Biological Control Program
Annual Report
2002

California Department of Food & Agriculture

BIOLOGICAL CONTROL PROGRAM

2002 SUMMARY

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Cover developed by Baldo Villegas. From top clockwise; Water hyacinth infestation near Stockton; *Neochetina bruchi* weevil and adult feeding damage; weevil larvae damage to roots and stems; water hyacinth flower cluster.

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PREFACE

The winds of good fortune are fickle. Last year, I announced the addition of new staff to the Biological Control Program. Pat Akers and Syed Khasimuddin had filled recent vacancies. Also, Bill Roltsch had just transferred to Sacramento from the Imperial Valley. These new individuals had energized the Program and taken off running in setting up their newly assigned projects. Unfortunately, it was not to last. The budget shortfall faced by the State of California resulted in a significant budget cut to the California Department of Food and Agriculture. The result was a cut of nearly 50 percent to the personnel and operating funds of the Biological Control Program. Funding for eight permanent staff was cut. Funds were also substantially reduced for all temporary (hourly) staff. Three of the permanent staff, Pat Akers, Syed Khasimuddin, and Debbie Mayhew were transferred to vacancies in other programs within the Department. Kris Godfrey and Don Joley were offered and accepted positions in other programs. The remaining three permanent positions and all temporary staff are now supported with outside funding. It was a great disappointment to have received this reduction in our funding. Last year, the Biological Control Program had made great strides in many of its projects. Some problems in the rearing of lygus bug were solved and natural enemy production levels were steadily increasing. Insects recently released on squarrose knapweed in northern California were showing rapid population increases and concomitant decreases in plant densities. A new parasite species for the pink hibiscus mealybug (PHM) was received and its production and release were just getting underway. New biological control agents on olive fly, yellow starthistle (YST), and Russian thistle, were nearing approval and pre-release studies were being initiated.

The reduction in staff and funding necessitated a re-prioritization of projects pursued by the Biological Control Program. Currently, the Biological Control Program consists of two scientists working on insect pests and two scientists working on weed projects, an agricultural biological technician, and a program manager. The insect projects include the PHM, lygus bug, cotton aphid, and olive fly. A small amount of time is directed at completing the red gum lerp psyllid and silverleaf whitefly projects. The weed projects include propagating and releasing the YST rust disease recently approved for introduction into the western United States, and biocontrol efforts against squarrose knapweed, water hyacinth, and purple loosestrife. Workshops for the redistribution of the YST insects have been discontinued as the insects are now widespread throughout the state and occur wherever YST is found growing. The Program has been very fortunate to receive funding for several projects including olive fly, PHM, lygus bug, water hyacinth, and the YST rust. These funds have helped greatly and provide support for the amount of staff and operating expenses necessary to perform these projects.

TABLE OF CONTENTS

Insect Projects

Weed Projects

Releases of Parasitoids for Control of the Olive Fruit Fly

C. H. Pickett, R. Rodriguez, D. Asakawa[1], and R. Messing

Activities for the biological control of olive fruit fly project in 2002 included, additional releases of *Psyttalia concolor* in Santa Barbara, monitoring potential release sites in Yolo County, sampling a former release site in Riverside, and starting an olive fruit fly rearing system at the Meadowview facility in Sacramento.

Releases in Santa Barbara began September 10, and were made weekly for four weeks. Approximately 500 adults were released on each date, alternating between one of two private properties on Mission Canyon Road. The initial release was caged while subsequent releases were both caged and open. On October 7, fruit from inside the sleeve cage was collected and yielded two *P. concolor*, showing within-season recovery. Two adult *P. concolor* were reared from approximately 200 fruit collected on September 24, at one of the Mission Canyon release sites, which had not received any releases in two years. These could represent parasitoids that have permanently established in this area. An additional 899 fruit were collected on November 5, from four other year 2000 release sites to confirm the establishment of *P. concolor*. No parasitoids were recovered eight weeks after they were placed in paper buckets and held at room temperature (23 °C).

In October, fruitfly parasitoids were collected in Kenya by Dr. R. Wharton, (Texas A & M University) and Robert Copeland. They discovered a large population of *Psyttalia lounsburyi* and *Utetes africanus* in a natural forest of wild olive trees at the base of Mt. Kenya. A small number were permitted for release after clearance through the University of California, (UC) Riverside quarantine facility. On October 22, approximately 90 *P. lounsburyi* and 10 *U. africanus* were released into separate sleeve cages at a single property on Mission Canyon Road in Santa Barbara. Seven days later, cages were removed. Approximately 20 of the *P. lounsburyi* were still alive inside the cage when it was opened allowing parasitoids to disperse.

Three locations were monitored in Yolo County for the presence of olive fruit fly. Fruit was collected and traps were placed (ChamP®) in five trees at each location. We plan on releasing *P. concolor* in this area and need background data on the fly population (Table 1). In addition to fruit flies, staff have reared a large number of *Pteromalus* sp. from fruit collected at Jurupa Cultural Center and in Santa Barbara (identified by S. Heydon, UC, Davis).

Table 1. Olive Fruit Fly Fruit and Trap Field Data 2002, no *P. concolor* were Recovered from the Fruit

Location	Date Collected	Number Fruit Collected	Flies/ Fruit	Flies/ Gram	Flies/Trap/ Day[1]
Davis 1, Yolo County	August 29, 2002	121	0.008	0.013	0.048
	September 25, 2002	147	0.0	0.0	0.177
	November 12, 2002	209	0.191	0.012	----
Davis 2, Yolo County	August 29, 2002	96	0.0	0.0	0.004
	September 25, 2002	144	0.0	0.0	0.037
Jurupa Cultural Center, Riverside County	February 24, 2002	62	0.677	0.713	----
	September 10, 2002	396	0.075	0.096	----
Mission Canyon Road, Santa Barbara County	September 17, 2002	299	1.033	0.798	----
	November 5, 2002	899	0.427	0.390	----
University of California, Wolfskill Field Station, Solano County	August 29, 2002	113	0.0	0.0	0.02
	December 3, 2002	201	0.0	0.0	----
University of California, Davis Bee Biology	September 3, 2002	100	0.01	0.006	----

[1]Traps were not used where data are missing

Pink Hibiscus Mealybug Biological Control in Imperial Valley, California, 2002 Update

W. Roltsch, D. Meyerdirk[1], E. Andress[3], J. Brown, K. Carrera, and J. Zuniga

The pink hibiscus mealybug (PHM), *Maconellicoccus hirsutus* (Green), was first detected in Imperial Valley, during August 1999. Population densities of PHM on mulberry, carob, silk oak, hibiscus and natal plum were determined to be high in several urban communities in southern Imperial Valley (Figure 1). Two parasitoid species, *Anagyrus kamali* Moursi and *Gyranusoidea indica* Shafee, Alam and Agarwal, were released at 10 sites in the fall of 1999. Subsequently, an insectary was established in El Centro for additional parasitoid production. The two species were then produced locally and released beginning in 2000. The culture of *A. kamali* produced up through 2001, originated from collections in China and Hawaii that were combined. *G. indica* was a combination of populations from Egypt, Pakistan, and Australia.

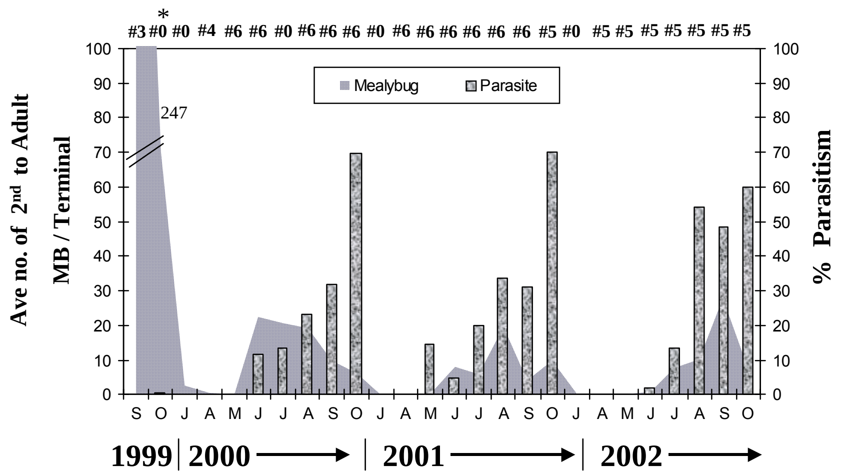
Production and Release: In 2002, a new culture of *A. kamali* was started. This population was collected in the very warm and dry climate of Upper Egypt, by Dr. Dan Gonzalez (UC, Riverside) in 2001. We produced and released approximately 213,000 *A. kamali* in 2002 (Table 1). Parasitoids were either released in Imperial Valley and provided to Mexican authorities for release in Mexicali Valley or sent to state and federal authorities in Florida for release against the recent PHM infestation in that state. In terminating the culture of *G. indica*, a final release of 13,800 parasitoids was made in California during January 2002.

Table 1. Destinations of PHM Parasitoids Produced at the California Department of Food and Agriculture Insectary, El Centro, CA in 2002

	Imperial Valley, California		Mexicali Valley, Mexico	Florida	Total
Month	**A. kamali***	**G. indica**	**A. kamali**	**A. kamali**	
January	1,200	13,800			15,000
February	3,100				3,100
March	9,050				9,050
April	10,150				10,150
May	9,100		3,500		12,600
June	0		12,400		12,400
July	1,850		15,250	9,600	26,700
August	2,650		7,100	12,000	21,750
September	34,500			12,000	46,500
October	26,250			9,600	35,850
November				9,600	9,600
December				10,800	10,800
Total	**97,850**	**13,800**	**38,250**	**63,600**	**213,500**

*From February 2002 onward, *A. kamali* culture was from Egypt, prior to that time it represented a combined culture of collections from China and Hawaii.

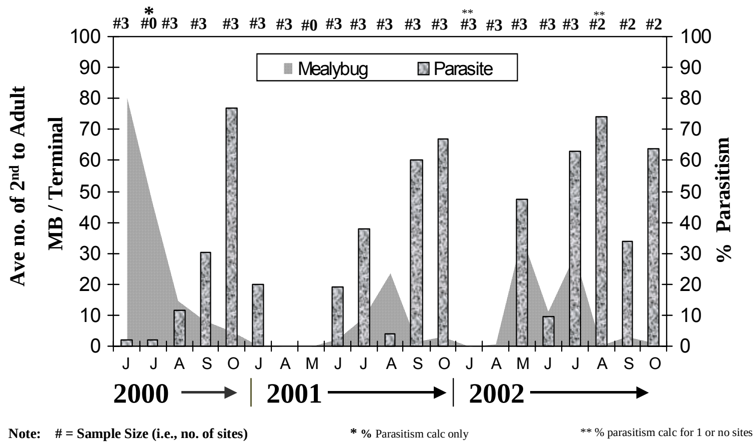
Figure 1. Pink Hibiscus Mealybug on Mulberry
Imperial Valley, California



Note: # = Sample Size (i.e., no. of sites)   Mulberry terminal samples not available in January   * % Parasitism calc only

Population monitoring: We continued PHM population density and parasitism monitoring on mulberry and carob trees at the same sites selected at the inception of the PHM project. Population densities on infested mulberry trees averaged over 200 mealybugs/terminal in September 1999 (Figure 1). Corresponding with the broad establishment of *A. kamali*, PHM densities have been consistently low for three consecutive years. *G. indica* is also established in Imperial Valley, however the numbers are typically low during the year, particularly during the warmest months from June through September. In 2002, less than 10 percent of all parasitoids collected during the year were *G. indica*, however, *G. indica* represented 21 percent of the primary parasitoids collected in October. Similar results have been recorded at three study sites consisting of carob trees (Figure 2). PHM densities were high initially, but with the onset of parasitism, population densities have been considerably lower.

Figure 2. Pink Hibiscus Mealybug on Carob Tree
Imperial County, California



Hyperparasitoid activity against introduced species: The impact of hyperparasitoid species **native** to Imperial Valley on newly introduced primary parasitoid species is being monitored. A hyperparasitic species (*Marietta* sp.) was first collected in July of 2000. At that time, its occurrence was quite rare. Dissected samples confirmed that the primary parasitoid, *A. kamali* is under attack by *Marietta* sp. (Aphelinidae) and, to a lesser extent, by *Chartocerus* sp. (Signiphoridae). *Marietta* sp. was common through the remainder of 2000, as represented by the percent of PHM mummies, from which hyperparasitoids emerged (mean percentage): early August, 11 percent, five sample sites; late August, 51 percent, six sample sites; September, 10 percent, six sample sites; and October, 38 percent, nine sample sites. Hyperparasitoid attack of *A. kamali* has remained approximately the same in 2001 and 2002.

Non-target impact of parasitoids: A number of samples of two resident species of mealybug have been collected over the past two years to monitor for non-target impacts. Ten separate collections of the solenopsis mealybug (*Phenacoccus solenopsis* Tinsley) and 13 collections of the striped mealybug, *Ferrisia virgata* (Cockerell) have been made in Imperial Valley. The former species is native, whereas the later is not a native species. To date, neither *A. kamali* or *G. indica* have been recovered from either mealybug species, thereby demonstrating that they are either moderately or highly host specific.

Area-wide survey: For the third year, an extensive survey was implemented to identify the extent of the PHM infestation in Imperial Valley and to determine if it was present in agricultural crops. In total, 1,863 urban sites and 1,490 agricultural sites were surveyed for PHM infestations. The regional distribution of PHM continues to be limited to the town of Imperial and areas south to Calexico. The PHM distribution has changed remarkably little from the spring of 2000 to the fall of 2002 and has not been found in any commercially grown crops.

The PHM was detected at approximately eight percent of the urban sites (mainly home yards) in Calexico in 2002; similar in value to 10 percent of the sites surveyed in 2001, down from 38 percent in 2000. In contrast, the PHM was detected at approximately 28 percent of the sites monitored in El Centro, up from nine percent in 2001 and 15 percent in 2000. The percent detection in the remaining communities in 2002 was similar or lower than recorded in previous years. It is noteworthy to point out that PHM density on mulberry trees and other host plants at the El Centro Naval Facility continued to be elevated when compared to most other locations in Imperial Valley. Over 50 percent of the mulberry and hibiscus host plants (more than 100 plants) had detectable populations of PHM. Follow up field samples determined that the population densities were among the highest in Imperial Valley. Furthermore, the native mealybug, *P. solenopsis*, exhibited elevated population densities as well. The combination of these two events strongly suggests that biological control is being disrupted at this location. This may be due to mosquito abatement insecticide application that is common at that site.

<u>Summary for 2002:</u> The two biological control agents released against the PHM have become widely established throughout infested areas of Imperial Valley, and at least one species has had considerable impact to date. Overall regional densities of PHM have decreased markedly since 1999 and its distribution has been unchanged and continues to be restricted to urban locations.

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Importation and Establishment of *Lygus hesperus* Nymphal Parasitoids

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

Lygus hesperus is a serious pest of several field crops across the United States. In California it causes about 30 million dollars in damage to cotton each year (Goodell, pers. comm.), and is a serious problem to growers of strawberries along the central coast of California at an estimated cost of 40.3 million dollars (www.ipm.ucdavis.edu, Zalom, pers. comm.). It is also a pest to numerous seed crops including alfalfa (UC Cooperative Extension 2000). Currently lygus is managed on most crops through applications of broad-spectrum insecticides. Cultural and biological alternatives are not considered useful. Although several natural enemies attack this pest, they are not economically effective. Importation of nymphal parasitoids in the eastern United States during the 1980's, successfully reduced *Lygus lineolaris* (a close relative of *L. hesperus*) infesting alfalfa. This project proposes doing the same for *L. hesperus* in central California. Lygus is highly polyphagous, moving between numerous plant species throughout the year. Therefore, one approach to reducing this pest's damage in cotton would be to reduce its densities in other preferred host plants. The objective is to reduce regional populations of *L. hesperus*, thus reducing the numbers that migrate into cotton each spring.

<u>Accomplishments Through Fall 2002:</u> The California Department of Food Agriculture's (CDFA) Biological Control Program has been actively importing and releasing *Peristenus* spp. (Hymenoptera: Braconidae) over the last five years. The United States Department of Agriculture, Agricultural Research Service (USDA-ARS) and CABI Bioscience explorers have collected parasitoids in southern France, northern and central Italy, and eastern Spain and south to the province of Granados (Table 1). Parasitoids, shipped as cocoons, have been sent to either the USDA-ARS quarantine facility in Newark, Delaware, or the Agriculture Agri-Food Canada quarantine in London, Ontario. Both agencies stored cocoons through the winter, then reared and cleared the adult parasitoids for shipment to the CDFA in Sacramento, California. Collections have been made in increasingly more southern sites, starting with southern France then moving to southern Spain, which matches most closely with the climate of central California.

The CDFA has reared *Lygus* sp. and *Peristenus* spp. following methods developed by the USDA-ARS in Newark, Delaware and others. Parasitoids received from these two quarantine facilities were either released directly into study plots of alfalfa or reared for future release. We received approximately 100 to 500 parasitoids each summer. Approximately 1,100 parasitoids were released in fall of 1998, then 6,000, 15,000, and 14,710 during summers 1999, 2000, and 2001, respectively. Almost 20,000 were released in 2002 (Table 2). However, over half of these were parasitized nymphs moved from our Sacramento release site. They have been released at 11 locations, from Kern County in the south to University of California, Davis in the north (Table 3). By maintaining year-round production of parasitoids in Sacramento, the overall numbers of parasitoids released each year, as well as initial field releases earlier in the year have increased. The earlier in the summer that releases are made, the greater the number of parasitoid generations produced, and hence, the higher the probability for permanent colonization. Most populations of *P. stygicus* and *P. digoneutis* were released at most sites. After four years, releases of *Peristenus* spp. ceased at the first release site (North B Street, Sacramento, CA) summer 2001.

Table 1. Locations for Releases of Parasitoids, 1998 to 2001

Species and Population Released Country (Province, nearest City)	Location Released	Lat/Long		Year Released
Peristenus stygicus, France (Hérault, Lattes)	North B Street, Sacramento	38º35.607'N	121º29.519'W	1998 1999 2000
	UC/USDA, Shafter			2000
Peristenus stygicus, Italy (Veneto, San Dona' di Piave)	UC, Davis	38º32.403'N	121º45.919W	1999 2000
	UC Kearney Ag Center			2000
Peristenus stygicus, Spain (Cataluña, Navata)	Fong's Farm, Yolo County	38º41.145'N	121º53.574'W	1999 2000
	Sander's Farm, Kern County			1999 2000
	Triple S Farms, Merced	37º08.508'N	120º18.604'W	2000 2001
Peristenus stygicus, Italy (Umbria)	UC/USDA Shafter	35º31.952'N	119º16.701'W	2000 2001
	North B Street, Sacramento	38º35.607'N	121º29.519'W	2001
	UC Kearney Ag Center	36º35.863'N	119º30.646'W	2001
	Poplar Avenue, Kern2	35º33.4'N	119º17.64'W	2001
Peristenus stygicus, Spain (Andalucia, Granada)	Triple S Farms, Merced	37º08.508'N	120º18.604'W	2001
	Madera	36º59.54'N	120º20.724'W	2001
	Poplar Avenue, Kern2	35º33.4'N	119º17.64'W	2001
	Castroville	36°46.012	121°42.887	2002
Peristenus digoneutis, (Veneto, San Dona' di Piave)	North B Street, Sacramento	38º35.607'N	121º29.519'W	1999
Peristenus digoneutis, Italy (Umbria)	North B Street, Sacramento	38º35.607'N	121º29.519'W	2001
	UC/USDA, Shafter, main	35º31.952'N	119º16.701'W	2000 2001
	Poplar Avenue, Kern2	35º33.4'N	119º17.64'W	2001
	Madera	36º59.54'N	120º20.724'W	2001
	Coast, Santa Cruz			2001
Peristenus digoneutis, Spain (Catalognia)	Coast, Santa Cruz			2001
	Castroville	36°46.012	121°42.887	2002
	UC/USDA, Shafter	35º31.952'N	119º16.701'W	2002
	UC Kearney Ag Center	36º35.863'N	119º30.646'W	2002
	UC, Davis			2002

Table 2. Peristenus Field Release Data Summary, 2002

Site	*P. stygicus*		*P. digoneutis*		
	Granada, Spain Released	Umbria, Italy Released	Catalonia, Spain Released	Nymphs Transferred	Total Insects Released
Castroville	0	100	776	1,056	1,932
UC KAC	703	0	268	4,603	5,574
Madera	204	0	0	0	204
Merced	316	0	0	0	316
SREC	0	0	0	1,778	1,778
SREC-Main	0	441	419	540	1,400
SREC-S40	674	0	344	1,400	2,418
UC, Davis	57	596	1,101	4,450	6,204
Total	1,954	1,137	2,908	13,827	19,826

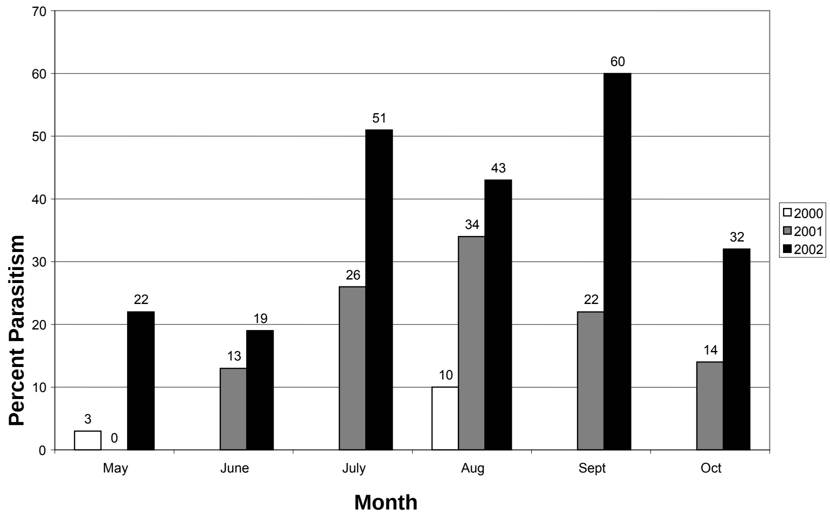
Results of recoveries at our first release site in Sacramento are encouraging. *Peristenus* were first recovered in May 2000. Each subsequent year parasitism levels have climbed at this location reaching a high of 60 percent in September 2002 (Figure 1). Both *P. stygicus* and *P. digoneutis* were recovered, over 16 months after the last releases at this location. Identities were verified by Henri Goulet (Ottawa, Ontario, Agriculture and Agri-Food, Canada*). P. stygicus* was dominant at the beginning of the summer, but both species were equally represented by October 2002. This is the first report of *Lygus* nymphal parasitoids successfully persisting and building in population size, in California. Furthermore, these two species have been firmly established.

