BIOLOGICAL CONTROL PROGRAM

California Department of Food and Agriculture

BIOLOGICAL CONTROL PROGRAM

1995 SUMMARY

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DIVISION OF PLANT INDUSTRY
INTEGRATED PEST CONTROL BRANCH

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The staff would like to thank Linda Heath-Clark for the drawing of *Eustenopus villosus* and for incorporating it into the cover art.

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Introduction

L. G. Bezark

The Biological Control Program of the California Department of Food and Agriculture has expanded its involvement in many new projects and issues in recent years. Several new insect projects were added this year including cotton aphid and giant whitefly. The recent addition of a new staff scientist, Kris Godfrey, has allowed the program to expand into biological control of the cotton aphid, an increasingly important pest of cotton. New weed targets such as squarrose knapweed and gorse have been added, broadening our involvement in weed biocontrol. The primary targets for the program, however, continue to be yellow starthistle and silverleaf whitefly (also known as sweet potato whitefly, biotype B), two pests of great importance to California agriculture. The project summaries contained in this annual report highlight the depth and breadth of the program's activities. Each summary is directed toward our mission, which is the introduction, establishment and evaluation of natural enemies of agricultural insect and weed pests. The establishment of effective biological control agents can reduce the amount of pesticides needed, serve as an alternative to pesticides and provide less costly and more enduring control strategies to growers and the environment.

The staff of the Biological Control Program meets its objectives through the following activities:

- Establishing natural enemy field nursery sites and facilitating the statewide distribution of new and established natural enemies

- Rearing, releasing and evaluating new biological control agents

- Developing new sampling and evaluation techniques

- Serving as an information resource to the department and other agencies

- Assessing the state's immediate and long range needs for new natural enemies and candidates for importation

These objectives are aided, in part, by a contract research program administered by the Biological Control Program. Research contracts are awarded to outside researchers for basic research on natural enemies and their hosts and to initiate foreign exploration for incipient projects or the continuation of existing pest control programs. Contracts are awarded through a competitive grant program as specified under AB 4176. This bill addresses pest management and, specifically, general fund monies applied to pest management research projects.

Currently, topics of interest to the Biological Control Program are developed and subsequently advertised in the State Contracts Register. In conjunction with the topics being advertised, letters are sent to research institutions, notifying researchers that proposals are being accepted. Submitted proposals are subjected to two levels of review prior to funding. The Pest Science and Technology Screening Committee determines the scientific validity of proposals and passes those which meet prescribed criteria to the Pest Management Research Committee which then assesses the research in the context of departmental and industry priorities.

Research contracts awarded in 1995 fund the following projects:

1. Search for and Obtain Natural Enemies of Russian Thistle, *Salsola australis*, in Europe and Asia

2. Identification of Species and Biotypes of Russian Thistle, *Salsola australis*, in California Using RAPD Assays

3. Marking Studies to Measure the Movement of Native Aphelinids from Refugia to Adjacent Crops Infested with Silverleaf Whitefly

4. Development of an Illustrated Key to Nominal North American Species of *Eretmocerus* and Species Reared from *Bemisia* in the United States

5. Survey of the Natural Enemies of Certain Homopterous Pest Insects in Florida for Possible Introduction into California for Biological Control

The following two projects were funded in 1993 and 1994 and are scheduled for completion in June of 1996 :

1. Development of DNA-based Assays for Identifying Parasitoids of Sweetpotato Whitefly

2. Curation and Archiving of Biological Control Vouchers and Development of Identification Expertise.

The following report summarizes research activities and implementation efforts performed by the Biological Control Program during 1995, including cooperative work with a number of other governmental agencies involved in biological control projects. Summaries of the program's insect projects begin on page 3. Six of these 15 projects are designed to explore the viability of new biological control agents. Weed project summaries begin on page 21. Eight of the 17 weed projects test new agents, and four reports summarize activities regarding redistribution efforts where established agents are collected and moved to infested areas in cooperation with County Agricultural Department personnel. Summaries of contracted research projects begin on page 48.

Exotic Species Introductions in Imperial Valley to Control the Silverleaf Whitefly

W. J. Roltsch, K. A. Casanave, J. A. Brown, and J. A. Goolsby[1]

Several exotic species of parasitoids have been released in the Imperial Valley during the past few years to control silverleaf whitefly. Multiple accessions have been released and monitored for establishment. Two species of parasitoids in the genus *Eretmocerus*, were released during 1994 by CDFA in four field sites where whitefly host plants are continually grown. Host plant species included collard, sunflower, kenaf, cotton and cantaloupe. In addition, small numbers (approx. 500-1,000) of each species were released at two private residences in Brawley. All parasitoids were produced at greenhouse facilities in Sacramento, having received parent stock from the Mission Biological Control Center (MBCC) in Mission, Texas. *Eretmocerus* nr. *californicus* (M94003)[2] native to Mission, Texas, was released from February 1994 through February 1995. Twelve releases of adults were made in each field site for a total of 28,000 parasitoids. The sex ratio of this biparental species was approximately 1:1. The second *Eretmocerus* species (M94002) from College Station, Texas was released from mid-July through mid-January at the same field sites. This species is uniparental and uniquely identifiable from other known *Eretmocerus* by its uniformly darkened, reddish-brown thorax. Approximately 13 releases of M94002 were made in each field site for a total of 91,000 parasitoids.

Samples were collected from all field sites during the spring and summer of 1995. The species M94003 must be slide mounted to identify and separate it from the *Eretmocerus* native to Imperial Valley. Thus far, no positive identification of this species has occurred, however not all collected material has been examined. During the fall of 1994, M94002 was common in field collections at each site. In several small field cages (1 m^3), numbers of this species increased readily during August and September (1994). However, during the following spring (1995), only three individuals of M94002 were found in April and one specimen in May. None have been found in subsequent collections.

During 1995, two additional species were released in large numbers (>100,000 ea.). One species is a uniparental *Encarsia* nr. *hispida* (M94056) from Brazil and the second, a biparental Old World species of *Eretmocerus* (M94023). These were released into the same field sites as in 1994, in addition to nine private residences. The *Encarsia* species (M94056) was released from June of 1995 through May of 1996. The *Eretmocerus* species (M94023) was released from July through November 1995. Three release modes were used. They were released as adults in vials, as pupae on excised *Hibiscus* leaves and as various life stages on live *Hibiscus* plants transplanted into each field site during the late fall of 1995 (M94056 & M94023) and spring of 1996 (M94056).

Three additional species were released in small numbers (i.e., several thousand). The species released in small numbers were all biparental, adelphoparasites in the species complex *Encarsia* nr. *transvena*. These included populations from Thailand (M94047), Malaysia (M94041), and Spain (M93003). The parasites were received as pupae, held for emergence, then released as adults. Because material received from MBCC yielded very few males, measures were taken to continually produce males by exposing a portion of the received material to excess pupae available from other cultures of *Encarsia* and *Eretmocerus*. As a result, releases generally consisted of 5-10% males. Reproduction has been observed in the field for all 1995 species/strains releases, however an in-depth assessment is pending.

