	26 (45)	0 (7)	-	-	-	-	-
Aug 3	-	--	12 (25)	-	-	-	-
Aug. 8	-	-	-	0 (12)	-	-	-
Aug. 13	34 (32)	0 (20)	-	-	-	-	-
Sept. 5	-	-	0 (16)	-	-	-	-
Sept. 14	22 (40)	0 (40)	-	-	-	-	-
Sept. 17	-	-	-	-	-	0 (5)	0 (25)
Oct. 3	-	-	-	50 (2)	14 (7)	-	-
Oct. 22	-	-	-	-	-	-	5 (17)
Oct. 24	-	-	-	-	-	0 (2)	-
Oct. 26	14 (50)	-	-	-	-	-	-
Oct. 29	-	0 (6)	-	-	-	-	-

*overwintering parasitoid

Table 4. Recoveries of released parasitoids by rearing to adults. All were *P. stygicus*.

	Percent emerged parasites (#nymphs) by location		
Date	Sac. North B	UC Davis	Kearny Ag Center
April 13	3 (110)	-	-
June 25	25 (60)	0 (50)	-
July 16	18 (100)	-	-
Aug. 3	-	-	0 (30)
Aug. 13	4 (95)	0 (30)	-
Sept. 7	-	-	0 (20)
Sept. 14	2 (50)	0 (46)	-
Oct. 26	6 (46)	-	-

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

W. J. Roltsch, J. Encalada-Fleytes and E. Andress[1]

An intensive effort was made to establish effective biological control agents on silverleaf whitefly (SLW) in the desert southwest from 1994 to 1999. Exotic species and strains of *Eretmocerus* and *Encarsia* were greenhouse-reared and released in large numbers (exceeding several million for many species) in commercial fields, refuge nursery plots and urban yards by State, Federal and University scientists. This report represents a brief review and update on the establishment of introduced SLW parasitoids in Imperial Valley. Specifically, the update represents a three year survey in commercial cotton fields, a limited set of data collected from two long-term refuge field plots and two home sites in Brawley, CA. Species identification was accomplished using recently published morphological keys and by DNA analysis (RAPD-PCR) conducted by the USDA-APHIS, Plant Protection Center, Mission, TX.

Each year, from 1994 through 1997, exotic parasitoids were released into long-term refuge field plots (1/2 to 1 acre) on multiple occasions each year. Plots were located at the Imperial Valley Research Center near Brawley, and at an organic farm at the south end of Imperial County. During the warm season, the 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 planted cantaloupe. Leaf samples were taken approximately 8 times each year from 1994 to 2001 to determine parasitoid population increase and persistence. Our report last year, reviewed the establishment process in detail. Table 1 summarizes the progressive increase in exotic *Eretmocerus* over six consecutive years averaged across all plant species by year, within the refuge field plots. This is based on field collections of *Eretmocerus*, and the determination of the percentage of exotic compared to the native *E. eremicus*. Historically, *Eretmocerus eremicus* has been a very common native SLW parasitoid within the region and is particularly common in mid to late summer, parasitizing SLW on many plant species. During 2001, SLW densities on most plant species in the field plots, with the exception of cantaloupe, were particularly low. In contrast to the previous three-year period, the majority of *Eretmocerus* were native *E. eremicus*, reflecting a one-year decline in exotic *Eretmocerus* spp. activity. Of the two home sites monitored, one had few exotic parasitoid species whereas exotics were dominant at the other.

Surveying conventionally managed cotton fields from 1998 to 2000 provided further evidence regarding the extent of exotic parasitoid establishment. Leaf samples were obtained from three edges of cotton fields each year during September and October. Exotic *Eretmocerus* were detected in 10 of the 23 fields (43%) sampled in the fall of 1998, 31 of 42 fields (74%) sampled in the fall of 1999 and 23 of 24 (96%) sampled in 2000 (Figs. 1a-c). In those fields where exotic *Eretmocerus* were detected, 4% of the *Eretmocerus* were exotics in 1998 and 21% were exotic in 1999 and 48% were exotic in 2000. Similarly, an increase in *Encarsia sophia* was noted from 1998 to 2000. *Encarsia sophia* was detected in only one of 23 cotton fields (4%) in 1998. However, *E. sophia* was detected in 27 of 42 cotton fields (64%) in 1999, and 24 of 32 (75%) fields in 2000. Over a three-year period, exotic *Eretmocerus* spp. and *Encarsia sophia* became widely established throughout Imperial Valley. The survey was discontinued for 2001. However, the second author did conduct an extensive survey of cotton fields in 2001, as part of an area-wide, multi-crop parasitoid impact assessment. Results from that study also indicate that

parasitism by exotic parasitoid spp. was lower then would be expected for 2001 (given the results presented above from the 1998 to 2000 survey).

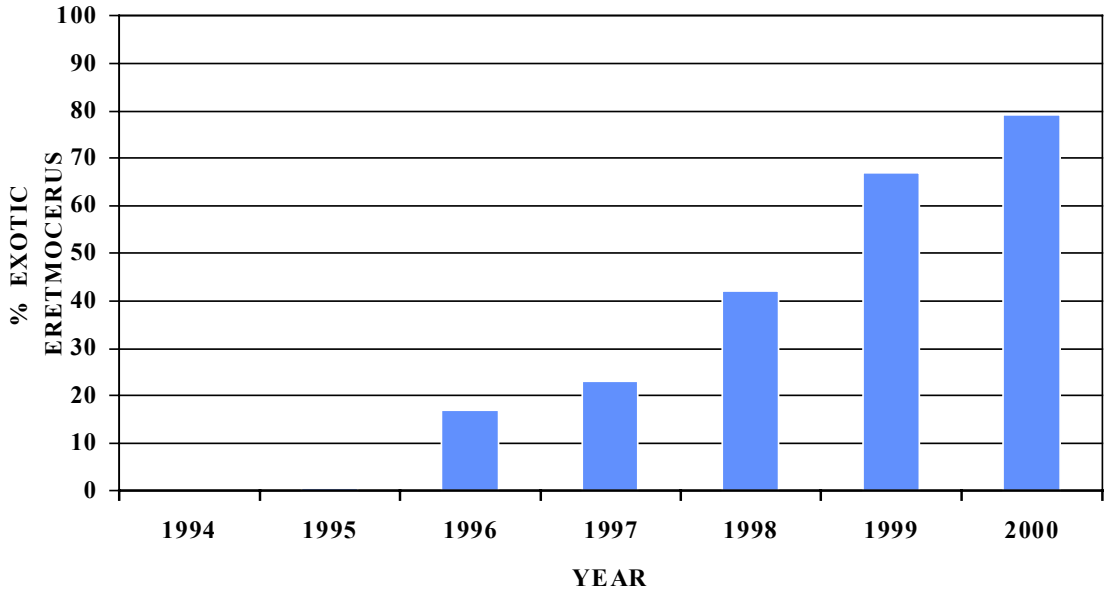
DNA analysis of specimens identified over the past several years indicates that most exotic *Eretmocerus* in this desert region are *Eretmocerus* sp. nr. *emiratus* (from: Ethiopia). and not *E. emiratus* Zolnerowich & Rose (from: United Arab Emirates). *Eretmocerus mundus* Mercet was collected infrequently in refuge field plots and commercial cotton fields. *Encarsia sophia* (=*Encarsia transvena*) from Multan, Pakistan was commonly collected in 1999 and 2000, however, its level of activity also appeared to be less in 2001.

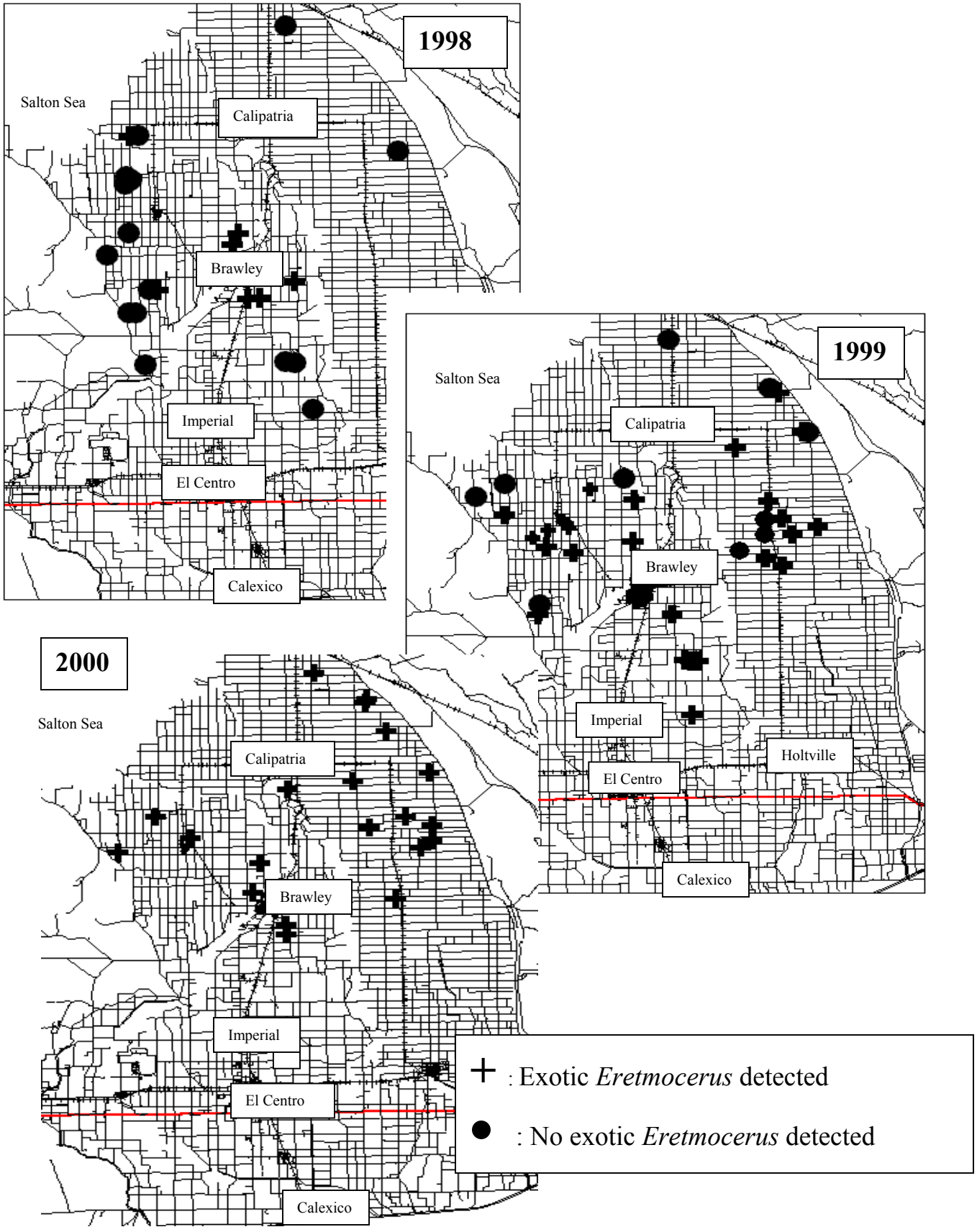
In summary, up to four exotic species/strains of silverleaf whitefly parasitoids are well established in Imperial Valley. Exotic parasitoid populations appeared lower in 2001 than in the previous three years. This may represent year-to-year variation driven by weather effects, or be viewed as a one year unusual event as part of the long-term process of establishment of introduced species.

---

[1] USDA-APHIS, PPQ, Phoenix PPC, Imperial Valley Research Center, Brawley, California

Fig. 1. ESTABLISHMENT OF SILVERLEAF WHITEFLY EXOTIC PARASITOIDS IN THE GENUS *ERETMOCERUS* IN FIELD PLOTS IN IMPERIAL VALLEY, CA
(NO RELEASES IN STUDY PLOTS FOLLOWING 1997)




1998
Salton Sea
Calipatria
Brawley
Imperial
El Centro
Calexico
1999
Salton Sea
Calipatria
Brawley
Imperial
Holtville
El Centro
Calexico
2000
Salton Sea
Calipatria
Brawley
Imperial
El Centro
Calexico
+ : Exotic *Eretmocerus* detected
● : No exotic *Eretmocerus* detected

Releases of Exotic Parasitoids of the Silverleaf Whitefly for Permanent Establishment in Central California

C. H. Pickett, J. Brown, G. Simmons[1], J. Goolsby[2] and B. Abel[3]

The silverleaf whitefly, *Bemisia argentifolii,* was an increasingly important pest of cotton in the San Joaquin Valley from 1994 through 1997 when this project was initiated. Field studies suggested that citrus had become an important overwintering site for this whitefly. Cotton has the highest incidence of whitefly infestations in areas of the Valley with a mix of citrus orchards and cotton fields. We report here on large-scale releases of *Eretmocerus emiratus* (M95104, U.A.E.), *Eretmocerus* sp. (M96076, Ethiopia), *E. mundus* (M92014, Spain), *E. hayati* (M95012, Pakistan), and trace numbers of *Encarsia sophia (= transvena;* M95107, Pakistan) into four citrus orchards. The study had two goals: (1) to determine if exotic parasites released into citrus during the fall would overwinter in this habitat and move into cotton the following spring; and (2) to permanently establish new populations of exotic parasites specific for the silverleaf whitefly.

Three study sites were identified initially, one each in Fresno, Tulare, and Kern Counties. A fourth was added because one of the original growers stopped farming cotton (Kern Co.). Sites consisted of cotton grown directly adjacent to the citrus, with both managed by the same owner. We began releasing parasites in early August or September 1997, 1998, 1999, and 2000 when migrating whitefly nymphs were first recorded from citrus leaves. Typically, over 100,000 parasites were released weekly at each location and a total of 4.05 million were released in 1997, over 10 million in 1998, 3.2 million in 1999, and 124,000 in 2000. The dispersal of the released parasites was monitored using sticky cards to trap insects. Identifications were based on the adult males since they could be readily distinguished from native *Eretmocerus* while on the sticky cards.

Parasitism of silverleaf whitefly on citrus was generally low, averaging 28% overall. However this value is quite high with respect to a survey on cotton from the same region around 1994 in which less than 1.5% of nymphs examined were found parasitized. Whitefly densities on citrus remained very low, usually less than 0.1 nymphs per cm^2 of leaf. During years in which exotics were being released, most of the parasitoids recovered from weed samples taken within 1 mile of citrus orchards were exotic, 81% to 95%. Two years after the last releases of exotic *Eretmocerus* spp., the proportion of exotic parasitoids sampled from weeds dropped to 11%. Although primarily *Eret. emiratus* (M95104 + M96076) was released, *Eret. mundus* (M92014) was the dominant species recovered in fall 2001. The density of silverleaf whitefly on weeds has varied about the same from 1998 to 2001, most samples ranging from 0.1 to less than 20 per gram dry weight.

[1]USDA-APHIS PPQ, Oswell St. Biological Control Facility, Bakersfield, California
[2]USDA-ARS, Australian Biological Control Laboratory, Indooroopilly, Queensland, Australia
[3]USDA-APHIS PPQ, Shafter, California

Susceptibility of Rice and Wild Rice to the Cereal Leaf Beetle

K. Godfrey, S. Blodgett[1] and P. Denke[1]

The cereal leaf beetle (*Oulema melanopus* (L.); Coleoptera: Chrysomelidae) has recently invaded the northwestern United States, and is expected to enter California in a few years. Throughout the United States where this insect is present, it is a pest of small grains and has some feeding activity on a variety of non-crop grasses. In the currently infested areas, rice makes up only a very small portion of the crop acreage, and wild rice is not grown. Since both of these crops are important in California, a study was conducted in Montana, a state that is infested with the cereal leaf beetle (CLB), to determine the potential threat of CLB to rice and wild rice production in California.

Susceptibility of rice and wild rice to CLB was evaluated in a greenhouse at Montana State University in Bozeman. Fourteen varieties of rice (provided by Dr. K. McKenzie, California Cooperative Rice Research Foundation, University of California, Biggs), one variety of wild rice (provided by Nor-Cal Wild Rice, Woodland), and one variety of barley (cv. 'Moravian 22'; known host of CLB) were tested. The rice and wild rice were germinated on moist filter paper prior to being planted. Half of all rice and wild rice was placed in a wading pool in which the water was kept about 3 inches above the top of the soil, and the other half, placed in pools to which water was added regularly, but the plants were not submerged. The barley was grown in pots under standard greenhouse conditions on the bench top.

Cereal leaf beetle adult and larval host acceptance was assessed on 3-week old plants. For adult CLB acceptance, two plants of each variety of rice and wild rice and eight plants of barley were placed in a cage with 50 CLB adults. Adult feeding damage and oviposition were assessed after 5 days. To determine larval acceptance, at least one CLB larva was placed on each plant using a paintbrush. The plants were examined after 24 hours and 5 days to determine if the larvae were still on the plants. Larval feeding damage and development were assessed 5 days after larval placement.

The CLB adults in this study did not feed or oviposit on any of the rice varieties (Table 1). Adult CLB fed lightly upon one wild rice plant and also deposited one egg on a single plant (Table 1). In contrast, the barley plants were almost completely defoliated by the adult CLB after 5 days and over 100 eggs were deposited (Table 1).

The CLB larvae were present on all plants after 24 hours, but disappeared after 5 days on the rice and wild rice (Table 1). There was some light feeding on several varieties of rice, but no larval feeding on wild rice (Table 1). No CLB larvae developed on any of the varieties of rice or wild rice (Table 1). On the barley, CLB larvae readily fed and developed (Table 1).

The results of this study suggest that the threat of CLB to rice and wild rice is minimal. However, because this study was conducted under greenhouse conditions, these crops should still be monitored for the presence of CLB. Under natural conditions, the rice and wild rice may be more acceptable hosts to CLB.

Table 1. Plant growth ratings, CLB adult feeding and oviposition ratings, and CLB larval feeding and development ratings on rice, wild rice, and barley in greenhouse host acceptance trials.

Plant and	Plant	CLB Adults		CLB Larvae	
Variety	Growth	Feeding	Oviposition	Feeding	Development
Rice					
S102	Poor	0/2 plants	0/2 plants	0/2 plants	0/2 plants
L204	Good	0/2 plants	0/2 plants	2/2 plants	0/2 plants
L205	Good	0/2 plants	0/2 plants	1/2 plants	0/2 plants
M104	Poor	0/2 plants	0/2 plants	0/2 plants	0/2 plants
M202	Good	0/2 plants	0/2 plants	1/2 plants	0/2 plants
M204	Poor	0/2 plants	0/2 plants	0/2 plants	0/2 plants
M205	Good	0/2 plants	0/2 plants	1/2 plants	0/2 plants
M401	Poor	0/2 plants	0/2 plants	0/2 plants	0/2 plants
M402	Poor	0/2 plants	0/2 plants	0/2 plants	0/2 plants
Akitokomachi	Poor	0/2 plants	0/2 plants	0/2 plants	0/2 plants
Calhakari	Poor	0/2 plants	0/2 plants	0/2 plants	0/2 plants
Calmati 201	Poor	0/2 plants	0/2 plants	0/2 plants	0/2 plants
Calmochi 101	Poor	0/2 plants	0/2 plants	0/2 plants	0/2 plants
Koshihikari	Good	0/2 plants	0/2 plants	1/2 plants	0/2 plants
Wild Rice					
NC-5	Good	1/2 plants	1/2 plants	0/2 plants	0/2 plants
Barley					
Moravian 22	Good	8/8 plants	8/8 plants	8/8 plants	6/8 plants

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Insectary Production of *Psyllaephagus bliteus* for the Control of the Eucalyptus Red Gum Lerp Psyllid

W. J. Roltsch, J. Brown, R. Morris, K. Casanave, J. C. Ball and S. Khasimuddin

Eucalyptus is a commonly planted tree in warm regions of the Southwest. There are more than 500 species of eucalyptus, most of which are native to Australia and Tasmania. A limited number of species are found in the Philippines, Java and New Guinea. The introduction of over 80 *Eucalyptus* species into California began before 1900. The majority of species presently grown are represented by approximately 20 species. Until the discovery of the eucalyptus longhorn beetle in California in 1984, eucalyptus was nearly pest-free within the western hemisphere.

The red gum lerp psyllid (RGLP) *Glycaspis brimblecombei* Moore, was first reported in California in June 1998 in El Monte, Los Angeles County. This sap-feeding pest, whose immature stages live under an excreted dome-shaped cap referred to as a lerp, has been found on several species of eucalyptus. Those most affected include: *Eucalyptus camaldulensis* Dehnh. (red gum), and *E. tereticornis* Smith, which are highly susceptible, and *E. rudis* Endl (flooded gum), which is considered moderately to highly susceptible. Within two years, the RGLP spread throughout the state and is currently one of the most serious pests attacking eucalyptus in California.