Table 3. Recoveries of Released Parasitoids

	Maximum Parasitism (n)		
Location	**2000**	**2001**	**2002**
Sacramento	10.0	34.0(32)	60.0 (30)
UC Davis	0.0	4.0 (50)	2.0 (50)
Merced	--	14.0 (7)	0.0 (0)
Madera	--	59.0 (2)	0.0 (14)
UC Kearney	24.0 (25)	12.0 (25)	10.0 (40)
UC/USDA Shafter1	--	5.0 (17)	0.0 (40)
UC/USDA Shafter2	--	--	0.0 (40)
Castroville[1]	--	--	--

[1]releases-initiated summer 2002

Figure 1. Percent Parasitism at Sacramento Site



Recoveries at other locations have been less than that in Sacramento (Table 4). Although we have made overwintering recoveries at most of these sites, there have not been consistent increases in parasitism over the last three years. There may be several reasons for these results. One may be current alfalfa management practice. We have recently learned that the parasitoids overwinter as cocoons below the duff of alfalfa. The alfalfa at our Sacramento release is cut, not baled. Thus a rich organic layer has built up over the last four years. Alfalfa at all other release sites has been baled, thus sites have little mulch, which stabilizes soil microhabitat, important to many ground dwelling predators. In January, we sampled for overwintering *Peristenus* in this plot. One adult *Peristenus* spp. emerged per 1.2 square feet of duff sampled. Both *P. stygicus* and *P. digoneutis* were recovered (6:4). Also, flood irrigation may be a problem in contrast to sprinkler irrigation, as used at our Sacramento site (parasitoids form cocoons in soil). Cultural practices such as alternate cutting and planting of nearby insectary flowers (present at the Sacramento site) may also contribute to differences in establishment of parasitoids. Climate could be a deciding factor. Sacramento between 100 and 300 miles north of the other release sites has somewhat cooler summers and wetter winters. Lastly, more time may be needed. Parasitoids were not recovered until five years after releases were made on the east coast.

While it appears these parasitoids will only survive in California under conditions described above, their initial colonization may depend on optimal conditions for year-round survivorship. Therefore, success at the original release site in Sacramento warrants additional effort in this direction.

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Production and Field Establishment of *Psyllaephagus bliteus* for Control of Red Gum Lerp Psyllid on Eucalyptus

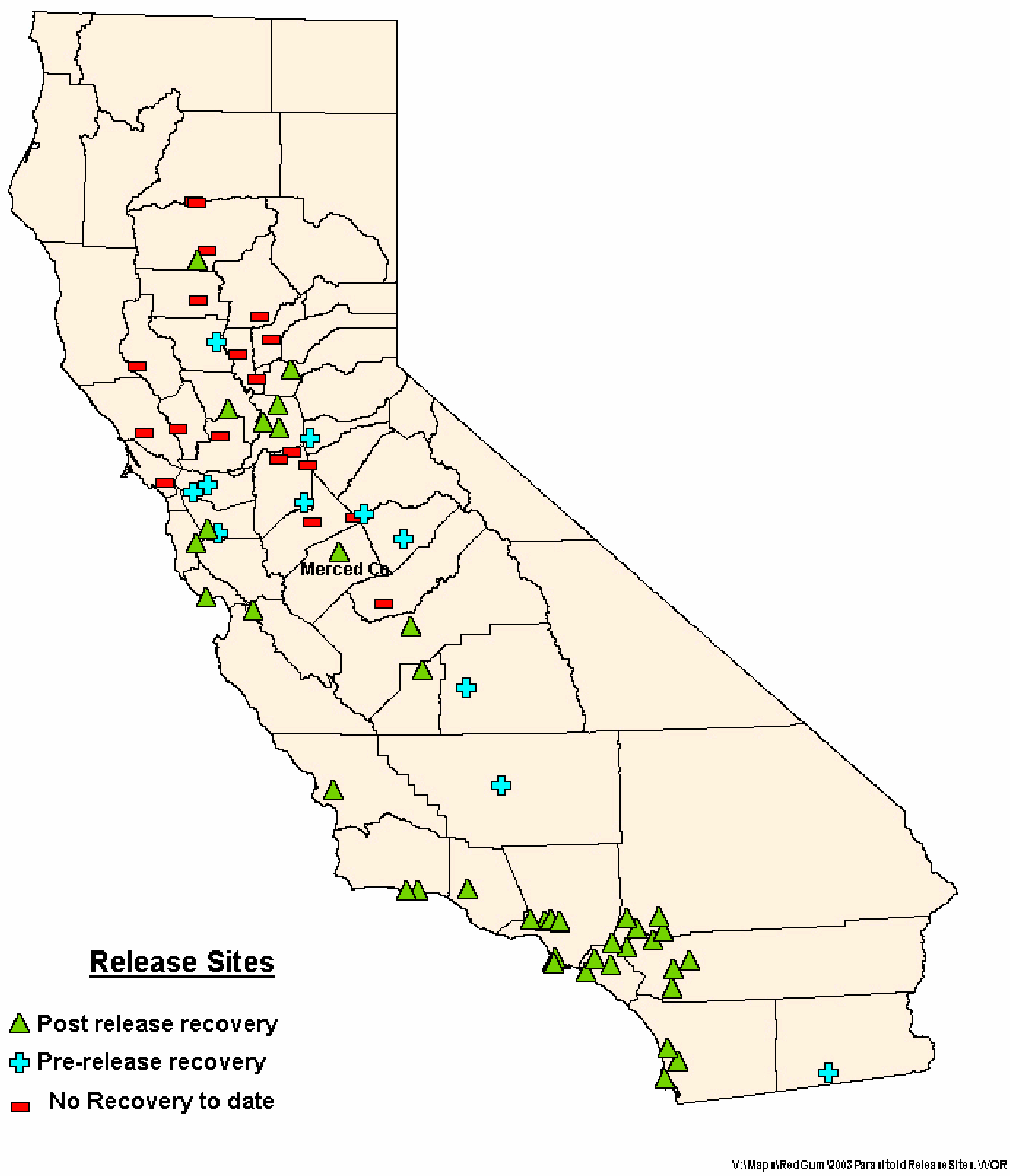
W. J. Roltsch, S. Khasimuddin, D. L. Dahlsten[1], J. Brown, B. Wusstig, and R. Morris

Progress toward the biological control of the red gum lerp psyllid (RGLP), *Glycaspis brimblecombei* Moore, continued through 2002. It is predominantly a pest of red gum eucalyptus, *Eucalyptus camaldulensis* Dehnh, in California. The primary objective during this year was to increase rearing facility productivity, and release the encyrtid parasitoid, *Psyllaephagus bliteus*, in all counties desiring to control the psyllid pest. In addition, monitoring activities were expanded to determine if the parasitoid is establishing in the many geographical locations where it has been released.

Rearing procedures were continued as described in the 2001 Biological Control Program Report. In 2002, over 31,000 parasitoids were produced at the California Department of Food and Agriculture (CDFA) Meadowview greenhouse facility for field release. This provided the means for scientists at the CDFA and University of California (UC) Berkeley to release a minimum of 400 parasitoids in at least one location in each affected county that had not received parasitoids prior to 2002 (Table 1). At the time of release, a 10-minute inspection for parasitoid exit holes was conducted at each site. In addition, leaf collections were made prior to the release of parasitoids to determine if parasitoids were already present at the site. Fifteen branches containing leaves infested with the RGLP were placed in a large paper bag, returned to the lab, and held for parasitoid emergence. Pre-release samples were of particular importance during 2002 because a number of releases had occurred (predominantly in coastal areas) in the previous eighteen months. The UC Berkeley program (i.e., Dr. Don Dahlsten and associated UC Cooperative Extension scientists) determined that by the fall of 2001, previous releases had resulted in the establishment of *P. bliteus* in the counties of Los Angeles, San Diego, San Mateo, and Ventura. In addition to the California releases, 928 parasitoids were sent to the Florida Department of Agriculture for release in that state during July of 2002.

Monitoring for post release parasitism was conducted by the CDFA in 22 counties. The majority of these sites represented locations where *P. bliteus* had been released during the nine months prior to the fall of 2002, primarily in the interior valley regions of California. The sample period ran from August to October. With the exception of lower desert regions, this is the seasonal time period when RGLP populations reach peak abundance, and red gum eucalyptus demonstrates considerable leaf loss and stress if under extensive attack. Samples consisted of approximately 15 branch terminals, 30 to 45 centimeters in length, from approximately five trees at each site. The intent was to obtain 100 leaves containing a minimum of 100 occupied lerps for population counts and for detecting signs of parasitism. Counts on 50 leaves were made and psyllids were inspected for signs of late stage parasitoid development. In addition, 50 leaves or more were held in screened cages for parasitoid emergence for 30 days. This was an additional practice used for detecting parasitoid establishment.

Red Gum Lerp Psyllid Parasitoid Release Site Status
UC Berkeley and CDFA, Fall 2002



By the fall of 2002, populations of *P. bliteus* were determined to have persisted at 54 of the 72 release sites (Figure 1). Summary results represent data collected from the 22 counties monitored in the fall of 2002, data from the UC Berkeley program, and pre-release records pertaining to 2002 late-summer/fall release sites. Pre-release samples during 2002 releases determined that the parasitoid was present at 12 locations prior to release. The adaptability of this parasitoid throughout the geographic range of red gum eucalyptus remains unclear. For example, although parasitoid populations have appeared to become established in Sacramento County, densities were low throughout 2002. In contrast, a prominent population of *P. bliteus* was recorded nine months following its November 2001 release in Merced County (Figure 1). County biologist R.J. Aguilar determined its spread to have exceeded 20 miles east and west, and at least 12 miles north and south of the original release site by August of 2002. It was found in 46 of the 54 sites surveyed in Merced County. During August and October, releases were made in western Modesto and Tuolumne Counties (east and northeast of Merced County). Parasitoid populations were determined to be present prior to release in each instance. The Merced population represented the closest, well-established population in the region, and was most likely the source for these Sierra foothill populations that existed prior to the 2002 releases in late summer.

Table 1. *Psyllaephagus bliteus* Release Data by County from 2000 through 2002

County	Release Dates/ Periods	Number of Release Sites	Number Released
Alameda	6/14/2000 - 8/23/2001*	2	697
Amador	8/30/2002 – 9/6/2002	1	802
Butte	7/9/2002	1	735
Calaveras	6/20/2002	1	1047
Colusa	7/17/2002	1	408
Contra Costa	5/10/2002	1	654
Fresno	6/30/2000 -8/30/2001	1	535
Glenn	9/13/2001	1	569
Imperial	6/25/2002	1	998
Kern	7/23/2002	1	245
Kings	5/29/2002	1	522
Lake	9/13/2002	1	625
Los Angeles	10/2/2001	8	4,659
Madera	6/5/2002	1	752
Marin	5/15/2002	1	571
Mariposa	8/2/2002	1	728
Merced	11/9/2001	1	582
Monterey	6/21/2001	1	763
Napa	4/30/2002	1	650
Orange	11/6/2000 - 12/13/2001	3	1,846
Placer	7/2/2002 – 9/25/2002	2	1,514
Riverside	10/30/2001 – 3/29/2002	6	4,596
Sacramento	7/27/2001 – 11/14/2002	4	3,039
San Benito	8/7/2002	1	587
San Bernardino	10/18/2001 – 3/6/2002	5	3,775
San Diego	9/15/2000 - 11/27/2001	3	2,035
San Joaquin	4/18/2002 – 9/20/2002	2	1,070
San Luis Obispo	9//27/2001 – 1/11/2002	1	2,734
Santa Barbara	2/6/2001 – 7/26/2001	2	270
Santa Cruz	8/16/2002	1	610
Shasta	6/25/2002	2	2,002
Solano	9/28/2001 – 7/3/2002	1	1,405
Sonoma	9/19/2001 – 10/26/2001	1	768
Stanislaus	5/24/2002	1	836
Sutter	5/24/2002 - 6/20/2002	2	1,465
Tehama	6/19/2002	2	1048
Tuolumne	8/21/2002 - 10/3/2002	2	875
Tulare	6/11/2002	1	800
Yolo	6/11/2002	1	573
Yuba	6/26/2002	1	955
Total		**71**	**49,466**

*Where more than one release was made, the release period is presented.

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Overwintering Recovery of Introduced Cotton Aphid Parasites

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

The cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae), can attain pest status in a variety of crops in the San Joaquin Valley. A cooperative biological control project among the United States Department of Agriculture, Agricultural Research Service (USDA-ARS), the California Department of Food and Agriculture (CDFA) Biological Control Program, University of California Cooperative Extension, and the University of Arkansas was initiated in 1996. 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* (ANP) and *Aphelinus gossypii* Timberlake (AG) (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.

In 2002, 10 nursery sites were maintained. The nursery sites were located as close as possible to the 10 nursery sites used in 2000 and 2001. One site was in Merced County, three in Madera County, and six in Kern County. The Merced County site did not have cotton, but was maintained because overwintering by ANP had occurred there for two years. Most of the sites have a variety of habitats that are favorable for the cotton aphid throughout the year. Beginning in early July, aphid populations were sampled by examining 40 to 80 cotton plants within each site for the presence of aphids. As soon as cotton aphids were found at a site, parasite releases began and continued until cotton was harvested. During 2002, a total of 17,700 ANP and 109,850 AG were released in the nursery sites. Weekly sampling to determine if the parasites were using the cotton aphids began two weeks after the first release. Any aphids or mummies recovered from the sampling were returned to the laboratory and held for parasite emergence. The sampling of sites continued until two weeks after cotton harvest, when sampling shifted to four-to-six week intervals sampling of adjacent areas that might harbor cotton aphid.

From samples collected in the winter and early spring (2001/2002), ANP was recovered at the Merced nursery (three adults) in February, and from a Kern nursery in April (two adults). Recovery of ANP in both nurseries occurred before any 2002 releases of ANP, and therefore represents overwintering by ANP. This is the second year of overwintering recoveries of ANP from the Merced nursery site, and the first year for the same Kern nursery. At the garden site maintained at Shafter Research and Extension Center, AG was recovered in February. This recovery represents overwintering by AG. At another Kern nursery, an intact black mummy was recovered in April, prior to the release of any parasites. The adult within this mummy failed to emerge, so its identity is not known. In May, AG was recovered after a release was made in the nursery. Therefore, AG is known to overwinter at only one site in Kern County.

Black mummies, indicative of the released parasites, were recovered at eight of the 10 nursery sites. More parasites were recovered from the nursery sites in Kern County. The nursery sites in Merced and Madera Counties had very few aphids throughout the entire cotton season. At one other Kern nursery sites; black mummies were recovered in November on Shepard's purse, suggesting that the parasites moved from the aphids on cotton to those on surrounding weeds.

Research supported with a grant from the California Cotton Pest Control Board
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Distribution of *Bemisia* Parasitoids in Central California

C. H. Pickett, R. Rodriguez, D. Keaveny, D. Overholt, and P. Akers

Large scale releases of *Eretmocerus emiratus* (M95104, U.A.E.), *Eretmocerus* nr. *emiratus* (M96076, Ethiopia), *E. mundus* (M92014, Spain), *E. hayti* (M95012, Pakistan), and fewer numbers of *Encarsia sophia (= transvena;* M95107, Pakistan) were made into four citrus orchards from 1997 through 2000. Three study sites were identified initially, one each in Fresno, Kern, and Tulare Counties. A fourth was added because one of the original growers changed farming practices. Sites consisted of adjacent citrus and cotton acreage managed by the same owner with a history of silverleaf whitefly (SLWF) problems. Parasite releases begin 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 with 4.05 million released in 1997, over 10 million in 1998, 3.2 million in 1999, and 124,000 (*E. sophia*) in 2000.

Parasitism of SLWF on citrus was generally low during years of releases, averaging 28 percent overall. However this value is quite high with respect to an earlier survey, in which less than 1.5 percent of nymphs examined on cotton and other weedy plants from the same region were found parasitized. During years in which exotics were being released, most of the parasitoids recovered from weed samples taken within one mile of citrus orchards were exotic, 81 percent to 95 percent. Two years after the last releases of exotic *Eretmocerus* spp., (fall 2001), the proportion of all parasitoids that were exotic dropped to 11 percent on weed samples. Although primarily *E. emiratus* (M95104 + M96076) was released, *E. mundus* (M92014) was the dominant species recovered in fall 2001. The density of SLWF on weeds has varied about the same from 1998 to 2001, most samples from 0.1 to less than 20 per gram dry weight.

Additional monitoring of the parasite population was conducted in 2002 to determine the presence and distribution of released parasitoids. The San Joaquin Valley is very large, over 300 miles long. Many years will be needed before the parasitoids have spread to their ultimate range. Not until they have spread out across the Valley will their full impact be realized. Additionally, continual monitoring will be needed to determine exactly what whiteflies are attacked by the released parasitoids due to concerns regarding non-target impacts.

<u>Materials and Methods:</u> Two sampling protocols were conducted in late summer to early fall. The first utilizes an existing sampling program managed by the Pink Bollworm (PBW) Program, California Department of Food and Agriculture (CDFA). Twice monthly from July through September, close to five percent of PBW trap sites in the San Joaquin Valley were sampled. There were 89 sites in Kern County, 72 in Kings, 57 in Tulare, 125 in Fresno, 29 in Merced and 13 in Madera. Ten leaves from 10 separate plants were selected from each site. Leaves are taken from the fifth mainstem node below the terminal. Leaves from each site are placed in a separate container labeled with the county, site, number, and date of collection. Plants are within 10 meters of a PBW trap. Leaves with high numbers of whiteflies (50 or more per leaf) were retained in one-pint paper cans, and shipped to the CDFA's Biological Control Program in Sacramento. Paper cans were held at room temperature for at least five weeks. The numbers of emerged parasitoids, native and exotic, as well as whiteflies were recorded.

The second sampling effort focused on identification of host whiteflies. Parasitoids are reared from isolated whitefly hosts to insure identification of host species. Once during August or September, six cotton fields were sampled for SLWF infested cotton leaves in the southern

San Joaquin Valley. Four of these fields were near each of the four original release sites, and two were distant. Since most cotton fields are sprayed with insecticides, weedy plants were sampled from an additional two locations within two miles of release sites. Leaves were shipped to Sacramento and processed for the presence of exotic parasites. Up to 40 nymphs from each sample location that appeared parasitized were carefully removed from leaves. Nymphs were placed into plastic emergence trays (Pro-Bind™ assay plate, 96, 0.3 ml wells, u-bottom, by Falcon®), one per well and incubated at room temperature. An absorbent paper cloth was placed between the top and bottom of the tray to prevent emerging insects from moving into adjacent wells. Trays and a dish of salt slurry were placed in a plastic food container to maximize humidity. Recovered parasitoids and host exuviae were identified to species or genus. Mr. Ray Gill, CDFA identified whitefly exuviae.

<u>Results:</u> Collections of leaves came from Fresno, Kern, Kings, Merced, and Tulare Counties. Most came from the latter three. Cotton leaf samples were retained by the PBW Program from 30 sample sites, placed into paper one-pint containers and shipped to Sacramento. For the more detailed survey, whitefly nymphs were recovered from cotton, nightshade (*Solanum* sp.), spurge (*Euphorbia* sp.), holy hock, purple potato vine, squash (Curcurbitaceae), and sunflower (*Helianthus*), and bean (Table 1).

Table 1. Parasitoids, Host Whitefly and Plants, 2002

Species	Sex	Host plant	Host whitefly
***Eret*. native**	♀	Cotton	*Bemisia tabaci*
***Eret*. native**	♀	Purple potato vine	*B. tabaci*
***Eret*. native**	♀	Purple potato vine	*Trialeurodes abutilonea*
***Eret*. native**	♀	Sunflower	*B. tabaci*
***Eret*. native**	♀	Holly hock	*B. tabaci*
***Eret*. native**	♀	Egg plant	*B. tabaci*
***Eret*. native**	♀	squash	*B. tabaci*
***Eret*. native**	♀	Mulberry	*Tetraleurodes mori*
Eret. mundus	♀	Cotton	*B. tabaci*
Eret. mundus	♀	Purple potato vine	*Trialeurodes abutilonea*
En. meritoria	♀	Cotton	*B. tabaci*
En. meritoria	♀	Holly hock	*Trialeurodes abutilonea*
En. coquillet	♀	Cotton	*B. tabaci*
***Eret*. native**	♂	Cotton	*B. tabaci*
***Eret*. native**	♂	Purple potato vine	*Trialeurodes abutilonea*
***Eret*. native**	♂	Mulberry	*Tetraleurodes mori*
En. pergandiella	♂	Cotton	*B. tabaci*
***Eret*. exotic**	♂	Cotton	*B. tabaci*
***Eret*. exotic**	♂	Nightshade	*B. tabaci*[1]
***Eret*. exotic**	♂	Sonchus	*B. tabaci*[1]
***Eret*. exotic**	♂	spurge	*B. tabaci*[1]
***Eret*. exotic**	♀	Bean	*B. tabaci*

[1]Host not identified by Mr. Gill

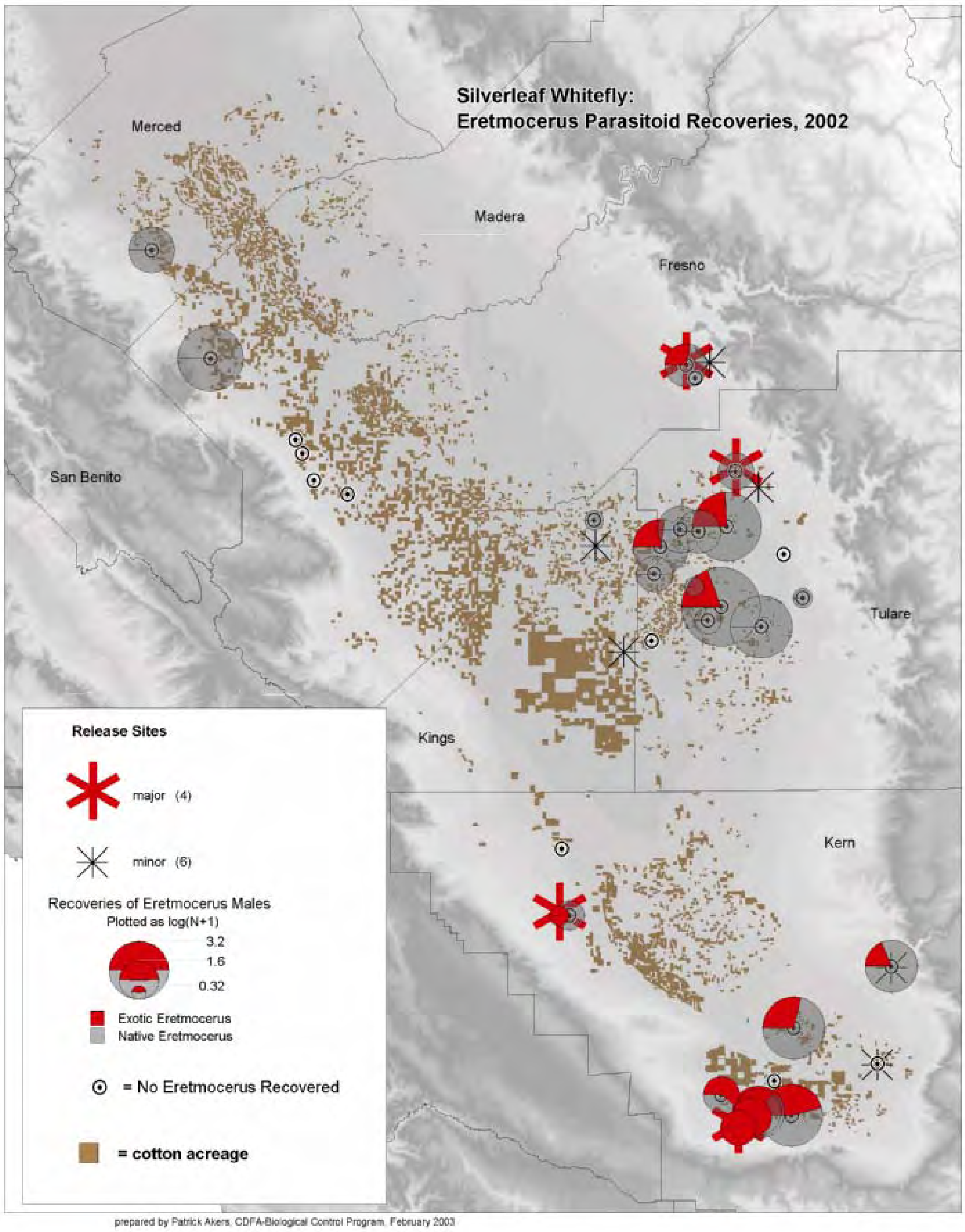
The only exotic parasitoid recovered with certainty was *E. mundus*. Three other species, *E. emiratus*, *E. hayati*, and *E. sophia* were released from 1995 to 2000. *E. mundus* was recovered primarily from *Bemisia tabaci* infesting cotton. However, it may also attack *B. tabaci* on the weeds collected in this survey, from which exotic, male *Eretmocerus* were recovered. Unfortunately, they cannot be identified to species. One record came from banded wing whitefly, *Trialeurodes abutilonea* infesting purple potato vine.