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[1]USDA-APHIS-PPQ, Mission Biological Control Center, Mission, Texas
[2]Refers to accession numbers used by USDA-APHIS for identifying populations

Importation and Release of *Delphastus* spp. for Control of the Mulberry Whitefly and the Silverleaf Whitefly in California

C. H. Pickett and W. J. Roltsch

Beetles in the genus *Delphastus* Casey (Coleoptera:Coccinellidae) are native to the Western Hemisphere, and are widely recognized as important in whitefly biological control. From 1992 to 1993, *D. catalinae* (Horn) (previously believed to be *D. pusillus* (LeConte)) was mass reared for studies on augmentative biological control and for permanent establishment in Imperial Valley in southern California. Approximately 1,000 adults were also released for colonization at each of two locations in central California, one private residence in Lindsay and a second, in Exeter. Adult beetles were released into mulberry trees that have been heavily infested by the mulberry whitefly, *Tetraleurodes mori* (Quaintance) (Homoptera: Aleyrodidae), over the last ten years or more. Prior observations over the past several years by the Tulare County Biologist, Dennis Haines, have never revealed the presence of *Delphastus* spp., and no previous records exist for members of this genus in central and northern California. Two years after the 1993 releases, large numbers of *Delphastus* sp. were found feeding on mulberry whitefly in mulberry trees in the two towns where beetles were released. Beetles were identified as *D. dejavu* Gordon, a closely related beetle with a western United States distribution. Since only a limited number of voucher specimens were identified (to *D. catalinae*), we are uncertain whether the recovered beetles are the same as those we released or that we released a mixed population. The latter is plausible since the source for our initial *Delphastus* culture was misidentified and none of the recent shipments of *Delphastus* originating from Florida have been identified by the leading authority for this genus, Dr. Robert Gordon (retired from the Systematic Entomology Laboratory, USDA, ARS). Private insectaries have been shipping *Delphastus* (all believed to be *D. pusillus*) back and forth across the country over the last five years. This example further emphasizes the importance of correct identification of biological control agents to the practice of classical biological. Without a large pool of experienced systematists, classical biological control cannot function.

A second effort to establish a new *Delphastus* species in southern California was undertaken this past year. Two species of *Delphastus*, *D. dejavu* and *D. sonoricus* Casey, have been reported feeding on silverleaf whitefly in the Phoenix area of Arizona. Since *Delphastus* spp. are not native to Imperial Valley and these species currently reside in an hot, arid desert region similar to Imperial County, we felt that importation of these beetles was worthwhile. Furthermore, efforts to permanently establish *D. pusillus* (actually *catalinae*) have failed. In September, 1995, the senior author traveled to Arizona to collect and import beetles to Imperial Valley. With the help of Dr. George Butler of the USDA-ARS in Phoenix, *D. sonoricus* (identified by Dr. Robert Gordon), were found widely distributed throughout Mesa on hibiscus and lantana. With the help of Dr. Michelle Walters of the USDA-APHIS in Phoenix, Arizona several hundred beetles were shipped and released into Brawley (Imperial County). Some of these were used for initiating a culture in Sacramento and will be released into Imperial County in spring 1996.

Release of Aphelinids for Control of Silverleaf Whitefly, *Bemisia argentifolii* in the San Joaquin Valley of California

C. H. Pickett, J. A. Goolsby[1], W. L. Abel[2], and G. Boyd

During fall 1994, six species/populations of aphelinids reared by the USDA-APHIS-PPQ Mission Biological Control Center (Mission, Texas) were released at five locations in or near Bakersfield, in central California in an attempt to establish permanent populations of new silverleaf whitefly, *Bemisia argentifolii* (Homoptera: Aleyrodidae), natural enemies. Parasites were released primarily at private residences that served as year-round field insectaries or refuges, free of pesticides and untimely cultivation. Sites were selected that contained woody perennial plants susceptible to silverleaf whitefly (e.g. hibiscus, lantana) and had home vegetable gardens with year-round plantings attacked by silverleaf whitefly (e.g. okra, melons, broccoli). Releases were made so that each site received species combinations that could be separated morphologically or through genetically unique DNA patterns. A grand total of 99,500 parasite pupae were released, 9,000 to 32,000 of each species/population. Three Old World species/populations of *Eretmocerus*, one Old World strain of *Encarsia* and two New World species of *Eretmocerus* were released onto hibiscus, lantana, or one of several herbaceous plants (Table 1).

During fall 1995, 28 species/populations of aphelinids, 26 reared by the USDA and two reared by the Biological Control Program, were released at 20 locations, mostly private residences, in Kern and Tulare Counties in central California (Table 2). A grand total of 183,714 pupae were released, 600 to 34,500 of each species/population. All released parasites were of Old World origin.

Post release monitoring has included recovery and identification of released parasites and whitefly density estimates. Adult parasites were identified using traditional morphological techniques and RAPD-PCR (randomly amplified polymorphic DNA - polymerase chain reaction) techniques. Two of the six species/populations released in 1994 were recovered as late as August 1995, at one of two release sites in Bakersfield, both private residences. At one of these sites all recovered male *Eretmocerus* were identified as an exotic parasite with accession number M93005 originally collected from Thirumala, India. The second recovered parasite, *Eretmocerus mundus* (M92014), was imported from Murcia, Spain. This is the first known report for year-round establishment of an introduced *B. argentifolii* parasite in California.

[1]USDA-APHIS-PPQ, Mission Biological Control Center, Mission, Texas
[2]USDA-APHIS-PPQ, Bakersfield, California

Table 1. Species/ populations of parasites released in southern San Joaquin Valley, 1994				
Accession Number	**Species**	**DNA Pattern**	**Country of Origin**	**# released**
M92014	Eretmocerus mundus	ERET-1	Murcia, Spain	10,000
M92019	Eretmocerus sp. A	ERET-1	Padappai, India	32,000
M93003	Encarsia sp.	ENC-7	Murcia, Spain	9,000
M93005	Eretmocerus sp.	ERET-2	Thirumala, India	12,000
M94002	Eretmocerus sp.	----------	College Station (TX), U.S.A	22,000
M94003	Eretmocerus sp.	ERET-6	Mission (TX), U.S.A.	14,500

Table 2. Species/ populations of parasites released in southern San Joaquin Valley, 1995				
Accession Number	**Species**	**DNA Pattern**	**Country of Origin**	**# Released**
M92014	Eretmocerus mundus	ERET-1	Murcia, Spain	5000
M92017	Encarsia formosa	ENC-2	Angelohori, Greece	2000
M92018	Encarsia sp. A	ENC-1	Parbhani, India	600
M92019	Eretmocerus sp. A	ERET-1	Padappai, India	7400
M92027	Eretmocerus sp.	ERET-1	Cairo, Egypt	1400
M93003	Encarsia transvena	ENC-7	Murcia, Spain	4600
M93005	Eretmocerus sp.	ERET-2	Thirumala, India	5400
M93058	Eretmocerus sp.	ERET-1	Tainan, Taiwan	6000
M93064	Encarsia lutea	ENC-10	Mazotos, Cyprus	1750
M94016	Encarsia transvena	ENC-11	Shan-Hua, Taiwan	200
M94023	Eretmocerus sp.	ERET-3&8	Sai Noi Klong Ha Roi, Thailand	34,500
M94040	Eretmocerus sp.	ERET-3	Kampang Saen, Thailand	6100
M94041	Encarsia transvena	ENC-5	Chiang Mai, Thailand	2400
M94047	Encarsia transvena	ENC-5	Kuala Lumpur, Malaysia	8700
M94055	Encarsia nr. pergandiella	ENC-15	Sete Lagoas, Brazil	4998
M94056	Encarsia nr. hispida	ENC-16	Sete Lagoas, Brazil	24,500
M94085	Eretmocerus sp.	ERET-1	Frascati, Italy	250
M94092	Eretmocerus sp.	ERET-1	Italy, mix	5700
M94103	Eretmocerus sp.	ERET-1	Gat, Israel	1800
M94105	Eretmocerus sp.	ERET-1	Gat, Israel	1200
M94107	Encarsia lutea	ENC-10	Mediterranean mix	1450
M94120	Eretmocerus sp.	ERET-1	Israel	28000
M94124	Eretmocerus sp.	ERET-1	Negev Desert, Israel	1600
M94125	Eretmocerus sp.	ERET-1	Golan, Israel	2800
M94129	Encarsia lutea	ENC-10	Mazarron, Casas Nueva, Spain	4200
M95012	Eretmocerus sp.	ERET-10	Multan, Pakistan	21166

Release of Natural Enemies for Control of the Giant Whitefly, *Aleurodicus dugesii*, in San Diego County

C. H. Pickett and D. Kellum[1]

The giant whitefly, *Aleurodicus dugesii* Cockerell, (Homoptera: Aleyrodidae), was first discovered in San Diego, California on October 15, 1992. *A. dugesii* has been previously reported from north central Mexico (Zacatecas) and the southern tip of Baja California. The original finds in San Diego were mainly from hibiscus, but the whitefly has now spread to a broad range of subtropical perennial trees and shrubs and herbaceous monocots. The giant whitefly has been found reproducing on 27 families and 30 genera of plants at the San Diego Zoo, and 32 families and 39 genera of plants in San Diego County. This list includes the important agricultural crops avocado, citrus, apricot, and prunes. The most commonly reported plants infested with giant whitefly in urban San Diego are hibiscus, bird of paradise, xylosma, avocado, and citrus. In addition to direct affects to plants, feeding results in sooty mold developing on honeydew excreted by whiteflies. This whitefly also poses a serious respiratory health problem to the general public. The nymphal stages produce long waxy hairs up to 4 inches long that can break off and float in the air, carrying honeydew with them. Leaves with high densities of giant whitefly are flocked with this white material.