In 1999, Dr. Donald Dahlsten (University of California, Berkeley) collected several parasitoids attacking RGLP in Australia. These were held in quarantine until a protocol of host testing was performed to determine the safety and specificity of the parasitoids. One species, *Psyllaephagus bliteus*, was successfully reared, determined to be a host specific primary parasitoid, and released from quarantine.

The CDFA, Biological Control Program, obtained *P. bliteus* in 2000 and began to set up an insectary. It soon became apparent that both the RGLP and the parasitoid could not be easily reared. Initially, the basic rearing scheme for the parasitoid included 1-3 plants infested with a broad range of RGLP life stages placed into a 3 cu. ft. cage. Parasitoids were released into cages at a 40-1 to > 100-1 host to parasitoid ratio for each host life stage, including 2nd to 5th instars. The resulting first generations were consistently very small with well over 50% males (Fig. 1). When cages that contained several plants were kept active for approximately 6-8 weeks, it was found that parasitoid production improved, as did the parasitoid sex ratio. As a result of these findings, the following rearing procedure was implemented for the remainder of the year. Four female and four male *P. bliteus* were released into each newly setup cage containing 2-3 plants. In total we had 20 cages setup by September of 2001. Each cage was used for many months, requiring the removal of excess psyllid adults, or their addition if sufficient numbers were not present in a cage. Parasitoids were collected two or three times per week. On each collection date, all parasitoids were removed with the exception of four female and four males. Over time, plants in poor condition were replaced with new psyllid-infested plants.

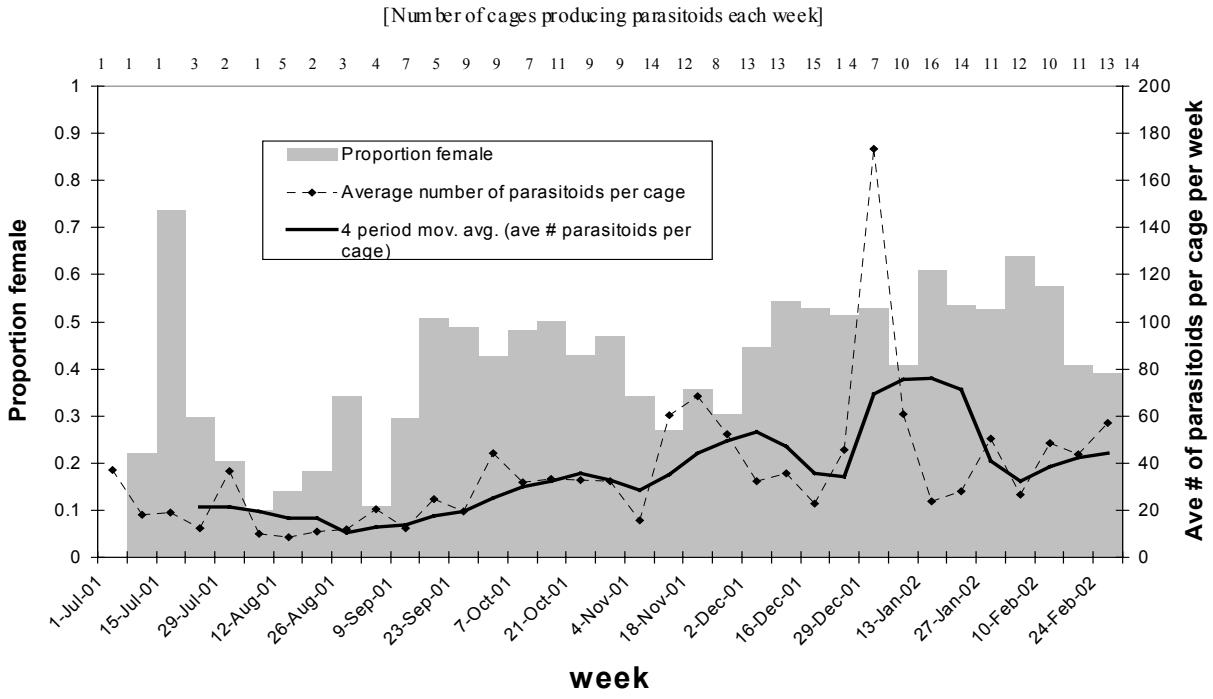
Improvements to the greenhouse facility were completed by September 2001, including improved temperature and lighting control. Consequently, a narrow temperature range (23 – 29^{o} C), and a constant long day length of 16:8 L/D were achieved using high-pressure sodium lights. The results of the modifications are apparent in Figure 1. After approximately 6-8 weeks, following several generations, 30 to 60 parasitoids emerged on average from each cage per week.

Typically, 9 to 15 cages produced parasitoids on a given week. Overall, the moving average presentation of per cage production illustrates a marked improvement in production by late-September. This coincided with the completion of facility improvements. In addition, detailed records showed that the parasitoid sex ratio was relatively stable at 1:1 from mid-September onward (Fig. 1). The moving average was calculated by averaging the current value with values of the three previous weeks. Total parasitoid production in the insectary increased greatly in the fall as a result of greater production per cage and an increased number of total cages. In mid-December the removal of females was relaxed on several occasions. Following this period, there was a marked increase in production in late December. During this week, a total of nearly 1800 *P. bliteus* were collected. This would seem to illustrate the potential of this rearing system when a greater number of females are left in the cages during each collection date with suitable numbers of immature psyllids.

For 2002, adult psyllids will be removed from cages and destroyed. Psyllids from the psyllid insectary will be added as necessary. This will be done to prevent the selection of a parasitoid resistant psyllid population within the parasitoid production cages.

In 2001, over 10,265 *P. bliteus* were produced and released into field nursery sites. Because the increase in production occurred in the fall, the majority of this material was released in the southern counties of California during mid-fall through winter. During late spring and summer of 2002, releases will be completed in each of the affected counties where releases have not yet taken place. Dahlsten (personal communication) has reported that establishment has occurred in eight coastal counties from the San Francisco Bay Area to San Diego County.

Fig. 1. Sex ratio and average weekly production of P. bliteus per cage



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

C. H. Pickett and P. Brennan[1] and R. Messing[2]

The olive fruit fly, *Bactrocerus oleae* (Gmelin), is a major pest of olives in Mediterranean countries, and was found for the first time in California (and the US) in October 1998. Olive fruit fly spread from central to northern California this last year and is now found in most olive growing regions of the state. An olive fly parasitoid, *Psyttalia concolor* (Szepligeti) (Hymenoptera: Braconidae) was collected off of olives infested by olive fruit fly in Tunisia and later cultured at the University of Hawaii. It was released at sites in Los Angeles and Santa Barbara in September 1999, and in Riverside County in fall 2000 (see previous annual report). *Psyttalia concolor* was not recovered in post-release samples during 2000 and 2001. We report on releases of the same parasitoid at the same location but earlier in the year than in 2000. Releases were made at the Jurupa Cultural Center, Riverside County.

Psyttalia concolor was cultured at the University of Hawaii, as in previous years, and shipped overnight to California for field release. A total of 1,744 adults were released between October 3, 2001 to December 11, 2001. Most of the parasitoids, (75 to 300 per release) were released uncaged into olive trees. On November 27, 2001 and December 11, 2001 three cages were placed on olive branches bearing fruit that was heavily infested with flies. Twenty-five adults were added to each of three cages. On January 10, 2002, sleeves were removed, and a sample of ca. 62 olives from the branches was placed into a paper can. Three weeks later, 11 dead and 7 live adult *P. concolor* were found in the paper can. Forty-two adult olive fruit fly had emerged during the same period of time. These results show that *P. concolor* is capable of attacking olive fruit fly in the field under California environmental conditions.

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Acknowledgements; We thank David Morgan for help with releasing parasitoids.

Foreign Exploration for Natural Enemies of the Olive Fruit Fly

K. A. Hoelmer[1], D. Coutinot[1] and C. H. Pickett

Explorations for olive fruit fly and its natural enemies were made by European Biological Control Laboratory (EBCL) staff in southern Spain (September 2000), Greece (Thessaloniki, Crete, Rhodes; June & December 2000), northern Tunisia (November 2000 & 2001) and South Africa (March 2001) in regions where wild and/or unsprayed olives occur. Collections of fly-infested olives were made from wild and cultivated olives, and parasitoids have been reared from them in quarantine. Collections from Greece included the chalcidoids *Eupelmus urozonus* and *Pnigalio mediterraneus* and from the islands of Rhodes and Crete, the braconid *Psyttalia concolor*. Collections of olive flies infesting wild olives in Tunisia yielded a population of *P. concolor* from a site in the Parc National d'Ichkeul. The South African collections yielded a variety of braconid and chalicidoid parasitoids which are currently being examined for identification. EBCL is presently establishing a colony of olive flies to enable rearing and laboratory studies of natural enemies. Further exploration is planned for northern, eastern and southern Africa, and (eventually) southwestern Asia. Cooperative arrangements have been developed with Bob Wharton, Texas A&M for fruit fly parasitoid biology and taxonomy and with Russ Messing, Univ. of Hawaii for fly rearing & parasitoid evaluation. These activities have been funded by several CDFA grants for foreign exploration and biological studies of olive fruit fly natural enemies.

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Biological Control of the Glassy-winged Sharpshooter

C. H. Pickett, D. J. W. Morgan[1], G. S. Simmons[2] and L. G. Bezark

Biological control is one of several strategies being investigated to reduce regional populations of the glassy-winged sharpshooter, *Homalodisca coagulata* (Say) (Homoptera: Cicadellidae). This pest invaded California around 1990 and is now widely established in the southern California counties of Los Angeles, Orange, Riverside, San Bernardino, San Diego, and Ventura. Kern and Santa Barbara Counties are partially infested, and small isolated infestations are present in Butte, Fresno, Imperial, Santa Clara, and Tulare Counties. The sharpshooter transmits the bacterium *Xylella fastidiosa*, which causes Pierce's disease of grapes and other serious plant diseases. During 2001, several milestones were achieved in the biological control of glassy-winged sharpshooter. New agents were discovered, two new rearing facilities became operational, rearing methods were advanced, and new staff hired.

The primary goal of this project is the importation of new biological control agents specific to the glassy-winged sharpshooter. Augmentative biological control using imported or native parasitoids and native predators will also be investigated (see G. Simmons, this volume). At least three native egg parasitoids have been found attacking glassy-winged sharpshooter in California, however additional species are needed. Although parasitism of glassy-winged sharpshooter eggs typically exceeds 80% in late summer, this pest still reaches very high levels during this time of year. Unfortunately, even small populations of the vector can spread Pierce's disease. The glassy-winged sharpshooter feeds on a broad range of plant species growing over a wide geographical range. A high diversity of natural enemies increases the likelihood of this pest being attacked and populations reduced year-round throughout the entire state. Collecting trips in 2001 were made to areas within the native range of glassy-winged sharpshooter: Texas, Florida, and northeastern Mexico. Collections were also made in Argentina and Chile where closely related species reside. In previous years foreign exploration also included Louisiana. Parasitoids were cleared through the quarantine facility at UC Riverside.

Releases of exotic parasitoids have focused primarily on one species, *Gonatocerus triguttatus* (Hymenoptera: Mymaridae), widespread in south Texas and northern Mexico, where glassy-winged sharpshooter populations are very low. Several biotypes and possibly new species have been discovered. A Mexican strain of *G. morrilli* was released but in low numbers. At least 9 new populations or species of *Gonatocerus* were discovered attacking species in the genus *Tapajosa*. Like *Homalodisca* spp., these species are all in the tribe Proconini. These parasitoids will be considered for further testing and possible field release following host testing in quarantine.

We now have two centers for insect rearing: the Oswell Street Biological Control facility located in Bakersfield and the Mount Rubidoux station in Riverside. Having two rearing centers provides flexibility and backup for the rearing of multiple species. The Oswell Street facility is a former seed company experiment station and was occupied April 2001. With much renovating by the USDA-APHIS, and the addition of two sea-vans (walk-in rearing), this site is near fully operational. Last summer it produced the majority of the 139,000 parasitoids released in California. The Mount Rubidoux station is a former USDA-ARS experiment station. Two temperature-and-light-controlled rearing rooms have been established, plus outdoor cages. Six

greenhouses are scheduled for renovation. Two portable greenhouses were constructed at Biotactics, a nearby private insectary from which we are leasing land.

One of the major challenges we faced last year was rearing parasitoids and their hosts. All host eggs used in parasitoid production came from field-collected adult glassy-winged sharpshooter. These insects have two discrete generations a year and overwinter almost entirely as adults. We lost the small culture of *Gonatocerus* spp. last year due to lack of host eggs in December. This hurdle has now been overcome. Two of us (GSS and DJWM) developed a multi-element approach for providing a continuous population of all host life stages. Several hundred parasitoids and glassy-winged sharpshooter eggs were maintained through this last winter. Using insulated sea-vans, with customized heating, cooling, and lighting we were able to maintain summer conditions from late summer 2001 until spring 2002. A high intensity lighting system was developed using high-pressure sodium plus metal halide lamps. These provided the complete spectrum of light required by plants. A new method for rearing nymphs was developed by Dr. Isabelle Lauziere (USDA-APHIS PPQ, Mission, Texas) and integrated into our rearing system. Also, as a stopgap, an egg storage method was tested and proven effective for winter storage of both glassy-winged sharpshooter eggs and developing *Gonatocerus*. Additional eggs for production have been provided through contract with private industry. Foothill Agricultural Research produced eggs during 2001. Buena Biosystems was awarded the egg production contract for 2002. These helped start the culture last spring and will be used to supplement summer production this year.

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[2]USDA-APHIS PPQ, Oswell St. Biological Control Facility, Bakersfield, California
Acknowledgements: Serguei Triapitsyn and Mark Hoddle (UC Riverside) participated in foreign exploration. Svetlana Myartseva (University of Tamaulipas) and Alejandro Rio (USDA-APHIS) of Ciudad Victoria, Tamaulipas provided invaluable assistance while collecting in Mexico. The following technicians and seasonals have provided the help critical for keeping rearing operations running: Patrina Brennan, Olga Fimbres, Andy Padilla, Lila Higgins, Joanne Vasquez, Humesh Kumar, Stephany Rill, Jason Welch, Joe Garcias, Jaime Cuevas, David Orso, Aime Long, and Chris Colazzo.

Cold Storage of Glassy-winged Sharpshooter Parasitoids

D. J. W. Morgan and P. M. Brennan

In fall 2000 our glassy-winged sharpshooter culture at the Mount Rubidoux Field Station went extinct. It had been maintained by field collected adults. However, over time, oviposition rates declined to negligible levels. To prevent loss of natural enemy colonies in 2001, we began to stockpile glassy-winged sharpshooter egg masses in cold storage for use at a later date and evaluate techniques to maintain viability. A number of parameters were evaluated, including temperature, whole plants or excised leaves, lighting regimes, and host plant species.

Temperature

Constant temperature incubators with light and humidity controls were used to evaluate temperatures sufficiently low to effectively halt egg development but high enough for eggs to survive and to maintain vigor of the plant in which the eggs were laid. Excised *Euonymus japonica* leaves containing glassy-winged sharpshooter eggs were stored in zip-loc bags to retain humidity and placed in incubators. One bag containing a minimum of 30 eggs in 4 masses from each of three incubators (3°C, 6°C, and 9°C) was removed at each time interval and placed in an incubator at 27°C. Emergence rates were calculated by counting the number of glassy-winged sharpshooter instars emerging within one week. Observations were made throughout the experiment on leaf and egg condition and on eclosion prior to removal from cold storage.

Leaves stored at or below 3°C lost turgidity after two days. Once removed from cold storage, these leaves either senesced or desiccated prior to wasp eclosion. Eggs in leaves started to eclosion within 24 days while being stored at 9°C. At 6°C, leaves and eggs remained in suspended animation for the full 48 days of the trial.

Host plant

Once an acceptable storage temperature was found (6°C), three species of preferred ovipositional host plants were tested to their resilience to cold storage. Whole plants containing glassy-winged sharpshooter egg masses were placed into a walk-in seed bank set at 6°C. At weekly intervals, one plant was removed and used in the wasp production process to obtain wasps (sting chamber for 5 days, emergence chamber for 10 days at 30°C, 70% RH, 16:8 L:D). Vigor of the plants and parasitism rate were monitored throughout the process.

Citrus and hibiscus fared poorly at low temperatures. Both species wilted rapidly (within five days) and the citrus started to shed leaves after two weeks. Once taken out of cold storage, the citrus recovered fairly well, but the hibiscus took longer to recover and lost many of its fully expanded leaves where the glassy-winged sharpshooter eggs were present. Once the leaves had fallen, eggs rapidly desiccated resulting in 100% mortality of eggs and parasitoids.

In contrast, *Euonymus japonica* survived cold storage excellently over the one-month test. When removed from the cold storage, plants were turgid and all eggs either emerged as glassy-winged sharpshooter nymphs or were successfully parasitized.

Since this study, we have successfully stored 60 *E. japonica* at 6°C for 3 months with no ill effect. After 4 months we started to see chlorotic coloration, probably due to the poor lighting rather than the temperature, and also fungal infection of eggs leading to egg mortality.

Whole plants *versus* excised leaves

We looked into the possibility of storing parts of plants rather than whole plants. This would be considerably more hygienic and would negate the need to water plants. Rather than using whole plants, we excised individual *E. japonica* leaves with egg masses and placed them in sealed Ziploc bags with a piece of moist tissue paper. Leaves survived well for up to one month at 6°C. Beyond one month, we started to observe fungal infection in the eggs and leaf senescence, both factors leading to egg mortality.

Lighting and other variables

For cold storage of whole plants, watering was necessary every week to prevent desiccation due to the conditioning system. While no rigorous studies were carried out, we found that the inclusion of a light into the cold room decreased chlorosis of the leaves but did result in plant growth, which had been negligible beforehand.

Beauveria bassiana Infection of Argentine Ants in Vineyards

K. Godfrey, D. A. Mayhew, K. Daane[1], J. Stimac[2] and L. Wood[2]

Several species of mealybugs can become pests in California vineyards. The pest problems with these species are exacerbated by the presence of ants that tend the mealybugs to obtain honeydew. Several species of ants, including the Argentine ant [*Linepithema humile* (Mayr) (Hymenoptera: Formicidae)] protect the mealybugs that they are tending from parasites and predators. Controlling ants in vineyards can be done with either tillage or insecticides applied in the spring. These treatments are not always effective in controlling ants. More alternatives for ant control are needed.

A strain of the fungus *Beauveria bassiana* (Balsamo) Vuillemin was collected from the red imported fire ant [*Solenopsis invicta* Buren (Hymenoptera: Formicidae)] near Cuiabá, Mato Grosso, Brazil, in the late 1980's (Stimac et al. 1987). Laboratory and field studies conducted in Brazil and Florida demonstrated the efficacy of this strain of *B. bassiana* (Bb447) against the red imported fire ant (Stimac et al. 1989, Oi et al. 1994, Stimac and Alves 1994). The ability of Bb447 to infect Argentine ant has been demonstrated under laboratory conditions (J. Stimac, unpublished data). However, its ability in the field to infect Argentine ant has not been investigated.

A study was conducted in a vineyard in Sonoma County, California to determine if Bb447 could infect Argentine ant under field conditions. The Argentine ants interfered with the natural enemies attempting to attack the grape mealybug, resulting in a severe grape mealybug problem within the vineyard. On 3 July, 15 vines were selected and assigned to one of the following five treatments: bait powder, gumdrop trap, cat food trap, tube trap, and control (no Bb447). In the bait powder treatment, 50 gm of a bait powder that contained 20% Bb447 by weight was placed in four piles near the base of vine. For the gumdrop trap, a gumdrop candy was glued to the bottom of a petri dish and 50 gm of the bait powder was placed around the gumdrop. The petri dish was then secured near the base of a vine. For the cat food trap, about 8 pieces of dry cat food were glued to the bottom of a petri dish, and then 50 gm of bait powder placed around the cat food. The trap was then secured near the base of a vine. In the tube trap, a mixture of table sugar and Bb447 spores (10% spores by weight) was placed into micro-centrifuge tubes. Four tubes were placed near the base of a vine. The gumdrop, cat food, and table sugar were used as attractants in an attempt to get the Argentine ant to come in contact with the Bb447 spores. At 3, 7, 14, and 24 days after application of the Bb447, each vine was visited and a sample of ants was taken from each vine. These ants were assayed for the presence of the fungus.