Recovery of parasitoids from the PBW survey came entirely from cotton leaves infested with *B. tabaci*. All the recovered parasitoids were species of *Eretmocerus*. The percentage of male *Eretmocerus* that were exotic varied from zero to 47 percent, with one exception of 100 percent;

however only one parasitoid was recovered in that sample. In eight of 30 samples taken from Fresno, Kern, Merced, and Tulare Counties, we recovered exotic, male parasitoids.

Combining data from both surveys, the map in (Figure 1) shows the distribution of recoveries and relative magnitude of parasitoids and whiteflies. The greatest concentration of exotic parasitoids is at the southern region of Kern County. Note: the large stars represent the original release sites in citrus groves where more than one million parasitoids were released. The minor release sites were non-crop annual plants or cotton into which less than 50 thousand parasitoids were released (see Biological Control Reports for 1998, 1999). Most of the recovered parasitoids near the original release site were exotic. These parasitoids have spread at least 10 miles. A larger percentage of *Eretmocerus* were native at our original release site in Fresno and Tulare Counties. No parasitoids were recovered from four sites in western Fresno County, while some natives were found at two sites north of these (Figure 1).

Figure 1. Distribution of Recovered Parasitoids and Whiteflies from the Pink Bollworm and Host Survey Data



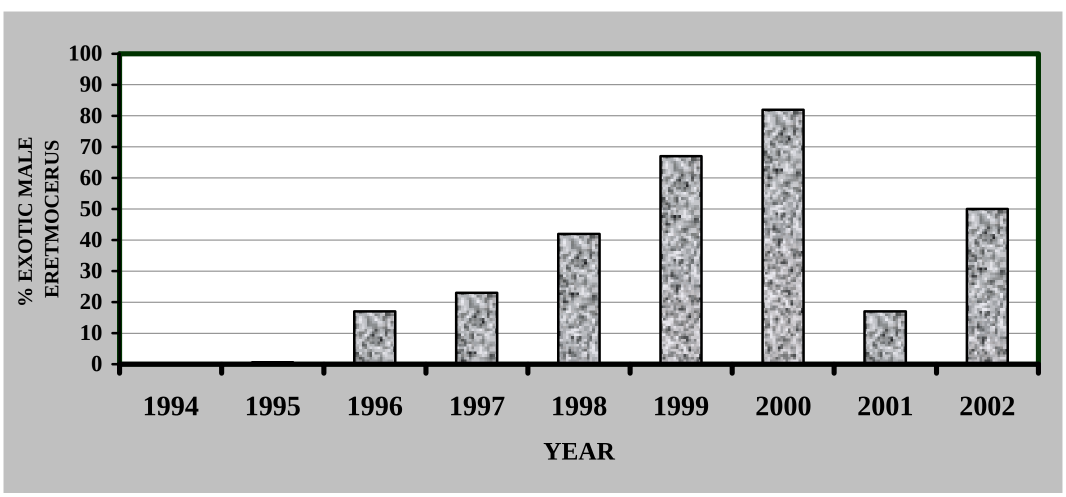
Establishment of Introduced Parasitoids of the Silverleaf Whitefly in Imperial Valley, CA

W. J. Roltsch and J. Encalada

An intensive effort was made to establish biological control agents in the desert southwest from 1994 to 1999. During this time, species and strains of exotic *Eretmocerus* and *Encarsia* were evaluated in laboratory and field cage tests. Within the Imperial Valley, greenhouse-reared parasitoids were released in large numbers (exceeding several million for many species) in commercial fields, refuge nursery plots and urban yards. This report provides a limited yearly examination of exotic parasitoid activity in the Valley, reflecting species establishment and relative abundance. The update primarily provides information collected from two pesticide-free refuge field plots (0.3-0.5 ha. each) located at the Imperial Valley Research Center, Brawley.

Similar to previous years, our 2002 field plots contained two beds of collard (planted in the fall of 2001), as well as okra, basil, cantaloupe and cotton (planted in March of 2002). Samples were collected approximately monthly from April to July (collard) or October (other plant species). In total, 464 *Eretmocerus* parasitoids were isolated for identification during 2002, along with *Encarsia* species. Of the entire collection of *Eretmocerus*, 50 percent of the male (Figure 1) and 41 percent of the female *Eretmocerus* were identified as representing introduced, exotic species. These findings are notable given that the native species, *E. eremicus*, is a commonly occurring biological control agent in the region. Species determination of exotics collected in 2002 is pending. From samples taken in 1997 to 2001, over 95 percent of the exotic *Eretmocerus* have been *E.* sp. nr. *emiratus* (M96076) and *E. emiratus* (M95104). The predominant *Encarsia* species collected, *E. sophia* (M95107), was very common by mid to late summer in the field nursery plots. Furthermore, *E. sophia* was the predominant parasitoid found on basil and collard in July and August, grown in a home garden in Brawley by a cooperating homeowner. *E. sophia* was also noted (by its distinct black pupal stage) as being particularly abundant on mulberry in October in the town of El Centro during 2002. In summary, several exotic species have been well established in Imperial Valley for over five years.

Figure 1. Establishment of Silverleaf Whitefly Exotic Parasitoids in the Genus *Eretmocerus* in Field Plots in Imperial Valley, CA



Seasonal Dynamics of the Citrus Peelminer in Tulare County

K. Godfrey, D. A. Mayhew, K. Daane[1], and D. Haines[2]

The citrus peelminer (CPM) [*Marmara gulosa* Guillen and Davis; Lepidoptera: Gracillariidae] is a small moth native to the southwestern United States and northern Mexico. It feeds by mining just below the surface of succulent stems, fruit, and occasionally leaves of its host. Historically, CPM has been a minor pest of citrus in the San Joaquin Valley (SJV), primarily in grapefruit in Kern County, with infestation rates rarely exceeding five percent. The CPM has a large host range but has remained an economic problem almost exclusively in citrus. Since the winter of 1998 through 1999, CPM infestation rates in grapefruit and other citrus varieties in the SVJ have increased dramatically (30 to 80 percent of the crop). Additionally, the host range of CPM has expanded to include 67 species in 31 plant families, including crops such as citrus, walnuts, stone fruit, almonds, melons, cotton, olives, and grapes. Therefore, because of the differences observed in the behavior of the current infestation, the source of the infestation was traced. After the freeze in 1998, bulk citrus from the Hermosillo and Ciudad Constitucion areas of northern Mexico, arrived at packinghouses in the Lindsay/Strathmore area. This fruit was infested with an insect that morphologically was identical to CPM found in the Coachella Valley. Given the differences in host range of this insect compared to that in the Coachella Valley, it was assumed that the CPM in the current infestation is a new strain or biotype. Studies comparing the molecular biology of the insects from the SJV with those from the Coachella Valley are currently underway at University of California, Riverside.

A study was conducted to determine the seasonal sequence of habitat use by CPM in Tulare County. One objective was to determine if there are habitats in which management tactics could be applied to reduce the pest pressure from CPM. Five sites located on the east side of Highway 99 from Earlimart to Farmersville, were selected for study. Each site had at its center a mature table grape vineyard (over three years of age). Sites were chosen that had large densities of CPM in 2001 (D. Haines, unpublished data). Each site was sampled at two-week intervals from late May through the end of October, and about once per month in November, December, and January.

Ten vines were selected at random on each edge of the vineyard. On each vine, the number of active (i.e., mines with live CPM larvae) and inactive (i.e., mines without live CPM larvae) mines found on up to one-meter length of a green cane (i.e., first year cane) was recorded. When clusters were present, one cluster near the sampled green cane was examined and the number of berries within the cluster containing active or inactive mines was counted. In habitats adjacent to the target vineyard, a 10-minute search was conducted. The number of active and inactive mines found, and the plant parts with mines were recorded.

The results from this study are consistent with two general patterns of seasonal dynamics. In one pattern (Sites 1, 2, and 3), cotton and dry beans influence CPM dynamics in grapes, and for the other pattern (Sites 4 and 5), citrus and nursery plants influence the dynamics.

The first pattern of CPM abundance in a table grape vineyard, as demonstrated by the data from Site 3, was characterized by a small peak in abundance in June, followed by a broad peak in abundance from mid July through September (Figure 1). Mining of green canes by CPM could be found as early as May. Mining of the main stem was found from mid June through October. No berry mines were found in this vineyard. The peaks in CPM density in the table grape vineyard appeared to be related to the density changes found in the surrounding habitats (Figure 2). The pattern in table grapes closely followed the pattern from cotton. CPM density in cotton declined in late June through mid-July probably due to the application of the insecticide aldicarb to the cotton crop. This insecticide treatment is part of the standard management program for controlling early season pests in cotton. In late July, as the effects of the insecticide diminished, the density of CPM again increased through August and September with mining found in main stems, lateral branches, and bolls.

Figure 1. The Mean Number of Active CPM Mines Per Centimeter of Green Cane at Sites 3 and 4 in Tulare County in 2002

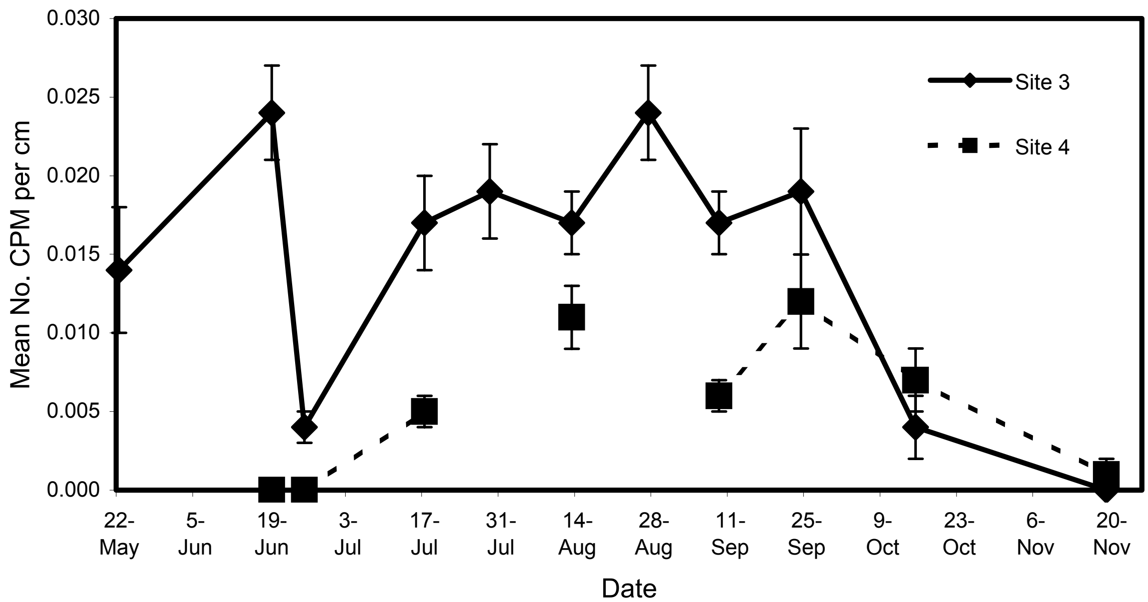
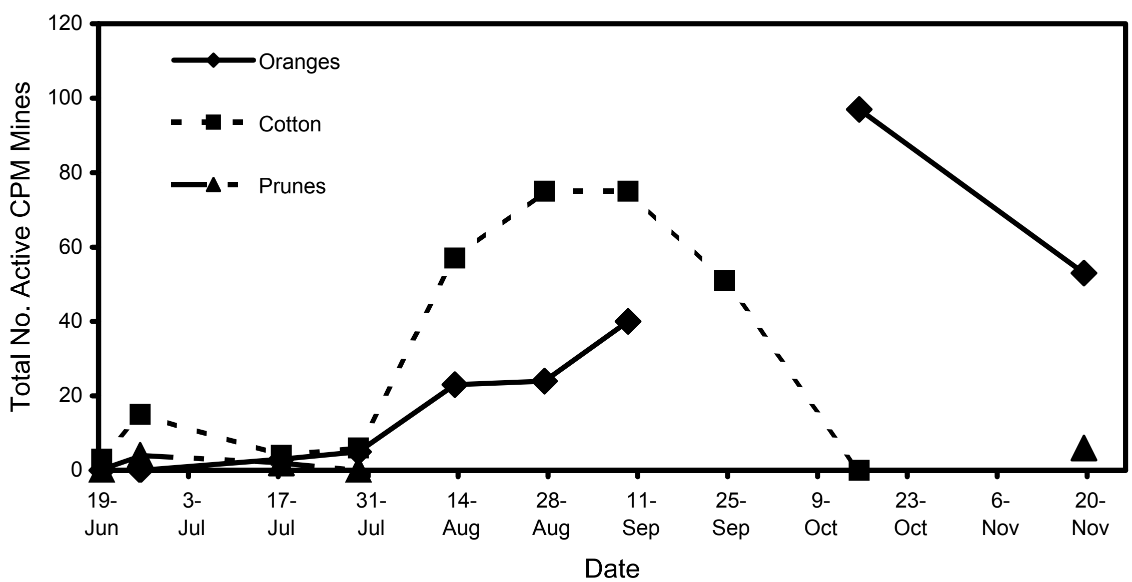


Figure 2. The Total Number of Active CPM Mines found in a 10-Minute Search of Habitats Located Adjacent to Site 3 in Tulare County in 2002



Movement of CPM from cotton and table grapes into oranges and prunes was also evident (Figure 2). For the oranges, the density of CPM began to increase in early August, peaking in mid October. This coincided with the timing of the senescence of the cotton and table grapes. Mining in the stems of prunes was found early in June and again in November and January, suggesting that CPM may overwinter in this host (Figure 2).

The second pattern of CPM abundance in a table grape vineyard, as demonstrated by the data from Site 4, was characterized by three general peaks in abundance: mid-July, mid-August, and late September (Figure 1). In addition, CPM appeared in this vineyard about a month later than that seen at Site 3. Rachis mining occurred from early July through October. Berry mining was found in July, September, and October, although the number of berries mined was extremely low (less than one percent of the clusters examined had berry mines).

The pattern of abundance in the surrounding habitats reflected the pattern found in the table grape vineyard (Figure 3). The adult CPM appeared to arrive at this site in early July with mining beginning in the oranges and mature grape habitats at about the same time. The density of CPM mines continued to increase into September. In mid-October, cheeseweed in the orange grove was extensively mined. In the almond grove, inactive mines were found in June, and active mines were found in July. This pattern of activity suggests that almonds may be used as a host in the winter through early summer.

There are hosts of CPM that are thought to harbor CPM throughout the year, and these include oleander and willow. At Site 5, one of the adjacent habitats is an oleander hedge. On all sampling dates June through January, active CPM mines could be found in the hedge (Figure 4).

The data collected in the studies conducted in Tulare County in 2002 suggest a pattern of movement among various habitats by CPM (Table 1). It appears that there are habitats in which CPM is present year-round at varying densities. For example, there appears to be resident CPM populations in oleander and citrus, and may be one in willow (data not collected). In late spring, CPM move out of year-round habitats and begin to infest walnuts, grapes (mature), plums, dry beans, early cotton, and weeds. In early summer, CPM mines can also be found to a limited degree in almonds and prunes. In mid to late summer, densities of CPM peak in dry beans, cotton, and grapes (mature and young). In addition, CPM mines can be found in kiwi stems, and pistachio stems and hulls. Throughout the fall, CPM densities increase in citrus, and it moves into possible overwintering hosts (e.g., weeds and prunes).

Table 1. Proposed pattern of seasonal movement of CPM from habitat to habitat. Habitats in italics are assumed to harbor CPM, but data has not been generated. Crops in bold show when CPM reaches peak density within a crop.

SPRING	SUMMER	FALL	WINTER
Almonds			
Citrus	Citrus	**Citrus**	Citrus
Cotton	Pistachio		Weeds
Dry Beans	Kiwi		Walnuts
Grapes	**Dry Beans**	Weeds	Almonds
Oleander	**Oleander**	**Oleander**	Oleander
Plums	**Cotton**		Plums
Walnut	**Grapes**	Prunes	Prunes
Weeds			
Willow	Willow	Willow	Willow

Figure 3. The total number of active CPM mines found in a 10-minute search in habitats located adjacent to Site 4 in Tulare County in 2002.

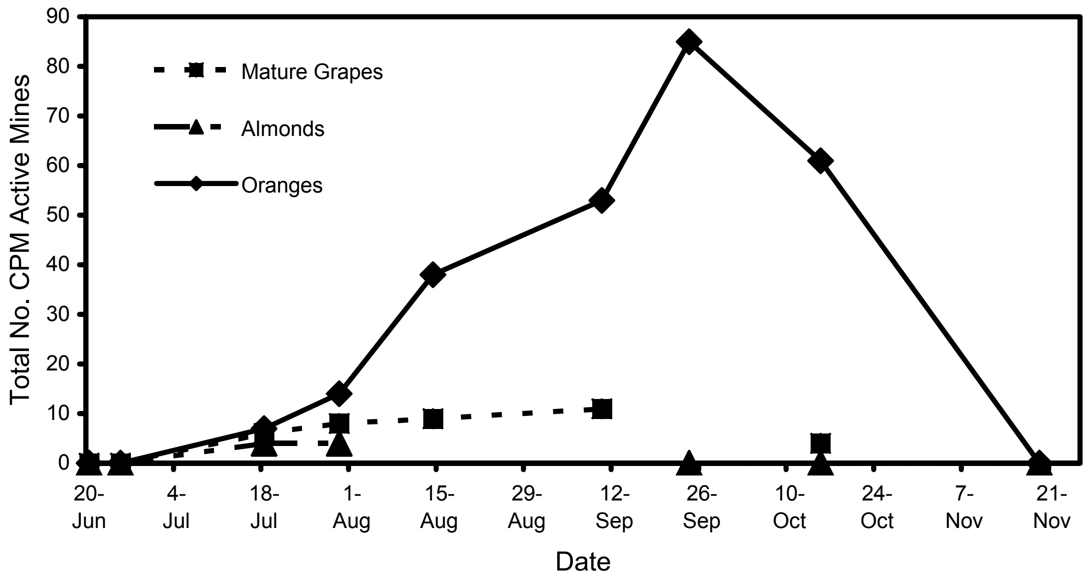
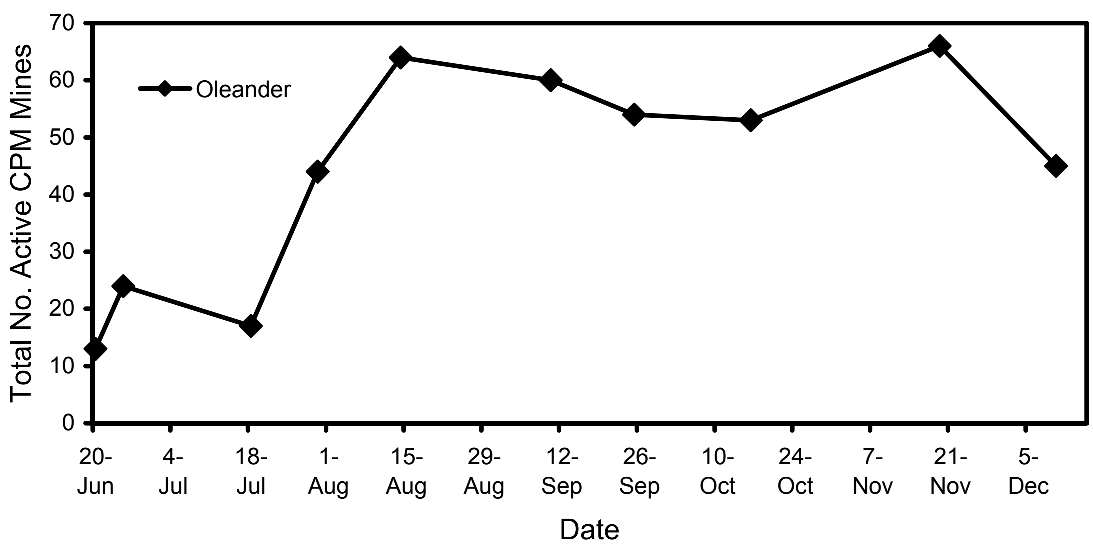


Figure 4. The total number of active CPM mines found in a 10-minute search of an Oleander hedge at Site 5 in Tulare County in 2002.