Delphastus spp. (Coleoptera: Coccinellidae) are arboreal, whitefly-specific predators. In an attempt to establish effective natural enemies of giant whitefly, 3,000 *D. catalinae* (Horn) (initially thought to be *D. pusillus*), were purchased from a local private insectary and released at two different sites in Carlsbad (San Diego County), California, September 29, 1995. They were released onto avocado, citrus, and hibiscus. On October 27, 1995, several larvae were observed on avocado.

This whitefly has also recently invaded and established populations in eastern Texas and Louisiana. Mike Rose of Texas A&M University found a parasite belonging to the genus *Entedononecremnus* (Eulophidae) in Texas attacking the giant whitefly in October 1995. The parasite was found in high numbers attacking the giant whitefly suggesting that it is a specific natural enemy of this pest. A total of 3,300 adult parasites were shipped to San Diego County from October to November 1995. Parasites were released at eight different locations including private homes and public properties. Most of the host plants were hibiscus. Monitoring of the whitefly and both natural enemies will continue through fall 1996.

[1] Department of Agriculture, San Diego County

Introduction of *Encarsia variegata,* a Parasite of the Nesting Whitefly

J. C. Ball, H. W. Browning[1] and D. Kellum[2]

The nesting whitefly, *Paraleyrodes minei* Iaccarino (Homoptera: Aleyrodidae), was discovered in San Diego in 1985, and has since spread to Los Angeles, Orange, and San Bernardino Counties. It attacks avocados, citrus, and several ornamental plant species.

In late 1993, the aphelinid parasitoid *Encarsia variegata* Howard, was collected in Florida and released at five locations in San Diego County. There was no recovery from these releases, and another attempt was made in 1994 with releases at three locations in the county. Although adult parasites were initially recovered in small numbers at the three locations by April 1995, only a single parasitized nesting whitefly nymph (mummy) was found at one site.

A third series of *E. variegata* releases was initiated in July 1995. Releases were made on three citrus trees at a single property in San Diego County. The same location had been used in the 1994 releases. This site was selected because there were many host plants in the immediate area infested with nesting whitefly. Research has shown that parasite establishment success is proportional to the numbers released. Since we were limited to relatively few parasites, we hoped that by concentrating the releases at a single site with many hosts, our chances might improve. As in 1994, releases of *E. variegata* adults were staggered in order to synchronize the availability of appropriate hosts (female parasite pupae) and insure mating in the field. This synchrony is a requisite of the autoparasitic habit. Some of the parasites were confined within cloth sleeves caging tree branches, while others were released without confinement. At approximately weekly intervals, between July 20 and October 25, 1995, a total of 515 female and 76 males were released.

In order not to interfere with parasite establishment, we did not sample the site until November. Then, we collected a 20 leaf sample from each tree and a 10 leaf sample from each confined branch. Two mummies with exit holes were the only evidence of parasitization recovered in the samples. However, 11 developing mummies were seen during a one hour visual inspection of the trees.

We will continue to monitor this site for establishment of *E. variegata*, but do not anticipate further releases. Should *E. variegata* become established and show sufficient population growth, field collection and redistribution will be attempted.

[1] University of Florida, IFAS, CREC, Lake Alfred, FL.
[2] Department of Agriculture, San Diego County

Establishment and Distribution of the Tachinid Fly, *Trichopoda pennipes* for the Biological Control of the Squash Bug, *Anasa tristis*

S. E. Schoenig and C. H. Pickett

The squash bug, *Anasa tristis* (DeGeer) (Hemiptera: Coreidae) is a frequent problem for producers of organic squash and pumpkins in California. The squash bug attacks all stages of the plant and is especially damaging to the seedling stage. In California the only recorded parasitoid of squash bug is an encyrtid which achieves low levels of parasitism on host eggs. A biotype of *Trichopoda pennipes* (Fabricius) is a nymphal-adult parasite of squash bugs and occurs in the eastern United States. It has been reported to parasitize up to 84% of overwintering bugs.

Efforts to establish this parasitoid in California were initiated in 1992. This project has two objectives: 1) establish field nurseries for *T. pennipes* in northern California, using flies collected by Dr. Michael Hoffmann (Cornell University) from upstate New York; and 2) measure the impact of released *T. pennipes* on populations of the squash bug.

In 1992 and 1993, a total of 841 pupae were shipped to our Sacramento facility. Of these, 486 successfully produced adult flies. A total of 243 adult flies were released at four nursery sites in Solano, Yolo, and Sacramento Counties over the same period of time. Also a total of 3,387 adult squash bugs, parasitized at our facility, were released at some of these sites. In 1993, parasites were recovered at a site near Winters and a vegetable garden near Davis (Yolo County). Intensive sampling of squash bug population densities was carried out at two sites in 1992 and 1993 to measure pre-release levels of the bugs.

T. pennipes successfully reproduced at four sites and has been recovered annually through 1995. Of these sites, two represented successful overwintering for three consecutive years by flies released in 1992. Parasitism has varied between 1.3% and 92.2%. In 1993, a nursery site was established at the Student Experimental Farm, University of California, Davis (SEF), and late season releases of parasitized bugs were made at this site. In May 1995, the first of three plantings of zucchini and kabocha squash was initiated which eventually covered two acres. Bugs collected elsewhere in Yolo County were moved to the SEF nursery site in June. The goal of this effort is to establish a large self-sustaining population from which parasitized bugs will be collected and distributed to uninfested areas in California.

The first distribution efforts occurred in 1995. Reintroduction attempts into the Capay Valley site were made with a total of 5,000 parasitized bugs released late in the season from the SEF site. Parasites were also released in Loomis, Placer County. In 1996, parasitized bugs will be moved to new sites, primarily from the SEF site. Population densities and percent parasitism of the squash bug will be monitored at release sites throughout the season.

Low Temperature Oviposition Rate of Aphelinids: a Technique for Determining Winter Tolerance of Exotic Parasites for Release in Imperial Valley

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

The USDA-APHIS-PPQ Mission Biological Control Center in Mission, Texas has over 30 species/populations of *Eretmocerus* and *Encarsia* (Hymenoptera: Aphelinidae) in culture. Several field and laboratory tests are underway to compare the relative merits of each of these parasite cultures for silverleaf whitefly control. The ability of these parasites to survive and reproduce under winter conditions may play an important role in their permanent establishment in the desert regions of the southwestern United States. In an ongoing project, we are measuring and comparing the oviposition rates of several candidate parasites at three temperatures representing winter conditions in southern California and Arizona, 45°, 55°, and 65° F (7.2° 12.7, and 18.3° C, respectively). This range of temperatures includes the lowest one that parasites would be expected to oviposit in the field.

Petri dishes containing leaves infested with silverleaf whitefly and parasites were placed inside an environmental chamber under controlled conditions of temperature and light (12L:12D). Three adult parasites of each species/population were placed into each of three dishes for each temperature setting. Therefore, each chamber represented one experimental unit with three subsample petri dishes for each culture of parasite being tested. The number of eggs per nine adults of each species was recorded after 60 to 72 hrs. A total of five different species or populations were tested over a six month period.