The results of this study demonstrate that Bb447 will infect Argentine ant under California conditions (Figure 1). The amount of infection was low and none of the traps appeared to work better than the original bait powder. The apparent decrease in infection through time was probably the result of recruitment from other Argentine ant nests to the monitored nests that had members succumb to the fungus (Figure 1). Unlike many other ant species, Argentine ants will cooperate with Argentine ants from other nests. Additional studies are needed to determine the efficacy of Bb447 against Argentine ant.

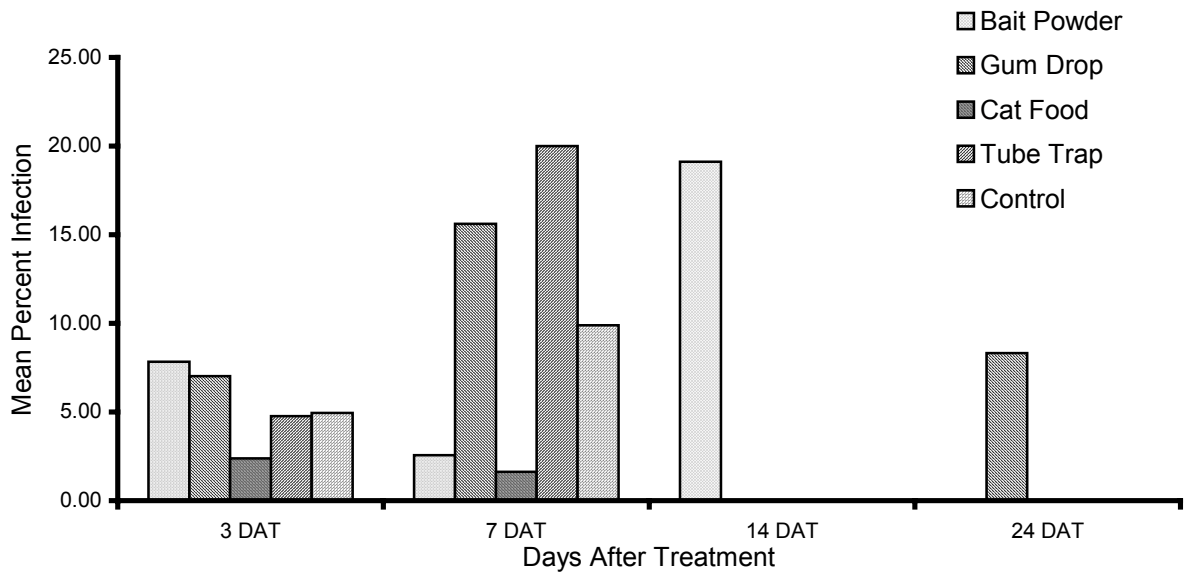

Figure 1. The mean percent infection of Argentine ants by Bb447 in a trial conducted in Sonoma County in 2001.

Oi, D., R. Pereiva, J. Stimac, and L. Wood. 1994. Field applications of *Beauveria bassiana* for control of the red imported fire ant (Hymenoptera: Formicidae). J. Econ. Entomol. 87: 623-630.

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Beauveria bassiana, Treatment of Red Imported Fire Ants in Almonds

K. Godfrey, D. A. Mayhew, J. Stimac[1] and L. Wood[1]

The red imported fire ant [*Solenopsis invicta* Buren (Hymenoptera: Formicidae)] has been found sporadically in almond orchards in northern California. When found, these populations are subjected to multiple insecticide applications in an attempt to eradicate the ants. The insecticides currently registered for use against the red imported fire ant (RIFA) are restricted as to the habitats in which they can be applied. Effective alternatives to these insecticides are needed for those habitats in which the current insecticides cannot be used.

A strain of the fungus *Beauveria bassiana* (Balsamo) Vuillemin was collected from RIFA near Cuiabá, Mato Grosso, Brazil, in the late 1980's (Stimac et al. 1987). Laboratory and field studies conducted in Brazil and Florida demonstrated the efficacy of this strain of *B. bassiana* (Bb447) against RIFA (Stimac et al. 1989, Oi et al. 1994, Stimac and Alves 1994). This strain of fungus has the potential to provide an effective alternative to the current insecticides used in California against RIFA.

A study was conducted in an infested almond orchard in Fresno County to determine if Bb447 would infect RIFA under California conditions. On June 21, active RIFA nests were treated with 100 gm of a bait powder that contained 20% Bb447 spores by weight. The bait powder also contained ground peanuts that served as an attractant to the ants. The nests were sampled 4, 7, 14, and 27 days after the initial application to determine the amount of infection by Bb447. The ants were assayed for fungus in California and then sent to Florida for confirmation of Bb447.

The treatment of the RIFA nests with Bb447 resulted in ants from 19 of the 21 nests demonstrating some level of infection by the fungus. For two of the nests, no samples could be taken because the nests died between the day of treatment and 4 days after treatment, possibly as a result of two treatments with insecticides in the previous year. In general, the treated nests showed a moderate level of infection 4 days after treatment (range 0 –86%) with a subsequent decline in ant activity and infection over the next three sampling dates. During the activity sampling, a lack of aggression by the ants on 7, 14, and 27 days after treatment was noted in nests that had modest levels of infection at 4 days after treatment. J. Stimac has documented this change in ant behavior associated with infection by Bb447 in the laboratory and field. The nests with ants that could be sampled during the course of the trial fell into one of five categories: no impact (two nests); weak activity and low to moderate infection throughout the study (seven nests); moderate activity declining to low activity and low to moderate infection (eight nests); moderate activity and infection (one nest); and moderate activity to low activity resurging to moderate activity and low to moderate infection (one nest).

In summary, Bb447 did infect RIFA in the almond orchard in Fresno County. The levels of infection were low to moderate, and a related decline in the activity of the ants was noted. Determination of the efficacy of this fungus for use in an eradication program will require further study.

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Oi, D., R. Pereiva, J. Stimac, and L. Wood. 1994. Field applications of *Beauveria bassiana* for control of the red imported fire ant (Hymenoptera: Formicidae). J. Econ. Entomol. 87: 623-630.

Stimac, J. and S. Alves. 1994. Ecology and biological control of fire ants. pp. 353-380. *In* D. Rosen, F. Bennett, and J. Capinera, eds. Pest management in the subtropics: Biological control – a Florida perspective. Intercept, Andover, U.K.

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Pink Hibiscus Mealybug Parasitoid Rearing and Release In Imperial Valley

W. J. Roltsch, E. Andress[1], D. Meyerdirk[2] and J. Brown

For a second year, two parasitoid species of the pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) (PHM) were mass reared for field release. During 2001, *Anagyrus kamali* Moursi and *Gyranusoidea indica* Shafee, Alam & Agarwal were again produced at the El Centro insectary in Imperial Valley, CA. Rearing details were described in last years report. In 2001, over 350,000 parasitoids were reared (Table 1) and over 78,000 *Anagyrus kamali* and 126,000 *Gyranusoidea indica* were released (Tables 2). The difference between production and release reflects those used to initiate new production cages. For 2001, much of this material was delivered to Mexican cooperators for release in Mexicali Valley, which borders Imperial Valley.

In December 2001, an insectary culture consisting of a new population of *A. kamali* from Egypt was initiated using material received from USDA-APHIS in Mission, TX. This population was obtained (collector: D. Gonzalez, U.C. Riverside) from a climate very similar to that found in Imperial Valley. Therefore, because of its close climatic match, it may provide even better control of the PHM than the former population of *A. kamali* that originated from Hawaii and China. An additional parasitoid species (*Allotropa* sp. nr. *mecrida*, Hymenoptera: Platygastridae) is presently in quarantine (USDA-APHIS, Plant Protection Center, Mission, TX) and is being tested to determine host range breadth (USDA-ARS, Newark Delaware). A narrow host range is desired. To date, tests have shown that the solenopsis mealybug (*Phenacoccus solenopsis*) is not a suitable host. This mealybug species is a prominent species native to southern California. Following the completion of additional tests, we are optimistic that *Allotropa* sp. nr. *mecrida* will become available for rearing and release during mid-summer of 2002. Additional candidates for host range evaluation include the longtailed mealybug (*Pseudococcus longispinus*), obscure mealybug (*Pseudococcus viburni*), citrus mealybug (*Planococcus citri*) and striped mealybug (Ferrisia virgata). Arrangements have been made to obtain populations of these mealybugs from cultures at the University of California, Berkeley and the University of Florida.

Table 1. El Centro insectary production of pink hibiscus mealybug parasitoids in 2001.

Month	*Anagyrus kamali*	*Gyranusoidea indica*	Total Production
Jan	15,500	14,700	30,200
Feb	17,900	25,200	43,100
Mar	17,050	39,500	56,550
Apr	9,900	15,100	25,000
May	235	1,450	1,685
June	3,200	6,952	10,152
July	13,500	22,450	35,950
Aug	10,000	16,050	26,050
Sept	14,400	13,575	27,975
Oct	24,050	30,350	54,400
Nov	15,000	19,950	34,950
Dec 1-8	1,800	4,250	6,050
Total	142,535	209,527	352,062

Table 2. EL Centro pink hibiscus mealybug insectary delivery records for 2001 (through October 2001).

Date	Sent to	*Anagyrus kamali*	*Gyranusoidea indica*	Total
Jan 01	Imperial Valley	4,500	1,500	6,000
Feb 01	Imperial Valley	7,000	9,200	16,200
6 Feb 01	St. Martin, French West Indies	5,000	3,000	8,000
16 Feb 01	"	800	6,000	6,800
27 Feb 01	St. Barthelemy, French West Indies	1,800	1,000	2,800
Mar 01	Imperial Valley	8,000	27,500	35,500
Apr 01	Imperial Valley	2,600	1,600	4,200
9 April 01	Bahamas	3,000	3,000	6,000
19 April 01	Mexicali	0	2,100	2,100
23 April 01	Mexicali	2,000	2,800	4,800
3 July 01	Mexicali	400	7,750	8,150
12 July 01	Mexicali	1,550	6,000	7,550
1 Aug 01	Mexicali	8,050	9,600	17,650
8 Aug 01	Mexicali	1,700	5,100	6,800
15 Aug 01	Mexicali	1,300	3,600	4,900
24 Aug 01	Mexicali	1,500	3,600	5,100
30 Aug 01	Mexicali	1,450	1,600	3,050
6 Sept 01	Mexicali	2,600	1,600	4,200
13 Sept 01	Mexicali	2,050	2,000	4,050
20 Sept 01	Mexicali	2,350	2,750	5,100
27 Sept 01	Mexicali	2,650	3,025	5,675
3 Oct 01	Mexicali	3,900	8,050	11,950
11 Oct 01	Mexicali	5,000	5,500	10,500
17 Oct 01	Mexicali	4,000	5,000	9,000
25 Oct 01	Mexicali	4,900	3,500	8,400
Total		78,100	126,375	204,475

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Pink Hibiscus Mealybug Biological Control in Imperial Valley

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

The pink hibiscus mealybug (PHM), *Maconellicoccus hirsutus* (Green), was first detected in Imperial Valley during August of 1999. Two parasitoid species in the family Encyrtidae, (*Anagyrus kamali* and *Gyranusoidea indica*) were released against this new pest in September of 1999. Parasitoids were received from USDA, APHIS, PPQ and the Puerto Rico and U.S. Virgin Islands Departments of Agriculture insectary operations. A total of 3,400 *A. kamali* and 900 *G. indica* were initially released, followed by extensive releases from insectary production supported by CDFA, USDA and Imperial County in El Centro, CA. Pink hibiscus mealybug densities and parasitism in mulberry trees at three residential locations have been monitored since 1999. Three more sites with mulberry trees were added to the list of monitored sites in January of 2000, along with three carob tree (non-deciduous) sites in June of 2000.

The PHM was nearly undetectable at most sites until mid-June in Imperial Valley (Fig. 1). Densities were somewhat lower overall in 2001 than in 2000. However, what has been most noticeable is that numbers have been dramatically lower for two consecutive years (2000-01) compared to those in the fall of 1999, when mean numbers per terminal were 247-second instar to adult mealybugs. On average, PHM densities have declined 96% on mulberry and 93% on carob since initial counts were taken. Consistent with increasing summer population densities of PHM, parasitism increased during both years, demonstrating a strong density dependent response (Fig.1). Parasitism was difficult to assess prior to June in 2001, because PHM densities were very low. Percent parasitism was variable across sites, increasing consistently from June to October (Table 1). The very high levels of parasitism in the October samples may be exaggerated, because they coincide with fall conditions that cause a slowing of PHM reproduction and development, and the characteristic movement of PHM from branch terminals to the bark of large branches and trunks of deciduous trees. Although little is known about how these events influence mealybug and parasitoid activity, they could cause an “accumulation” of parasitized individuals by reducing the mobility of parasitized mealybugs. Percent parasitism is based on mealybugs collected as third instars to adults, excluding mummies. Nearly all parasitism (>95%) was caused by *Anagyrus kamali*. However, elevated levels of *Gyranusoidea indica* have been recorded in the late fall (Nov.- Dec.) of each year, based on corrugated cardboard band sampling of PHM on the bark of large tree limbs.

Hyperparasitism (principally of *Anagyrus kamali* by *Marietta* sp). reached 34% by October of 2001. This result was nearly identical to that found in October of 2000 (est. 38%). Hyperparasitism was calculated as the number of hyperparasitoids emerging from a sample divided by the number of hyperparasitoids plus the number of primary parasitoids that emerged. These levels from late season collections may be biased in an upward direction because nothing emerged from many of the *A. kamali* mummies in many of the samples. It is unknown whether these parasitoid species (primary and secondary parasitoids) exhibit some degree of winter diapause.

Fig. 1. Pink Hibiscus Mealybug on Branch terminals of Mulberry Trees

Imperial Valley, California

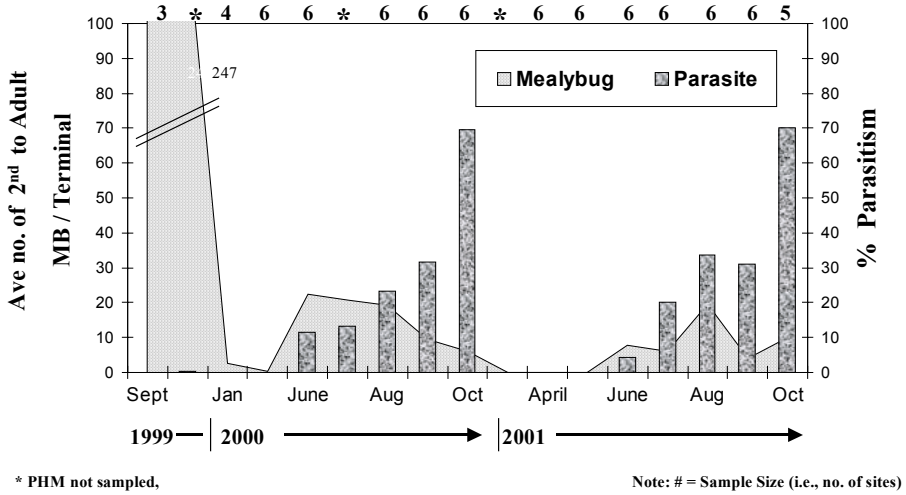


Table 1. Percent parasitism at nine release sites in Imperial Valley. Number in parentheses is the number of specimens.

Site & Host sp	Jan. 2001	April 2001	May 2001	June 2001	July 2001	Aug. 2001	Sept. 2001	Oct. 2001
A- Mulberry	-	-	-	-	20 (70)	75 (4)	33 (6)	58 (36)
B- Mulberry	-	-	-	0 (12)	-	61 (80)	0 (14)	
B- Hibiscus	-	-	-	-	0 (3)	-	-	-
C- Mulberry	-	-	-	-	7 (100)	12 (8)	42 (62)	41 (29)
C- Hibiscus	-	0 (7)	33 (3)	25 (4)	7 (14)	33 (60)	52 (124)	-
D- Mulberry	-	-	-	-	0 (4)	12 (34)	28 (36)	100 (3)
E- Mulberry	-		-	10 (19)	49 (100)	60 (73)	64 (51)	86 (29)
F- Mulberry	-	-	-	4 (69)	6 (50)	15 (13)	17 (18)	65 (20)
G- Carob	-	-	-	48 (100)	64 (72)	26 (3)	80 (5)	-
H- Carob	-	-	-	-	0 (40)	89 (3)	40 (5)	66 (6)
I- Carob	-	-	-	8 (36)	49 (100)	-	-	-
Mean				15.8%	20.2%	42.5%	39.5%	69%

[1]USDA-APHIS, National Biological Control Institute, Riverdale, Maryland

Dynamics of a Citrus Peelminer Population in an Orange Grove in Tulare County

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

The citrus peelminer (*Marmara gulosa* Guillén and Davis; Lepidoptera: Gracillariidae) began causing problems for the citrus industry in the San Joaquin Valley in 2000. Typically, this moth that is native to the southwestern United States and northern Mexico was considered only a pest of citrus and was found on a limited number of citrus varieties. However, the citrus peelminer in the current infestation is acting very differently. This insect is polyphagous, and will feed on more than 43 species of plants, including citrus, cotton, grapes, pumpkins, peppers, plums, walnuts, oleander, and willow (D. Haines, Tulare County Agricultural Commissioner's Office, unpublished data). It feeds by mining just below the surface of succulent stems and on fruit. It is the mining on the fruit of fresh market crops that causes economic damage. In past outbreaks of the peelminer in the San Joaquin Valley, grapefruit were the primary host damaged with generally less than 5% of the fruit mined. In the current outbreak, all varieties of citrus are attacked, especially pummelos, grapefruit, and Fukomoto oranges; in some groves, as much as 70% of the fruit is mined and unmarketable.

We have been investigating population density changes of the citrus peelminer through time in a grove of Fukomoto navels located near Strathmore, Tulare County. The grove, situated near the possible source of introduction of the peelminer, had been mapped for peelminer severity damage in 2000. The changes in the infestation of the peelminer within this block were followed through the 2001 season to determine if the infestation was increasing in severity and if native parasites were beginning to utilize the peelminers within the grove. Sampling was conducted in the northwest portion of the grove in a block that was comprised of 17 rows, each with 8 trees. Samples were taken at 3-week intervals from 28 June through 12 September, and at 2-week intervals from 12 September through 25 October. On each sample date, ten fruit from each of 40 trees were assessed for peelminer damage. The assessment included the number of mines on each fruit and a damage rating based on the amount of surface area mined. The damage rating was as follows: 0 = no mining; 1 = 1 –25% of the surface area mined; 2 = 26 - 75% of the surface area mined; and 3 = > 75% of the surface area mined.

Prior to harvest (7 and 8 November), the grove was again mapped for peelminer infestation. The peelminer damage was assessed by examining 12 fruit per tree (three fruit on each compass point), and recording the damage rating for each fruit. The number of damaged fruit per tree and the average damage rating per tree were then used to assign each tree to a citrus peelminer infestation category. The categories were as follows: none = citrus peelminer infestation nearly undetectable with most of the fruit marketable; light = citrus peelminer infestation detectable with about 67% of the fruit marketable; moderate – citrus peelminer infestation easily detectable with about 50% of the fruit marketable; and severe = citrus peelminer heavy with less than 33% of the fruit marketable.

The density of citrus peelminer in the block of oranges began to increase in late August (Figure 1). Initially, a few fruit were found with very few, small mines (Figure 1). In late September and October, the density of citrus peelminer larvae and the severity of the damage increased rapidly (Figure 1). This increase coincided with the maturation and senescence of other host plants of the citrus peelminer. In particular, fields of two alternate hosts, cowpeas and

cotton, located north of this block, were maturing. Both fields had active infestations of citrus peelminer. No parasites were recovered from the peelminers within this block of navel oranges.