This research was supported in part by a grant from the California Table Grape Commission.
[1]Division of Insect Biology, University of California, Berkeley, CA
[2]Tulare County Agricultural Commissioner's Office, Tulare, CA

Citrus Peelminer Density Reduction with Bt-Cotton

K. Godfrey, D. A. Mayhew, S. Wright[1], and P. Goodell[2]

The citrus peelminer (*Marmara gulosa* Guillen and Davis; Lepidoptera: Gracillariidae) has been causing great concern in Fresno, Kern, and Tulare Counties since 2000. In the current infestation, citrus peelminer (CPM) has greatly expanded its previously known host range to include 67 plant species in 31 families. Although it has extensively mined cotton stems and bolls since 2001, no economic damage to the cotton crop has occurred. During cotton variety trials in 2001, it was noted that CPM could not effectively mine or survive in cotton plants transformed to include the gene for one of the *Bacillus thuringiensis* endotoxins. (Hereafter, those plants will be referred to as Bt cotton). The Bt cotton did not deter oviposition by CPM, so a study was conducted to determine if Bt cotton could be used to reduce densities of CPM within an area.

Assessment of Bt cotton in reducing densities of CPM in an area was conducted in a cotton field located near Plainview, Tulare County. Habitats adjacent to this field included black-eyed beans (south), kiwis and citrus (east), pasture (north) and alfalfa (west). At the south end of the cotton field, six row plots were established alternating between Bt cotton and non-Bt cotton. (Seed provided by Delta and Pine Land Company). The first plot was located immediately adjacent to the black-eyed beans. The plots were sampled approximately every two weeks beginning May 22. On each sample date, five black-eyed bean plants on the row adjacent to the cotton were examined for the presence of CPM. The number of active (mines containing live larvae) and inactive (mines with no larvae or dead larvae) mines on the stems and leaves, and the height of the stems were recorded. For each plot of cotton, five plants (one per row) were examined for the presence of CPM. The number of active and inactive mines on the stems and bolls were recorded. In addition, the height of the cotton stem and the diameter of the bolls were recorded. This sampling continued until September 24 for the black-eyed beans when the beans were harvested, and until October 15 for the cotton plots (plots were harvested).

The density of CPM in the black-eyed beans peaked in mid-July (Figure 1). All of the mining occurred in the stems, except for one leaf mine in late May. The pods were not mined. This crop appears to be a bridge for CPM from late spring weeds to mid-season cotton.

For the two types of cotton, a dramatic difference in density of CPM can be seen (Figure 1). The Bt cotton, in general, had very low densities of CPM with the majority of plants not being mined at all. The non-Bt cotton demonstrated a gradual increase in CPM density through early September (Figure 1). Much of the mining was located on the main stem. However, mining of lateral branches was found beginning at the end of July. Boll mining was found in both types of cotton, although substantially more mining was found in non-Bt cotton (Table 1). Boll mining began in mid-August when bolls were 2.5 to 5 centimeters in diameter. No mining was observed on squares or blooms.

In summary, the black-eyed beans appear to act as a transition crop for CPM. In cotton, CPM is able to build to very large densities over the summer and moves out of the cotton beginning in mid to late September. The Bt cotton supported drastically less CPM than non-Bt cotton.

Figure 1. The mean number of active CPM mines per cm of stem in the Bt cotton study in Tulare County in 2002. The bars represent the standard error of the means.

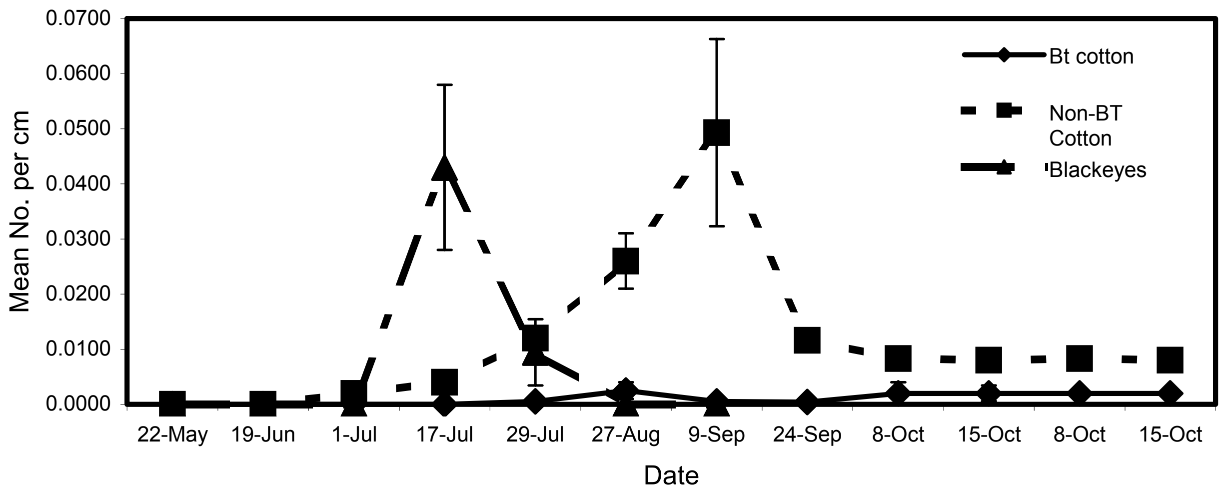


Table 1. The proportion and percent of bolls with active mines in the Bt cotton study in Tulare County in 2002. An attempt was made to sample 60 bolls on each date. At the end of the season, many bolls were open, so the total number of sampled bolls was less than 60.

	Non-Bt cotton		Bt cotton	
Date	Proportion	Percent	Proportion	Percent
August 13[a]	5/60	8.3	0/60	0
August 27	20/60	33.3	2/60	3.3
September 9	28/60	46.7	0/60	0
September 24	12/45	26.7	0/57	0
October 8	6/49	12.2	1/47	2.1
October 15	4/27	14.8	1/33	3.0

[a]From May 22 through July 29, no mined bolls were found.

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This research supported in part by a grant from the Citrus Research Board

Phenology of Damage by Citrus Peelminer Population in an Orange Grove

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

The phenology of damage and estimation of losses from citrus peelminer (CPM) (*Marmara gulosa* Guillen and Davis; Lepidoptera: Gracillariidae) has been investigated in a block of Fukomoto navels in Tulare County since late 2000. The northwest corner of the block (17 rows by eight trees) was sampled at approximately two-week intervals from May 22 through October 23. During the first half of the summer, May 22 through July 29, five stems on 20 trees selected at random were examined for evidence of CPM. The number of active (mines with live larvae) and inactive (mines with no or dead larvae) mines on each stem was recorded. For the last half of the summer, August 13 through October 23, 10 fruit on each of 40 trees selected at random were examined. Each fruit was given a damage rating and the number of mines on each fruit was counted. The ratings were: 0 = no mining; 1 = 1 to 25 percent of surface area mined; 2 = 26 to 50 percent of surface area mined; 3 = 51 to 75 percent of surface area mined; and 4 = > 76 percent of surface area mined.

The entire block (32 rows by 24 trees) was mapped on November 6, 7, and 14, prior to fruit harvest. Twelve fruit on each tree (one fruit at the lower, middle, and upper portion of the tree on each compass point) were assessed for damage using the rating scheme given above. Each tree was then assigned to a damage category. Damage categories were: none equals CPM infestation nearly undetectable with most of the fruit marketable; few equals CPM infestation detectable with about 67 percent of the fruit marketable; medium equals CPM infestation easily detectable with about 50 percent of the fruit marketable; and lots equals CPM infestation heavy with less than 33 percent of the fruit marketable. Chi-squared analysis was applied to a portion of the data (odd-numbered rows from nine through 31) to determine if CPM was found evenly throughout a tree.

The damage ratings on the fruit increased gradually from the end of August through October (Figure 1). In samplings earlier than this, only one active mine was found on a stem near a fruit (July 17). In terms of the distribution of fruit in each damage-rating category, a gradual increase in damaged fruit was seen from the middle of August through the end of October (Figure 2). (Note that for the August 13 and 27 sampling dates, only 200 fruit were examined due to time constraints). Fruit with the highest damage rating (four) were not found until the final sampling date, and only two of the 400 fruit were this damaged.

The final mapping for 2002 had far fewer trees in the extreme damage categories (medium and lots) than in previous years (Table 1). The trees with the heaviest damage were found in the northwest corner and along the west side. This is not surprising considering that the major source of infestation, a cotton field, was located immediately northwest of the block. The decrease in damage in this block as compared to previous years may have been due to a combination of placement of experimental pheromone traps within the block at a time of maximum moth flight, and the rapid picking, shredding, and disking of the cotton field. In other navel orange groves that were routinely sampled in other studies, more extensive damage to the fruit occurred than seen in this block.

The chi-squared analysis of the damage ratings in the final mapping revealed that more damaged fruit were found on the east side of the tree than would be expected if there was a proportional distribution of mined fruit ($X^2 = 40.8$, df = 3; $P < 0.01$). This side of the tree would be the “protected” side of the tree from the prevailing winds (usually out of the northwest). In terms of the height of the fruit on the tree, a greater proportion of damaged fruit was found on the lower and upper portions of the tree (X^2 = 23, df = 23; P < 0.01). These positions on the tree may be somewhat more protected than the middle part of the tree. As the fruit enlarges, branches on the bottom of the tree tend to touch the ground, providing a protected place for the CPM. Fruit on the upper parts of the tree tend to be either in the interior of the tree, or the branches bend down with the weight of the fruit, creating protected areas for the CPM. Female CPM seems to prefer to oviposit on fruit or stems that either have many leaves around them or are shaded from the sun.

Table 1. The number and percent of trees in each of the damage categories in a Fukomoto block in Tulare County from 2000 through 2002.

Year	None	Few	Medium	Lots
2000	303 (39.5%)	191 (24.9%)	249 (32.5%)	24 (3.1%)
2001	102 (13.3%)	376 (49%)	200 (26.1%)	89 (11.6%)
2002	295 (38.5%)	346 (45.1%)	97 (12.6%)	29 (3.8%)

Figure 1. The mean damage rating on fruit from the northwest corner of a block of Fukomoto navels in Tulare County in 2002.

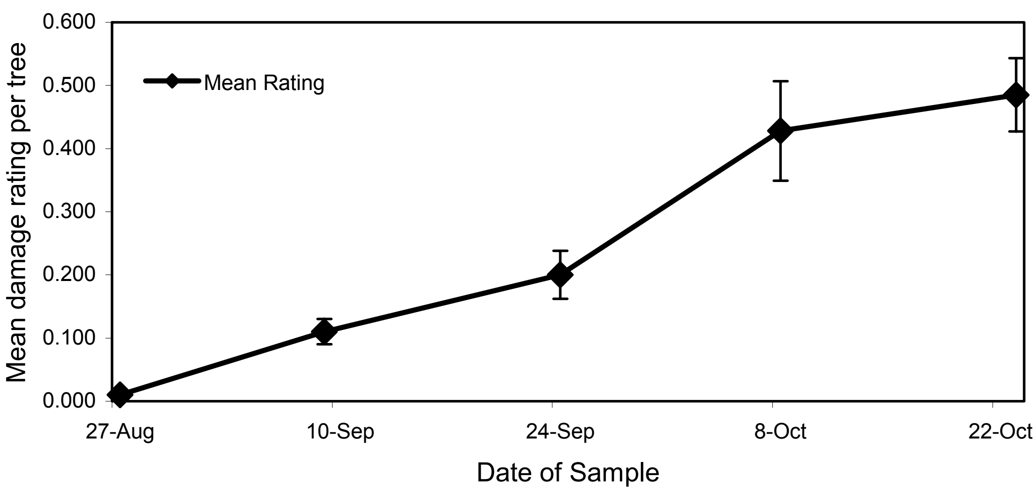
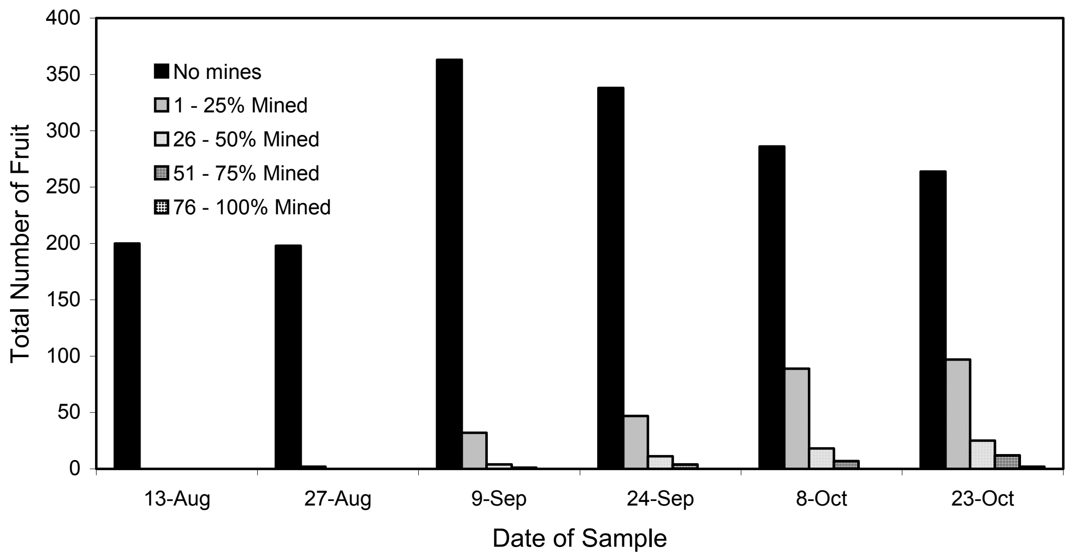


Figure 2. The total number of fruit in each damage-rating category on each sample date in the northwest corner of a block of Fukomoto navels in Tulare County in 2002.



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[1] University of California – Riverside, Kearney Agricultural Center, Parlier, California
[2] F. O. Fisher Farms, Strathmore, California

Other Projects in the Early Stages of Development

<u>Palm Leaf Skeletonizer:</u> 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 [*Homaledra 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 appropriate biological control agents for this insect. A survey of the natural enemies attacking palm leaf skeletonizer began in 2002 in Florida. From this survey, the following parasites were tentatively identified: *Phytomyptera* spp. (Diptera: Tachinidae); *Conura* sp. (Hymenoptera: Chalcididae); *Horismenus* nr. *ignotus* Burks (Hymenoptera: Eulophidae); *Arachnophaga costalis* Gahan, *Brasema* spp. (Hymenoptera: Eupelmidae); *Goniozus nigrifemur* Ashmead, and *G. scitulus* Evans (Hymenoptera; Bethylidae). In addition, Dr. Kellum traveled to Florida to learn about the palm leaf skeletonizer and its parasites.

<u>Citrus Root Weevil:</u> Project Participants: K. Godfrey, E. Grafton-Cardwell (University of California (UC), Riverside, Kearney Agricultural Center, Parlier, California), C. McCoy (University of Florida, Lake Alfred, Florida), J. Pena (University of Florida, Homestead, Florida), and R. Luck (UC, Riverside, California). The citrus root weevil [*Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae)] is a serious threat to the citrus and nursery industries in California. This weevil has been intercepted numerous times in shipments of plants to California with the most recent interception occurring in 2001. Because of the threat from this weevil, funding was obtained from the UC Exotic Pests and Diseases Research Program to educate producers as to what to expect from this weevil when it becomes established in California and to develop suitable biological control agents for this insect. During 2002, three workshops were held in Temecula, Tulare, and Ventura to educate producers. Dr. McCoy and Dr. Pena traveled to California to speak at the workshops. In addition, an educational booklet and web site were developed for use in the workshops. Studies are also underway in Florida to identify strains of fungi and an efficient delivery system that could result in epizootics in the adult weevil population. Egg parasites of the weevil are also being studied to determine their biologies, the best methods to rear large numbers of the parasites and the most efficient method for field release and establishment.

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, 2002, program staff reared natural enemies for control of the Pink Hibiscus Mealybug, *Maconellicoccus hirsutus*, cotton aphid, *Aphis gossypii,* the western tarnished plant bug, *Lygus hesperus,* and the red gum lerp psyllid, *Glycaspis bliteus*.

Natural Enemy	Host	Source Population	Agency Receiving Shipment	Project Description	Stage Delivered	Total Insects Delivered
Psyllaephagus Bliteu sp	Red gum lerp psyllid	Australia	UC CDFA	Colonization in nursery sites, state-wide	adults	31,448
Aphelinus nr paramali	Cotton aphid		Merced County	Colonization in nursery sites	Adults and pupae	600
			Madera County	Colonization in nursery sites	Adults and pupae	2,300
Aphelinus gossypii			Kern County	Colonization in nursery sites	Adults and pupae	14,800
Aphelinus gossypii			Merced County	Colonization in nursery sites	Adults and pupae	1,200
			Madera County	Colonization in nursery sites	Adults and pupae	8,600
			Kern County	Colonization in nursery sites	Adults and pupae	100,050
Peristenus stygicus	lygus	Italy (Umbria) Spain (Granada)	UC CDFA USDA	Open release, alfalfa, central, northern CA	adults	3,091
Peristenus igoneutis		Spain, Catalognia	UC CDFA USDA	Open release, alfalfa, central, northern CA	adults	2,908
Anagyrus kamali	Pink hibiscus mealybug	Egypt	CDFA Imperial Valley	colonization	adults	97,850
Anagyrus kamali		Egypt	Mexicali Valley	colonization	adults	38,250
Anagyrus kamali		Egypt	Florida	colonization	adults	63,600
Gyranusoidea indica		Egypt Pakistan Australia	CDFA Mexico French West Indies	colonization	adults	13,800

Current Status of Biological Control of Squarrose Knapweed in California

D. M. Woods

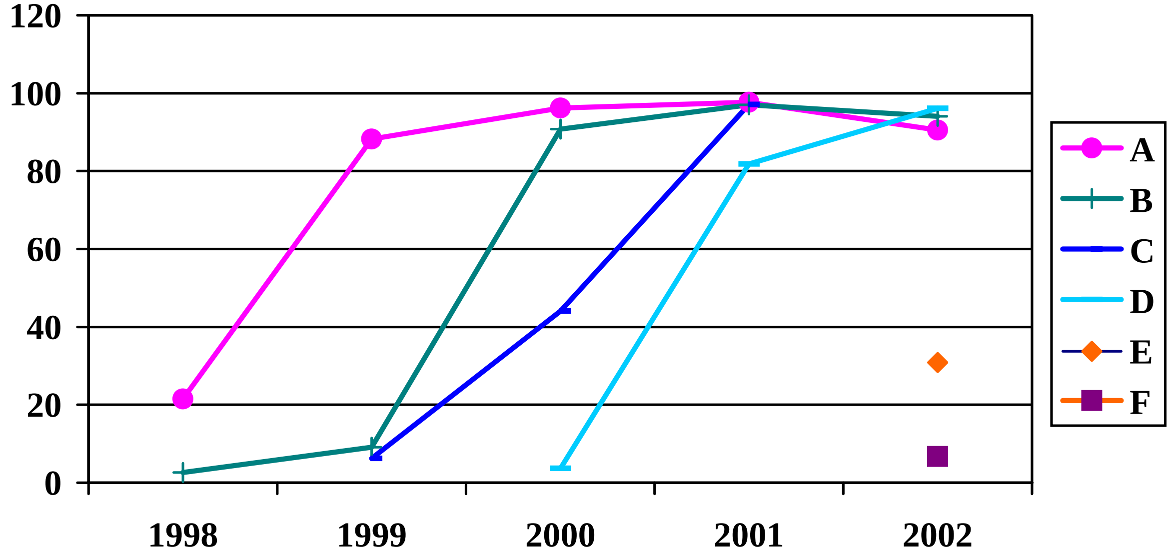
The Biological Control Program, in connection with partnering agencies is currently in the middle of a biological control project on squarrose knapweed. Program staff are actively distributing biological control insects throughout infested areas and evaluating their impact. Early results indicate a highly successful project. A combination of biological control insects appears to have caused a visible and quantifiable impact on this invasive “A” rated weed. The insects are spreading and rapidly increasing in number with an associated near halt in seed production and an increase in the number of dying plants.

Squarrose knapweed is related to yellow starthistle, diffuse knapweed and spotted knapweed. The largest infestations in the United States occur in Utah, and are estimated at over 120,000 acres. California has the second largest acreage, with much smaller amounts in Oregon and Washington. In California, squarrose knapweed occurs along roadsides, in natural areas, and in rangeland, where it can develop into nearly monotypic stands. The California Department of Food and Agriculture and other agencies have a long-standing management program utilizing chemical and physical methods, with the intention of plant reduction, and a goal of eradication. The Biological Control Program initiated biological control efforts in Siskiyou County in 1996, and near the county borders of Lassen, Modoc, and Shasta in 1998. Over a period of several years, five species of insects collected in Oregon were intentionally released as biological controls of squarrose knapweed. Additionally, at least one other insect species has established as a result of natural immigration from other knapweeds. The Siskiyou site was subsequently treated by the Agriculture Commissioner’s office as part of an aggressive weed eradication program so biological control was discontinued. We began focusing on the second site and noted a dramatic and rapid buildup of the released biological control insects.

Samples were collected samples at the end of each summer (beginning in 1998) to assess the percentage of squarrose knapweed seedheads that were attacked by biological control insects. Most sites started with a small percentage (two to six percent) of the seedheads being attacked by the gallfly *Urophora quadrifasciata*. This insect seems to have moved unaided from other knapweed species, probably in Oregon. It has over several years been released and distributed in many states as a biological control of diffuse and spotted knapweed. It is a strong flier so it self-distributes. The insect seems to have spread to and established at a low level over a broad area of Northern California, wherever knapweeds are present. At this low level of attack, its impact is minimal.

Two seedhead feeding weevil species, *Larinus minutus* and *Bangasternus fausti*, have been released by the Biological Control Program and seem to have established rapidly. Results are shown in (Figure 1). The most significant result is the speed of population buildup. Essentially, the seedhead weevils increase rapidly, attacking at least 90 percent of the seedheads in a local area within two years of arrival at the site, either by release or natural movement. Monitoring is being conducted at a few additional sites but laboratory analysis of the samples is not yet complete. Since Sites A through D are similar in features and relatively close together, at additional sites. Sites E through F were established several miles away. They will be monitored to confirm the early data and interpretation.