No significant differences were found among tested populations when pooling oviposition rates across all temperatures, (ANOVA, $p = 0.84$, $F = 0.45$; range: 0.37 to 0.94 eggs per adult; $n = 2$ to 9). No significant differences were found among tested species at each temperature level (7.2°, 12.7°, and 18.3° C, respectively) (Table 1). One unexpected result, however, was the change in oviposition rate over the course of the study, despite constant rearing and experimental conditions (Fig. 1). Initially, none of the populations oviposited at 7.2°C. However, the oviposition rate beginning in October increased for all tested species and at all three temperatures. They peaked in early November and appear to be decreasing. The reason for the change in oviposition rate is unknown. These studies will continue, but the number of replicates and tested populations will increase.

[1]USDA-APHIS PPMC, Brawley, CA

Table 1. Mean eggs per parasitoid (n) measured at different temperatures.

Treatment	ANOVA, p-value, F, d.f.	M92014 (Spain)	M94023 (Thailand)	M94055 (Brazil)	M94056 (Brazil)	M95012 (Pakistan)
7.2°	0.39, 1.10, 5	0.65 (7)	0.10 (8)	0.00 (2)	0.29 (8)	0.33 (2)
12.7°	0.99, 0.10, 5	0.70 (7)	0.44 (7)	--	0.59 (9)	0.69 (2)
18.3°	0.74, 0.58, 5	1.06 (7)	0.70 (7)	1.25 (2)	1.44 (9)	1.78 (2)

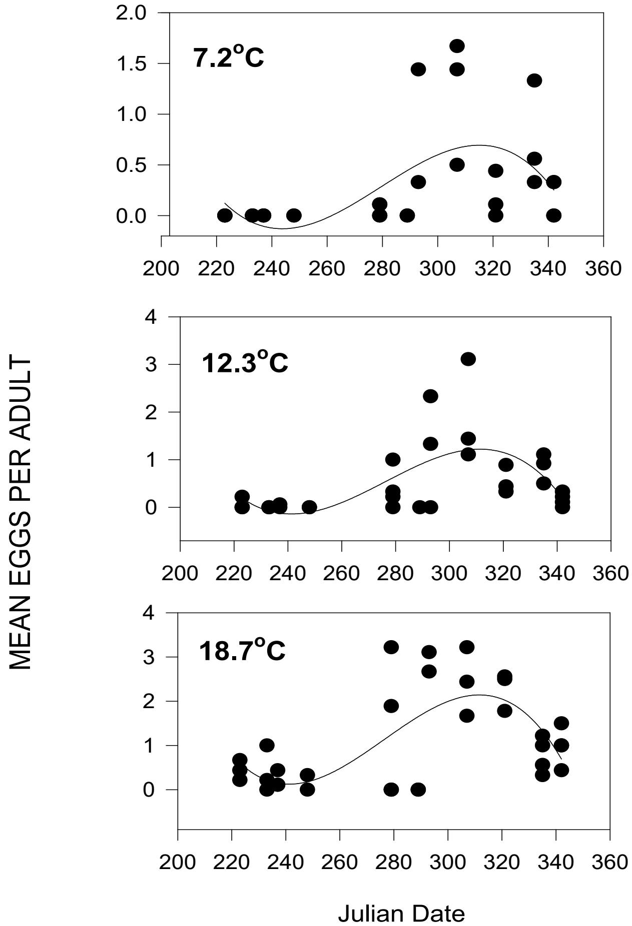

Fig. 1. Mean eggs per adult vs. time at which replicate was conducted. A third degree polynomial regression was fitted through data points. Data for all five species/populations were plotted.

Insect Natural Enemies Mass Reared for Research and Colonization Projects

K. A. Casanave and J. A. Brown

Each year one or more insect natural enemies are mass reared for a variety of projects conducted by our 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 all natural enemies were reared for control of silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring (Homoptera: Aleyrodidae) (= sweet potato whitefly strain B).

Natural Enemy	Agency Receiving Natural Enemy	Project description	Number of insects delivered
Encarsia nr. hispida from Brazil (M94056)	CDFA	Establish in refuges, Imperial County	213,000
	USDA-APHIS-PPQ	Augmentation in Imperial County	369,000
	USDA-APHIS-PPQ	Evaluations in field cages, Imperial County	7,300
	USDA-APHIS-PPQ	Field releases, Texas	4,400
	USDA-APHIS-PPQ	Urban releases, San Joaquin Valley, CA	28,500
Eretmocerus sp., from Thailand (M94023)	CDFA	Establish in refuges, Imperial County	242,500
	USDA-APHIS-PPQ	Urban releases, San Joaquin Valley, CA	45,700
	USDA-APHIS-PPQ	Augmentation studies in Imperial County	100,000

Insectary Production of Silverleaf Whitefly and Its Parasitoids

J. C. Ball, S. E. Schoenig, and J. A. Brown

Forcasting production of mass reared parasitoids of the silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring, (Homoptera: Aleyrodidae), is at best difficult. To reduce uncertainity in forcasting production, an "Equilibrium Method" parasite production system may be employed that relates the systematic sequencing of hosts and parasites to provide a more predictable age distribution and supply. Irrespective of the technical problems of confinement, dispersion, and removal, developing such a system requires knowledge of the whitefly's reproduction, development, and preferred host stages.

In a preliminary study, several plant species were screened for whitefly host-preference and adaptability to isolation cage experiments. This was summarized in the 1994 annual report. This year, we looked at whitefly development on hibiscus, the host plant most commonly used in our insectary. To estimate developmental rates on hibiscus, 100 to 300 adult whiteflies of each sex were introduced onto 30 individually caged plants for a 24 hr oviposition period. The plants were pruned to two or three young, true leaves that had not fully expanded. At approximately daily intervals, a single plant was sacrificed and the number of whitefly in each stage recorded. From these data, a non-parametric survival analysis will be used to estimate the average developmental times for each stage. These estimates will be incorporated into a computer model to simulate whitefly population growth as a function of degree days.

The second component of the production system, superimposed on the whitefly development, is the host-stage preference and host-stage mediated development of the parasites. Host-stage preference tests were run on several aphelinid species reared at our greenhouses in Sacramento. These were *Encarsia luteola*, *En.* nr. *pergandiella*, *En.* nr. *hispida*, *Eretmocerus* nr. *californicus*, and *Eret. sp.* ex. *Pakistan*. In general, *Encarsia spp.* preferred the later nymphal instars, whereas *Eretmocerus spp.* showed preference for the younger nymphs. The most complete study was run on *En.* nr. *hispida* and *Eret.* ex. *Pakistan*. This study was conducted on rooted sweet potato leaves, using a technique reported by W. A. Jones and T. S. Bellows, Jr. Caged leaves were infested at intervals with whitefly adults to obtain a range of stages. Excised leaves were placed inside a screen-covered 50 dram vial, their petiole immersed in a water filled floral pick, protruding through the base of the cage. Five female *Eret.* ex. *Pakistan* and five to eight female *En.* nr. *hispida* were then introduced into the vials for 24 hour oviposition. To determine host-stage preference, all whitefly nymphs on selected leaves were dissected. For host-stage mediated development, the whiteflies were allowed to complete development after the parasites were removed. A mark was placed with india ink on the leaf next to the nymph, so whitefly and parasite development could be followed.

Under conditions of the experiment (exposure of parasites to all nymphal stages but not equal numbers in each stage) *Eret.* ex. *Pakistan* oviposited on 83% of the 1st instars present, 92% of the 2nd instars, 114% (result of supernumeries) of the 3rd instars, 47% of the early 4th instars, and on none of the late 4th instars. *Encarsia* nr. *hispida* oviposited in 2% of the 1st instars, 4% of the 2nd instars, 36% of the 3rd instars, 66% in the early 4th instars, and 50% in the late 4th instars present. In general, for both species, parasite developmental time took longer when younger whitefly nymphs were attacked. There also appeared to be greater early whitefly mortality in 1st and 2nd instars exposed to *Eret.* ex. *Pakistan* than those exposed to *En.* nr. *hispida*.