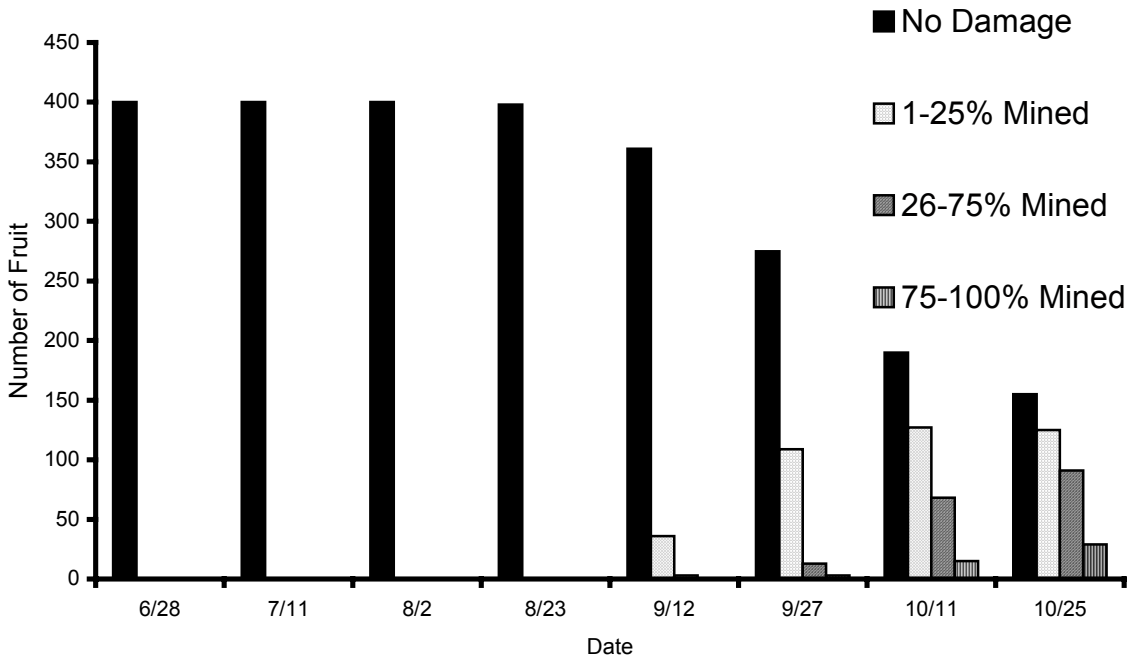
The severity of the citrus peelminer problem within this grove increased from November 2000 to November 2001 (Table 1). The most striking differences between years were the general increase in severity of the citrus peelminer problem throughout the grove, and the increase in citrus peelminer densities on the north and west side of the grove. The north and west sides were located closest to the field of alternate hosts. It appears that the moths coming from the alternate hosts were initially colonizing the trees on these edges.

During 2001, one release of *Cirrospilus coachellae* (Hymenoptera: Eulophidae), a parasite native to the Coachella Valley that provides good biological control for citrus peelminer, was made in this block. Sampling of this citrus peelminer population will continue next year to determine the extent of peelminer infestation and to attempt to recover the introduced parasite.

Table 1. The proportion of trees prior to harvest in each citrus peelminer infestation category in a grove of Fukomoto navels in Tulare County in 2000 and 2001.

Infestation Category	2000	2001
None	0.395	0.133
Light	0.249	0.49
Moderate	0.325	0.261
Severe	0.031	0.116

Figure 1. The number of fruit in each damage-rating category for samples taken in a grove of Fukomoto navels in Tulare County in 2001.



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Augmentative Biological Control of the Glassy-winged Sharpshooter in Citrus with Releases of Green Lacewing Larvae

G. S. Simmons[1], C. H. Pickett and J. Welch[1]

In recent years, the glassy-winged sharpshooter, *Homalodisca coagulata* (Say) (Homoptera: Cicadellidae), has dramatically increased in pest status in California (see Pickett et al., this volume). There are extensive programs under development for both insecticidal and classical biological control. In some commercial crops (e.g., citrus under IPM, organic grapes) as well as urban gardens, there may be grower or public resistance to the application of the most effective pesticides. In addition, it will take time before newly introduced biological control agents begin to exert control. Augmentative release of natural enemies could suppress populations of glassy-winged sharpshooter in localized areas while leaving other areas to chemical suppression as part of region-wide efforts to contain this pest.

In the spring and summer of 2001, two replicated field cage trials of augmentative biological control were conducted in citrus in Kern County. Commercially available green lacewing nymphs, *Chrysoperla rufilabris* (Neuroptera: Chrysopidae), were released at high and low densities onto caged citrus trees inoculated with uniform numbers of first and second instar sharpshooter nymphs. Sharpshooters nymphs were added to the caged trees as background densities of nymphs were low with only 5 to 10 nymphs per tree.The first trial was conducted from May 15 to June 12, 2001. Trial 2 was conducted from September 14 to October 17, 2001. Each trial consisted of 12 caged Washington Navel trees with three treatments replicated four times in a randomized complete block design. Each caged tree represented an experimental unit. Trees were ca. 1 meter in height and enclosed in 1.5 x 1.5, by 1.8 meters high cages constructed of 52 X 52 threads per square inch lumite mesh screen. In each cage, 50 first and second instar sharpshooter nymphs reared from laboratory colony eggs were put on the tree and allowed to settle and feed. Third instar lacewing larvae (obtained from a commercial insectary) were added to each release cage five days after glassy-winged sharpshooter introduction. The three treatments were high, low and no lacewing release. In trial one, the high and low release rates were 100 and 20 third instar lacewing larvae per cage. In trial two, the high and low release rates were 60 and 30 third instar larvae released per cage. About 30 days after lacewing release, all glassy-winged sharpshooter nymphs and adults were collected from each enclosed plant by shaking insects off branches into a 0.6 meter diameter canvas sweep net. The collected glassy-winged sharpshooters were taken to the laboratory for counting. Impact of treatments on sharpshooter nymphal density was evaluated using ANOVA. Sharpshooter counts were log transformed before analysis. Means were separated using a LSD mean separation test.

In the first trial, releases of both low (20) and high (100) third instar lacewing larvae per cage resulted in about equal reductions of glassy-winged sharpshooter (65% and 64% respectively), both significantly lower than the no-release control (Table 1). In the second trial, there were no statistically significant reductions of glassy-winged sharpshooter densities relative to the control for either release rate of 60 or 30 lacewing larvae per plant. Laboratory observations of the third instar lacewing larvae used in trial 2 showed that a high proportion of larvae stopped feeding and began pupating within a day after releases were made. This may have contributed to the lack of differences between treatments obtained in the second trial, as the lacewing larvae released in the field may have also stopped feeding and pupated within a short time after release into cages. To eliminate this problem, further work will be necessary using younger lacewing larvae. It will also be of interest to document which glassy-winged

sharpshooter life-stages are susceptible to predation by the green lacewing. Preliminary observations in the laboratory showed that eggs through fifth instar nymphs are preyed upon by both second and third instar lacewing larvae. Rates of feeding on eggs ranged as high as 45% and from 75 to 100% on first though fifth instar nymphal stages.

In 2002, work will continue in cages with release rate testing as well as testing younger instars of green lacewing to determine if these are efficacious for release against glassy-winged sharpshooter adults, nymphs and egg masses. Other work will include open release trials against natural populations of glassy-winged sharpshooter

Table 1. Mean ± SEM of glassy-winged sharpshooter nymphs and adults per treatment. For trial 1, the high and low release rates were 100 and 20 lacewing larvae, for trial 2, the high and low release rates were 60 and 30 lacewing larvae.

	Trial 1	**Trial 2**
Release Treatment	No. of Glassy-winged Sharpshooter	No. of Glassy-winged Sharpshooter
No-release	22.5 ± 6.2a	11.0 ± 1.6a
Low Release	8.0 ± 2.6b	12.0 ± 4.1a
High Release	7.8 ± 3.9b	25.0 ± 12.3a

Means within the same column followed by the same letter are not significantly different ($P = 0.05$, LSD test)

[1]USDA-APHIS-PPQ Oswell St. Biological Control Facility, Bakersfield, California

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Evaluation of *Aphidius colemani* as a Parasite for the Cotton Aphid in the San Joaquin Valley

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

A cooperative project was initiated in 1996 to increase the amount of biological control exerted on populations of the cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae), in the San Joaquin Valley. This aphid is of great importance within the valley because it can attain pest status in a variety of crops. Since the initiation of the project, five species of natural enemies have been or are being investigated. The aphidiid parasite, *Aphidius colemani* Viereck (Hymenoptera) from Chile, is currently being evaluated in caged trials for inclusion in the introduced complex of natural enemies in the San Joaquin Valley.

Evaluation of *Aphidius colemani* (AC) for inclusion in the complex includes investigation of the ability of this parasite to reduce densities of the cotton aphid in sleeve cages in citrus and cotton at the Shafter Research and Extension Center. For citrus, sleeve cage studies were initiated in May on six branches with both new growth and cotton aphid populations. Ten AC adults were added to each of four sleeve-caged branches, and the remaining 2 caged branches served as control (i.e., no parasites added). The cages were left undisturbed for 14 days, and then examined to determine the number of aphids, mummified aphids, and parasites in each cage. For each cage, approximately 100 aphids were held in petri dishes in the laboratory to allow the development of any additional parasites.

A similar sleeve cage study was conducted on cotton from 2 July through 23 October 2001. Sleeve cages were placed on individual branches of 15 cotton plants along a row. Four replicates of the study were established. Cotton aphids were added to each cage, then after 5 days, 10 AC adults were added to each treatment cage (6 cages in replicate 1, 3 cages in replicate 2, and 10 cages in replicates 3 and 4). The remaining cages were left as controls. After 7 days, the cages were harvested, and the number of aphids, mummies, and parasites were counted. Additionally, approximately 100 aphids from each cage were held in petri dishes in the laboratory to allow the development of additional parasites.

The field cage trials did not produce consistent results, although for some of the replicates, fewer cotton aphids were present in the treatment cages than in the controls (not statistically significant, $P = 0.05$; Table 1). In citrus, no aphid density reduction occurred and no mummies were produced (Table 1). Ants invaded the cages and may have interfered with the parasites. In cotton, no statistically significant reduction in aphid density was seen, however, in two of the replicates, the cages with parasites had a lower mean density of cotton aphid than the controls (Table 1). In all of the replicates in cotton, cages with parasites produced AC mummies, suggesting that the cotton aphids on cotton were acceptable hosts (Table 1). Additional field trials will be required to more fully investigate the ability of AC to reduce cotton aphid densities in cotton.

Table 1. The mean number (± std. error) of aphids, the number of *Aphidius colemani* (AC) mummies found at harvest, and the total number of *A. colemani* adults produced in each treatment in sleeve cage trials conducted in citrus and cotton at Shafter Research and Extension Center in 2001.

Crop/Replicate	Treatment	Mean No. of Aphids[a]	Total No. of AC Mummies	Total No. of AC Adults
Citrus				
Replicate 1	Parasite	683.25 ± 181.41 (n = 4)	0	0
	Control	286.5 (n = 2)	0	0
Cotton				
Replicate 1	Parasite	136 ± 21.36 (n = 6)	7	14
	Control	443.33 ± 200.46 (n = 3)	0	0
Replicate 2	Parasite	605.33 ± 171.41 (n = 3)	0	1
	Control	No data		
Replicate 3	Parasite	248.3 ± 103.53 (n = 10)	20	25
	Control	No data		
Replicate 4	Parasite	1161.5 ± 246.93 (n = 10)	45	95
	Control	1302.5 ± 475.5	0	0

[a]± std. error (n = number of cages)

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

Maintenance 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 management 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. Two parasite species, *Aphelinus* near *paramali* and *Aphelinus gossypii* Timberlake (Hymenoptera: Aphelinidae), have been identified as useful in the initial construction of the introduced natural enemy complex. Distribution of these two parasite species throughout the San Joaquin Valley began in 2000 with the establishment of ten nursery sites.

In 2001, the maintenance of the ten nursery sites continued. The nursery sites were located as close as possible to the nursery sites used in 2000. However, several of the nurseries were moved because of changing cropping patterns, with one nursery in Merced County, three nurseries in Madera County, 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 mid June, 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. During 2001, a total of 41,600 ANP and 41,650 AG were released within the nursery sites. Weekly sampling to determine if the parasites were using the cotton aphids that were present began approximately 2 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. Adults of ANP were recovered from all ten nursery sites, and the recoveries continued into November. The other parasite, AG, was recovered only at two nursery sites (one site in Madera County and one site in Kern County). The reasons for the lack of success with AG are unknown. This study will continue for another year.

Research supported with a grant from the California Cotton Pest Control Board

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

Other Projects in the Early Stages of Development

K. Godfrey

1. Palm leaf skeletonizer. Project participants: K. Godfrey, F.W. Howard (University of Florida, Ft. Lauderdale, Florida), and D. Kellum (Agricultural Commissioner's Office, San Diego, California). The palm leaf skeletonizer [*Homoledra sabalella* Chambers (Lepidoptera: Coleophoridae)] was identified from samples of palm fronds from private residences in San Diego County in 2000. This insect is native to Florida and feeds exclusively on palms. In Florida, biological control maintains densities of this insect well below economic levels. Therefore, a project was initiated to identify and import the appropriate biological control agents for this insect. Surveys of the biological control agents attacking this insect in San Diego County and Florida were initiated in 2001.Outreach materials were developed and distributed.

2. Citrus Root Weevil. Project Participants: K. Godfrey, E. Grafton-Cardwell (University of California – Riverside, Kearney Agricultural Center, Parlier, California), Clayton McCoy (University of Florida, Lake Alfred, Florida), J. Pena (University of Florida, Homestead, Florida), and R. Luck (University of California, Riverside, California). The citrus root weevil [*Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae)] was found in nursery plants delivered to two locations in Sacramento area in 2001. At one site, the plants were stored such that the citrus root weevil may have left the plants and moved into the surrounding area. Since this pest causes problems in both the citrus and nursery industries, a project was initiated to educate producers in both industries on what to expect when this weevil becomes established in California and to continue to develop suitable biological control agents for this insect. Funding for this project was granted by the University of California Exotic Pests and Diseases Research Program.

Insect Natural Enemies Mass Reared for Research and Colonization Projects

K. Casanave, J. Brown, D. A. Mayhew, L. Brace and R. Rodriguez

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,* the western tarnished plant bug, *Lygus hesperus,* the red gum lerp psyllid, *Psyllaephagus bliteus*, pink hibiscus mealybug, *Maconellicoccus hirsutus*, and the glassy-winged sharpshooter, *Homalodisca coagulata*.

Natural Enemy	Host	Source Population	Agency Receiving Shipment	Project Description	Stage Delivered	Total Insects Delivered
Psyllaephagus sp	Red gum lerp psyllid	Australia	UC Berkeley & Counties	Nursery Sites	adults	10,265
Aphelinus nr paramali	Cotton aphid		USDA-ARS, Shafter/Kern Co	Nursery Sites	Adults & pupae	23,800
			CDFA/Madera & Merced Co	Nursery Sites	Adults & pupae	17,800
Aphelinus gossypii			USDA-ARS, Shafter/Kern Co	Nursery Sites	Adults & pupae	16,500
			CDFA/Merced & Madera Co	Nursery Sites	Adults & pupae	25,150
Aphidius colemani			USDA-ARS, Shafter/Kern Co	Field cage, cotton & citrus	Adults & pupae	500
Peristenus stygicus	lygus	Italy (Umbria)	CDFA, North B St	Open release, alfalfa	adults	1520
			UC/USDA Shafter Field Station	Open release, alfalfa	adults	1732
			CDFA, Madera	Open release, alfalfa	adults	1173
			UC, KAC	Cage, Open release, alfalfa	adults	2054
		Spain (Granada)	CDFA, Merced	Open release, alfalfa	adults	600
			CDFA, Madera	Open release, alfalfa	adults	1050
			Kern, Poplar Ave	Open release, alfalfa	adults	1974
		Spain (Catalognia)	CDFA, Merced	Open release, alfalfa	Adults/larvae	1724/550
			CDFA, Kern, Poplar Ave	Open release, alfalfa	adults	655
		Spain (Catalognia, Navata; nr. Figueras	UC Santa Cruz	?	adults	1100
Peristenus digoneutis		Italy (Umbria)	CDFA, Sacramento North B St.	Open release, alfalfa	adults	795
			CDFA, Madera	Open release, alfalfa	adults	123
			UC, KAC	Open release, alfalfa	Adults/larvae	1736/600
			UC/USDA Shafter	Open release, alfalfa	adults	1732
			CDFA, Kern Poplar Ave	Open release, alfalfa	adults	479
		Spain, Catalognia	UC Santa Cruz	Open release, alfalfa	adults	53
Gonatocerus triguttatus	glassy-winged sharpshooter	Weslaco, Texas	CDFA, PDCP	Nursery site, open release	adults	110,125
Gonatocerus morrilli		Mexico (Nuevo Leon & Tamaulipas	CDFA, PDCP	Nursery site, open release	adults	755
Gonatocerus nr. asheadmi		Mexico (Nuevo Leon & Tamaulipas	CDFA, PDCP	Nursery site, open release	adults	26,800
Anagyrus kamali	Pink hibiscus mealybug	China/Hawaii	CDFA, Mexico French West Indies	Open release, urban sites	adults	78,100
Gyranusoidea indica		Egypt, Pakistan, Australia	CDFA, Mexico French West Indies	Open release, urban sites	adults	126,375

Distribution of and Colonization by Biological Control Agents of Squarrose Knapweed

D. M. Woods and B. Villegas

Squarrose knapweed is the most widely distributed knapweed in California. It has been difficult to eradicate on a statewide level and has maintained substantial populations in many parts of northern California. Consequently, we are attempting to establish biological control agents that might reduce the rate of spread. 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, have shown potential to attack squarrose knapweed. We began releasing these species on squarrose knapweed in 1995-6 and continued through 2001. The first releases were near Hawkinsville in Siskiyou County. Recently, an expanded program has been instituted in northeastern California centered around Pittville. Adult weevils were field collected in Oregon in conjunction with the Oregon Department of Agriculture, and then released on squarrose knapweed in California. Limited releases of other agents have also been made but have not been emphasized as they seem to offer less promise of impact. These include, the knapweed rootboring weevil *Cyphocleonus achates,* (Coleoptera: Curculionidae), the knapweed flatheaded borer, *Sphenoptera jugoslavica* (Buprestidae), the gall flies, *Urophora affinis* and *Urophora quadrifasciata*, and the seedhead fly *Terellia virens* (Diptera: Tephritidae). Colonization and establishment are monitored by visual and sweep net monitoring during the summer, and by dissecting field collected seedhead samples in the fall. Distribution, colonization and establishment results are shown in Table 1. Colonization is considered successful when a population maintains a presence from 1-2 years. Establishment is defined as population maintenance at least 3 years.

Table 1. Status of biological control agents on squarrose knapweed in California.

Site	County	Agent	Release date	Colonized/established
Hawkinsville #1	Siskiyou	*Cyphocleonus achates*	6/95	-/-
		Bangasternus fausti	6/97	+/+
Hawkinsville #1	Siskiyou	*Bangasternus fausti*	7/96, 6/97	+/+
		Larinus minutus	7/97	+/+
Pittville	Lassen	*Bangasternus fausti*	7/98	+/+
		Larinus minutus	7/98	+/+
		Urophora affinis	7/98	-/-
		Urophora quadrifasciata	7/98	+/-
		Cyphocleonus achates	7/98	-/-
		Sphenoptera jugoslavica	7/98	+/-
		Terellia virens	7/98	-/-
Nubieber #2	Lassen	*Bangasternus fausti*	6/99, 6/2000	+/-
		Larinus minutus	7/99, 7/2000	+/-
		Urophora affinis	7/2000	+/-
		Cyphocleonus achates	7/2000	-/-
Nubieber #3	Lassen	*Larinus minutus*	7/99, 7/2000	+/-
Wild rice site	Lassen	*Bangasternus fausti*	6/2000	+/-
Nubieber #5	Lassen	*Bangasternus fausti*	6/2000	Not surveyed
Nubieber #6	Lassen	*Bangasternus fausti*	6/2000	Site destroyed
Nubieber #7	Lassen	*Larinus minutus*	7/2000	+/-
Nubieber #8	Lassen	*Larinus minutus*	7/2000	+/-
Nubieber #9	Lassen	*Larinus minutus*	7/2000	+/-
Lookout #1	Modoc	*Larinus minutus*	7/2000	+/-
Lookout #2	Modoc	*Larinus minutus*	7/2000	-/-
Lookout #3	Modoc	*Larinus minutus*	7/2000	+/-
South Pittville	Shasta	*Larinus minutus*	7/2001	Not surveyed

Renewed Biological Control Program against Purple Loosestrife in California

B. Villegas and D. B. Joley

Four biological control agents of purple loosestrife, *Lythrum salicaria* L., have been released in small numbers in California since 1996. These were released in Butte, Kern, Nevada, San Joaquin, Shasta and Siskiyou Counties, but little or no recoveries have taken place. The insects released are two leaf-feeding beetles, *Galerucella calmariensis* L., and *G. pusilla* (Dufft.) (Coleoptera: Chrysomelidae); a root boring weevil, *Hylobius transversovittatus* Goeze and the flower-bud weevil, *Nanophyes marmoratus* (Goeze) (Coleoptera: Curculionidae). The biological control agents were either collected or obtained from cooperators in Oregon, Washington and Cornell University in New York.