Figure 1: Percentage of Infested Squarrose Knapweed Seedheads at Monitored Sites



Site A received a large number (2000) of insects in 1998
Site B received a smaller number (200) of insects in 1998
Site C received a moderate number of insects (400) in 1999
Site D is 0.5 mile from sites A and B and received no releases
Site E received a moderate number of insects (500) in 2002
Site F is 0.2 miles from site E and received no releases

In addition to their rapid population buildup, the seedhead weevils have a dramatic impact on the plant itself. Infested seedheads produce almost no viable seed. Less than one percent of the attacked seedheads produce a single seed. Two methods: (bagged and unbagged seedheads) are being used to monitor the impact of the seedhead insects. The results are consistent with both methods. The total seed destruction is estimated to be 90 percent in the original release area based on measured destruction in monitored heads and the sitewide percentage of attacked seedheads (Figure 2). Seed destruction at the new 2002 release Site E was estimated at 24 percent directly around the insect release area. Based on previous results, this should increase greatly next year.

Two root-feeding insects have been released on squarrose knapweed in Northern California. The root-feeding weevil, *Cyphocleonus achates* has yet to establish, while the beetle, *Sphenoptera jugoslavica*, established easily and rapidly. Within four years of release, the root beetle attacked over 80 percent of the plants in the release site. Recently, multiple beetle larvae have been observed within the roots of many of the squarrose knapweeds. Knapweed plants appear to be declining in vigor as a result of the attack. Most of the plants attacked by root-feeding insects now have dead stems and some plants seem to have died as a result of this attack.

Evaluations have been initiated to measure the impact of the biological controls on plant density. During the past two years, dramatic reductions in total knapweed plants per square meter have been measured in two locations within the original 1998 release area (Figure 3).

Figure 2: Percentage of Seed Destroyed by Biological Control Insects

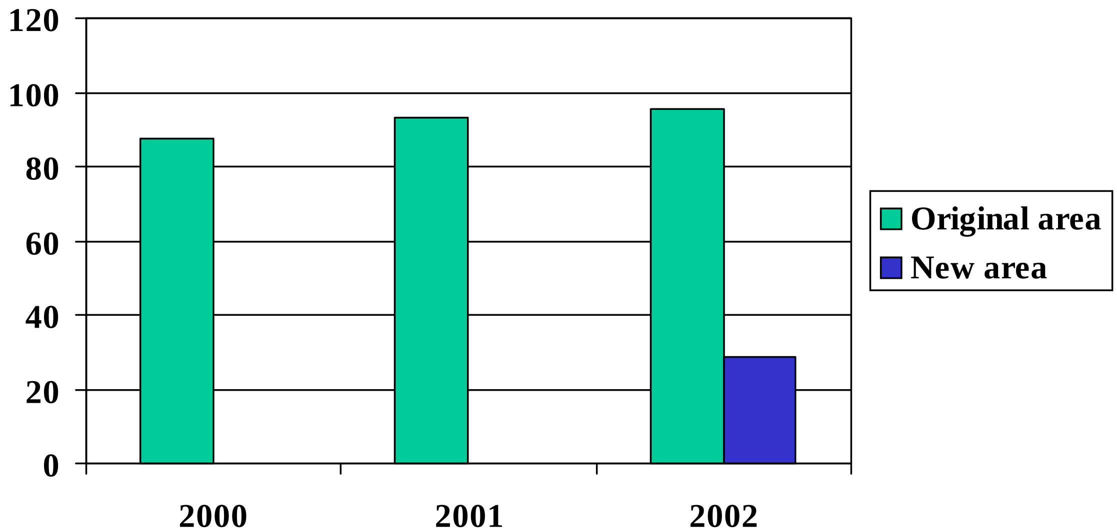
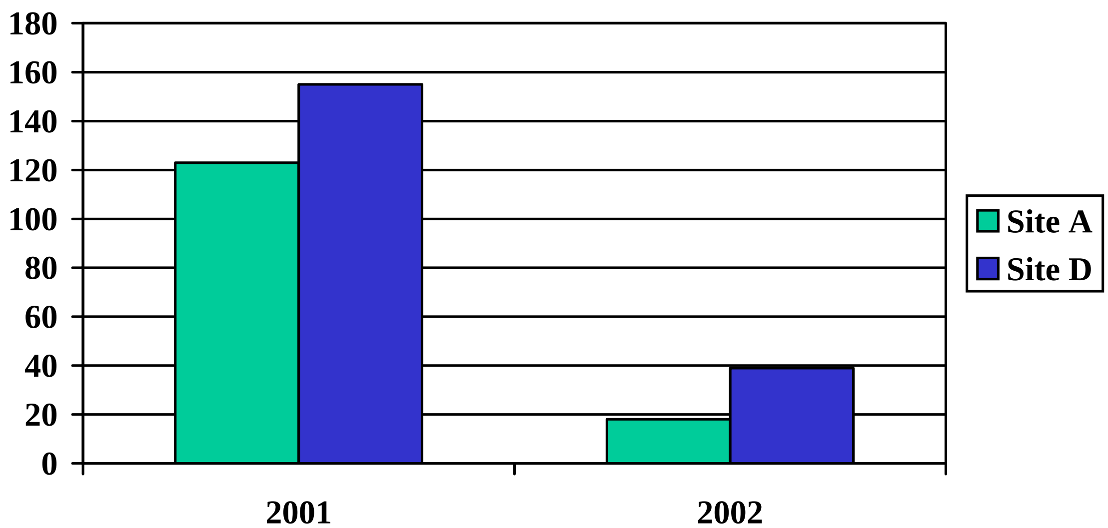


Figure 3: Squarrose knapweed plant density at two locations within the original (1998) Biological control release area.



Redistribution of Biological Control Agents of Squarrose Knapweed in Northern California

B. Villegas and C. Pirosko[1]

Squarrose knapweed (*Centaurea squarrosa* L.) is the most common knapweed in California. Most of the infested acreage is located in Lassen, Modoc, and Shasta Counties in Northern California. Another large infestation was located in Siskiyou County north of Yreka, but aggressive herbicide treatments by the Siskiyou County Department of Agriculture greatly reduced the infestation.

The first intentional releases of biological control agents on squarrose knapweed in California was made in Siskiyou during 1995 to see if these insects would impact squarrose knapweed as infestations were expanding in northern counties. In 1997 through 1998, establishment of with the two seedhead weevils, *Bangasternus fausti* (Reitter) and *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae) was noted at release sites in Siskiyou County. These weevils and other available biological control agents were then released in Lassen County starting in 1998. The decision to expand biological control releases to adjacent infestations in Modoc, Shasta and other sites in Lassen County was made after encouraging recoveries were made at the initial 1998 release sites in Lassen County. The status of the seven released insect species is shown in (Table 1).

Table 1: Natural Enemies Released in Northern California for the Biological Control of Squarrose Knapweed, 1995 through 2002

Species Name	Common Name	Impact to Plant?	Summer 2002 Status
Bangasternus fausti (Reitter)	Broadnosed knapweed seedhead weevil	Seeds	Widely established
Larinus minutus Gyllenhal	Lesser knapweed flower weevil	Seeds	Widely established
Cyphocleonus achates (Fåhraeus)	Knapweed rootboring weevil	Roots	Did not establish
Sphenoptera jugoslavica (Benberger)	Bronze knapweed rootboring beetle	Roots	Widely establish
Urophora affinis Frauenfield	Bandedwing knapweed gall fly	Seeds	Did not establish
Urophora quadrifasciata (Meigen)	UV knapweed Gall fly	Seeds	Established
Terellia virens (Loew)	Green clearwing knapweed seedhead fly	Seeds	Did not establish

All of the insects released on squarrose knapweed in Northern California were initially collected in Oregon on diffuse and spotted knapweed. The biological control agents were mass collected with assistance of the Oregon Department of Agriculture and the United States Department of Food and Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine personnel. In 2002, a survey was conducted to determine establishment at all release sites in the infested areas of Lassen, Modoc and Shasta Counties (Table 2). The release sites in Siskiyou County were not included in this survey as squarrose knapweed, which is under eradication action and no suitable sites could be found during site visits in 2000 through 2001. The two seedhead weevils, *B. fausti* and *L. minutus*, have been recovered at all sites where they were released either individually or in mixtures of the two species. Surprisingly, the bronze knapweed rootboring beetle, *S. jugoslavica* was recovered over a wide area from its initial release site in 1998. Adult beetles were noted up to four miles from the initial release site but root damage was only noticed within one-quarter mile of the initial 1998 release site. Since only 40 adults were released at that site, it is hoped that similar results will be found with further small releases of the beetles. The rest of the insects listed in (Table 1) were not found during our 2002 field surveys. The knapweed rootboring weevil *Cyphocleonus achates* (Fåhraeus) (Coleoptera: Curculionidae) and the seedhead fly *Terellia virens* (Loew) (Diptera: Tephritidae) were never recovered. The two tephritid flies, the gallflies, *Urophora affinis* Frauenfield and *Urophora quadrifasciata* (Meigen) were recovered at the initial release site in 1998 at low levels. *U. affinis* has not been recovered since 1998 and *U. quadrifasciata* has been recovered at low levels at other sites over the three-county infestations. It appears that the two seedhead insects, *B. fausti* and *L. minutus*, once established at a site, will kill larvae of the two flies even if they are within enclosed galls.

Three insects, *B. fausti*, *L. minutus*, and the bronze knapweed rootboring beetle, *Sphenoptera jugoslavica* (Benberger) (Coleoptera: Buprestidae), were found in high enough numbers to mass-collect and redistribute to other sites. Approximately 111 *S. jugoslavica* beetles were collected and distributed between one site in Modoc County and three locations within a large Shasta County infestation. Also, a mixture of approximately 5,500 *B. fausti* and *L. minutus* were collected and released at a site in Modoc, two sites in Lassen and six sites in Shasta County. All the releases occurred July 15 through 17, 2002.

Initial efforts were made in 2002 to map the infestation of squarrose knapweed in Lassen, Modoc, and Shasta counties in order to make better utilization of control methods. Chemical control will be made along highways and high risk areas of the three-county area and biological control releases of the three promising beetles, *L. minutus*, *B. fausti*, and *S. jugoslavica* will be made in heavily infested areas where chemical control applications are not feasible.

Table 2: Status of Biological Control Agents Released against Squarrose Knapweed in Northern California during 1995 through 2002

BC Agent	**County**	**Sites**	**Number Released**	**Date**	**Status**
Sphenoptera jugoslavica	Lassen	1	40	July 16, 1998	Established
Sphenoptera jugoslavica	Modoc	1	24	July 16, 2002	New Release
Sphenoptera jugoslavica	Shasta	1	35	July 15, 2002	New Release
Sphenoptera jugoslavica	Shasta	2	52	July 17, 2002	New Release
Subtotal		**5**	**151**		
Cyphocleonus achates	Siskiyou	1	100	June 1, 1995	Not Established
Cyphocleonus achates	Lassen	1	100	July 10, 2000	Not Established
Subtotal		**2**	**200**		
Urophora affinis	Lassen	1	100	July 10, 2000	Not Established
Urophora affinis	Lassen	1	50	July 16, 1998	Not Established
Subtotal		**2**	**150**		
Urophora quadrifasciata	Lassen	1	100	July 10, 2000	Low Recovery
Urophora quadrifasciata	Lassen	1	50	July 16, 1998	Low Recovery
Subtotal		**2**	**150**		
Terellia virens	Lassen	1	20	July 16, 1998	Not Established
Bangasternus fausti	Siskiyou	1	100	July 17, 1996	Established*
Bangasternus fausti	Siskiyou	5	1,100	July 12, 1997	Established*
Larinus minutus	Siskiyou	1	200	July 10, 1997	Established*
Larinus minutus	Modoc	3	1,500	July 10, 2000	Established
Larinus/Bangasternus mix	Modoc	1	300	July 16, 2002	New Release
Larinus/Bangasternus mix	Shasta	8	4,000	July 15, 2001	Established
Larinus/Bangasternus mix	Shasta	6	4,000	July 17, 2002	New Release
Bangasternus fausti	Lassen	1	300	July 16, 1998	Established
Bangasternus fausti	Lassen	1	270	June 9, 1999	Established
Bangasternus fausti	Lassen	4	1,600	June 10, 2000	Established
Larinus minutus	Lassen	1	2,000	July 16, 1998	Established
Larinus minutus	Lassen	2	800	July 15, 1999	Established
Larinus minutus	Lassen	5	2,000	July 10, 2000	Established
Larinus/Bangasternus mix	Lassen	2	1,200	July 16, 2002	New Release
Subtotal		**41**	**19,370**		

In 1999 the Siskiyou County Department of Agriculture treated a wide spread infestation of squarrose knapweed. The status of the weevils since this action was taken is unknown.

[1]CDFA Integrated Pest Control, Weed and Vertebrate Control, Burney CA

Progress on the Biological Control Program Against Purple Loosestrife in California

B. Villegas

Four biological control agents of purple loosestrife, *Lythrum salicaria* L., have been released in small numbers in California since 1996. 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). They were either collected or obtained from cooperators in Oregon, Washington and Cornell University in New York.

Surveys for establishment by the root-boring weevil were made at two selected sites in Shasta County. In order to minimize destructive sampling, a transect through each of the two release sites was established and 10 plants were dug up and their roots examined for boring damage by the weevil larvae. At one site that received releases of 100 weevils during 2000 and again in 2001, four of 10 plants displayed damage. At a second site that received a release of 100 weevils in 2001, only two of 10 plants had weevil larval damage. These are the first recoveries of this weevil in California. Similar transects will be done at other release sites in the future. No releases of *Hylobius transversovittatus* were made during 2002.

The flower-bud weevils (*Nanophyes marmoratus)* have been released at several sites in Butte and Shasta counties. The weevils damage purple loosestrife through adult feeding of the foliage and larval feeding that causes flower-bud abortion thus reducing the seed output by the plants. It appears that they have more than one generation per year. They readily establish and at one site in Butte County have been recovered in low numbers. The weevils also quickly colonized in Shasta County and were recovered in fair numbers during 2002 at a 2001 release site. Approximately 1,600 flower-bud weevils, received from Oregon on August 2002, were released at three sites in Shasta County.

Approximately 10,000 leaf-feeding beetles, *Galerucella calmariensis* and *G. pusilla* were released at five sites in Butte, Kern, and Shasta counties during 2000. The leaf beetles were collected in the Moses Lake area of central Washington in late May 2002, and delivered overnight to California. Butte and Kern each made 1,000 leaf beetles releases. This is the second year of a renewed program to release large numbers of these leaf-feeding beetles in California. Recoveries of the leaf beetles have been very encouraging in Kern and Shasta counties. The Butte County 2001 release site was chemically sprayed out by the property owner. Initial recoveries have been made at all sites released during 2001 in Kern and Shasta counties. Some recoveries were made in 2002 at some of the 1998 through 2000 release sites. At previous release sites, 1998 through 2000 where additional large releases were made, recoveries as well as damage were more pronounced. Adults were noted at the monitoring sites from May through July. It is assumed that the beetles go into diapause sometime after the F1 generation emerges in July. Preliminary observations in Kern County in early May 2003 suggests establishment there. Initial releases occurred in Kern County in 1999. Monitoring studies to quantify the damage by the beetles will be developed during 2003 and implemented at selected sites in Kern and Shasta counties.

Acknowledgments: Dr. Bernd Blossey (Cornell University), Eric Coombs (Oregon Department of Agriculture) and Gary W. Brown and Kerby Winters (United States Department of Food and Agriculture, Animal and Plant Health Inspection Service), Portland, OR for supplying some of the purple loosestrife biological control agents in the past and during 2002.

Water Hyacinth Biological Control
Status of Weevils in the Sacramento/San Joaquin Delta

P. Akers

In the early 1980's, two weevil species, *Neochetina eichhorniae* and *N. bruchi*, were released in the Sacramento/San Joaquin Delta for the control of water hyacinth. Over a two-to-three year period, approximately 3,000 *N. bruchi* were released at three sites, and 7,500 *N. eichhorniae* at two sites (one site received both). Follow-up studies indicated that *N. eichhorniae* had taken up residence in the Delta, while the fate of *N. bruchi* appeared more uncertain. Long-term monitoring had not been completed.

The State Legislature requires the California Department of Boating and Waterways (CDBW) to control water hyacinth in the Delta. Historically, this control depended upon herbicides, but recent public and regulatory concerns have prompted the CDBW to explore alternative options. Biological control may prove useful in waterways within the Delta that are more or less isolated from the general channel system. The CDBW is limited in treating many of these areas yet hyacinth populations are extremely dense.

During 2002, we investigated options to obtain weevils and move them into dense isolated patches of water hyacinth. One possible source for weevils is to field collect them from areas where the populations are high, such as in Florida. While this is economical, an average of four to nine percent of weevils in the southeast have a chronic protozoan infection, known as microsporidian disease. The disease decreases lifespan and, especially, egg production. Infection rates can become much higher if the population is stressed or crowded. While keeping the disease out of California is desirable, producing disease-free weevils is expensive. Therefore, we established a field survey of the Delta to determine if weevils were still present and if they were already infected.

The primary goal of the survey was to collect 200 to 300 weevils to screen for microsporidia disease. These numbers were determined by considering the likely infection rates and the probability of falsely concluding there was no microsporidian disease in the Delta. For example, *N. bruchi* has an average infection rate of about four percent in the Southeast. If a conservative assumption was made that the true infection rate was as low as one percent, there would be a probability of 0.134 (or about one in eight) of collecting 200 weevils without microsporidia, and thereby falsely concluding there was no disease. If 300 weevils were screened, the probability decreases to 0.049, or about one in 20.

The survey covered a two-week period, September 24 to October 3, 2002. All collections were made from an airboat or a conventional boat. At each collection site plants were examined for adult weevils, which is the easiest life-stage to find. During the first portion of the survey, weevil collections were combined for microsporidia screening. It was soon determined that weevils were much more common in the Delta than previously suspected. Therefore, during a second set of more extensive site visits, care was taken to note the number of weevils collected at each location, and to keep these different collections separated. For this second portion of the survey, the crew visited 23 locations (Map 1). Twenty to 50 plants were searched at each location, depending on the amount of damage and the rate at which weevils were recovered. The intent was to recover no more than 75 weevils from any one site. Fewer plants (generally 20) were searched at sites where there was very little damage or the rate of finding weevil was low. Fewer plants were also searched at sites where many (five to 15) weevils were recovered from each plant, as the limit of 75 was quickly reached.

In selecting survey sites, an attempt was made to cover the navigable Delta east to west and north to south. A site was selected if it had at least a few patches of hyacinth and was several miles from previous sites. The selection of search sites was relatively unbiased in that a patch was not usually scanned for damage before landing the boat and searching at least 20 plants. The survey therefore should reasonably represent the relative weevil distribution. Within a site, plants with heavier weevil damage were selected for searching, so the density of weevils per plant may be overestimated. (Map 1) shows the number of weevils normalized to a basis of 100 plants searched, for comparison.

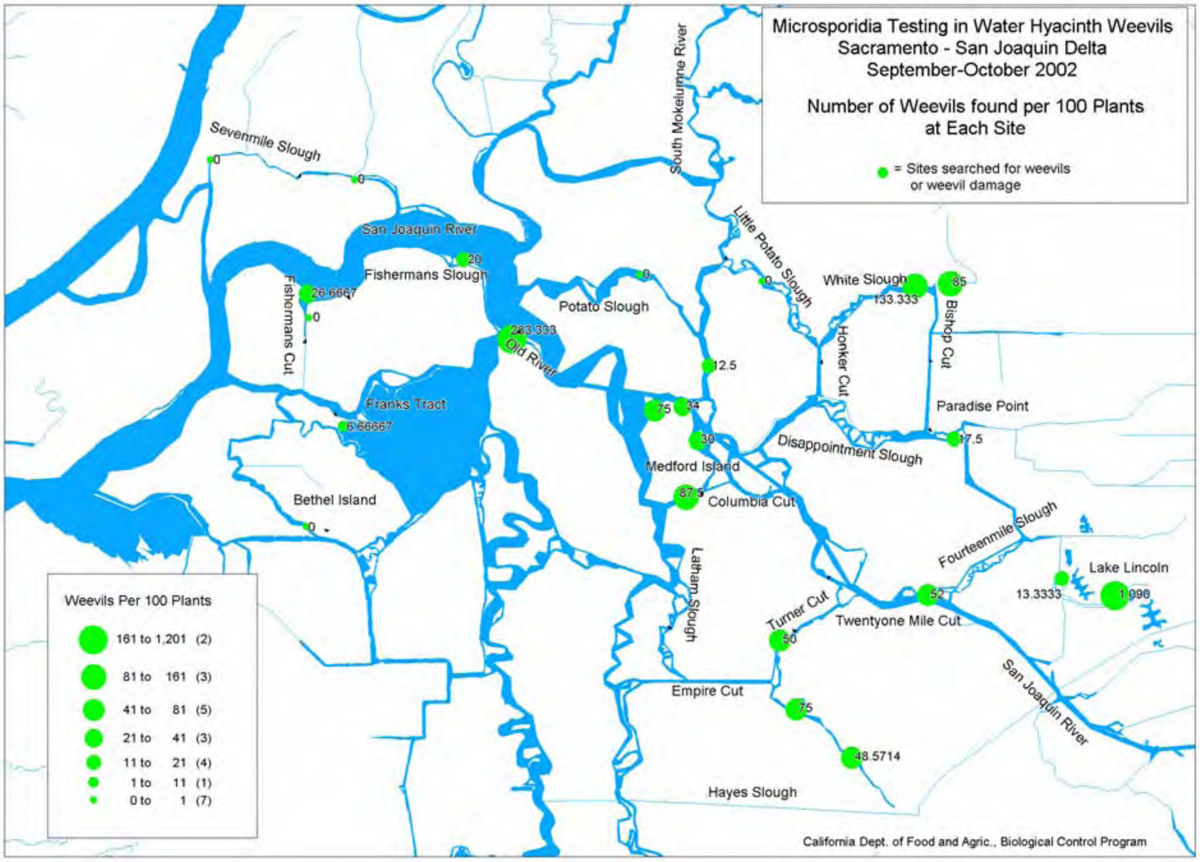
All collected weevils were identified to species. Preliminary identifications suggested *N. eichhorniae* might be uncommon in the collections, yet it was originally the more abundant species. Therefore, special care was taken in the identifications. Specimens were compared to keys, descriptions, and voucher specimens. An insect systematist who had worked with the two weevils was also consulted. A total of 322 weevils were submitted for microsporidia screening to Mr. Bud Thomas, Consulting Diagnostic Service, Berkeley.