Silverleaf Whitefly Natural Enemy Refuges in the Imperial Valley

W. J. Roltsch, C. H. Pickett, and M. Rose

Natural enemy refuges consisting of several species of annual plants grown continuously throughout the year, were evaluated for their potential in building and maintaining populations of parasitoids and predators of the silverleaf whitefly, *Bemisia argentifolii*, Bellows & Perring (Homoptera: Aleyrodidae). The study was conducted from the summer of 1993 to the fall of 1995. Two refuge plots (1 & 2 acres) and corresponding check sites were evaluated at the USDA-ARS field station in Brawley, and one refuge plot (5 acres) and check site were present at a commercial farm (organic) in southern Imperial County. Plots consisted of two rows (i.e., strips) of refuge plants alternating with 20 rows of crop (melon or cotton). Refuge strips were replanted twice each year at each field site; the first in late February (interplanted sunflower, collard, and kenaf [1994] or roselle[1995]) and the second planting in early October (sunflower and collard). On sample dates, leaves consisting primarily of fourth instar whitefly nymphs were collected. Counts were conducted on two 1 cm^2 leaf disks using a dissecting microscope. Percent parasitism was based on the dissection of fourth instar whitefly nymphs. Whitefly density was estimated from direct counts; immature parasitoid density was estimated by multiplying whitefly density by percent parasitism.

The activity of native *Eretmocerus* sp. was enhanced to a limited extent by this refuge system. In most instances, parasitoid numbers in cantaloupe within refuge plots were initially greater than those in the control plots. Marking studies (see Corbett this volume) determined that much of the population that was produced on collard during late winter moved to the adjacent cantaloupe. However, sufficient levels of native *Eretmocerus* were not achieved because this species did not produce high densities on collard (crucifers in general), and sunflower phenology and its susceptibility to whitefly did not coincide ideally with winter conservation needs. The impact of the refugia on neighboring crops may have been greater given better overwintering host plants. Sunflower was senescent by mid-winter, thereby providing little if any overlap with spring crops. The native *Eretmocerus* sp. caused relatively low levels of parasitism on whiteflies on refuge collard, and although native *Encarsia* spp. may reach levels of parasitism as high as 40% on collard they seldom attacked whitefly on cantaloupe in adjacent fields.

Although alternatives to collard are needed to create a successful refuge, parasitoid species capable of performing well on crucifers are needed since a large acreage of broccoli, cabbage and other cruciferous crops are grown in the Imperial Valley during winter months. Currently these crops serve as a "refuge" for whiteflies since they are essentially void of effective natural enemies. Preliminary results from field cages suggested that exotic species capable of reaching high densities on collard could have a significant impact on spring crops (see Roltsch and Goolsby, this volume).

Field Cage Evaluations of Exotic Parasitoid Species of the Silverleaf Whitefly

W. J. Roltsch and J. A. Goolsby[1]

Two cultures of *Encarsia* nr. *transvena* (Hymenoptera: Aphelinidae) were evaluated on cotton, and three were compared in a fall evaluation on broccoli during 1995. These studies are being done in tandem with those conducted by Kim Hoelmer (USDA-APHIS), who is evaluating six cultures of *Eretmocerus* and *Encarsia*. All parasites were was provided by the USDA-APHIS, Mission Biological Control Center. Field cages used for this study were 2 m x 1.5 m x 2 m, and constructed of 52x52 mesh Lumite screen. Cages were located in a well-drained field site at the USDA-ARS station in Brawley.

Field cages were set up 4-7 days prior to transplanting greenhouse grown seedlings into the field. Three cages on each host plant were provided as replicates for each parasite species/strain evaluated, and for one control (i.e., only receiving whitefly). Whiteflies, (approx. 200 females per cage for broccoli, 120 on cantaloupe, and 200 on cotton) were introduced 10-14 days following transplanting. When early fourth instar whiteflies were present, 50 female and 10 male parasitoids were released into each treatment cage. Approximately 14 days later, when early stage parasitoid pupae were present, 20 additional females along with 2-5 males were introduced into each cage, and this was repeated one week later. Second and third introductions were designed to create a staggered age distribution and favorable sex ratio of the parasitoids. The assessment of the F_1 production was accomplished by collecting half leaf samples at first emergence of the F_1 parasite generation. The number of parasite pupae, immature whiteflies as well as and contaminant species were counted.

The summer cotton evaluations of M94047[2] and M94041 were inconclusive due to low whitefly densities. However, in the fall study on broccoli, F1 production of M93003 was clearly superior to that of M94047 and M94041. The F1 generation was sampled on 16 November 1995, by removing half of each broccoli leaf containing F1 pupae. The F1 parasitoid pupal densities per cage were estimated at 204 (M94041), 304 (M94047), and 648 (M93003). The within treatment variation among cages was very low during the first sample. Females of M930003 were 2-3 times more productive than either of the other two. Assuming that all 50 females lived long enough to reproduce, the average M94041 female produced 4.1 offspring, while M94047 produced 6.1 offspring and M93003 produced 13.0 offspring. One week following the release of 50 female parasitoids, several individuals were found alive in each treatment. A second broccoli sample was taken from each cage on 19 January 1996**.** Whole leaves were sampled and the study was terminated. Parasitoid numbers had declined from those found in November**.** In part, this may have been due to low whitefly densities in each cage. Means were 11, 8, and 87.7 parasitoid pupae per cage for M94041, M94047, and M93003, respectively. In addition, pupal counts may have been affected by the presence of two native *Encarsia* species. *E. luteola* and *E. meritoria*. These parasites were found in two of the control cages during the first sample period. During the second sample period, these "contaminants" were found in all cages: mean of 57 in M94041 cages, 124 in M94047 cages, 42.7in M93003 cages, and 69.3 in the control cages.

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[1]USDA-APHIS-PPQ, Mission Biological Control Center, Mission, Texas

[2]Refers to accession numbers used by USDA-APHIS for identifying populations of imported parasitoids

Survey for Parasitoids of Sunflower Moth, *Homeosoma electellum*

D. M. Woods and L. D. Charlet[1]

The sunflower moth, *Homeosoma electellum* (Hulst) (Lepidoptera: Pyralidae), is one of the world's most important insect pests of sunflower. It is the only sunflower pest in California that warrants consistent pesticide applications. Parasitoids of the sunflower moth may be naturally occurring in some areas of California. Identification and evaluation of these parasitoids may benefit both planting seed producers in California and oil seed producers in the central United States. Commercial sunflower fields planted for seed production were surveyed for damage caused by the sunflower moth in northern California during 1992, 1994, and 1995. Sunflower heads with evidence of insect damage were sent to Dr. Laurence D. Charlet, Research Entomologist, USDA-ARS, Fargo, North Dakota, for evaluation. Larvae were extracted from the heads, reared on artificial diet, and evaluated for parasitoids. The results for the 1995 survey are presented below. Infestation levels were much lower in samples from 1995 than in previous years as was percent parasitism. Samples collected in 1995 emphasized the northern region of California sunflower production. The only samples in which parasitism occurred in 1995 were collected in the southern part of the region. Sunflower moth infestation rates were also higher in the southern area. Higher ambient air temperatures in the northern area, cultivar variation or local pest control practices may all contribute to the geographic variation.

Sample	Location	No. Heads	Plant Stage	Mean # Larvae per Head	% Parasitization
1	Esparto	4	R9	0	0
2	Woodland	10	R9	1.5	0
3	Yolo	9	R9	7.2	0
4	Clarksburg	6	R8	2.2	0
5	Orland	9	R8	1	0
6	Artois	8	R9	1.8	0
7	Willows	8	R7	15.5	0
8	Hamilton City	6	R9	0	0
9	Willows	4	R9	0.5	0
10	Elmira	14	R8	31.2	6.3
11	Dixon	7	R8	19.7	0
12	Vacaville	10	R8	20	3

[1]USDA-ARS, Northern Crop Science Laboratory, Fargo, North Dakota

Survey of "Native" Parasitoids of Vine Mealybug, *Planococcus ficus*, in the Coachella Valley

J. C. Ball, K. E. Godfrey, D. Powell[1], E. Reeves[2], D. Gonzalez[1], and S. Triapitsyn[1]

In 1994, grape growers in the Coachella Valley (Riverside County) in southern California, reported that a mealybug was reducing the quality and yield of their grapes. Specimens of this mealybug sent to the California Department of Food and Agriculture, Plant Pest Diagnostic Laboratory and the US National Museum were identified as the vine mealybug, *Planococcus ficus* (Signoret) (Homoptera: Pseudococcidae), a species not previously reported from California. This mealybug is an economic pest of grapes in the Mediterranean region, the Middle East, South Africa, Pakistan, and Argentina. In those areas of the world, the vine mealybug has also been reported to attack fig, avocado, mango, and pomegranate, although it has not been found on those hosts in the Coachella Valley.