In spring 2001, a renewed three-year biological control program was initiated on purple loosestrife infestations located in Butte, Kern, and Shasta Counties. The renewed effort called for larger releases of the four biological control agents at fewer release sites. Approximately, 27,000 leaf beetles were collected in the Moses Lake area of central Washington and released at six sites in Shasta County and at one site each in Butte and Kern Counties. Also, 500 root-boring weevils were received from Dr. Bernd Blossey (Cornell University, Ithaca, NY) and released at 3 sites in Shasta County. About 800 flower-bud weevils were received from Gary Brown (USDA-APHIS, PPQ, Portland, Oregon) in July and released at one site in Shasta County. We anticipate that the higher release rates of the biological control agents will result in greater and faster establishment of the insects in the study areas.

The released sites will be surveyed for establishment during the 2002 season in order to evaluate this renewed biological control program. However, it takes at least two full seasons to do an adequate evaluation. The *Galerucella* leaf beetles and the *Nanophyes* bud weevils are fairly easy to check for establishment because of their external feeding habits, but evaluation of establishment by the root-boring weevil, *Hylobius transversovittatus,* is destructive in nature as sampling and destruction of the roots is required in order to check for larval tunneling and damage.

Table 1. Releases of Biological Control Agents on Purple Loosestrife (PLS) in California, 1996-2001

County	Nearest City	Sites	Releases Made	Year	Previous Releases	2001 Releases	Grand Total
Galerucella calmariensis and *G. pusilla*, the PLS leaf-feeding beetles							
Butte	Oroville & Palermo	9	16	1998-2001	4,100	4,000	8,100
Kern	Onyx	1	4	1999-2001	2,000	4,000	6,000
Nevada	Bear River	2	5	1998	1,900	0	1,900
San Joaquin	Lodi	1	1	1998	1,000	0	1,000
Shasta	Glenburn	7	11	1998-2001	2,400	18,000	20,400
Siskiyou	Tulelake	3	3	1998	1,400	0	1,400
Hylobius transversovittatus, the PLS root-boring weevil							
Butte	Oroville & Palermo	4	4	1996-2000	465	0	465
Shasta	Glenburn	5	5	1996-2001	611	500	1,111
Nanophyes marmoratus, the PLS flower-bud weevil							
Butte	Oroville & Palermo	3	3	1997-2000	180	0	180
Shasta	Glenburn	2	2	1997-2001	150	800	950

Acknowledgments: We would like to thank Dr. Bernd Blossey (Cornell University) and Gary W. Brown (USDA APHIS Plant Protection and Quarantine Officer), Portland, OR and his technicians Laurie Hewitt and Kerby Winters for supplying some of the purple loosestrife biological control agents.

Puccinia centaurea, a Naturally Occurring Pathogen of Tocalote

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

Tocalote, *Centaurea melitensis* L., Asteraceae, also called Napa thistle is an exotic weed closely related to yellow starthistle. Although not common in northern California, tocalote is widespread in the southern San Joaquin Valley, where it is sometimes mistaken for yellow starthistle. Natural enemies of tocalote have not been investigated sufficiently to import agents for a classical biological control program. Therefore, we have begun evaluating natural enemies fortuitously occurring on *C. melitensis* in California. In 1998, we began investigating natural enemies of yellow starthistle that were naturally spreading to tocalote. As part of our survey efforts, on January 27, 2001 we detected rust pustules on a limited population of *C. melitensis* in the Berryessa hills west of Winters, California. A sample of the rust was tentatively identified as *Puccinia centaurea* by Bill Bruckart, USDA-ARS FWRDIC.

The rust has been easy to propagate in the greenhouse on tocalote but we have been unable to infect yellow starthistle or bachelor's button, *Centaurea cyanus*, a common host for most rusts of Centaurea species. *Puccinia centaurea* has been described from several species of Centaurea and other Cynarea but we could not find evidence of it reported on *C. melitensis*. Thus we feel that this is a newly described host association.

We began preliminary field evaluations of the impact of the rust in April 2001. Tocalote plants had bolted and essentially all plants had rust pustules. One hundred plants were selected for further study. Each plant was marked with a small flag, the height recorded and leaves and stem evaluated for rust infection by two rating methods. For the first method, each plant was inspected for the vertical distribution of rust pustules. Plants with pustules only in the lower third of the plant were rated 1; those with rust in the lower 2/3 of the plant were rated 2; and a 3 was given to plants with pustules as high as the upper third of the plant. The second method was a rust intensity based system evaluating the amount of rust on infected areas. A rating scale from 0 to 5 was developed, with 0= no rust to 5 = > 75% coverage on infected leaves. Plants were evaluated a second time at plant maturity (May 31, 2001) for vertical distribution of rust pustules. Plants height was measured, then plants were harvested and weighed.

The results presented in Table 1 indicate that the rust continued to spread vertically throughout the plant between April and May as all plants ended the season with about the same average height of rust pustules. However, the early appearance of rust pustules on the upper parts of the plant seems to have had an important impact on further plant growth and development. Plants with pustules limited to lower reaches of the plant early in the season generally increased in size more than plants with rust in the upper portions. Similarly, more seedheads were produced on plants with limited rust. The rust intensity scale data, (Table 2) is not easy to evaluate. Analysis was severely limited by the lack of data for the highest and lowest rating categories. It is possible that the most severely rusted plants could die early but our sample size was too small to assess it. This study was a small preliminary study, designed to familiarize us with this new rust disease. These results do however, indicate that a heavy, early infection with the rust fungus, *P. centaurea,* tends to suppress further growth and reproduction by *C. melitensis*.

Table 1. Effect of early season rust distribution on growth of tocalote

	Mid season height of pustules		
	1	2	3
Final height of pustules	2.21	2.177	2.65
Mean seedheads per plant	3.25	2.02	1.13
Mean percent increase in plant height	31.3	18.6	10.5

Table 2. Effect of early season rust intensity on further growth of tocalote.

	Mid season rust rating					
	0	1	2	3	4	5
Number of plants	1	40	43	12	2	0
Mean seedheads per plant	1	1.95	2.39	2.45	0.5	-
Mean percent increase in plant height	0	2.3	3.4	3.9	-2	-

Releases of Biological Control Agents onto Black and Meadow Knapweeds in Northern California

B Villegas, G. W. Brown[1] and E. Coombs[2]

Several species of knapweeds have been introduced into California and become established as noxious weeds. Black knapweed, *Centaurea nigra* and meadow knapweed, *C. x pratense*, exist in several counties in northern California primarily along disturbed roadsides and forest clearings. Black knapweed appears to be increasing, particularly in Del Norte and Siskiyou Counties as well as areas of southern Oregon that border these two counties. Meadow knapweed, *C. x pratense*, is a recognized hybrid of black knapweed, *C. nigra* and brown knapweed, *C. jacea* and is often misidentified as either black or brown knapweed. Brown knapweed is by far the least common of the three knapweeds in California.

There are currently no biological control agents that were specifically selected for and tested against black, brown, or meadow knapweeds. There are, however, several biological control agents available for both diffuse and spotted knapweeds that may have potential in impacting both black and meadow knapweed. In July 2001, two seedhead weevils, *Bangasternus fausti* (Ritter) and *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae) were mass collected from diffuse knapweed south of The Dalles in northern Oregon and shipped to Del Norte and Siskiyou Counties for release on black knapweed (Table 1). In Del Norte, the weevils were released at six sites located along Highway 199 from Gasquet to the Oregon State Line. In Siskiyou County, the insects were released at six sites along Indian Creek Road from Happy Camp to the Oregon State Line. A second collection of *Larinus minutus* was made from spotted knapweed from more moist areas near Mount Hood in northern Oregon. These weevils were transported and released at two sites near Mount Shasta that were heavily infested with meadow knapweed (Table 2). In August, a third insect, *Cyphocleonus achates* (Fåhraeus) (Coleoptera: Curculionidae), a root-boring weevil, was collected from spotted knapweed in the Bend area of central Oregon and released on black and meadow knapweed in Siskiyou County (Table 1 & 2).

It should be noted that personnel from the Siskiyou County Department of Agriculture released about 300 seedhead weevils in June 1999 at one of the six sites selected for the 2001 releases. Those weevils, consisting of both *B. fausti* and *L. minutus,* were collected from squarrose knapweed, *Centaurea squarrose* Willd (Table 1) near Hawkinsville, California.

We are beginning to evaluate establishment and possible impact of the biological control agents on black and meadow knapweed in northern California. Preliminary sampling of the seedheads at three sites in July, September and December 2001 reveled that the knapweed gall fly, *Urophora quadrifasciata* Meigen (Diptera: Tephritidae), was well established on black knapweed in Del Norte County and on meadow knapweed at one site near Mount Shasta in Siskiyou County. Emergence holes attributable to the seedhead weevil, *Larinus minutus*, were readily noticed on meadow knapweed seedheads at the main release site in Mt Shasta. No exit holes were found at two sites infested with black knapweed in Del Norte and Siskiyou Counties. No sampling was done for the root-boring weevil, *Cyphocleonus achates* to avoid impacting population establishment.

Table 1: Releases of *Larinus minutus*, *Bangasternus fausti* and *Cyphocleonus achates* weevils against black knapweed infestations in Northern California.

County	Nearest City	Sites	BC Agent	Numbers Released
Del Norte	Gasquet	6	*L. minutus, B. fausti*	4,000
Siskiyou	Happy Camp	1	*L. minutus, B. fausti*	300
Siskiyou	Happy Camp	6	*L. minutus, B. fausti*	4,000
Siskiyou	Happy Camp	2	*Cyphocleonus achates*	200

Table 2: Releases of *Larinus minutus* and *Cyphocleonus achates* weevils against meadow knapweed infestations in the Mount Shasta area of Northern California.

County	Nearest City	Sites	BC Agent	Numbers Released
Siskiyou	Mt. Shasta	2	*L. minutus*	2,500
Siskiyou	Mt. Shasta	1	*Cyphocleonus achates*	60

[1] USDA-APHIS, PPQ, Portland, Oregon
[2] Oregon Department of Agriculture, Salem Oregon

Release and Recovery of Biocontrol Agents Associated with the Integrated Project on Yellow Starthistle at Fort Hunter Liggett

D. B. Joley, M. J. Pitcairn, B. Villegas, G. Wilber, J. Torrence[1], and J. DiTomaso[1]

In 1999, a cooperative project was initiated between the California Department of Food and Agriculture and the University of California, Davis, to demonstrate large-scale integrated control of yellow starthistle at Fort Hunter Liggett Military Reservation in Monterey County. A multi-year management plan was developed for each of five different habitats identified during previous vegetation surveys. 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. Due to concerns raised later by the U.S. Fish and Wildlife Service, alternate sites for herbicide treatments were established away from habitats potentially utilized by the endangered arroyo toad. Also, management plans for the purple amole and vernal pool habitats were changed to not include herbicide treatments.

Implementation of the integrated control effort began in 1999. Here we report on the biological control activities of that plan. Efforts were continued in 2001 to ensure that yellow starthistle biological control agents are established and increasing at all research sites at Fort Hunter Liggett. Two agents, *Eustenopus villosus* and *Larinus curtus*, were collected from California field sites by Biological Control Program staff and released at several sites during June. Release sites were located just outside the proposed treatment zones. Presumably, established agents will migrate into the treatment areas following treatment. Release histories for each demonstration plot are shown in Table 1.

Table 1. Number of released *Eustenopus villosus* (1999-2001) and *Larinus curtus* (2000-2001) on yellow starthistle at various sites at Fort Hunter Liggett, Monterey County.

	E. villosus			*L. curtus*		
Location of Sites	1999	2000	2001	1999	2000	2001
Military Use (TA15)	0	1,000	400	0	200	200
Hay Camp Road[1]	0	0	600	0	0	400
Oak Woodland (TA 27)[2]	0	1,000	400	0	200	200
Purple Amole[3]	600	200	400	0	0	0
Vernal Pools	800	400	0	0	0	700

[1]Current Oak Woodland site

[2]Previous Oak Woodland site; area burned then scraped in 2001.

[3]Burned in 2000 and 2001; current biocontrol agent nursery sites are located south of the treated area and west of the treated area (control area of original Oak Woodland)

Three additional natural enemies of yellow starthistle were found to be already present at the installation, and their numbers will not be augmented. *Chaetorellia succinea* is now well established, and should significantly impact seed production. *Bangasternus orientalis* and *Urophora sirunaseva* were also present at low levels, but neither is expected to significantly impact yellow starthistle seed production.

Distribution and relative abundance of biocontrol agents on yellow starthistle plants were determined in June 2001 using a sweep net at 25 release sites encompassing three years of releases. *B. orientalis* adults (generally in low numbers) or their eggs were found at 22 sites,

indicating they are widely distributed. *C. succinea* adults were found at 16 sites, but are likely distributed throughout the installation as they are strong fliers. *E. villous* adults were found at 14 sites; their numbers appear to be increasing where they were released in 1999. *L. curtus* adults were found at 6 sites (they were released only at 5 of the 25 sites prior to the 2001 survey).

Whole plant samples (9-15 plants) were collected at the end of the season (September or October) at release sites in 2000 and 2001 to determine the rate of attack by the bioagents in the seedheads. Results are shown in Table 2.

Attack rates of both *C. succinea* and *E. villosus* generally increased overall from 2000 to 2001, although some site/agent changes were very small. *L. curtus* and *B. orientalis* remained at very low levels (0-5 %), and are not expected to contribute significantly to seed destruction at Fort Hunter Liggett. Attack by all insects over the five sites reached 65% in 2001. Approximately 50% of those attacked heads contained *Chaetorellia* during both years.

Table 2. Percent of yellow starthistle seedheads infested with two exotic insects at Fort Hunter Liggett, Monterey County. The weevil, *E. villosus*, was released intentionally (Table 1), whereas *C. succinea* moved from nearby locations.

	E. villosus		*C. succinea*	
Location of Sites	2000	2001	2000	2001
Military Use (TA15)	2	17	30	47
Hay Camp Road	ND*	2	ND	42
Oak Woodland (TA 27)	4	12	44	44
Purple Amole	10	13	22	68
Vernal Pools	23	22	28	51

*ND - no data

Releases of *Eustenopus villous* are planned for June 2002, but will likely be made along the corridor of the San Antonio River. It is along this corridor that some of the greatest impact of yellow starthistle is felt, but which cannot be treated with herbicides for the foreseeable future.

[1]University of California, Davis, CA

Biological Control of Leafy Spurge along the Scott and Klamath Rivers of Siskiyou County

B. Villegas, D. B. Joley and P. Griffin[1]

Leafy spurge, *Euphorbia esula* L., is native to Eurasia where it ranges from Spain to Japan. In North America, leafy spurge is considered a noxious weed. It is a deep-rooted perennial and forms large patches through vigorous lateral root growth. The plant also produces latex which can cause dermatitis to humans and grazing animals. Leafy spurge is also of concern as it out-competes native grasses and forb species which are important components of productive rangelands and mountain meadows.

Leafy spurge has been known to exist in Scott Valley of Siskiyou County for many years. Infestations occur in both irrigated pastureland and within the riparian zone of the Scott River and its tributaries and along the Klamath River. The extent of the infestation along these two rivers was unknown until the 2000-2001 seasons when the Siskiyou County Department of Agriculture performed an intensive survey. A total of 92 sites were found in 62 miles of river surveyed. Most of these sites had less than 100 plants, but some sites were quite extensive and infesting several hundred feet of riverbank. Infestations were found along the Scott River and then along the Klamath River up to 26 miles below its confluence with the Scott River.

In 2001, the Biological Control Program and the Siskiyou County Department of Agriculture initiated a three-year biological control study. Our goal was to see if certain biological control agents could impact leafy spurge at selected sites along the Scott and Klamath River. Because many of the infested sites are located along the riparian zones in sandy shallow soils subject to flooding, careful selection of release sites is very importance in the success of this trial study.

Leafy spurge has been the target of biological control research in Canada and the United States for over 30 years. A complex of insects has been introduced and released in many parts of the United States and Canada (Table 1). Among the insects that have shown good biological control of leafy spurge in the Great Plains and Canada 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. Six species have been released in North America, but only two, *Aphthona lacertosa* (Rosh) and *Aphthona nigriscutis* Foudras, were readily available through cooperators with the USDA-ARS/APHIS Team Leafy Spurge located in Sidney, Montana.

On June 21-22, 2001 a total of 15 releases ranging from 3,000-5,000 flea beetles each were made along the surveyed infestations along the lower Scott River and Klamath River below the confluence with the Scott River (Table 2). Each release consisted of a mixture of the two species of flea beetles; roughly 95% *A. lacertosa* and 5% or less *A. nigriscutis*. The pre-selected release sites contained the largest infestations of leafy spurge ranging from a few hundred plants to over an acre in size. The sites will be visited during 2002 for signs of colonization and potential impact by the flea beetles. The flea beetles were obtained from Dr. Bob Richard (USDA-ARS/APHIS Team Leafy Spurge, Sidney, Montana) and collected on June 18-19, 2002 near Medora, North Dakota.

Table 1. Biological Control Agents released in North America for the Biological Control of Leafy Spurge, *Euphorbia esula* L. (Adapted from Julien and Griffiths, 1999. Biological Control of Weeds: A World Catalogue of Agents and their Target Weeds. CABI Publishing, 4th ed., pp 61-64. by B. Villegas)

Biological Control Agent	Source of Introduction	Damage to Leafy Spurge
Aphthona abdominalis (Duftschmidt) (Coleoptera: Chrysomelidae)	Italy	*Aphthona* adults feed on the foliage; larvae feed on the roots and root hairs
A. cyparissiae (Koch)	Austria, Hungary, Italy and Switzerland	
A. czwalinae Weise	Austria, Germany, Hungary and Russia	
A. flava Guillebeau	Eurasia; Hungary, Italy and Inner Mongolia	
A. lacertosa (Rosh)	Hungary and Yugoslavia	
A. nigriscutis Foudras	Hungary	
Chamaesphecia astatiformis Herrich-Schäffer (Lepidoptera: Sesiidae)	Yugoslavia	Larvae burrow into the stem and root-crown.
C. crassicornis Bartel	Hungary	″
C. hungarica (Tomala)	Hungry and Yugoslavia	″
C. tenthrediniformis (Denis & Schiffermüller)	Austria and Greece	″
Hyles euphorbiae L. (Lepidoptera: Sphingidae)	Hungry and Yugoslavia	Larvae feed on the leaves and flower bracts
Lobesia euphorbiana (Freyer) (Lepidoptera: Tortricidae)	Italy	″
Minoa murinata Scopoli (Lepidoptera: Geometridae)	Germany	″
Oberea erythrocephala (Schrank) (Coleoptera: Cerambycidae)	Hungry, Italy and Switzerland	Adult feeding and oviposition kills stem tips; larvae burrow into the stem and root-crown
Pegomya curticornis (Stein) (Diptera: Anthomyiidae)	Hungary	Larvae kill the growing tips affecting seed production
Pegomya euphorbiae (Kieffer)	Hungary	″
Spurgia capitigena (Bremi) (Diptera: Cecidomyiidae)	Italy	Larvae cause stem-tip galls reducing seed production.
Spurgia esulae Gagné	Italy	″

Table 2. Releases of *Aphthona lacertosa* and *A. nigriscutis* flea beetles along the Scott and Klamath Rivers, Siskiyou County.

Site #	River Drainage	Location Name	Release Date	Numbers Released
1	Scott River	Indian Scotty #1	6/21/2001	5,000
2	Scott River	Indian Scotty #2	6/21/2001	3,000
3	Scott River	Boulder Creek #1	6/21/2001	5,000
4	Scott River	Boulder Creek #2	6/21/2001	3,000
5	Scott River	White Water Ranch Site #1	6/21/2001	3,000
6	Scott River	White Water Ranch Site #2	6/21/2001	3,000
7	Scott River	Evans Property #1	6/21/2001	3,000
8	Scott River	Evans Property #2	6/21/2001	3,000
9	Scott River	Derry Hut Site #1	6/21/2001	3,000
10	Scott River	Derry Hut Site #2	6/21/2001	3,000
11	Scott River	Johnson Bar	6/21/2001	3,000
12	Klamath River	Cannon Property #1	6/21/2001	5,000
13	Klamath River	Cannon Property #2	6/21/2001	3,000
14	Klamath River	Portuguese Creek Site#1	6/22/2001	5,000
15	Klamath River	Portuguese Creek Site#2	6/22/2001	3,000
	Total *Aphthona* flea beetles released:			53,000

Passive Sorting Technique for Separating Biological Control Agents from Debris in Sweep Net Collections

B Villegas, G. W. Brown[1] and E. Coombs[2]

A passive sorting technique for mass collecting and separating large numbers of biological control agents was tested in July 2001 in order to expedite the collection and redistribution of beneficial insects for weed biological control. This collection technique takes advantage of the behavior of many insects to move upwards towards light. By using inexpensive widely available containers and materials, numerous insects can be collected by two-person teams in a relatively short period of time.