The four preliminary collections yielded 143 weevils, and the main survey yielded an additional 354 weevils. The survey showed that weevils are common in much of the Delta and can be relatively abundant in some locations. We found weevils at 17 of 23 sites, where the range ran from two to 109 weevils. Between 10 and 20 weevils were recovered from nine sites, between 30 and 40 weevils were recovered at two sites, only one site had more than 40 weevils, and the remaining five sites had less than 10 weevils. The distribution of the weevils in the Delta seemed to be lower in the west and higher towards the east. In part this seemed to parallel the abundance of hyacinth, but hyacinth abundance did not strongly affect the density of the weevils. For example, one of the largest sets of mats found was at Frank's Tract, toward the west of the search area, but only two weevils were found in 30 plants searched. In contrast, for the patch where over 100 weevils were found, field staff had seen only two or three other small patches in miles of traveling the water channel; the patch itself was only about 20 by 30 feet, and it had been treated with herbicide.

The identification of the weevils showed that only *N. bruchi* had been collected, implying that only *N. bruchi* has become established in the Delta. This is surprising, as fewer *N. bruchi* were released in the original introductions, the main release site for *N. bruchi* was destroyed a few years after the introductions, and *N. eichhorniae* is often more abundant in other areas of the United States. On the other hand, *N. bruchi* has a shorter life cycle than *N. eichhorniae,* which might make it more efficient in the highly dynamic conditions of the Delta.

None of the 322 weevils screened for microsporidia were infected. In addition, no signs of other pathogens were visible. If the true infection rate were one percent, there would be a probability of 0.039, or about one in 25, of collecting 322 clean weevils. If the infection rate were about 2.2 per 1,000, there would be a probability of about 50 percent of collecting 322 clean weevils. In other words, the weevils almost certainly had less than a one percent infection rate, but there is a 50 percent chance they had at 0.2 percent infection rate or less. It appears that the weevils in California are free of disease.

Map 1



Microsporidia Testing in Water Hyacinth Weevils
Sacramento - San Joaquin Delta
September-October 2002
Number of Weevils found per 100 Plants
at Each Site
= Sites searched for weevils
or weevil damage
Weevils Per 100 Plants
161 to 1,201 (2)
81 to 161 (3)
41 to 81 (5)
21 to 41 (3)
11 to 21 (4)
1 to 11 (1)
0 to 1 (7)
Sevenmile Slough
San Joaquin River
Fishermans Slough
Fishermans Cut
Franks Tract
Bethel Island
Old River
Potato Slough
South Mokelumne River
Little Potato Slough
White Slough
Bishop Cut
Honker Cut
Paradise Point
Disappointment Slough
Medford Island
Columbia Cut
Latham Slough
Fourteenmile Slough
Lake Lincoln
Turner Cut
Twentyone Mile Cut
Empire Cut
Hayes Slough
San Joaquin River
0
0
20
26.6667
0
283.333
6.66667
0
0
0
12.5
133.333
85
75
34
30
17.5
87.5
52
13.3333
1,090
50
75
48.5714
California Dept. of Food and Agric., Biological Control Program

Changes in the Biological Control Agent Complex of Yellow starthistle over Eight Years

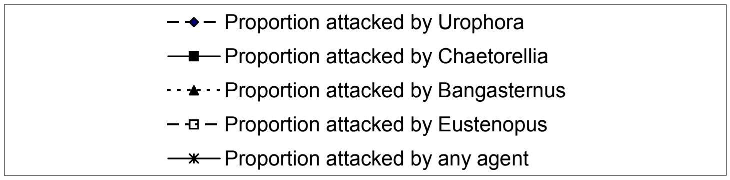
D. M. Woods, M. J. Pitcairn, D. B. Joley, and V. Popescu

A complex of five exotic insect species, all attacking the flowerheads, have been established in California for biological control of yellow starthistle (YST). Preliminary indications are that no single species will be the dramatic silver bullet in reducing YST abundance. Since all species are being distributed throughout the distribution of YST, we anticipate that changes in the species compositional makeup will occur over time and hope that the eventual mix will be effective on starthistle. We have been monitoring several sites in California repeatedly for seven to nine years for effects on YST density and the bioagent composition. This report describes a preliminary comparison of bioagent composition at five of these sites. Each site received some but not all species of insects, but all eventually supported some level of all insects as they immigrated from nearby locations. Entire plant samples were collected at the end of the season (September through October) and processed in the laboratory where each seedhead was examined for evidence of insect attack. Approximately 400 seedheads were evaluated per site per year. Four species, *Bangasternus orientalis* (Capiomont) (Coleoptera: Curculionidae), *Urophora sirunaseva* (Hering) (Diptera: Tephritidae), *Eustenopus villosus* (Boheman) (Coleoptera: Curculionidae), and *Chaetorellia succinea* (Costa) (Diptera: Tephritidae) are the primary focus in this report. The fifth agent, *Larinus curtus* Hochhut (Coleoptera: Curculionidae) is at such low levels as to be virtually undetectable.

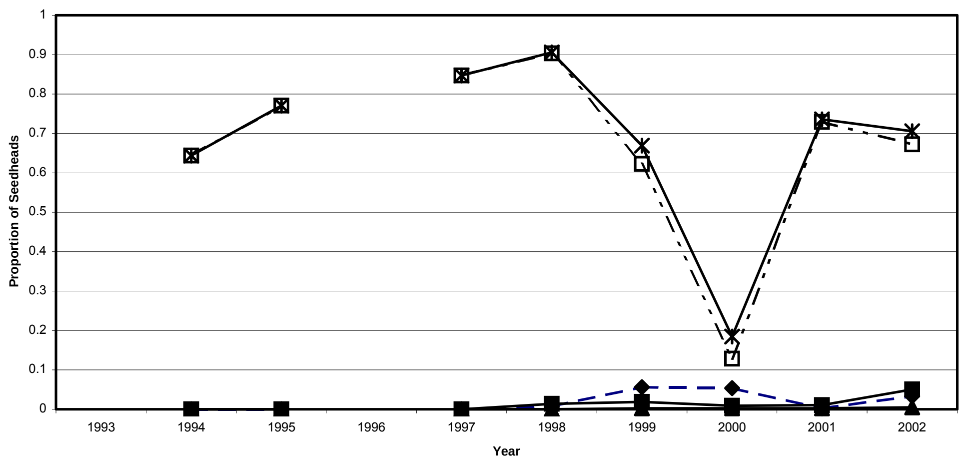
The Napa County site was one of the first in the nation for release of the hairy weevil, *E. villosus*. Weevils were released in 1991 and 1993. No other species of insect was intentionally released at this location. Three years after its release, the hairy weevil was attacking over 60 percent of the seedheads. Although all three of the other species of insect have successfully migrated to the area, they have not been able to establish substantial populations, attacking well below one percent of the total seedheads. A similar result occurred at the Nevada County site, which received a release of the hairy weevil in 1990. The hairy weevil attained and retains a dominating position today. Again, no other species was intentionally released at this location, but extensive intentional releases were made in the area. Interestingly, only the accidentally released *Chaetorellia succinea* has established more than a marginal presence.

The Placer County site is unusual in that all of the insect species had migrated to the site and established before we could release them. We made additional releases of *E. villosus, U. sirunaseva*, and *B. orientalis* in 1994 and 1995. However, *E. villosus* maintained an unassailable dominance of the seedheads at this site over the remainder of our monitoring.

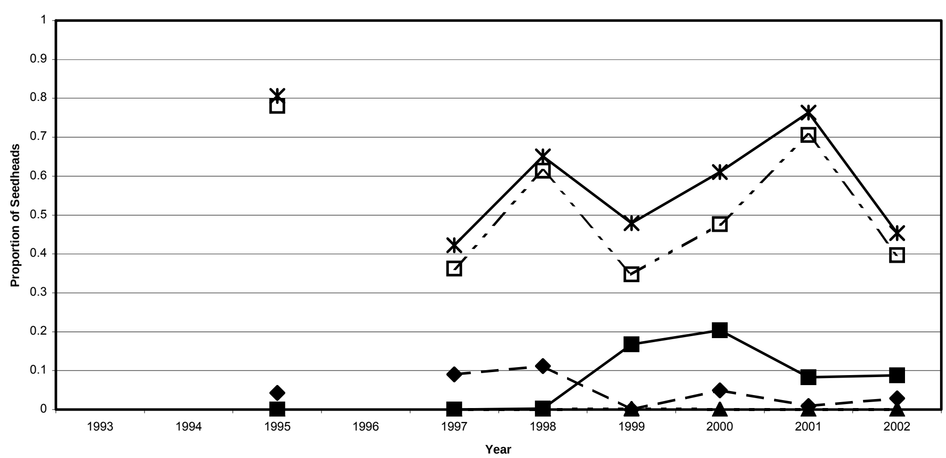
The Sonoma and Yolo County sites were essentially free of biological controls when monitoring first began. All agents except *C. succinea* were released in 1994 and 1995. *Chaetorellia* was released in 1995 at the Yolo County site, but naturally migrated to the Sonoma County site by 1997. The gallfly was able to establish a significant presence initially at both sites but seems to be gradually being displaced by the other biological control agents. The peacock fly seems to be able to make substantial inroads when *E. villosus* is at low levels (Yolo County site) and also slow but distinct inroads when *E. villosus* is at high levels (Sonoma County site).



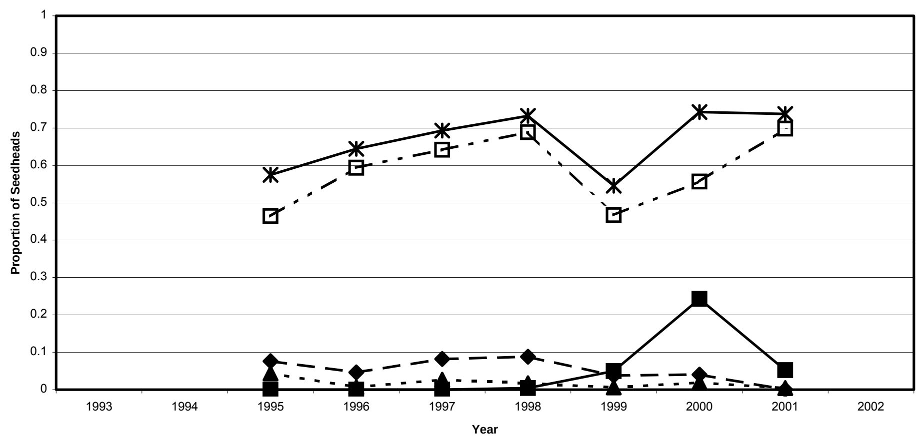
Napa County Site



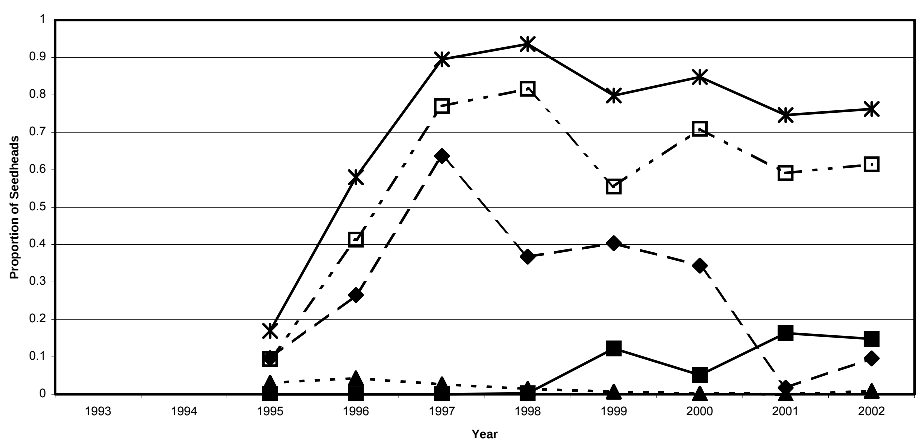
Nevada County Site



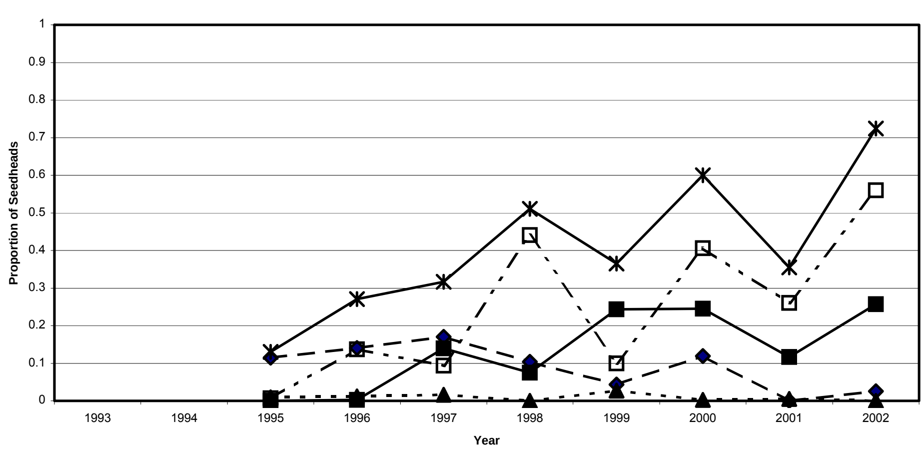
Placer County Site



Sonoma County Site



Yolo County Site



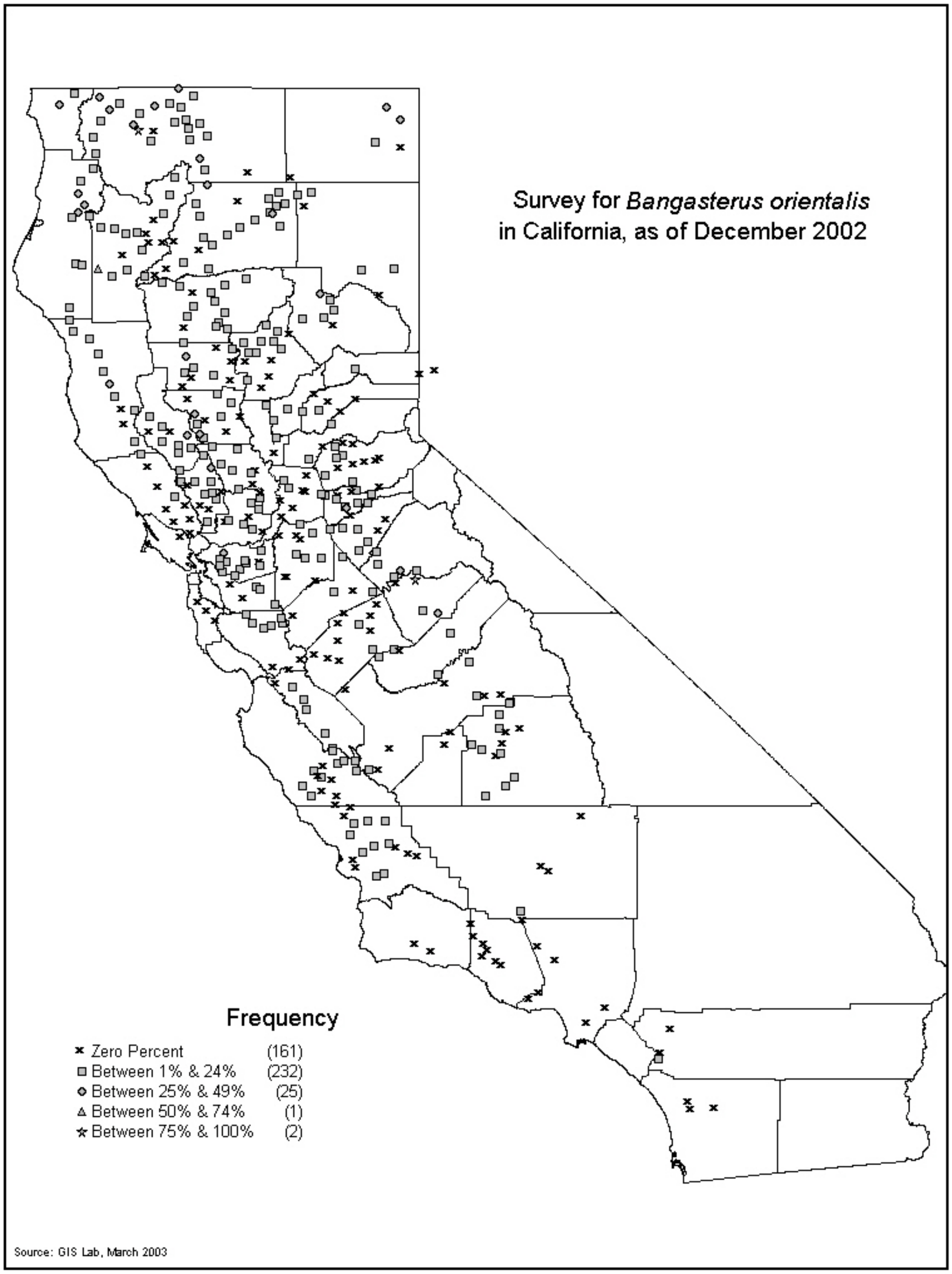
Statewide Survey of Yellow starthistle Biological Control Agents

M. J. Pitcairn, B. Villegas, D. Woods, G. Wilber, A. Duffy, and M. El-Bawdri

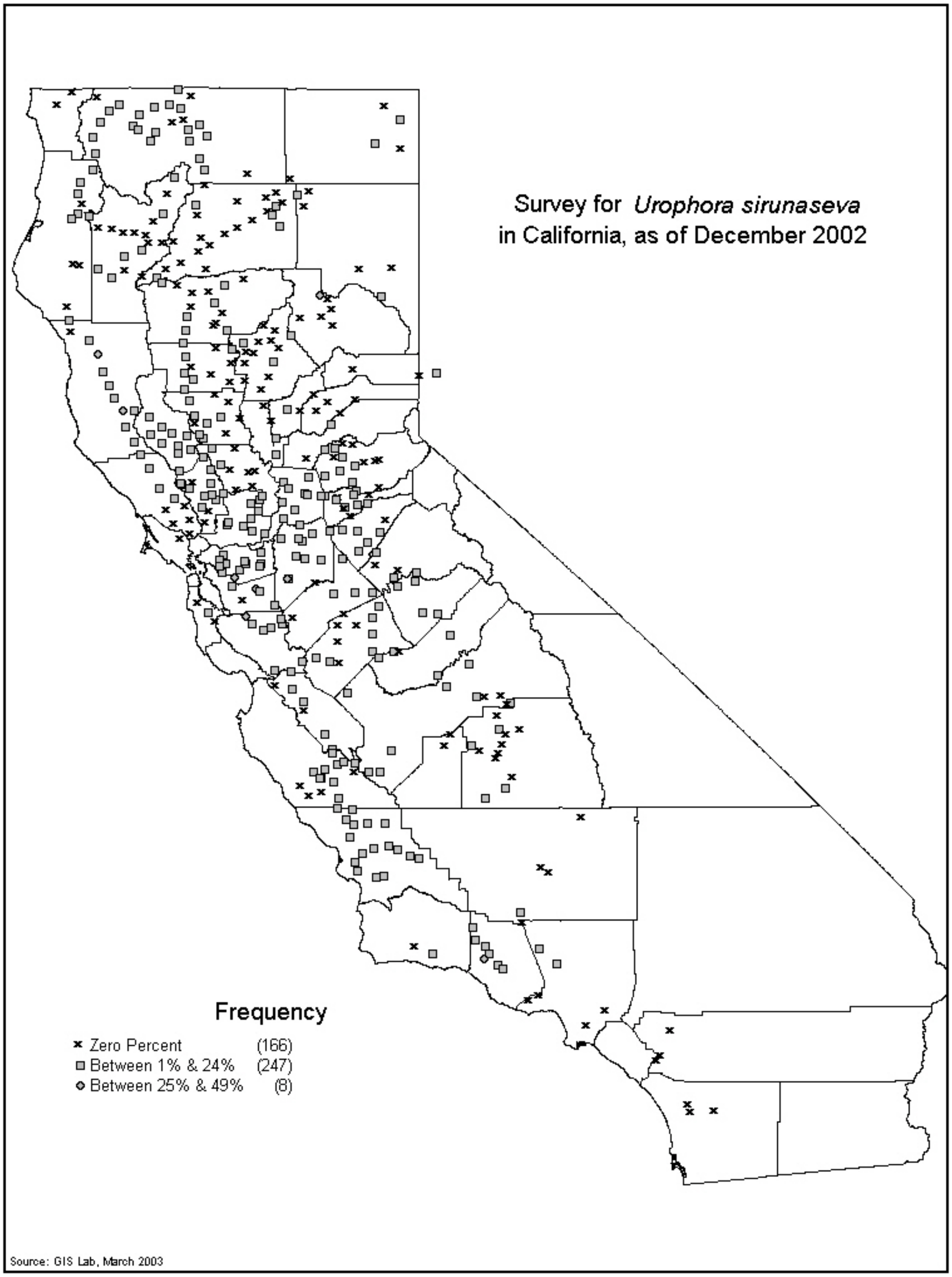
The California Department of Food and Agriculture, in cooperation with the United States Department of Agriculture, is actively involved in the release and establishment of biological control agents against yellow starthistle (YST), *Centaurea solstitialis* L. A total of six insects have been approved and released as biological control agents in California. Of these, five became established: two are uncommon and occur only at a few release locations, and three (the bud weevil, *Bangasternus orientalis*, the gall fly, *Urophora sirunaseva*, and the hairy weevil, *Eustenopus villosus*) are widespread. In 1988, the Biological Control Program initiated a distribution program to assist with statewide distribution of the biological control agents. Annual workshops were 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 collected available insects from nursery sites and returned to their counties to establish their own nursery sites for further distribution.