In a cooperative effort with the University of California at Riverside, the California Department of Food and Agriculture, and the Riverside County Agricultural Commissioner's Office, a survey was initiated to determine the species and relative abundance of parasitoids already present in the Coachella Valley that attack the vine mealybug. Samples were collected every one to two months throughout 1995 from four to five infested vineyards. All parts of the vine (roots, trunk, canes, leaves, and fruit) were sampled to provide information on mealybug and parasite phenology.

Anagyrus pseudococci (Girault) (Hymenoptera: Encyrtidae) and *Chartocerus* sp. (Hymenoptera: Signiphoridae) were the only naturally occurring parasites found in 1995, and the latter species is regarded as a hyperparasite. Parasitization by "native" parasites was low on average in the samples, but definite differences were found between exposed mealybug populations (those on leaves and fruit) and concealed populations (those on roots and buried canes). Parasitization reached 13% in fruit clusters and 22% on leaves, whereas, on roots and buried canes, parasitization was generally well below 1%. It is possible that the parasitization found in the root and cane samples actually occurred while mealybugs were on the more exposed parts of the vine. Parasitization of mealybugs on the trunk was not assessed.

Work will continue on vine mealybug in the Coachella Valley. Emphasis will be on the introduction and evaluation of exotic natural enemies and understanding the seasonal patterns of mealybug distribution and abundance on the vine.

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[1] Department of Entomology, University of California, Riverside.
[2]Agricultural Commissioner's Office, Riverside County, Riverside.

Survey of Indigenous Natural Enemies of the Cotton Aphid, *Aphis gossypii*, in the San Joaquin Valley

K. E. Godfrey and J. R. Brazzle[1]

The cotton aphid *Aphis gossypii* Glover, (Homoptera: Aphididae), is considered the most widespread problem affecting cotton growers in the approximately 1.25 million acres of cotton planted annually in the San Joaquin Valley of central California. Historically, the cotton aphid was an early season pest, but in recent years, densities of the cotton aphid have been increasing greatly in mid to late season. These increases in density have resulted in actual yield reductions, quality losses attributable to increased sticky cotton at harvest, and losses in profit due to the cost of additional insecticide applications to prevent anticipated increases in the amount of sticky cotton at harvest. Although insecticides are still effective against the cotton aphid, the length of time the insecticide can keep the aphid densities low is brief due to reinvasion of the fields by alate aphids within days after treatment. Many populations of the cotton aphid in the San Joaquin Valley also have demonstrated resistance to some of the insecticides used in their control. Management of the cotton aphid, therefore, will require the integration of management tactics such as biological, cultural, and/or chemical control rather than sole reliance on insecticides.

In an attempt to enhance one management tactic, biological control, a survey of the indigenous parasitoids attacking cotton aphid in the San Joaquin Valley was initiated in the fall of 1995. This survey is a necessary precursor to the introduction of exotic natural enemies that could enhance biological control of the cotton aphid. In this survey, twelve sites were established in Kern County in October 1995, that represent the variety of habitats occupied by the cotton aphid. These habitats include cotton, citrus, melon, cole crops, and non-crop plants such as cheeseweed [*Malva parviflora (Malvaceae*)]. Beginning in November 1995, the sites were visited monthly, and samples were taken when host plants of the cotton aphid were present.

At the conclusion of the December sample, only 6 of 12 sites had been sampled consistently due to cropping sequence or seasonality of the cotton aphid host plants. Parasites were recovered from aphids collected in cotton, citrus, sunflower, and cheeseweed. The majority of the parasites found were aphidiids (Hymenoptera) belonging to the genus *Lysiphlebus* (40% of the total parasites recovered in the November sample; and 55% of the parasites in the December sample). The remaining parasites were hyperparasites belonging to the families Cynipidae (*Alloxysta* sp.; 24% in the November sample and 44% in the December sample) and Pteromalidae (*Asaphes* sp. and *Pachyneuron* sp.; 36% in the November sample and 1% in the December sample). This survey will continue through the fall of 1996.

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[1]UC Cooperative Extension, Bakersfield

Biological Control of Euonymus Scale

R. K. Wall, C. H. Pickett, and D. J. Hamon[1]

Since its accidental introduction into the United States from Asia some 100 years ago, euonymus scale, *Unaspis euonymi* (Comstock) (Homoptera: Diaspididae), has been a problem to the growers of euonymus, a desirable ornamental plant. Today, euonymus scale is widely spread in the eastern United States. There are no effective native natural enemies of euonymus scale in the United States. Thus, efforts have been made to import natural enemies from Asia: *Cybocephalus* nr. *nipponicus* Endrody-Younga (Coleoptera: Nitidulidae), a member of the sap beetle family, and *Chilocorus kuwanae* (Silvestri) (Coleoptera: Coccinellidae), a lady beetle. These two beetles also attack San Jose scale, *Quadraspidiotus perniciosus* (Comstock) and white peach scale, *Pseudaulacaspis pentagona*, two related armored scales which are highly destructive pests of fruit trees throughout the U.S. In 1984, the USDA-APHIS introduced *Cy. nipponicus* and *Ch. kuwanae* from Korea to scale-infested euonymus plants at the U.S. National Arboretum in Washington, D.C. Both *Cy. nipponicus* and *Ch. kuwanae* reduced euonymus scale infestations to very low levels. Within six years, both beetles were established in nine eastern states and the District of Columbia.

Euonymus scale is only a problem in a few northern California counties. The effectiveness of the two introduced natural enemies on the east coast of the United States prompted interest in introducing *Cy. nipponicus* and *Ch. kuwanae* in California. For the past three years, the Biological Control Program, in conjunction with USDA-APHIS conducted limited releases of *Cy. nipponicus* and *Ch. kuwanae* at sites in Sacramento and Napa counties. In 1993 and 1994, a total of 2,647 adult *Cy. nipponicus* and 187 adult *Ch. kuwanae* were released at two sites, William Land Park, Sacramento, and the Decia Nursery in Napa County. In 1995, the Decia Nursery site was replaced with a site located on the California State University, Sacramento (CSUS) campus. Four hundred adult *Cy. nipponicus* and 100 adult *Ch. kuwanae* were released in 1995 at CSUS. In 1995, the USDA-APHIS continued to rear and ship *Cy. nipponicus* and *Ch. kuwanae* and began supplying *Encarsia* nr. *diaspidicola* and an *Aphytis* sp. for release at the Sacramento sites. Two thousand six hundred thirty adult *E.* nr. *diaspidicola* and 1,335 adult *Aphytis* sp. were released at both Sacramento sites.

Although only 62 *Ch. kuwanae* adults were released in January, 1994, at William Land Park, a healthy population of *Ch. kuwanae* adults and larva was observed at the release and control areas in August and again in October, 1995. No further releases are scheduled for any site in northern California in 1996. Detailed monitoring will continue for at least another year to determine the effectiveness and establishment of these four introduced natural enemies of the euonymus scale.

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[1]USDA-APHIS-PPQ, Sacramento, CA

Releases of the Peacock Fly, *Chaetorellia australis*, in California for the Biological Control of Yellow Starthistle

B. Villegas and C. E. Turner[1]

Yellow starthistle, *Centaurea solstitialis* L. (Asteraceae), is one of the most important and widespread weeds in California. It is estimated that Yellow starthistle, infests more than eight million acres in California alone. The peacock fly, *Chaetorellia australis* Hering (Diptera: Tephritidae), is one of five biocontrol agents that have been introduced into California from Greece for the biological control of yellow starthistle. *C. australis* was initially released at six sites in Contra Costa, Mariposa, Napa, Nevada, Plumas and Shasta Counties by the USDA, ARS, Biological Control of Weeds Laboratory in cooperation with the Biological Control Program from 1988-1995. The release information through 1995 is summarized in Table 1. No recoveries have been obtained at any of these sites to date.