The sorting technique is very simple and does not need previously constructed sorting screens or special collection containers. With the exception of a professional sweep net, most materials can be obtained inexpensively at most hardware stores. The insects separate themselves from sweeping debris by moving towards the light and into a clean container where they can be mass collected and separated into ready-to-release containers.

MATERIALS NEEDED:

1) A sturdy 18-inch diameter sweep net with a canvas bag.
2) Resealable plastic Ziploc bags at least 8 inches by 8 inches in size.
3) Flat clear plastic storage boxes with opaque resealable lids at least 1-2 inches larger than the Ziploc bags. The ones we used were approximately 11 inches wide x 16 inches long by 6 inches high and had a volume of approximately 3 US Gal (11.3L).
4) 4-6 pillowcases per person. Pillowcases made of nylon fabrics are best, as they do not allow the insects to cling to the sides of the fabric.
5) 1-2 48-quart or larger cooler for keeping insects cool while in the field. We use only AC/DC coolers which cool without condensation.
6) A roll of duct tape.
7) Clear vials for estimating the number of insects collected.
8) A fine point permanent marking pen, shipping containers, tissue paper, labels, and aspirators with filters.

This technique works best when used by a two-person team. Test sweeps should be taken at each collection site to insure that large numbers can be collected in a short period of time. For example in the collection of *Eustenopus villosus* weevils, if at least 200 weevils are not readily collected in five minutes of sweeping or in 100 sweeps, the collection site is not ready for mass collection. Similar guidelines can be used for other beneficial insect collection sites. After a site is determined to be a good collection site, the two-person team should concentrate on sweeping the host weeds. After a known number of sweeps or after a particular time period, the sweep net is emptied into a pillowcase, taking care to exclude excess foliage and sharp, dried plant stems. Pillowcases are ideal as they can be easily carried and they can be opened and closed easily. A different pillowcase should be used for each collection site. After collecting at one site, the pillowcases containing the sweepings are stored in an ice chest so that the biological control agents are not exposed to direct sunlight and protected from overheating.

After collections at several sites, passive sorting is initiated. First prepare a sorting box by covering three sides of a plastic storage box with duct tape to block the sunlight (Fig. 1). From the pillowcases, handfuls of sweepings are placed in the Ziploc type baggies, and each baggie is placed open, and on its side inside a closed plastic storage box. The objective is to position the open Ziploc bag in the "dark" side of the box with the opening oriented toward the untapped or clear end of the box. The clear side is then positioned towards the light and away from direct sunlight. Prepare all the sorting boxes the same way and stack them up one on top of the other (Fig 2). Designate one person to monitor the sorting boxes to ensure they are not overheated from any direct sunlight. Passive sorting can also be done indoors using artificial incandescent light which warms the container and speeds up separation.

After a while, the beneficial insects start to move from the sweepings to the opening of the Ziploc baggie (Fig 3). When most of the insects have moved away from the sweeping debris, remove the baggie from the storage box and properly dispose of it as nearly pure colonies of the beneficial insects remain in the box (Fig. 4). We recommend chilling the entire separator to slow the insects down, giving you more time to remove extraneous material. Minor removal of debris, host seedheads, weed seeds and other insects might be necessary depending on the collection site and time of the year. Once the final "cleaning" is achieved, the insects are ready for counting and separating into equal numbers.

Mass counting estimates are based on predetermined ideal release size. For example, the ideal release size for the knapweed seedhead weevils is 500 individuals. To rapidly partition insects into lots of their ideal release size, 500 weevils were hand counted and then transferred to a clear-plastic vial. A line was drawn on the outside of the plastic vial to mark the volume occupied by the 500 weevils. Once a line was established for each weevil species, the insects were scooped with the vial and filled up to the 500-weevil mark (Fig. 4). These "counted" weevils are then placed in individual release containers for redistribution. Release containers can be filled half way with crinkled tissue paper with a little water added to prevent dehydration of the weevils. As long as the release containers are kept in a cool place such as an ice chest, they can usually survive 48 hours without any further handling until ready for release.

This technique was tested in July 2001 on two seedhead weevils, *Larinus minutus* and *Bangasternus fausti*, swept from diffuse and spotted knapweed in northern Oregon. This same technique could be used in the mass collection of other weed biological control agent, including:

- *Aphthona* flea beetles used for the biological control of leafy spurge
- *Galerucella* leaf beetles used for the biological control of purple loosestrife
- *Bangasternus* and *Larinus* seedhead weevils used for control of knapweeds and yellow starthistle
- *Eustenopus* seedhead weevils used for the biological control of yellow starthistle.
- *Microlarinus* seed and stem weevils used for the biological control of puncturevine.
- Most other hardbodied insects like weevils and chrysomelid beetles used for the biological control of weeds.
- Some predatory insects such as coccinellid beetles which show the same orientation behavior of moving up towards a light source.

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Fig. 1. Sorting box with three sides covered with duct tape to block the sunlight; Fig. 2. Sorting boxes can be stacked for ease of monitoring; Fig. 3. Knapweed weevils moving from the sweepings to the opening of the Ziploc baggie; Fig. 4. A storage box with nearly clean collection of weevils sorted by this technique; Fig. 5. Clear-plastic vial with measuring lines on the outside for ideal release sizes for different biological control agents.

Collection Workshops and Releases of *Eustenopus villosus* and *Larinus curtus* for the Biological Control of Yellow Starthistle in California in 2001

B. Villegas

Two seedhead weevils, the hairy weevil, *Eustenopus villosus* (Boheman) and the flower weevil, *Larinus curtus* (Hochhut) (Coleoptera: Curculionidae), were mass collected during five workshops held at field collection sites located in Lindsay, Tulare County, Ione, Amador County, and at two sites in Weaverville and Mad River, Trinity County. The objective of these workshops was to train the participants on the biological control of yellow starthistle using the available biological control agents at centrally located sites.

The hairy weevil has been widely redistributed in the past by county and public agencies through similar collection workshops. It is estimated that at least 750 sites have been used as release sites throughout California in 50 counties. The hairy weevil was introduced from Greece in 1990 for the biological control of yellow starthistle, *Centaurea solstitialis* L. (Asteraceae). The first introductions of the flower weevil from Greece into California were in 1992. To date close over 90 releases of flower weevils have been made from weevils collected from the one site in northern Oregon and from Ione, Amador County, California.

Table 1 summarizes the releases made by participating counties. Eleven counties participated in three collection workshops held in Tulare and Amador Counties. In addition, the Trinity Resource Conservation and Development Council, Inc. in Weaverville and the US Forest Service in Mad River organized two yellow starthistle control workshops which were attended by approximately 30 Trinity County residents. Attendance to these workshops was low this year because of rainy weather during the two workshops. From Tulare and Amador Counties, approximately 1,885 hairy weevils and 2,200 flower weevils were collected and released at 15 sites as part of the Yellow Starthistle Control Demonstration implemented at the Fort Hunter Liggett Military Base in Monterey County. Additionally, 1,000 hairy weevils were collected from Tulare County and delivered to the Department of Defense Ammunitions Depot in Riverton, Stanislaus County to supplement their biological control efforts at this military facility.

Only four counties, Napa, San Benito, Shasta, and Tulare made within county releases from established populations of the hairy weevil. This year those releases were combined with those resulting from the collecting workshops.

Table 1: Releases of the hairy weevil, *Eustenopus villosus*, and the flower weevil, *Larinus curtus* in 2001

COUNTY	Hairy Weevils Released	Hairy Weevil Sites	Flower Weevils Released	Flower Weevils Sites
Alameda	800	4	2500	4
Alpine	100	2	100	2
Contra Costa	2,300	5	1,800	6
Glenn	400	2	400	2
Kings	400	2	0	0
Lake	400	2	400	2
Merced	500	2	500	2
Monterey	1,885	8	2,200	7
Napa	1,000	2	1,200	3
San Benito	750	3	0	0
Santa Barbara	800	4	327	1
Shasta	5,200	26	0	0
Sonoma	1,425	8	1,425	8
Stanislaus	1,000	1	0	0
Trinity	600	12	0	0
Tulare	1,000	5	0	0
Grand Totals	**17,560**	**88**	**10,852**	**37**

Seven-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

Five exotic insect species have been established in California for biological control of yellow starthistle. 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 locally abundant in the Pacific Northwest but are limited to isolated populations in California. A sixth species, the seedhead fly, *Chaetorellia succinea* (Costa) (Diptera: Tephritidae), was accidentally introduction into western North America in 1991 and 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 plant-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 1995-2001 are presented in Table 1.

Seven years after the initial releases, we have evidence that attack by these biological control agents has reduced seed production by yellow starthistle at all three sites. The weevil, *E. villosus*, has become the most abundant insect at all three 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 change in plant architecture with the damaged plant dominated by stem material. Instead of flowers born on the tips of stems, new flowers are produced on short stems (<1 cm) arising from the leaf axils along the main stems. The attack rates of *E. villosus* at the Placer and Yolo County sites increased steadily from 1995-1998, declined in 1999 and 2000, but increased in 2001 to levels similar to that observed in 1998. At the Sonoma County site, the attack rate also increased until 1998, declined in 1999, but increased in 2000. In 2001, the attack rate of this weevil decreased to 56%. Population build-up and attack by *E. villosus* may have now leveled off and the values for attack rate observed over the last 3-4 years may indicate the range of values to be expected by this species in the future.

The infestation rates of *B. orientalis* and *U. sirunaseva* were initially high 1995-97 but have declined to less than 1% in 2001 at all three 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 population densities initially increased steadily, attack rates declined in 2001 at the Placer and Yolo County sites but continued to increase at the Sonoma County site. Field observations suggest that *C. succinea* adults emerge before yellow starthistle plants have begun to produce seed heads. This lack of synchrony may be exacerbated by the destruction of young flower buds by adult *E. villosus*. The result is a long delay between fly emergence and the occurrence of flower heads in the appropriate stage for oviposition. In the absence of flower heads, gravid female flies likely will leave the area in search of host plants with heads ready for oviposition. The incidence of *L. curtus* has been low (<1%) at all three sites.

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* resulted in a steady decline in the number of flowers per plant and the number of seeds per head. The percentage of mature heads infested by at least one biological control insect increased from 25% in 1995 to 91% in 1998 and has remained high since then (range 68-82%). In addition, there has been a concurrent decrease in seed production (13,877 to 806 seed 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 (seeds/m^2), this insect was able to rebound in 2000 and maintain a high level of attack (71%) on a larger crop of seed heads. Attack by *C. succinea* increased to 13% in 2001.

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, seeds per head and total seed production (seeds/m^2) declined in 1999 through 2001.

The density of biological control agents at the Placer County site built up quickly but has shown little change from 1995-2001 (range 52-73%). The weevil, *E. villosus* is the most abundant insect, infesting 59% of the seedheads in 2001; the other biological control agents occurred at rates >1-7%. There has been little change in plant density and flower production at this site. Seed production in 2001 increased to 5471 seeds per m^2, a level similar to values observed in 1995 & 1996. Attack by *C. succinea* at this site declined in 2001. This was the last year of observations at this site as the property was sold in late, 2001. The new landowner did not wish to participate in this study.

These observations provide evidence that these natural enemies have reduced yellow starthistle seed production at two of 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 seedhead 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 parenthesis indicate percent survivorship of seedlings to adult plants.

Placer County

Plant		**95**	**96**	**97**	**98**	**99**	**00**	**01**	**02**
Seedlings/sq. meter		-	651	669	883	666	842	762	
Adult plants/sq. m		332	83(12.7)	108(16.1)	151(17.1)	54(8.1)	109(12.9)	138(18.1)	
Heads/ sq. meter		679	280	438	378	256	355	388	
Seed/head		8.4	18.4	15.1	7.8	17.0	11.2	14.1	
Seeds/square meter		5,704	5,152	6,614	2,948	4,372	3,976	5471	
Insect & release year									
B. orientalis	93	6.6%	0.7%	1.8%	1.6%	1.1%	0.5%	0.8%	
U. sirunaseva	93	5.4%	3.8%	10.4%	13.2%	2.9%	8.3%	0	
E. villosus	93	53.6%	56.1%	56.9%	65.4%	45.2%	46.2%	59.5%	
L. curtus	94	0	0	0.2%	0.4%	0.2%	0.1%	0	
C. succinea	-	0	0	0	0%	6.2%	18.4%	7.1%	
Heads w/ 1 or more sp		62.5%	60%	66.7%	73.5%	51.6%	63.1%	65.9%	

Yolo County

Plant		**95**	**96**	**97**	**98**	**99**	**00**	**01**	**02**
Seedlings/sq. meter		-	1095	1928	1076	642	992	840	187
Adult plants/sq. m		975	322(29.4)	180(9.3)	422(39.2)	72(11.2)	285(28.7)	43(5.1)	
Heads/ sq. meter		1181	369	343	830	249	439	64	
Seed/head		23	26	13	18	17	9	7.9	
Seeds/square meter		27,163	9,594	4,459	14,691	4,275	3,951	506	
Insect & release year									
B. orientalis	91	5%	2%	4%	3%	4%	2%	1.3%	
U. sirunaseva	93	13%	18%	17%	13%	11%	13%	1.3%	
E. villosus	93	5%	19%	23%	50%	24%	32%	58.4%	
L. curtus	94	0	0	0.2%	0%	0%	0%	0	
C. succinea	96	0	2%	8%	12%	23%	14%	2.6%	
Heads w/ 1 or more sp		19%	33%	31%	57%	36%	43%	66.2%	

Sonoma County

Plant		**95**	**96**	**97**	**98**	**99**	**00**	**01**	**02**
Seedlings/sq. meter		-	897	822	624	234	1020	310	234
Adult plants/square m		241	233(25.9)	222(27.0)	231(37.0)	64(27.4)	435(42.6)	28(9.0)	
Heads/ square meter		547	442	508	486	414	622	72	
Seed/head		25.4	11.9	7.8	7.0	13.3	7.5	11.2	
Seeds/square meter		13,877	6,609	3,979	3,386	5,484	4,649	806	
Insect & release year									
B. orientalis	94	5.6%	10.6%	4.6%	1.1%	0.5%	0.3%	0.6%	
U. sirunaseva	94	5.0%	17.1%	21.2%	23.3%	21.2%	21.7%	0.6%	
E. villosus	94	13.4%	37.1%	79.2%	81.8%	59.5%	71.4%	56.4%	
L. curtus	94	0	0.2%	0.9%	1.1%	0.5%	0.3%	0	
C. succinea	-	0	0	0	1.6%	9.5%	8.2%	12.8%	
Heads w/1 or more sp		25%	58%	86%	91%	76%	82%	68%	

Seasonal Impact of Yellow Starthistle Biological Control Insects

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

Six seedhead attacking insect species are established in California as biological control agents for yellow starthistle. We have been evaluating the impact of individual insect species as well as a combination of the current insects on yellow starthistle at several sites in California, and some of these results are presented elsewhere. Yellow starthistle has an extended flowering period in California with seedheads maturing from early summer through mid fall. We have been concerned that the attack rate on seedheads is not consistent throughout the flowering period. The results presented here are a preliminary analysis of data from one-site/year combination to investigate the seedhead attack activity of currently available biological control agents. These data are from a 1999 study in Sonoma County in which all maturing seedheads on 160 yellow starthistle plants were enclosed in cotton bags to contain developing seeds and seedhead insects. Seed production and specific insect attack were analyzed across the flowering period. The site is fairly cool and usually delayed from more inland areas of California.

Seed production across the flowering period is shown in Figure 1. Clean heads, (heads not attacked by any insects) averaged over one third more seed than heads attacked by insects throughout the sampling interval. Clean heads represented less than 25% of the total number of seedheads for the year. Seed production per seedhead decreased over the season, with sharp decreases after the first sampling and again at the end of the season. The difference in seed production per head of attacked versus clean seedheads is a measure of the impact of the insects. Thus for a single sampling period such as September 1, insect attack decreased the seed production per seedhead from 20 to 12 seeds per seedhead. The lack of apparent effect on Aug 1 is largely an effect of a near absence of clean seedheads at this date, with only very small seedheads escaping insect attack. Late in the season, the total seedheads bagged each week, both clean and infested, declined dramatically making results for this period less reliable.

The percent attack by the various agents allows us to investigate deficiencies in our guild of seedhead insects across the season (Fig. 2.). The weevils, *Bangasternus orientalis* (Capiomont) (Coleoptera: Curculionidae), and *Larinus curtus* Hochhut (Coleoptera: Curculionidae), are in relatively low abundance at this site, the former seems to have been displaced in previous years by other bioagents and the latter has never established well. During 1999, their activity was so low that we could not evaluate it from the collected seedhead samples.

The gall fly, *Urophora sirunaseva* (Hering) (Diptera: Tephritidae), appears to exhibit two distinct generations at this site. A peak in oviposition occur early each year, as soon as buds are well developed, and then again in late summer with oviposition for the overwintering generation.

The hairy weevil, *Eustenopus villosus* (Boheman) (Coleoptera: Curculionidae), established an abundant population at this site. Over 60% of the seedheads are attacked by *E. villosus* at the earliest sampling dates of the season. During the succeeding 6 weeks (3 sampling periods), over 80% of the seedheads were also attacked. Attack rate then declined until the end of the season when all the weevils have died. Fortunately, at this point in the year, (late October), only a small amount of yellow starthistle was still growing and producing seed.

The most recent agent to establish at this site, the false peacock fly, *Chaetorellia succinea* (Costa) (Diptera: Tephritidae), invaded these sites between 1996-1998. Preliminary data from

other sites indicate that this agent destroys a large proportion of seed in the head. The peak of activity appears to come just as the weevil, *E. villosus*, is becoming less important.

These observations provide further evidence that these natural enemies are reducing yellow starthistle seed production at this site and that attack is consistent from the onset of flowering through September. The weevil, *E. villosus,* is clearly the most important insect to date, however, activity is limited to primarily to summer (July-September) and that many flowerheads produced after mid-September are not attacked. We hope that the false peacock fly, *C. succinea*, will continue to increase in abundance and attack the late-season flowers.

Figure 1. Seed production of yellow starthistle. Seedheads flowering at various times during 1999. Results of all seedheads (filled circles) at the Sonoma County site are compared with seedheads that were not attacked by seedhead insects (open circles).