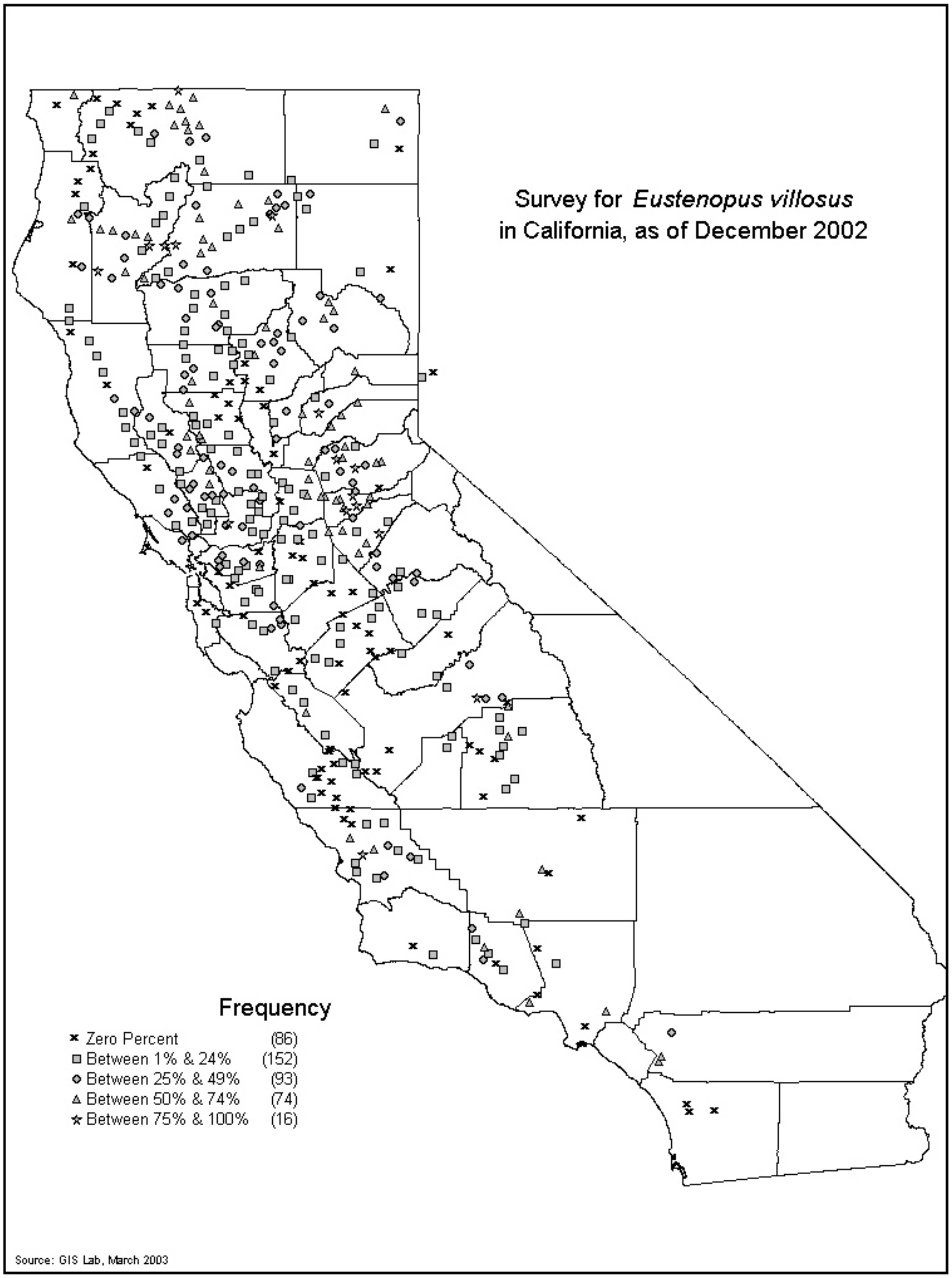
The objective of the distribution program is to establish self-sustaining populations of biological control agents in all areas of the state infested with YST. Hundreds of release sites have been established throughout the state as a result of this distribution program. Once established, the released insects will multiply and spread into nearby areas infested with YST. Presumably, insects have already begun to spread from these sites and now inhabit a much larger area of YST infested landscape. To determine the extent of area where these beneficial organisms now occur, a survey of roadside YST populations was performed in 2001 and 2002. The objective of this survey was to document the natural dispersal of these biological control agents. Using the map of townships infested by YST obtained in 1997 (see Biological Control Program 1997 Annual Summary), roads running through the townships rated with “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 and latitude and longitude for each location were recorded. All flower heads were returned to the laboratory and examined for the presence or absence of the biological control agents.

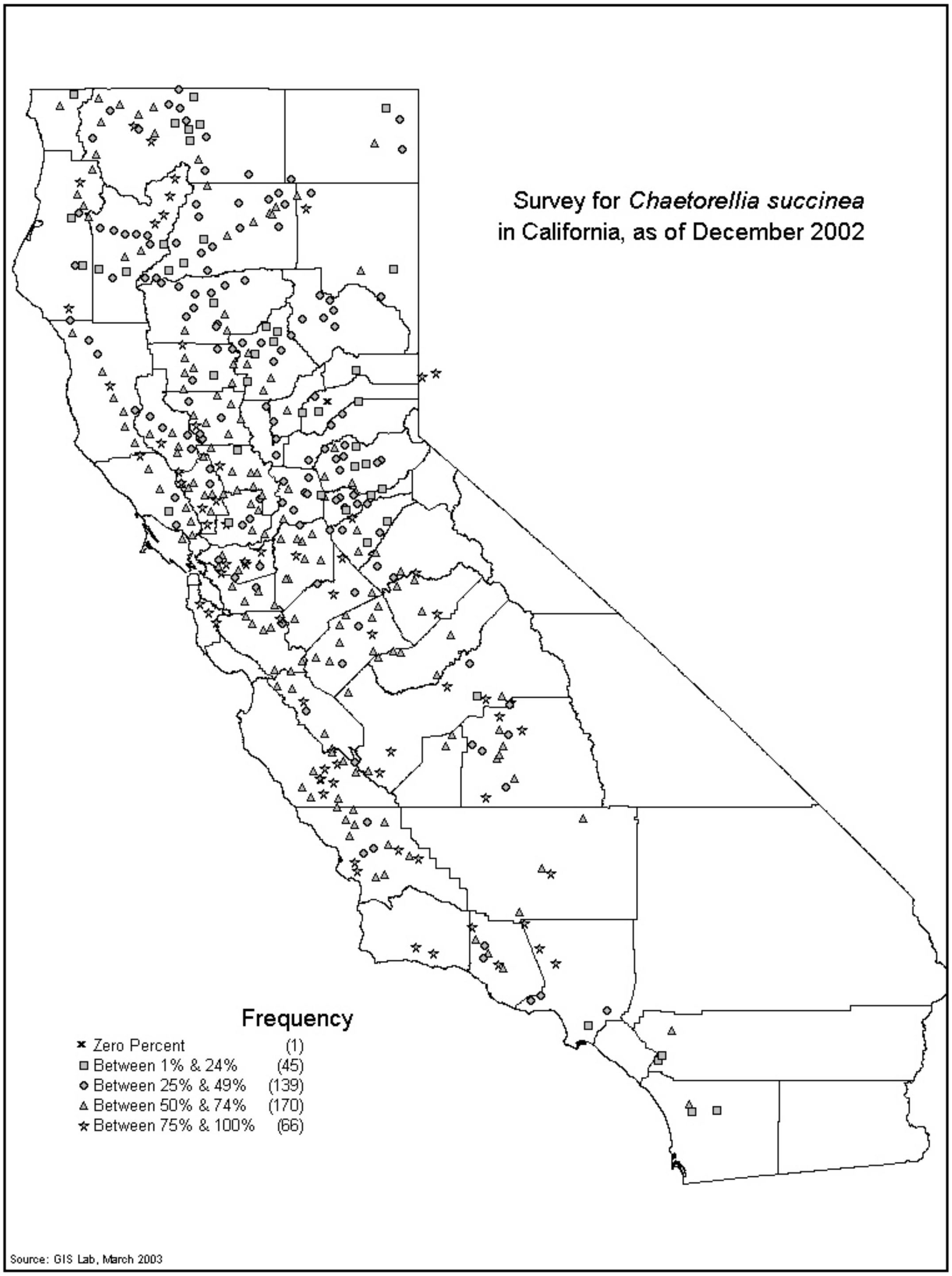
A total of 421 sites were sampled throughout the state. The results are shown in Figures 1 through 4 with different symbols indicating the frequency of occurrence of each agent at each site. In general, the most common seed head insect was *Chaetorellia succinea*, an accidentally introduced seedhead fly that has a particularly strong affinity for YST. This fly was found at 420 of the 421 (99.7 percent) locations sampled. This was followed by *E. villosus*, which occurred at 80 percent of the sampled locations, *B. orientalis*, which occurred at 62 percent of the locations, and *U. sirunaseva*, which occurred at 61 percent of the locations. When found, *B. orientalis* and *U. sirunaseva* occurred at low infestation levels, with usually less than 25 percent of the heads attacked (Figures 1 and 2). In contrast, both *E. villosus* and *C. succinea* occurred at much higher levels, with 43 percent and 89 percent of the survey locations having attack rates greater than 25 percent, respectively.



Survey for *Bangasterus orientalis*
in California, as of December 2002
Frequency
Zero Percent (161)
Between 1% & 24% (232)
Between 25% & 49% (25)
Between 50% & 74% (1)
Between 75% & 100% (2)
Source: GIS Lab, March 2003


Survey for *Urophora sirunaseva*
in California, as of December 2002
Frequency
Zero Percent (166)
Between 1% & 24% (247)
Between 25% & 49% (8)
Source: GIS Lab, March 2003


Survey for *Eustenopus villosus*
in California, as of December 2002
Frequency
Zero Percent (86)
Between 1% & 24% (152)
Between 25% & 49% (93)
Between 50% & 74% (74)
Between 75% & 100% (16)
Source: GIS Lab, March 2003


Survey for *Chaetorellia succinea*
in California, as of December 2002
Frequency
Zero Percent (1)
Between 1% & 24% (45)
Between 25% & 49% (139)
Between 50% & 74% (170)
Between 75% & 100% (66)
Source: GIS Lab, March 2003

Releases of Exotic Natural Enemies for the Biological Control of Yellow starthistle in California, 1984 through 2002

B. Villegas

Six insect species have been introduced into the United States for the biological control of Yellow starthistle (YST), *Centaurea solstitialis* L. These insects include two seedhead gall flies, *U. sirunaseva* (Hering), and *Urophora jaculata* Rondani, a seed feeding fly, *Chaetorellia australis* Hering, and three weevils: *Bangasternus orientalis* (Cap.), the YST seedhead weevil, *Eustenopus villosus* (Boheman), the hairy weevil, and *Larinus curtus* Hochhut, the YST flower weevil. An additional species, *Chaetorellia succinea* (Costa) was mistakenly introduced as *Chaetorellia australis* in 1991. It quickly became established and has become an important natural enemy of YST in the western states of California, Idaho, Nevada, Oregon, and Washington. It was justly named the false peacock fly.

In order to manage releases of these insects, the Biological Control Program, the County Agricultural Commissioners and Sealers Association and the United States Department of Food and Agriculture, Agricultural Research Service initiated a cooperative redistribution program in 1988. Newly introduced biological control agents were quickly released in California and once the insects were well established, collection workshops were set up at available sites and county personnel attending the workshops were able to collect their own biological control agents for release in county nursery sites. The county nursery sites were then monitored and when the insects were well established, personnel from that particular county were able to collect and redistribute the insects elsewhere in that county. Regional collection sites in Amador, El Dorado, Nevada, Placer, Sacramento, Shasta, Siskiyou, and Tulare counties were used for supplying insects to the rest of the counties in California. Additionally, counties like Amador, El Dorado, Glenn, Monterey, Napa, Placer, San Benito, Shasta, Trinity, and Tulare and have organized in-county redistribution collections and educational workshops for their county constituents.

(Table 1) summarizes all the releases that the Biological Control Program has been able to record from data forms supplied during the many collection workshops. This table, when used with distributional maps of YST in California, shows that most areas of California reporting acreage of YST have had some level of releases. This table can also be used with the recent YST survey that was done along major highways that passed through YST infested areas of California. To date, most of the workshops have been done for mass collecting the hairy weevil. A total of 1,182 individual releases have occurred in 50 counties with a grand total of 324,208 weevils released. The hairy weevil is a poor flyer and consequently distributional releases are encouraged. Only 33 releases were made with the two peacock flies, *Chaetorellia australis* and *C. succinea*. These flies are very strong fliers, particularly *C. succinea*. In field surveys conducted since it was released in 1994 through 1996, it quickly became established and moved from released areas very quickly as well. By the summer of 1999, *C. succinea* was recovered in all YST infested areas in California. This fly has also been recovered from tocalote, *Centaurea melitensis* L. and in some areas particularly in the San Joaquin Valley and Southern California; this weed might provide a bridge to isolated populations of YST.

Table 1: Natural Enemies Released Against *Centaurea soltstitialis* in California, 1984 through 2002

County	B. orientalis		E. villosus		L. curtis		C. sucsinea		U. sirunaseva	
	# Insects	# Releases	# Insects	# Releases	# Insects	# Releases	# Insects	# Releases	# Insects	# Releases
Alameda	785	3	1,300	6	2,500	4	0	0	0	0
Alpine	0	0	299	4	150	2	0	0	0	0
Amador	400	2	1,450	6	500	2	164	1	1,026	3
Butte	708	3	2,470	11	0	0	0	0	400	3
Calaveras	3,581	14	3,660	20	1,103	4	0	0	400	3
Colusa	335	2	800	3	0	0	0	0	0	0
Contra Costa	4,325	21	22,532	55	5,325	19	800	3	3,523	14
Del Norte	547	4	500	2	0	0	0	0	0	0
El Dorado	2,899	13	9,661	42	0	0	200	1	1,084	4
Fresno	1,400	4	4,050	13	0	0	300	1	1,300	5
Glenn	5,125	23	17,320	60	1,000	4	700	2	675	6
Humboldt	4,255	15	6,175	25	1,350	2	0	0	520	5
Imperial	0	0	0	0	0	0	0	0	0	0
Inyo	0	0	0	0	0	0	0	0	0	0
Kern	500	4	4,755	25	600	3	0	0	300	2
Kings	200	2	1,910	9	0	0	0	0	300	2
Lake	1,270	7	1,720	4	400	2	0	0	200	2
Lassen	408	2	3,470	16	200	1	0	0	0	0
Los Angeles	297	1	2,367	5	0	0	0	0	0	0
Madera	900	5	254	1	0	0	200	1	200	2
Marin	1,469	11	3,285	14	450	2	300	1	200	2
Mariposa	523	3	1,065	5	0	0	1,151	4	0	0
Mendocino	2,750	17	3,484	12	200	1	0	0	3,342	19
Merced	710	5	22,363	10	500	2	0	0	405	3
Modoc	600	4	1,350	6	0	0	0	0	200	1
Monterey	650	4	23,865	77	3,685	14	0	0	400	3
Mono	0	0	0	0	0	0	0	0	0	0
Napa	2,390	13	10,849	35	1,200	3	770	2	4,369	14
Nevada	3,924	25	400	2	200	1	322	2	200	1
Orange	0	0	0	0	0	0	0	0	0	0
Placer	2,470	21	3,765	20	580	3	460	2	1,169	5
Plumas	1,550	8	6,150	25	225	1	42	1	0	0
Riverside	300	1	700	5	0	0	0	0	0	0
Sacramento	750	4	7,516	24	1,350	4	972	2	425	2
San Benito	1,100	6	5,460	22	200	1	0	0	400	2
San Bernardino	0	0	0	0	0	0	0	0	0	0
San Diego	0	0	0	0	0	0	0	0	0	0
San Francisco	0	0	0	0	0	0	0	0	0	0
San Joaquin	1,179	5	1,550	7	0	0	0	0	680	4
San Luis Obispo	3,081	20	9,765	21	0	0	560	1	3,450	13
San Mateo	1,160	6	915	5	0	0	0	0	850	5
Santa Barbara	200	1	9,135	23	1,077	2	800	2	600	3
Santa Clara	1,885	8	5,902	27	358	2	0	0	450	3
Santa Cruz	216	1	0	0	0	0	0	0	0	0
Shasta	5,697	30	38,575	183	600	3	794	3	6,050	20
Sierra	250	1	650	4	200	1	0	0	0	0
Siskiyou	1,399	7	2,100	10	400	2	500	1	550	3
Solano	1,425	9	2,500	9	400	1	0	0	1,451	8
Sonoma	3,343	14	10,216	46	3,500	16	0	0	1,890	7
Stanislaus	1,024	6	6,750	20	0	0	0	0	1,158	3
Sutter	425	2	5,280	22	270	1	300	1	0	0
Tehama	2,025	14	11,920	54	600	3	0	0	200	2
Trinity	4,188	22	8,605	41	1,000	3	0	0	700	4
Tulare	365	3	23,475	101	500	2	300	1	300	2
Tuolumne	934	4	2,387	10	0	0	0	0	300	2
Ventura	0	0	630	2	0	0	0	0	0	0
Yolo	4,323	19	4,608	12	800	4	400	1	1,126	5
Yuba	1,331	9	4,300	21	200	1	0	0	1,200	2
Total Releases	**81,571**	**428**	**324,208**	**1,182**	**31,623**	**116**	**10,035**	**33**	**41,993**	**189**
No. Counties	**49**		**50**		**33**		**20**		**38**	

Identification and Mapping of Russian Thistle (*Salsola tragus*) and its Types

P. Akers, M. J. Pitcairn, Fred Hrusa, and Fred Ryan

Russian thistle, *Salsola tragus* L., is an annual tumbleweed infesting 41.3 million hectares in the western United States. Originally from southwest Asia, it was accidentally introduced into South Dakota in the 1870's. Since then, it has spread over most of the central and western United States and southern Canada. Russian thistle is now found in much of California, especially in the drier lands of the southern and western San Joaquin Valley. It causes considerable damage as a roadside weed, as a weed in certain crops and by fouling canals. It also acts as a reservoir for important insect pests, including the beet leafhopper. The leafhopper transmits curly top virus, a virus that infects several hundred varieties of ornamental and commercial crops, especially sugar beets, tomatoes, and melons in California. The problem has led to a control program costing approximately $1.2 million per year, and requiring extensive insecticide treatments of the leafhoppers in Russian thistle stands.

An Eriophyid mite is currently undergoing host safety testing as a possible biological control agent for Russian thistle. This is the first new effort against the weed since the release of two Coleophorid moths in the early 1970's. Both species can be found in most localities that have Russian thistle, but they have not had much impact on the plant. When the mite or other agents are ready for release, their impacts will need to be evaluated in order to measure the performance of the project. Work this past season prepares for managing the future release of natural enemies and documenting impacts on the Russian thistle population in California by; 1) identifying potential new species that have until recently all been identified as *S. tragus;* and, 2) mapping the distributions and density of Russian thistle and, if possible, its various types.

The plant commonly known as Russian thistle is actually a grouping rather than a uniform species. Earlier work had shown that there are at least two types of Russian thistle distinguishable by enzyme and DNA differences (Ryan and Ayres 2000). They are referred to as Type A and Type B, with Type A being the traditionally recognized *S. tragus*. However, there were questions as to whether the two types could be distinguished morphologically. Dr. Fred Hrusa, Senior Plant Taxonomist, California Department of Food and Agriculture, was working on a limited set of specimens from a common garden found several promising morphological characters of these two main types. This year's effort was intended to verify their usefulness in the field. It also provided an opportunity to gather more diverse specimens for the continuing search for useful characters. This was especially important for Type B, which was represented by only a few populations in the common garden material. A secondary objective was to begin accurate mapping of the weed, particularly any variants. Identifying cryptic species is important because biological control agents are often highly specialized, and an unrecognized species could possibly be unaffected by biocontrol agents, allowing it to simply replace the weed controlled by the introduced agents. Mapping is important to target the sites of releases, identify research locations, and follow changes in the weed population in order to document impact.

Isoenzyme assays were used to verify field (or herbarium) identifications of the type or species of field-collected specimens. These assays were based upon aspartate amino transferase (AAT) and 6-phosphogluconate dehydrogenase (6-PGDh) and give clear separations of Types A and B, providing evidence for the possible existence of cryptic species. For each plant collected and identified in the field, a herbarium specimen was prepared and tagged for later morphological study and a matching tagged sample was run through the isoenzyme assay.

Some plants were also run through RAPD DNA analysis in order to begin a more detailed study of the relationships of the types gathered during the course of the survey.

Because the morphological character sets need to be as broadly useful as possible, specimens were collected at many sites within the Central Valley and into the Mojave Desert. A broad survey also meets the need to map the distribution and density of the types about the state. A total of 89 sites were visited, of which 12 sites were in the Mojave. Generally, a site was selected to be at least 15 miles from a previous collection site. Areas where Type B had previously been reported were targeted for visits, as it was desirable to ensure that both types were well represented in the collections, and Type B appeared to be somewhat uncommon in earlier work. If possible, sites were selected that had at least several dozen plants, but it was common to cover many miles in the agricultural areas of the Valley without seeing any Russian thistle. In these cases, sites with even a single plant were visited. Unfortunately, there was no method to efficiently record the many locations where no Russian thistle was seen. Sites were scored for the distribution of plants relative to the roadside (roadside only, away from roadside only, or both), a visual estimate of the size of the patch, and a visual estimate of the relative abundance of the types. In the latter half of the survey, after enough Type B had been collected for morphological and isozyme studies and field staff could recognize Type B in the field, a point-line transect through many of the stands, noting the type or absence of Russian thistle at two meter intervals.

Field observations were made on 220 plants, of which 150 were collected for the herbarium and for isoenzyme assays. Plants were given a field identification as to Type or species (in the case of *S. paulsenii*), scored for the characters that promised to best separate Type A and B (length of anthers, subjective estimate of abundance of winged seeds, form of minor lobes on winged seeds, maximum diameter of the winged seeds, the placement of winged seeds on the plant, and hairs on stems), and for height and two width measurements, to give a rough measure of volume. Typically, two specimens were collected for each Type present. Plants were chosen to represent the range of variation at the site. If there was a wide range of variation of a Type at a site, more than two specimens were collected. At 17 sites, only Type A plants were found and they seemed to fall within the range of variation that had already been collected in nearby locations, so no plants were collected.

Type B was found to be generally uniform in its characters, and scientist learned to recognize it routinely and easily in the field (Table 1). On the other hand, Type A was highly variable and may grade into another type, Type C, and possibly into a form of *S. paulsenii* (the lax form). It may prove difficult to consistently identify these latter groups, at least in the field.