In previous release efforts between 1988 and 1994, neither colonization nor establishment of the peacock fly was observed in California. Establishment was confirmed at release sites in Oregon and Washington where bachelor's button was in close proximity of yellow starthistle, suggesting that a second host, bachelor's button, needed to be present in the same area infested by yellow starthistle. It appeared that the adult peacock flies from the overwintering population emerge before most yellow starthistle seedheads are available for oviposition by the flies. Bachelor's button flowers earlier than yellow starthistle and its seedheads are available for oviposition by the early-emerging flies. However, in the last two years, the peacock fly has been recovered from sites in Oregon devoid of any bachelor's button. Also, last November a naturalized population of peacock flies was found in the Willow Creek-Salyer Bar area of Humboldt and Trinity Counties in northern California.

In 1995, a new two-year colonization effort was made by the Biological Control Program in cooperation with the USDA, ARS, the Oregon Department of Agriculture, and the California Agricultural Commissioners and Sealers Association. Through this effort, mass collections of yellow starthistle seedheads infested with *C. australis* were made from the Merlin area of southern Oregon in April and June 1995 and transported to the Biological Control Program facility in Sacramento. The earliest collected material was placed in cold storage until early May in order to synchronize fly emergence with susceptible yellow starthistle seedheads at potential release sites in California. Once field plots of yellow starthistle had developed to a susceptible stage, the *C. australis* infested seedheads were transferred into sleeve cages and fly emergence was monitored daily. Adult fly emergence started within a week and continued for approximately three weeks. From this material, a total of 3,632 peacock flies were released at six sites located in Amador, Mariposa, Placer, San Luis Obispo, Sacramento, and Siskiyou Counties from May 12 through June 3, 1995. An additional collection of F_1 infested seedheads of bachelor's button, *Centaurea cyanus* L. (Asteraceae), was made in early June for an additional release of *C. australis* in Fall River Mills area of Shasta County. A total of 408 peacock flies were released there on June 21, 1995.

Table 1: Releases of the Peacock Fly, *Chaetorellia australis* Hering on Yellow Starthistle in California 1988-95

Year	1988	1989	1990	1991	1994	1995	TOTAL
Counties	1	3	1	1	1	7	11
Individual Releases	1	3	1	1	2	10	18
Total Numbers	200	362	122	86	175	4040	4,985

During fall 1995, all the release sites, (Fig 1), were visited and checked for colonization by *C. australis*. Evidence of damage as well as live puparia in overwintering hibernacula were found at all seven release sites. Impressive within-site movement of the peacock fly was noted at all sites especially in the Mariposa, Sacramento, and San Luis Obispo County sites. Small samples of infested seedheads were collected from each release site and taken to the Biological Control Program facility for confirmation of colonization by the peacock fly.

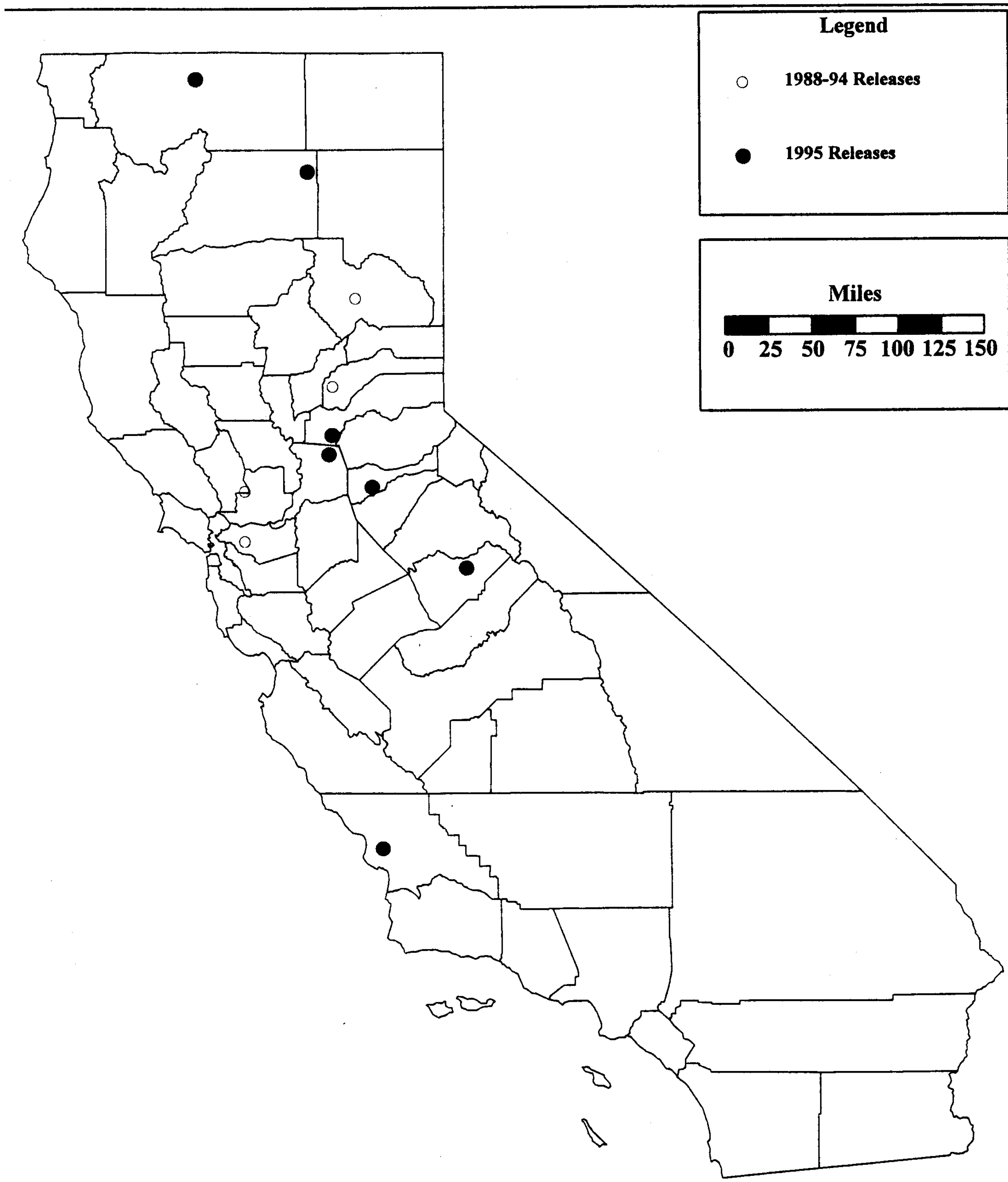
It should be noted that three of the sites had naturalized infestations of bachelor's button in close proximity to heavy infestations of yellow starthistle. These sites were located in Mariposa, Shasta and Siskiyou Counties. The other four releases were made onto yellow starthistle infested sites without any obvious bachelor's button plants in the immediate area. These sites are located in Amador, Placer, Sacramento and San Luis Obispo Counties. The four sites were chosen to see if the peacock fly would establish on yellow starthistle in the absence of bachelor's button.

The peacock fly appears to have two to three generations per year in southern Oregon. The females insert their eggs beneath the involucral bracts of closed seedhead buds, and the larvae feed as they tunnel through and destroy most of the developing seeds in the seedheads. If it can become established throughout California, the peacock fly may be a good biological control agent, remaining active throughout the long flowering season of yellow starthistle.

[1]USDA, ARS, Western Regional Research Center, Albany

Figure 1: Releases of the Peacock Fly in California in 1988-95

Biological Control Program, CDFA



Biological Control of Diffuse Knapweed, *Centaurea diffusa*

D. B. Joley, D. M. Woods, and C. E. Turner[1]

Diffuse knapweed, *Centaurea diffusa* Lamarck (Asteraceae), infests millions of acres of rangeland in the United States and Canada. Along with other knapweeds, diffuse knapweed has diminished quality of grazing lands and wildlife habitat. In California, diffuse knapweed is under active eradication in most areas of the state except in Trinity County, where chemical spray programs are precluded.