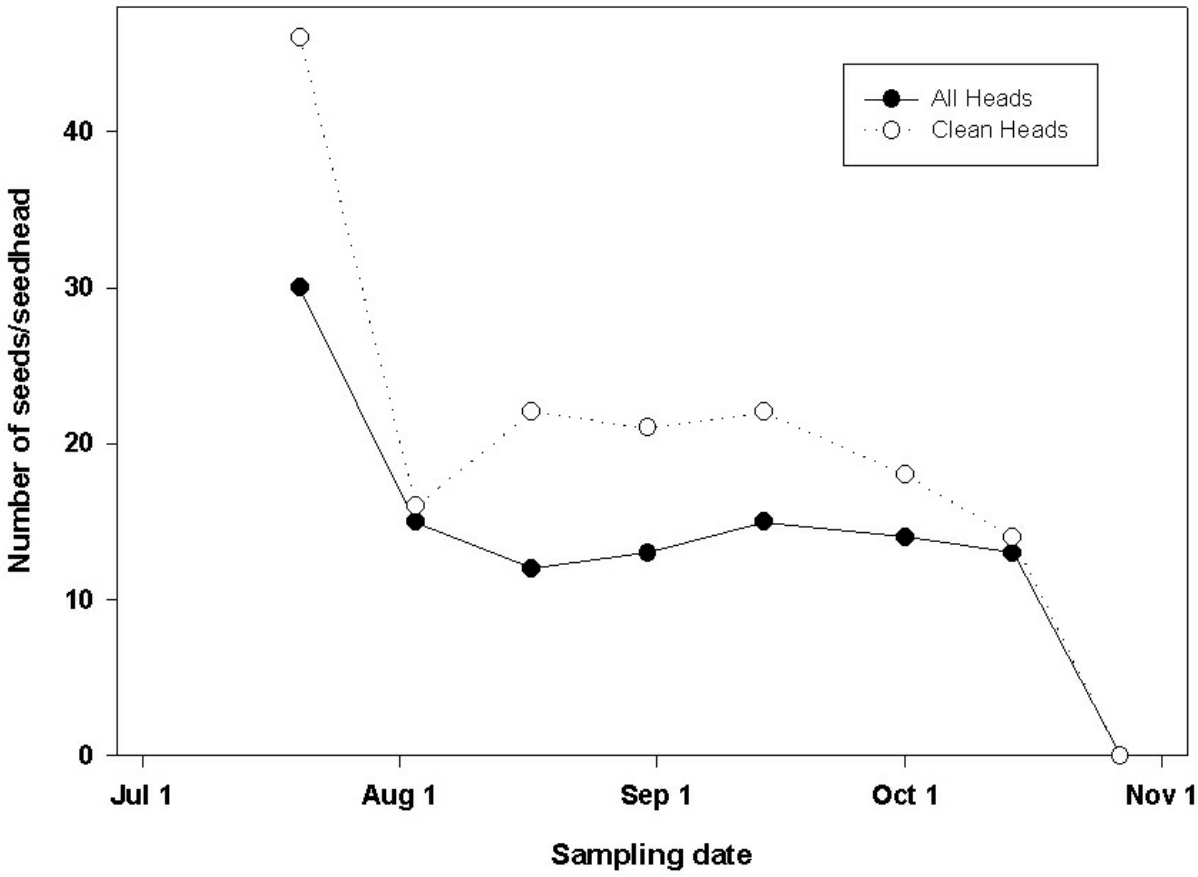
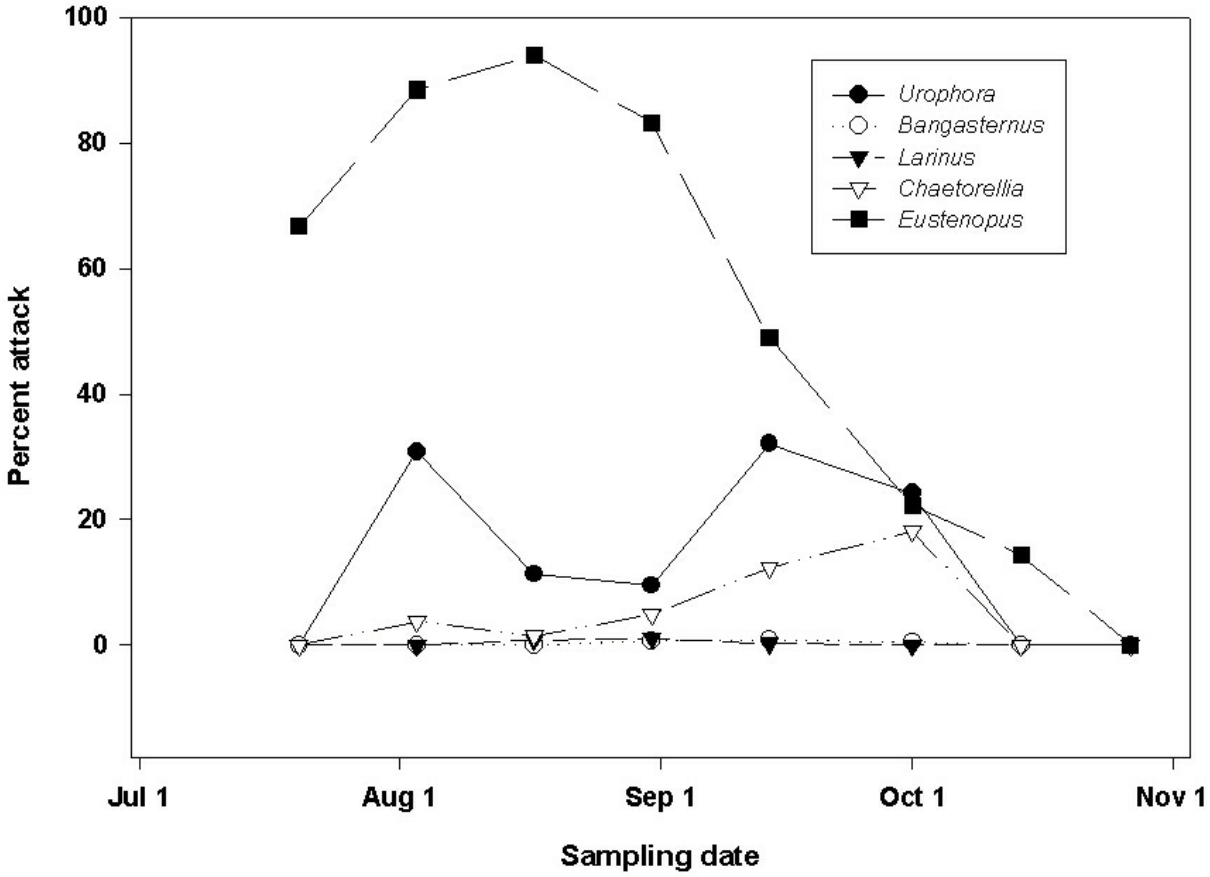


Figure 2. Percent attack of yellow starthistle by all biological control agents at the Sonoma County site in 1999.



Observations on the Overwintering Emergence of *Chaetorellia succinea* and *Urophora sirunaseva* in Central California

M. J. Pitcairn

The false peacock fly, *Chaetorellia succinea* (Costa), and the gall fly, *Urophora sirunaseva* (Hering), are two exotic fruit flies that attack the flower heads of yellow starthistle, *Centaurea solstitialis* L. The gall fly was introduced as a biological control agent in 1984; the false peacock fly was an accidental introduction probably in 1994. Both flies have a strong affinity for yellow starthistle flower heads and overwinter as diapausing mature larvae in the seedheads of their host. In spring, adults terminate diapause, pupate within the seedhead and emerge as adult flies. Following ovarian maturation and mating, females initiate oviposition on young yellow starthistle flower buds, usually 1-2 weeks before they flower.

Preliminary field observations suggested that the false peacock fly emerges very early in spring before many yellow starthistle plants have produced flower buds of the appropriate stage for oviposition. Lack of appropriate oviposition sites might cause flies to leave the area in search of plants in a more advanced stage of growth. This lack of synchrony may prevent the false peacock fly from achieving maximal impact on yellow starthistle seed production. To examine the relationship between overwintering emergence and plant phenology, yellow starthistle seedheads infested with both fly species were collected in October from four sites in central California: Placer County (elev. 430 m), Sacramento County (elev. 130 m), Solano County (elev. 20 m) and Sonoma County (elev. 400 m) and allowed to emerge in an outdoor insectary at Davis, CA. Approximately 400-800 seedheads were collected from each site. Seedheads from each site were maintained separately in screened containers exposed to natural day length and ambient temperatures. Containers were checked every 1-2 days and all emerged flies were removed, identified to sex, and counted. Information on yellow starthistle plant phenology was obtained from a concurrent study documenting the plant survivorship at a site approximately two miles south of Davis. Briefly, small 0.1x0.1 meter quadrats were placed randomly within a population of yellow starthistle plants and all plants within these quadrats were followed weekly throughout their development. From this study, the number of plants in the rosette, bolting, and budding stages were obtained.

The results from 2000 (Figure 1) showed that emergence of *C. succinea* began in early April and lasted approximately 3 ½ weeks. In contrast, emergence of *U. sirunaseva* began in late April and lasted approximately 4 ½ weeks. Emergence by *C. succinea* coincided closely with the initiation of bolting by yellow starthistle plants. However, no plants produced flower buds until early May, just as *C. succinea* completed emergence. Emergence of *U. sirunaseva*, however, began in early May and more closely coincided with the number of plants with flower buds.

Early emergence of *C. succinea* adults may lead to dispersal of the overwintering generation in search of oviposition sites. After a short period of mating and maturation of ovaries, *C. succinea* females begin search for oviposition sites. In the absence of yellow starthistle flower heads, it is likely that females will leave the area in search of plants with flower heads in the appropriate stage for oviposition (Bu-4 or “full spiny”).

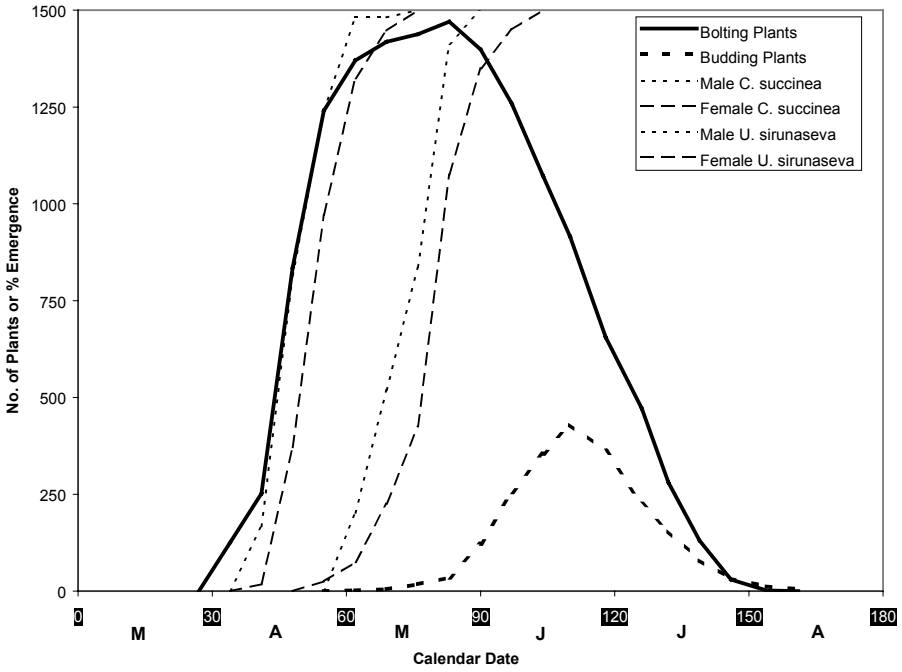
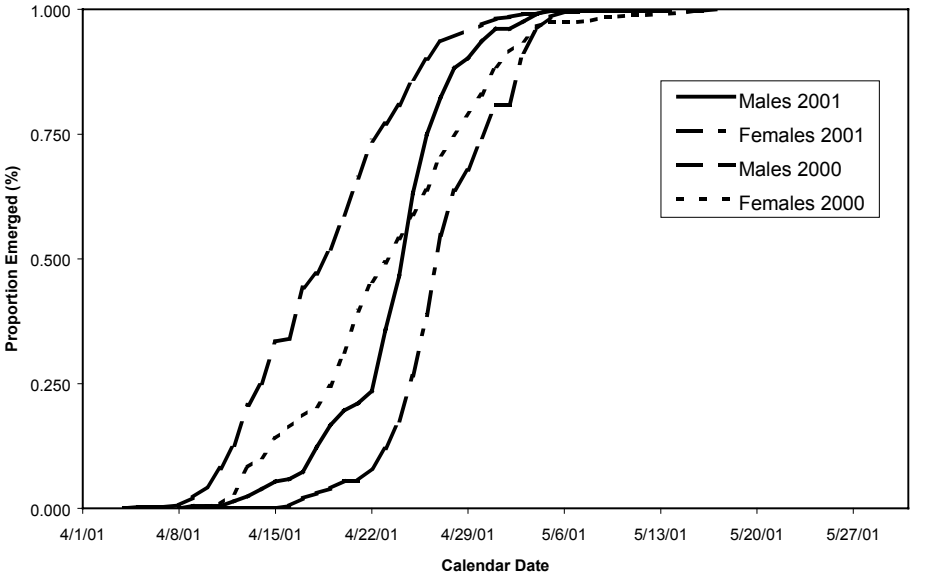


Figure 1. Proportion emergence (0-100%) of *Chaetorellia succinea* and *Urophora sirunaseva* as a function of calendar date near Davis, CA. The number of plants per stage (no. per sq. meter) was obtained from a concurrent study approximately 2 miles south of emergence monitoring location.

The emergence of overwintering *C. succinea* during 2000 and 2001 is shown in Figure 2. Males emerged approximately 1 week earlier than females during both years. The first male was observed April 5 in 2000 and on April 14 in 2001. Peak emergence (50%) occurred on April 17 and 25 for 2000 and 2001, respectively. Peak emergence for females occurred on April 22 and April 27 for 2000 and 2001, respectively. Replotting these data using degree-days above a lower threshold of 10^{o}C smoothed out the emergence curves and brought the two years closer together. Still, the difference between 2000 and 2001 is approximately 30 degree-days. Better prediction of emergence might be achieved through the use of a nonlinear biophysical model of development.



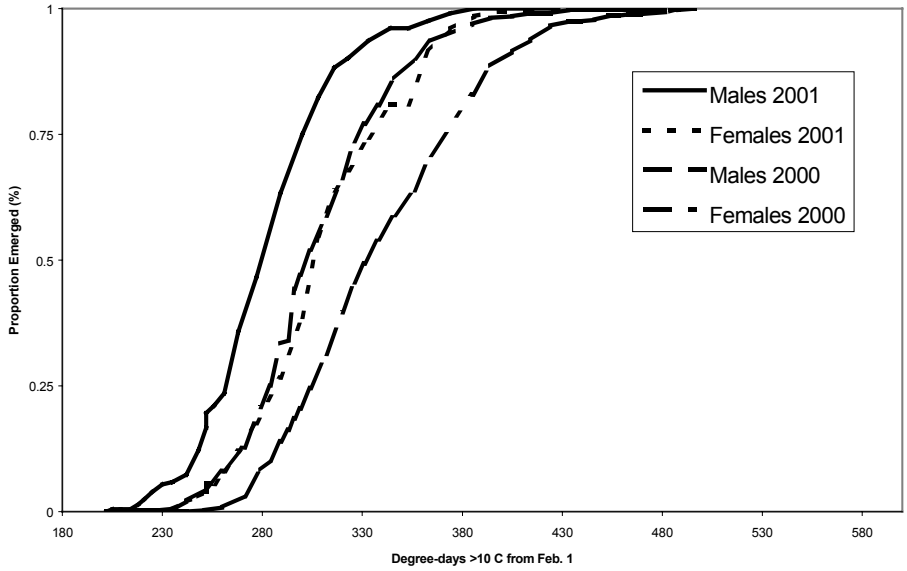


Figure 2. Emergence of *Chaetorellia succinea* adults from overwintering yellow starthistle seed heads at Davis, CA. A. Proportion emergence as a function of calendar date. B. Proportion emergence as a function of degree-days above a lower threshold of 10^{o}C. Degree-days accumulation began February 1 each year.

Statewide Survey of Yellow Starthistle Biological Control Agents

M. J. Pitcairn, B. Villegas, G. Wilber, R. Rodriguez and D. M. Woods

The California Department of Food and Agriculture in cooperation with the United States Department of Agriculture is actively involved in the release and establishment of biological control agents against yellow starthistle, *Centaurea solstitialis* L. A total of six insects have been approved and released as biological control agents in California. Of these, the bud weevil, *Bangasternus orientalis*, the gall fly, *Urophora sirunaseva*, and the hairy weevil, *Eustenopus villosus* are established and widespread. In 1988, the Biological Control Program initiated a distribution program to assist with statewide distribution of the biological control agents. Annual workshops are performed for staff of the California County Agricultural Commissioner Offices to train participants in the identification, collection, and release of these biological control agents. Participants collect available insects from nursery sites and return to their counties to establish their own nursery sites for further distribution.

The objective of the distribution program is to cover with biological control agents all areas of the state infested with yellow starthistle. Hundreds of release sites have been established throughout the state as a result of this distribution program (Table 1). We anticipate that all three insects will build up populations and spread outward once established at release sites. Presumably, insects have already begun to spread from these sites and now inhabit a much larger area of yellow starthistle infested landscape. A survey of roadside yellow starthistle populations was initiated in 2001 to document the natural dispersal of these biological control agents. Using the map of townships infested by yellow starthistle obtained in 1997, roads running through the townships rated "high infestations" were selected for survey. A minimum of 100 flower heads were collected at each sample location, then plants were swept with an insect net and the presence of any biological control insects were recorded. The date, latitude and longitude for each location were recorded. All sampled heads were returned to the laboratory and are currently being examined for the presence or absence of the biological control agents.

Table 1. Number of release sites to date for three biological control agents on yellow starthistle in California

Species	Year of introduction	No. of release sites	No. of Counties
Urophora sirunaseva	1984	191	38
Bangasternus orientalis	1985	428	50
Eustenopus villosus	1990	1090	50

A total of 278 sites were sampled throughout the state, with 67 sites processed to date. The preliminary results show that plants at 61% of the locations were infested with *U. sirunaseva*, plants at 82% of the locations were infested with *B. orientalis*, and plants at 63% of the locations were infested with *E. villosus*. In contrast, plants at 100% of the locations were infested with *Chaetorellia succinea*, an accidentally introduced seedhead fly that has a particularly strong affinity for yellow starthistle. When completed, the location data and attack rate by the biological control agents will be analyzed using a Geographic Information System to determine distance of movement of away from release sites and to predict their eventual spread throughout the state.

Tissue Carbon and Nitrogen in Yellow Starthistle in California

D. S. Spencer[1], M. J. Pitcairn, G. Ksander[1], V. Popescu and R. Wall

A number of factors can influence the success of weed biological control agents by regulating their abundance or the plant's response to them. Research in other plant systems has demonstrated that plant characteristics, especially nitrogen content, can strongly influence growth and developmental rates of insect herbivores. In particular, some studies have shown that the addition of nitrogen-containing fertilizer can result in an increase in insect herbivore abundance, including biological control agents. There have been few reports of nitrogen concentrations in yellow starthistle in the field and no studies examining the change in nitrogen content through the life of the plant. Knowledge of the seasonal fluctuations in N content of yellow starthistle may be useful in the timing releases of potential biological control agents that attack specific plant organs. For example, all else being equal, herbivores released earlier in the season would likely experience higher quality shoots or flowers as food sources (in terms of N content). Here we report on the results of an extensive survey of carbon and nitrogen in tissues of yellow starthistle from three field populations in central California.

Beginning in March 2000, we collected at least five plants (all above-ground growth and the top 15 cm of the taproot below-ground) from each of three sites located in Solano County (20 m elevation), Placer County (430 m elevation), and Sonoma County (400 m elevation). These sites were chosen to represent three different climatic regions where yellow starthistle occurs in California. Individual plants were collected within a few meters of each other. Samples were obtained at approximately weekly intervals during the growing season then monthly from October 2000 to March 2001 and taken to the laboratory for processing. In all samples, plants were separated into flowers, shoots, and roots. The carbon (C) and nitrogen (N) content of these samples were determined using a Perkin-Elmer model 2400 CHN analyzer with acetanilide used as a standard.

In late May 2001, one soil sample was collected from two depths (5-13 cm and 13 to 25 cm) at each of the three sites. Samples were sealed in plastic bags, air dried, and passed through a 2 mm sieve. The samples were sent to the University of California DANR Analytical Laboratory where selected soil properties were determined.

The results of this study showed that plant dry weight increased over the growing season. Plants at the Solano County site were larger than at the other two sites. Solano County plants began to substantially increase in size in mid-May while plants at the other sites were delayed approximately two to three weeks. Plants at the Solano County site started producing flowers earlier as well. At all three sites, the total dry weight of flowers per plant was nearly equal to shoot dry weight for plants surviving after August. Shoot tissue C changed over the course of the year (Figure 1). It was lowest in newly germinated seedlings, increased during the winter and spring and remained within a narrow range throughout the summer. For roots, tissue C generally increased later in the summer. Tissue C of flowers was similar to values measured for shoots.

In contrast with tissue C, the N content of shoot tissues was greater for seedlings and displayed a steady decline through time (Figure 1). Root tissue N displayed a similar pattern. Relative to shoots and roots, the N content of flowers was greater. Over the flowering period values were initially high, dropped slightly, then more or less constant. Except for young plants, the C:N ratio was quite high for shoots, roots, and flowers.

Examination of the ANOVA main effects (site and date) indicated that root and flower tissue C were not significantly different among the three sample sites, but they did differ through time. However, the significant interaction terms between date and site indicate that the overall effect was not consistent. This means that for some sample dates the values at the three sites were different (see table 1 for ANOVA results for Sonoma County). Shoot tissue C differed among the three sample sites and through time. However, the significant interaction terms indicate that for some sample dates the values at the three sites did not differ (Table 1).