Many of the important field characters involve the flowers and seeds. The winged seeds of Russian thistle are unusual among plants and an explanation may be helpful. The winged seeds look like flowers on casual observation. The true flower is nondescript and is chiefly marked by the expansion of the anthers. After flowering, the calyx remains wrapped about the developing seed. An auxiliary lobe on the sepal may develop into a “wing” that gives the appearance of a petal on a flower. Whether the lobes develop depends on the Type, on the individual plant, and on the location of the flower on the plant. Seeds can form without having any development of the sepal lobes. This is common in Type A. In seeds that do develop wings, not every sepal of the calyx develops equally. Three sepals will develop larger, sub-equal “major” lobes. Alternating with them, the lobes of the remaining two sepals will expand to a varying extent, depending on type and species. They are always clearly smaller than the major lobes, sometimes little more that triangular nubs. Type B has exceptionally large and obvious minor lobes.

In the field, the most useful characters for verifying Type B were the length of the anther and the form of the minor seed lobes. In Type B, the minor seed lobes are rounded, large, and lay on top of the major seed lobes, such that they are easily visible in the field, even without a hand lens. The anthers, when they are present, are small, 0.5 to 0.7 millimeters (mm). Other useful field characters were that Type B plants always have seeds with expanded wings (in the latter part of the season), the winged seeds appear both low and high on the plant and the internodes between bracts are generally long relative to the bract length. These latter characters give the plant an open, white-spangled appearance that is generally easily recognizable even from several meters. All these characters are in general distinctive from Type A and the other possible forms. In Type A, the minor lobes are much smaller than the major lobes and are generally hidden between or beneath them, such that they are hard to find in the field. They may be small triangular nubs, or oblong with slightly expanded tips.

Type A is highly variable in many of its characters. Some plants never develop any winged seeds or only very few, even at the very end of the season (November), though such plants are often quite large, over a meter in every dimension. This in itself will rule out a plant as Type B, as Type B always has winged seeds, at least after July. Type A plants without winged seeds often have an abundance of anthers, even late into the season. Seeds are present, even though they do not have wings. Plants without winged seeds generally have long internodes between bracts, these being congested only near the branch tips. Such plants appear relatively open and often have a lacy, soft appearance. At the other end of the scale, many Type A plants can have an abundance of winged seeds. In the latter plants, the winged seeds generally occur in the outer, upper parts of the plant, towards the outer half of the branches. This is unlike Type B, where the winged seeds also occur toward the base of the branches and plant. In Type A plants with abundant winged seeds, the internodes between bracts are short and the winged seeds arise close together, giving the plant a dense, bristly, compact appearance, again unlike Type B. The full range of variability of Type A can occur at a single site.

The only character that does not appear to vary much for Type A is the length of the anther, at approximately 1.1 to 1.3 mm, as opposed to 0.5 to 0.7 mm for Type B. If anthers are available, they alone can distinguish Type B from other types and species.

Winged seed diameter is not a good character for distinguishing a plant, at least in the field. Type B plants were consistent with diameters running five to eight millimeters. Type A plants were highly variable. Some plants with winged seeds had diameters in the two to three mm ranges, but plants with a range of four to seven mm were more common.

Stem hairs were occasionally useful. Type B appears to be always glabrous. Type A usually has stem hairs of some form, but glabrous individuals are common. In addition, in the field it is often difficult to clearly see far back on a stem or major branch.

Field identification of the different types generally agreed well with isozyme identifications, especially after a little experience with Types A and B in the field (Table 1). Isozyme BB plants were consistently identified as Type B in the field. Isozyme AA plants were nearly always identified as Type A (the two isozymes, AAT and 6-PGDh, cannot distinguish *S. paulsenii* from Type A and therefore the three *S. paulsenii* among the AA group in (Table 1) are not misidentified). Beyond Type B lays a range of other morphologic and isozyme types (Table 1) that are beginning to show some consistent relation to one another, although more work with the putative Type C and the forms of *S. paulsenii* is needed. Type C had been noted occasionally in earlier work and continued to appear in the current collections. Of the 21-isozyme California plants, 10 were identified in the field as Type A, but six of those 10 plants were from the first collecting bout, when we were first becoming familiar with variation in the field. That so many of the California plants caused difficulties in their identification, especially later in the collecting period, indicates that the field biologist was becoming aware of differences that distinguish this type. Many isozyme California group plants had minor wing lobes that were somewhat expanded beyond that typical for Type A, or had non Type B winged seeds that nonetheless occurred both low and high on the plant, or they were intermediate in the length of the anther. More collection and study of isoenzyme Type C plants may well lead to morphological characters than can distinguish this type.

Table 1: Comparison of Isoenzyme Identifications Versus Field Identifications of Individual Plants

Isozyme ID's: AAT + 6-PGDH	**Number of Plants**	**Field ID's and Number of Plants**			
		A	**B**	**P**	**Other**
AA[1]	73	66		3	B?[2] = 1
					A? = 3
BB	48		47		B? = 1
AB	3	2			B? = 1
CA	21	10			A? = 3
					A mwEx = 2
					A Low = 2
					A or P = 1
					B? = 3
A''A	3				A Low = 3
AA'	3	2			B? = 1
AB'	1	1			
AB*	2	2			
C'A	1			1	

[1] XX = the type identification provided by AAT plus the type identification provided by 6-PGDh. For example, AA means isozyme AAT identified the plant as Type A and 6-PGDh also identified it as Type A. A = Type A, B = Type B, C = Type C: A', A'', B', B*, and C' were possible variants from the main types.

[2] B? = The plant seemed most likely to be a B but the identification was difficult: A? is similar; P = *S. paulsenii*; A mwEx = a note was made that minor wing was somewhat expanded, intermediate between A and B; A Low = plants seemed to have A-type winged seeds (relatively small minor lobes) but they arose LOW on the plant, opposite to the usual pattern in Type A.

Mapping of the site collections confirms general impressions of Russian thistle as a roadside weed in most of the Central Valley and western Mojave. In heavily agricultural areas, Russian thistle was actually uncommon, even along the roads. It was generally common only along some of the major highways and railroads, and in the peripheries of towns. Usually it is found away from the roadside only near towns, in vacant lots and similar locations that receive occasional (not regular) disturbance. The only area where Russian thistle was growing abundantly is in the general landscape was in the area of the Elk Hills, in southwestern San Joaquin Valley. The search only went as far north as Marysville, but Russian thistle still occurred there. Type A was found throughout the survey area. Type B was only found south of Tracy, and not at all in the Mojave. An earlier survey had found Type B as far north as Marysville, but the current search in the same areas found none. The precise locations where Type B had been found previously were not visited during this survey.

Type B is generally not as common as Type A, but it seems to have a population concentration around the town of South Dos Palos and along Highway 133 south into Mendota. The two types can occur in pure or mixed stands. In mixed stands, Type A is generally more abundant. Line transects were performed at 26 collection sites and mixed populations were found at three of these sites. The average percent cover of Type B at the three sites was 3.2 percent, while it was 38.0 percent for Type A. While this is a very small sample, the results are consistent with subjective impressions. With regard to the frequency of finding sites with the different types, of the 89 sites visited in the survey, 49 were in the San Joaquin Valley south of Tracy. Of those 49 sites, 23 had Type A only (sometimes possibly mixed with Type C), 14 had Type B only (sometimes possibly mixed with Type C), and nine had mixed stands. Of the remaining three sites, one had *S. paulsenii* only and the other two possibly had only Type C. The results from these 49 sites almost certainly overstate the relative frequency of Type B because Type B was a conscious target of the survey. Still, Type B is not rare within its range, though not as common as Type A.

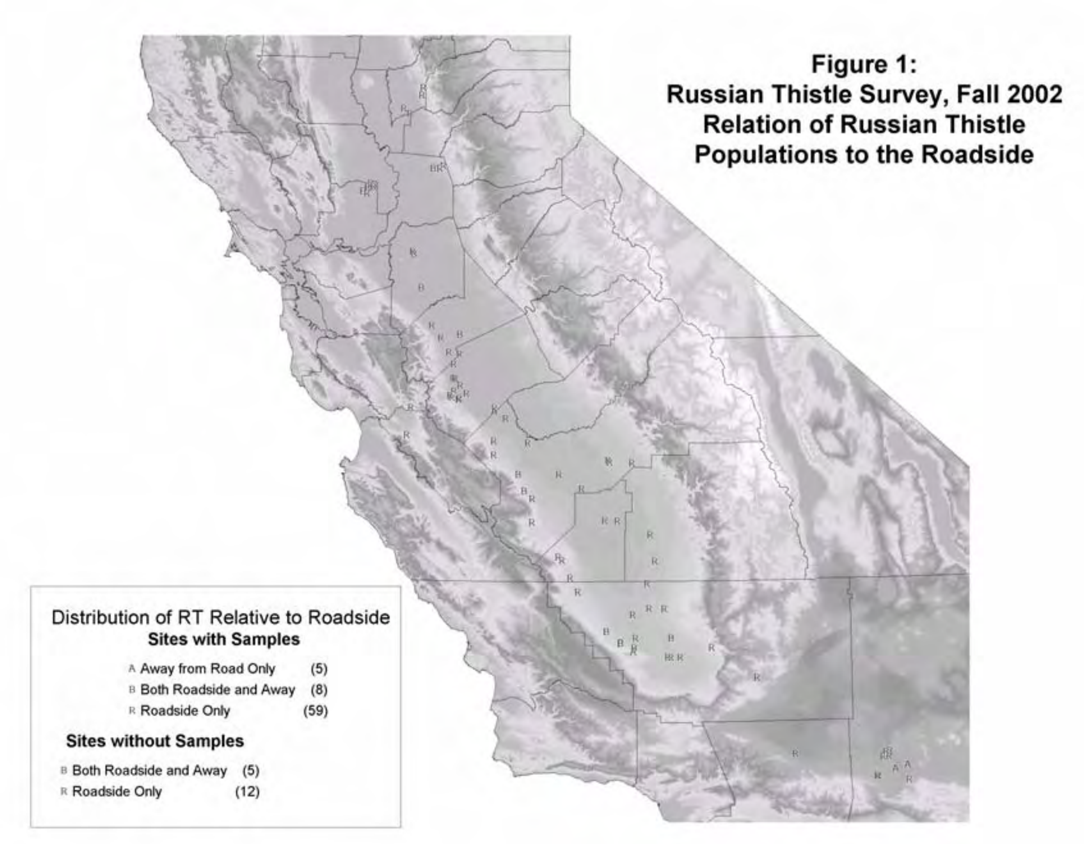


Figure 1:
Russian Thistle Survey, Fall 2002
Relation of Russian Thistle
Populations to the Roadside
Distribution of RT Relative to Roadside
Sites with Samples
A Away from Road Only (5)
B Both Roadside and Away (8)
R Roadside Only (59)
Sites without Samples
B Both Roadside and Away (5)
R Roadside Only (12)

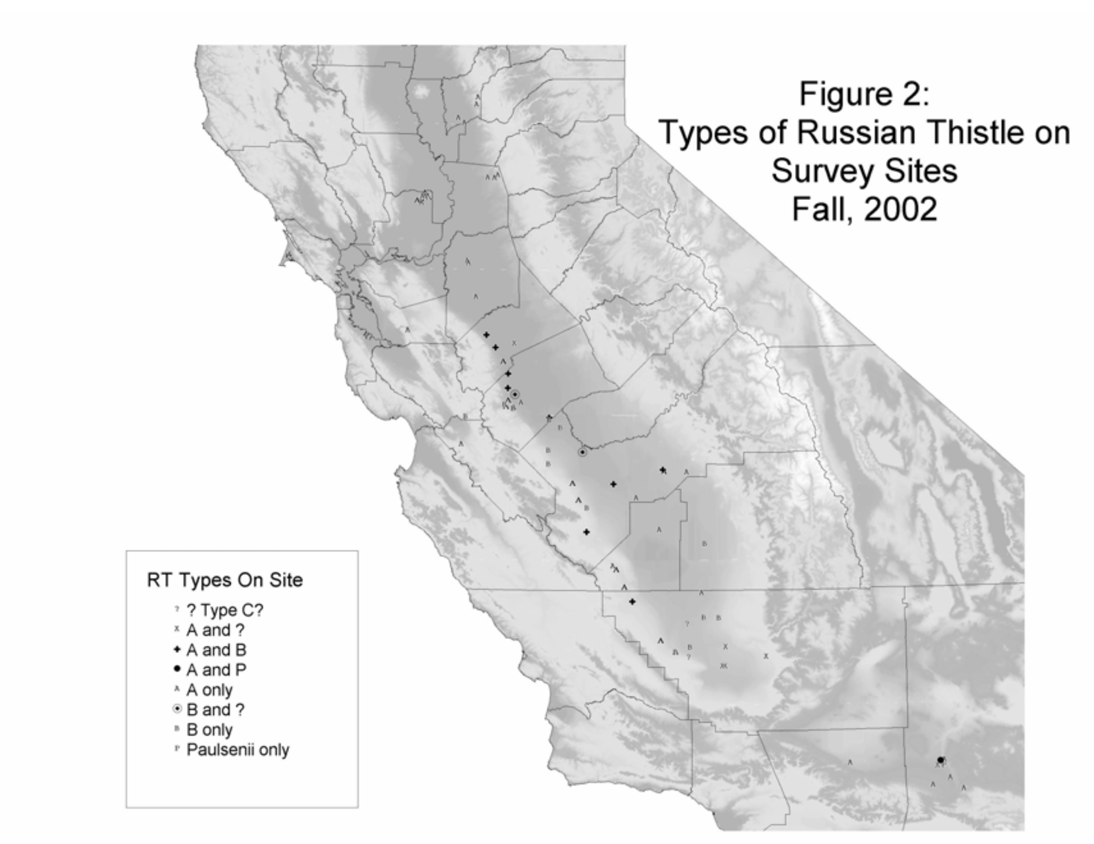


Figure 2:
Types of Russian Thistle on
Survey Sites
Fall, 2002
RT Types On Site
? Type C?
A and ?
A and B
A and P
A only
B and ?
B only
Paulsenii only

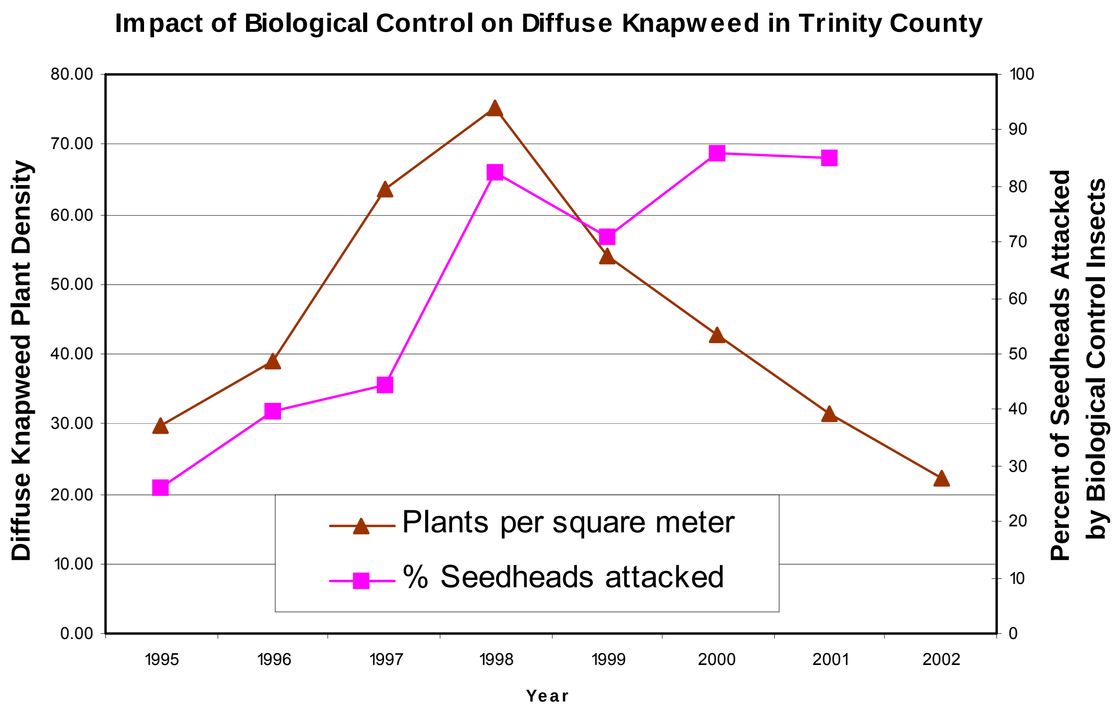
Apparent Decline in Density of Diffuse Knapweed Associated with Biological Control

D. M. Woods, D. B. Joley, 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 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 miles away. By 1998, both weevils could be found at the monitoring site in low numbers, and by 2001, both were well represented. Previous reports have discussed the phenomenon of clustering of the two weevils as well as preliminary analysis of seed destruction. In this report we discuss long-term changes in plant density concurrent with increased amount of the biological control insects.

Density measures of diffuse knapweed plants were made annually in permanent plots in the fall. A total of 30 quadrats, each one half meter by one half meter were evaluated along each of two transects established through an established stand of diffuse knapweed. Attack of the biological control insects was evaluated by collecting 10 randomly selected plants outside the transects each year, and evaluating each seedhead in the laboratory for evidence of attack.

From 1995 through 1998, the density of diffuse knapweed more than doubled within our plot area. Populations of the biological control insects were also dramatically increasing during this same period to nearly 80 percent of the seedheads attacked. Insects attack rates have remained high (over 70 percent) from that point forward. Diffuse knapweed plant densities have declined each year that the attack rate was at least 70 percent. While we cannot rule out all other factors at this point, competing vegetation has not increased within the plot during the course of the study. Consequently, we are optimistic that the decline in diffuse knapweed plant density is due to the biological control agents.


Impact of Biological Control on Diffuse Knapweed in Trinity County
Diffuse Knapweed Plant Density
Percent of Seedheads Attacked by Biological Control Insects
Plants per square meter
% Seedheads attacked
Year
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
100
90
80
70
60
50
40
30
20
10
0
1995
1996
1997
1998
1999
2000
2001
2002

Establishment and Impact of *Terellia virens* on Spotted Knapweed

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

The most recent addition to the complex of seedhead feeding insects on spotted knapweed in California is the green clearwing fly, *Terellia virens*, (Loew) (Diptera: Tephritidae). We first released *T. virens* in 1996 at the western edge (hill) of our spotted knapweed infestation along the Pit River in Shasta County. In 1997, staff released additional numbers of *T. virens* at this location as well as the 'plot' location some 400 meters east. By July 2000, adults were detected in the field during summer as well as overwintering larva in the end-of-season fall field collections. This report describes the population buildup as well as estimates of seed destruction from 1997 through the summer of 2002.

Population changes of the insect are estimates from our end of season field collections. Ten entire spotted knapweed plants were collected in early fall from each monitoring location. All seedheads were removed, mixed and at least 300 randomly chosen and examined for evidence of insect attack. Seed destruction data were obtained from a separate set of samples, using a methodology designed to collect maturing insects and developing seeds from individual seedheads. To do this, spotted knapweed seedheads with oxidized flowers were enclosed in cotton bags every other week during the flowering season over four years (approximately 50 bagged per site, each week, of each year). At the end of the season, the bagged, mature seedheads were collected, dissected and inspected for viable seed as well as evidence of attack by any seedhead insect.

The tephritid fly, *T. virens* did not establish well at the original two release sites (Table 1). Both sites already had established populations of other biological control insects at that time. In particular, the weevil, *Larinus minutus* had achieved over 70 percent attack rate of the seedheads at these sites. This level of destruction effectively precludes the fly from locating an open niche and escaping being eaten by the weevil larva. Fortunately, adult *T. virens* flies well and spread unaided ahead of the weevil's progression to a site 800 meters upriver (logjam) where it has established a strong population. Although *L. minutus* migrated to the site at nearly the same time, the strong establishment of *T. virens* may actually be limiting development or establishment of *L. minutus,* which currently has only infests 13 percent of the seedheads.

Table 1. Seedhead attack by *Terellia virens* at three sites along the Pit River

	1997	1998	1999	2000	2001	2002
Hill	0.4%	0	0	0.2%	0	0
Plot	4.7%	1.0%	0.4%	0.4%	0.3%	0.2%
Logjam	0	0	4.8%	10.2%	8.5%	21.7%

The larvae of *T. virens* consume seeds as well as other seedhead tissue. Unfortunately, the larvae are usually present singly and seem to consume only about a third of the seed compared to those present in clean seedheads (Table 2). Additionally, the low number of attacked seed severely limits any evaluation. It is possible that when multiple larvae develop within a seedhead, seed loss will be substantial. Until that can be confirmed, *T. virens* should be viewed as a small but significant contributor to biological control of spotted knapweed.

Table 2. Mean Seed Production per Seedhead (Number of Seedheads)

	1998		1999		2000		2001		2002	
	Number Insects	*Terellia* Only	Number Insects	*Terellia* Only	Number Insects	*Terellia* Only	Number Insects	*Terellia* Only	Number Insects	*Terellia* Only
Hill	14.9 (46)	10 (1)	11.5 (39)	7 (1)	13.6 (34)	-			10.7 (3)	-
Plot	8.6 (17)		4.3 (14)		12 (6)	8 (1)	11.9 (47)	4 (2)	0 (1)	-
Logjam							16.3 (68)	12 (12)	17.8 (24)	9 (11)

Weed Seed Bank

Patrick Akers

The Biological Control Program established a seed bank in 1979 in order to have seeds available for propagation of host plants for biological control projects. Wild-collected species are collected from several regions of the state representing different geographic and climatic conditions. The seed bank also includes accessions obtained from commercial seed suppliers. Each collection typically consists of between 200 to 3,000 seeds. The seed bank supports tests of biological control agents with potential for release in California. Local biotypes of the target species and non-target species, including both native plants and crop species are maintained. The seeds are used by the Biological Control Program and by other researchers who screen natural enemies for host specificity.

During 2002, the conversion of the seed bank database from File Maker Pro on a Macintosh to MS Access on a Personal Computer was completed and the database was improved. Several seed lots that had been collected but never entered into the seed bank were finished. Additional new collections were made to support several projects that are entering the host-testing phase. A total of 173 accessions were added to the seed bank, representing 91 species. The collection currently contains 387 accessions representing 156 species, up last year from 215 accessions representing 73 species.

The Seed Bank sent out 123 seed lots representing approximately 100 species to cooperating agencies/researchers. Recipients included Massimo Cristaforo, Rome, Italy, Dr. Andy Shepard's group at Montpelier, France, Dr. Lincoln Smith, United States Department of Agriculture (USDA), Albany, Dr. Bill Bruckart, USDA-Ft. Dietrick, MD, Dr. Richard Hansen, USDA, Bozeman, MT, and Keith Colpetzer (mile-a-minute). The seeds supported testing for Yellow starthistle, the weedy brooms, Russian thistle, and mile-a-minute weed.

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