Root, shoot, and flower tissue N differed among the three sample sites and through time. However, the significant interaction terms indicate that for some sample dates the values at the three sites did not differ (ANOVA results not shown). This is apparent from an examination of Figure 1. The tissue C:N ratio for shoots and flowers followed a pattern of significant differences similar to that described above for tissue N. Root C:N ratio was not significantly different among sites and was similar to results observed for root and flower tissue C.

Based on the limited number of soil samples we collected, soil properties at the three sample sites were quite similar, except that the Sonoma County site had higher levels of soil N and P. Mean weight of individual plants collected from these sites appeared to be inversely related to levels of soil N and P. This apparent anomaly may be explained by greater uptake of N and P to support the greater average plant weights. Unfortunately, we know of no other data on properties of soils in habitats where yellow starthistle is found, which would corroborate this observation.

Tissue carbon and nitrogen values for yellow starthistle plants (40% and 2 to 4%, respectively) were similar to values reported for plant species in other published studies. A review of published reports of other plant systems indicated that N content of different plant tissues ranged from 0.3 to 7.0% with highest concentrations (3 to 7%) occurring in growing tissues or in storage organs. Seasonal variation in yellow starthistle N content was quite large and generally followed the typical pattern described for other plant species. There were also differences among plant tissues. N levels were higher in flowers and shoots prior to the onset of flowering than for root samples. This agrees with the expected pattern of N concentration being higher in plant organs with higher turnover rates.

Shoot and flower tissue C displayed relatively small seasonal changes. However, root tissue C at each of the three sites displayed a sharp dip in April or May which may be related to bolting. Reduced root tissue C associated with early stages of flowering by yellow starthistle is consistent with the interpretation that stored materials in the roots may be important to reproduction. A practical implication of these observations is that biological control agents that attack underground organs early in the growing season may be useful in reducing flowering.

The variable tissue N levels for yellow starthistle plants in this study emphasize the need for research on the possible influence of N content on growth, reproduction, and survivorship of the insect biocontrol agents which have been released to date or which may be proposed for release in future. They also provide information for designing such studies so that their ecological relevance is maximized

[1]USDA-ARS, Exotic and Invasive Weed Research Unit, Davis, California

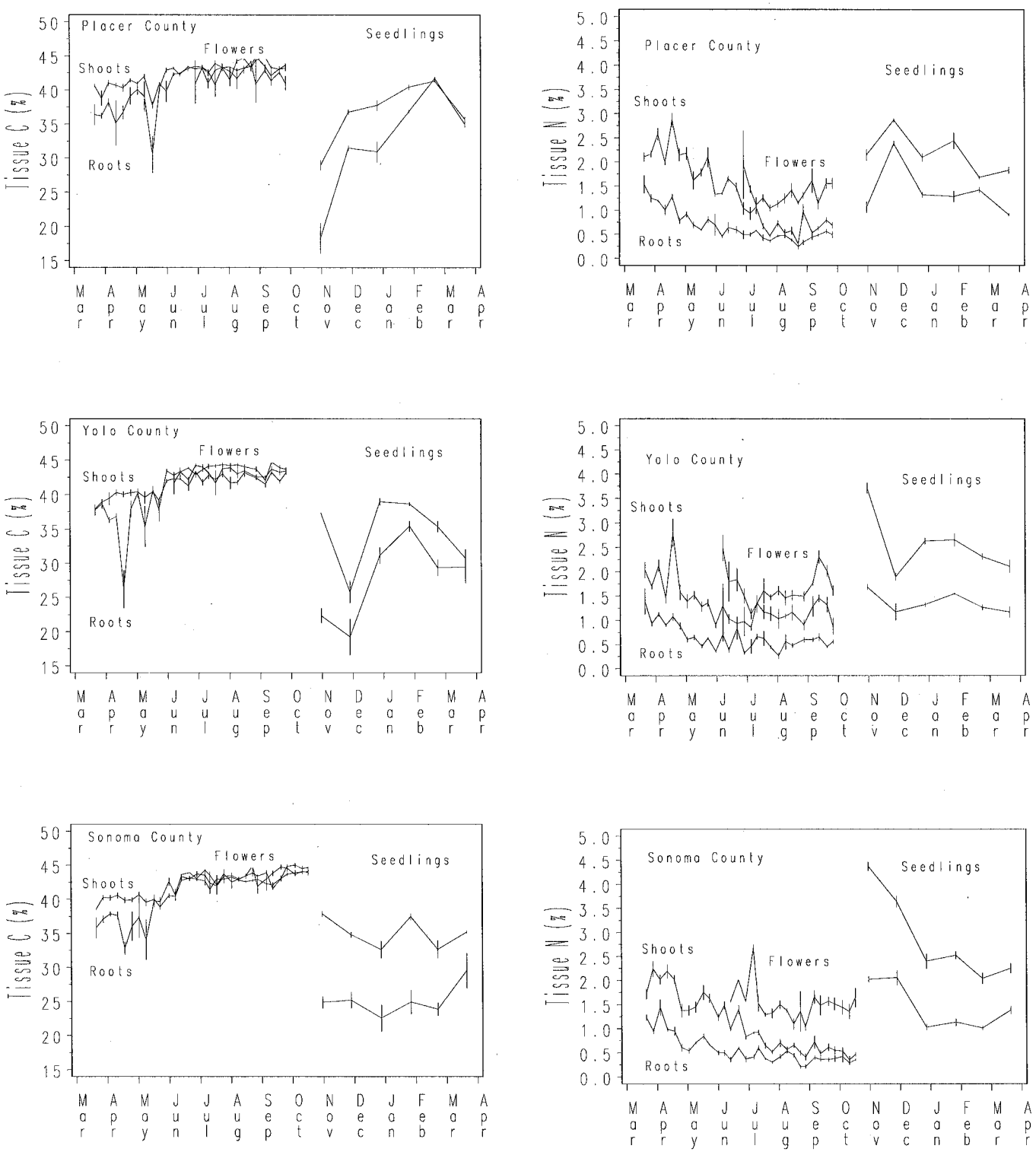


Figure 1. Tissue carbon (%) and nitrogen (%) for yellow starthistle plant parts from three sites in northern California: upper panels = Placer County, middle panels = Solano County, and lower panels are Sonoma County. Values plotted are the mean and 1 standard error.

Weed Seed Bank

P. Akers

The Biological Control Program established a weed seed bank in 1979 in order to have seeds available for propagation of host plants for biological control agents. The collection currently contains 215 accessions from 73 species of plants. They were collected from several regions throughout the state representing different geographic and climatic conditions. The seed bank also includes accessions from commercial seed suppliers. Each collection typically consists of between 200 to 3,000 seeds. The seeds are used by the Biological Control Program and by other researchers who screen natural enemies for host specificity. In this way, the seed bank supports tests of biological control agents with potential for release in California against local biotypes of the target species and for possible damage to non-target species, including both natives and crops. This year, the Seed Bank supplied seeds of nine species and varieties to four different laboratories. The species included purple starthistle (*Centaurea calcitrapa*), Sierra thistle (*Cirsium* sp.) snowy thistle (*Cirsium. occidentale*), diffuse knapweed (*Centaurea diffusa*), Sicilian starthistle (*Centaurea sulphurea*), squarrose knapweed (*Centaurea squarrosa*), and three varieties of safflower. Recipients included Tim Widmer of USDA at EBCL, Massimo Cristaforo at BBCA, Jorg Ochsmann at the Institut fur Plfanzengenetik und Julturpphlflanzennforschung, and Lincoln Smith at USDA Albany.

This year the seed bank database is being converted to from a FileMaker Pro database running on a Macintosh to MS Access running on Wintel computers, which will make it more generally accessible.

Impact of the Rust, *Puccinia carduorum* on Musk Thistle

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 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 May 26, 1999. Our goals include measuring impact of the rust on plant survival, growth and seed production. In previous years we have addressed impacts on rosettes and seedlings. This report addresses impacts on seed production.

We established adult plant monitoring transects at two musk thistle sites on Mt Shasta. The west transect was established through a dense stand of musk thistle along a berm established as a windrow in a pine plantation. The north transect was established in a shaded area under pine trees that had recently been cultivated for weed control. Musk thistle was widely spaced at this shaded site with individual plants about 1-3 meters apart. Twenty-five plants were selected along each transect and all seedheads enclosed in cotton bags throughout the summer to contain developing seeds. Plants were also rated for rust attack with a rust intensity scale (0-9) two times during the summer, with 9 = pustules covering nearly 75% of the infected leaves. A second rating system was used in late July, when the first seedheads were maturing. Plants were categorized for the height that rust pustules were produced on the plant, with rust in the lower third on the plant (1): the lower two thirds (2); or in all thirds (3). At the end of the summer, plants were harvested, weighed and seed production evaluated.

Sample processing is incomplete at this point but a partial analysis is shown in Table 1. We could not detect any clear relationship between our rust intensity rating and seed production (data not shown). Two explanations are; that rust intensity has no effect on plant reproductive success, or; that the rating system did not separate levels of plant damage. A modified rating system will be tested in 2003. Interestingly, however, the vertical distribution of the rust along the plant, may have a measurable effect on seed production. Seedheads on plants with rust in the upper two thirds of the plant produced significantly fewer seeds than plants with rust limited to the lower third. This result is particularly clear for the more open, west site. One possible explanation is that plant resources slated for seed production are derived primarily from the upper leaves, and this rating system better represents the level of plant damage caused by the rust.

Table 1. Seed production in musk thistle seedheads on plants with rust at different heights along the plant.

	Mid season height of pustules		
	1	2	3
Mean seeds/seedhead – west transect	131	89	84
Mean seeds/seedhead – north transect	177	162	165

Infestation Rates of *Rhinocyllus conicus* in Seedheads of Musk Thistle in Disturbed and Nondisturbed Areas at Mt. Shasta

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

The largest untreated infestation of musk thistle, *Carduus nutans* L., (Asteraceae), in California occurs in pine plantations on the western slope of Mt. Shasta, near Mt. Shasta City. The thistles grow predominantly on berms of soil remaining after the brush was bulldozed in the 1960's and later to make way for tree plantings.

The seedhead weevil, *Rhinocyllus conicus* (Froelich), was released on musk thistle in the area beginning in November 1974. Although musk thistle declined to less than 25% of its former abundance (Joley et al., unpublished data), significant thistle stands remain, especially in areas where there is little competing vegetation. These stands have remained at relatively dense levels during most years, and are a source of seed for both stand maintenance and initiating satellite infestations.

During 2001, we observed several large areas that had been cleared of understory brush (*Manzanita*, *Ceanothus*, and *Prunus spp*.) and now support reproductive stage musk thistle plants. At that time we observed very few *R. conicus* present on the flowerheads, similar to previous casual observations in newly disturbed sites. Since additional management of these plants was not anticipated, we decided to document the current level of *R. conicus* at one of these disturbed areas and to compare it to weevil levels at our non-disturbed monitoring sites. Additionally, we initiated a study to determine the amount of time required for *R. conicus* to recover to pre-disturbance levels. Documenting post-disturbance responses are important for management of musk thistle, especially where herbicides are precluded.

Twenty-five musk thistle plants were selected along single transects in both disturbed (North site) and non-disturbed (West site) areas. During the first visit all mature seedheads were bagged to capture insects and seeds. Many early heads had already shed seed. The remaining heads were bagged on two follow-up visits. When plants were senescent, bagged heads, stems, and rosette leaves were harvested. Only the seedheads were processed for this study. Florets were scraped from the receptacle and the receptacle diameter was measured. Heads were dissected carefully with hand shears and forceps, and the number of larval chambers counted. We also counted weevil larvae, pupae, and adults that were free in the bags or retained in the heads, and the total numbers used to adjust otherwise low counts of chambers. Table 1 compares various plant and weevil attributes for both sets of plants. These results will provide a baseline with which to compare during weevil recovery in succeeding years.

Table 1. Musk thistle seedhead production and attack by *R. conicus* at disturbed and undisturbed sites near Mt. Shasta, California

Site	Mean No. of Seedheads/Plant	Percent of Seedheads Infested by *R. conicus*	Mean No. of Weevil Chambers/Seedhead
North (disturbed)	5.0	16	<0.1
West (undisturbed)	6.2	53.2	5.2

Musk thistle seedheads in the recently disturbed north site virtually escaped attack by *R. conicus* during the first year of flowering, despite the presence of weevils in undisturbed areas less than 1 kilometer away. It seems reasonable to expect that the influx of fresh seed and lack of

plant competition will lead to a considerable resurgence in thistle density over the next few years. Interestingly, nearly all plants observed at the disturbed north site as well as those at other disturbed areas in 2001 were reproductive. Apparently, very little germination had occurred the second year following disturbance as there were few vegetative (first year) plants. Previously, we had observed almost no musk thistle in these areas for the past decade or more, presumably because of the presence of dense cover. It remains unknown whether the resurgence of musk thistle is a result of seeds already present as a dormant seedbank or seeds moved in by heavy equipment at the time of disturbance. A quick response to eliminate musk thistle plants before seeds were shed this year might have substantially reduced the thistle population at the north site and other disturbed areas, but now these areas will likely become heavily infested and remain that way for several years to come.

Further studies may be warranted to document the time required for invasion of *R. conicus* and the build up of competing vegetation returns musk thistle to levels comparable to undisturbed areas. This will provide land managers with specific information as to possible risks of vegetation removal on releasing noxious weeds such as musk thistle.

Impact and Interaction of *Bangasternus fausti* and *Larinus minutus*, on Diffuse Knapweed in Trinity County

D. B. Joley, D. M. Woods 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. One exception occurs in the South Fork Mountain area of Trinity County where the Biological Control Program has had an ongoing project to release and evaluate available biological control agents. Six agents are currently established in this area, with two (both capitulum weevils) *Bangasternus fausti* (Reitter) (Coleoptera: Curculionidae) and *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae) having increased to high levels at their respective release sites and appearing to be making an impact on seed production. *B. fausti* was first released in 1994 along Miller Road at a location named the 'monitoring' site. *L. minutus* was first released in 1995 approximately 0.5 mi away at the 'Upper Miller' site. By 1998, both weevils could be found at both sites in low numbers, and by 2001, both were well represented. For this report, we thought it would be useful to compare the distribution of these two weevils in seedheads of individual plants collected from the two sites over the last three years to evaluate whether or not there was an emerging pattern of dominance. In addition, we initiated a preliminary evaluation of seed destruction by the two weevils.

In the first study, whole plant samples were collected from the two sites along Miller Road during autumn 1999, 2000, and 2001. The infestation rate was estimated by examining under a microscope all heads from each plant for insects, empty pupal cells, or other evidence of larval damage. Although there is similarity in damage caused by the two weevils, damage can usually be ascribed to a single species, so percent infestation is reported for each weevil on each individual plant (Fig. 1).

Infestation rates of *B. fausti* and *L. minutus* showed 'tight patterns' at the two respective release sites in the 1999 plant samples. Thus, attack seems determined by the density of weevil species present with little interference occurring between the two weevils. Over the next two years, each species tended to maintain its predominance at its original release site, but also began to increase at the other site. It will probably require several more years of sampling to determine what kind of equilibrium will occur between the two weevils. In 2001 a single plant at each site was infested primarily with the weevil species released at the other site. This may indicate that it is possible there are cues that allow the two weevils to partially avoid each other. As noted in a previous annual report, there were seedheads with *B. fausti* eggs that contained adults or other stages of *L. minutus*, indicating some degree of interference.

Overall infestation rates were high at both sites. There were plants at both sites that tended to escape attack, but these plants had developed late, relative to weevil activities, and were still green and had numerous immature seedheads when harvested. It still 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

In the second study, 25 (post-anthesis) flowerheads of diffuse knapweed were covered using small cotton bags tied with drawstrings during July 2001 to capture seeds and insects at each of the two sites. Bagged heads were retrieved in mid-autumn and transported to the

laboratory for processing. Seedheads were examined and dissected; eggs (*B. fausti*), feeding damage, and life stages (by species) were recorded. Firm achenes were removed, saved, and cut later; those with a firm embryo were counted as viable. Only clean heads (no visible feeding on seeds or on the receptacle) and heads damaged by *B. fausti*, and *L. minutus* were used for comparing seed destruction.

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 two sites along Miller Road.

Seedhead Status	Ave. No. of Seeds/Seedhead
Infested with *B. fausti*	0.5
Infested with *L. minutus*	2.2
Clean[1]	10.0

[1]Includes heads with *B. fausti* eggs but no larval feeding in receptacle

The results are shown in Table 1. Both insects clearly caused significant seed destruction, compared to the clean heads, but neither species completely destroyed all seeds in every head they infested. It is somewhat surprising that *B. fausti* appears to decrease seed production slightly more than does *L. minutus* because we assumed that the latter, being larger, would be more destructive. However, there were too few samples to make a definitive comparison. Heads attacked by *B. fausti* were slightly smaller than those attacked by *L. minutus*, which may have accounted for the difference. Further sampling for a few more years is warranted and should include larger samples and later-developing seedheads.

Seasonal Incidence of Seedhead-Attacking Insects on Spotted Knapweed

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

There are currently five seedhead feeding biological control agents established on spotted knapweed, *Centaurea maculosa* Lamarck (Asteraceae), in California. Three of these, *Urophora affinis* Frauenfield (Diptera: Tephritidae), *Terellia virens*, (Loew) (Diptera: Tephritidae), and *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae), were intentionally released whereas *Urophora quadrifasciata* (Meigen) (Diptera: Tephritidae) migrated from knapweeds in Oregon and *Eustenopus villosus* (Boheman) (Coleoptera: Curculionidae), spread from yellow starthistle. This report describes some interactions of these insects occurring across the summer season, over a three-year period. Data were obtained from our bagging studies, using a methodology designed to collect maturing insects and developing seeds from individual seedheads. Seedheads with oxidized flowers of spotted knapweed growing along the Pit River in Shasta County, were enclosed in cotton bags every other week during the flowering season over three years (approximately 50 bagged per week per year). At the end of the season, the bagged, mature seedheads were dissected and inspected for viable seed as well as evidence of attack by the seedhead insects. Results are shown in Fig 1.

The tephritid fly, *T. virens*, has not established well enough at this site to evaluate seasonal and multi-year changes. Attack rates have always remained less than 3% for any single sampling period.

Galls produced by the gall fly, *U. quadrifasciata*, were very common and numerous in 1998 and in earlier years. In 1999, and 2000, the percentage of spotted knapweed seedheads sporting galls was dramatically down at all sampling periods during the year. Additionally, in 1998, galls of *U. quadrifasciata* were highly concentrated with several (3-9) galls in each seedhead. By 1999, most galled heads had less than three galls per seedhead. The reduction in galls is most apparent in the middle and later half of the season (July and August) when galls become somewhat rare. The other gall fly, *U. affinis*, has a single generation per year with peak attack percentage in mid July. After the peak the decline is quite rapid. The decline is also progressive over the years with fewer galls each year during the later half of July through August. This decline coincides with the increase in *L. minutus*.

The two weevil species seem largely responsible for the declines in gall flies. The papery *U. quadrifasciata* galls and enclosed larvae are almost completely consumed by both weevils. The woody *U. affinis* galls are incompletely consumed but the fly larvae almost always are consumed. Populations of the weevil, *L. minutus*, have greatly increased over the years, with the overall attack percentage increasing from 22% to 43% to 66% for the 1998 to 2000 seasons. *L. minutus* seems particularly successful during July-early August, infesting nearly every head and eliminating all other species. A single generation is completed each year, although adults seem to be active most of the summer. This gall fly emerges early in the summer, and it appears that the early emergence before *L. minutus* and *E. villosus* begin oviposition remains the only hope for survival. Likewise the hard gall producing *U. affinis* is becoming severely limited in mid July-August, with total galled plants nearing elimination

The hairy weevil, *Eustenopus villosus* is actually a yellow starthistle biological control agent that seems capable of attacking and completing its life cycle on spotted knapweed. All life stages have now been confirmed on spotted knapweed in our bagging studies. *Eustenopus*

villosus seems to prefer yellow starthistle to spotted knapweed. We have noticed *E. villosus* on yellow starthistle in the area for the past several years, but could not confirm attack on spotted knapweed until 1999. During 1999, the yellow starthistle population in the area of our plot was very low and surviving plants were extensively fed upon by *E. villosus,* so that the plants remained severely stunted until the fall. Without its preferred host during the summer, the weevil moved over to spotted knapweed and we achieved our highest attack levels to date. In 2000, attack levels declined, but all life stages were again detected.

Figure 1. Percent attack by biocontrol agents in seedheads of spotted knapweed for the years 1998(circle), 1999 (square), and 2000 (triangle).