Four biocontrol agents have been released on diffuse knapweed in Trinity County. *Urophora affinis* Frauenfield (Diptera: Tephritidae), was released from 1976 through 1981, and although established, the percentage of heads infested in 1995 remains very low (12%), compared to levels observed in other states and in Canada. A second agent, the buprestid root beetle, *Sphenoptera jugoslavica* Obenberger, was released in 1980 in very small numbers (8 adults), and little expectation was given for establishment. However, in 1995 numerous roots of diffuse knapweed, approximately one mile from the original release site, were found to contain larvae of an unidentified buprestid. Infested roots were collected and maintained for emergence of adult beetles. A single adult did emerge and was positively identified as *S. jugoslavica* by Richard Westcott, Oregon Department of Agriculture. Additional introductions of this agent no longer seems warranted.

The final two released biocontrol agents (both are weevils), are recent introductions (Table 1). *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae) was released in 1995 for the first time, and *Bangasternus fausti* (Rietter) (Coleoptera: Curculionidae), for the second consecutive year. A field check of seedheads in November indicated that *B. fausti* may be establishing.

Table 1. Biocontrol agents released on diffuse knapweed in Trinity County in 1995.

BIOCONTROL AGENT	DATE RELEASED	NUMBER RELEASED
Bangasternus fausti	June 30	200
Larinus minutus	June 9	500

Two additional agents have recently been detected without a history of intentional introduction. Seedheads were collected in November 1995 and placed in sleeve cages in the laboratory for emergence of *Urophora affinis*. In one of the sleeve cages, a single specimen of *Urophora quadrifasciata* (Meigen) (Diptera: Tephritidae), has emerged. Since this species was not released in Trinity County, it evidently has moved down through Washington and Oregon from Canada where it was accidentally released. Previously, *U. quadrifasciata* was known in California only on squarrose knapweed, near Hawkinsville, Siskiyou County. The rust, *Puccinia jaceae* (Otth.), also an immigrant from an accidental release in Canada, continues to infect diffuse knapweed plants in Trinity County at low levels.

[1]USDA, ARS, Western Regional Research Center, Albany

Release and Establishment of Natural Enemies for the Biological Control of Spotted Knapweed, *Centaurea maculosa* in California

D. M. Woods, D. B. Joley, and C. E. Turner[1]

Spotted knapweed, *Centaurea maculosa* Lamarck (Asteraceae), is currently under eradication in most of California. One location in the Big Bend area of Shasta County is, however, too large and remote for effective eradication. The Biological Control Program has initiated a ‘classical’ biological control effort in the core of this relatively remote and persistent infestation.

Five natural enemies have been released at the infestation in the Big Bend area over the past few years (Table 1). In 1993, three natural enemies were introduced: *Urophora affinis* Frauenfeld (Diptera: Tephritidae), a seedhead gall fly, *Agapeta zoegana* L. (Lepidoptera: Cochylidae), a root-boring moth, and *Cyphocleonus achates* (Fahraeus) (Coleoptera: Curculionidae), a root-boring weevil. Additional releases of *A. zoegana* and *C. achates* were made in 1994, and again in 1995. Two new natural enemies, *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae), and *Terellia virens* (Loew) (Diptera: Tephritidae), were introduced this year to augment the agents previously released on spotted knapweed (Table 1). Establishment of the gall fly, *U. affinis,* was confirmed this year by detection of galls in seedheads and adult flies in the field. Plant samples obtained in November were found to have galls in 17% of the heads. The weevil, *L minutus,* successfully infested several heads in the release area. Establishment of *A. zoegana* and *C. achates* has not been confirmed in spite of repeated field inspections. Measurements of spotted knapweed population and seedhead density were made during 1994 and 1995, as the baseline for evaluation of future impact by the biocontrol agents.

Table 1. Biocontrol agents released on Spotted Knapweed in Shasta County

Biocontrol agent	Total Released	Years Released	Status December 1995
Urophora affinis	327	1993	Established 17% infestation
Agapeta zoegana	578	1993, 1994, 1995	No recoveries
Cyphocleonus achates	210	1993, 1994, 1995	No recoveries
Terellia virens	97	1995	No recoveries
Larinus minutus	800	1995	Successfully colonized

[1]USDA, ARS, Western Regional Research Center, Albany

Biological Control of Squarrose Knapweed, *Centaurea virgata spp. squarrosa*: Initial Release of *Cyphocleonus achates*

D. M. Woods and D. B. Joley

Squarrose knapweed, *Centaurea virgata spp. squarrosa* Lamarck (Asteraceae), is a close relative of diffuse and spotted knapweeds. Infestations exist in several counties in California and are usually confined to disturbed roadsides. A few large infestations occur in the state that threaten parts of both Oregon and California. The Biological Control Program has initiated a biological control project in the core of one of these large, persistent infestations located north of Yreka in Siskiyou County.

There are currently no biocontrol agents available that were specifically selected and tested against this weed host. However, several agents currently available for diffuse and spotted knapweeds may have potential for use on squarrose knapweed. One of the knapweed gall flies, *U. quadrifasciata* Meigen (Diptera: Tephritidae), appears to have immigrated from Oregon and is established on squarrose knapweed in northern California. The infestation rate in 1995 at the Yreka site was estimated at 6% of the heads having at least one gall. In 1995, a shipment of *Cyphocleonus achates* (Fahraeus) (Coleoptera: Curculionidae), a root-boring weevil, was released in an attempt to establish additional agents on this troublesome weed. The weevils were provided by P.C. Quimby, USDA-ARS, Bozeman Montana, and sent from a field site in Corvallis, Montana. One hundred weevils were released along a dirt road through the infested area. Releases of other agents from diffuse and spotted knapweed will be made during 1996.

Biological Control of Bull Thistle, *Cirsium vulgare*:
Release and Colonization of *Urophora stylata*

D. B. Joley, B. Villegas, and C. E. Turner[1]

Bull thistle, *Cirsium vulgare* (Savi) (Asteraceae), is a widespread exotic biennial weed that is usually associated with a high degree of disturbance, such as overgrazed permanent pasture and woodland clearings. Despite the close taxonomic relationship between bull thistle and North American native *Cirsium spp*., a host specific bioagent, the gall fly, *Urophora stylata* (Fabricius) (Diptera: Tephritidae), has been approved, released, and monitored for several years in other western states.

Efforts to establish *U. stylata* in California began in 1993 and continued in 1994. In 1995, a total of 893 flies were released at 5 sites, (Table 1), in three counties for a three year total of 10 sites in 7 counties. Post-release surveys by Biological Control Program staff and county agricultural biologists have failed to find establishing populations at any release sites. Galls with larvae were found at some sites, but no evidence of *U. stylata* was found the following year. Observations by Eric Coombs, Oregon Department of Agriculture, suggest that establishment has been successful only at sites below 1,000 feet elevation. Introduction of *U. stylata* will continue in 1996, and releases will be concentrated in counties along the central and northern California coast and the Sacramento Valley.

Table 1. *Urophora stylata* releases in 1995.

COUNTY NEAREST TOWN	DATE RELEASED	NUMBER RELEASED
San Luis Obispo Cambria	July 20	290
Mendocino Ukiah	August 3	280
Mendocino Willits	August 3	50
Marin Tomales #1	August 11	234
Marin Tomales #2	August 22	39

[1]USDA, ARS, Western Regional Research Center, Albany

Terellia fuscicornis, A New Biocontrol Agent of Artichoke Thistle in California

D. M. Woods, M.J. Pitcairn, D.B. Joley, and C.E. Turner[1]

Artichoke thistle, *Cynara cardunculus* L. (Asteraceae) can be a troublesome weed in certain parts of California. Although historically a large effort was made in physically and chemically controlling the weed, most control programs in the state have been severely limited due to cost constraints. The importance of the phylogenetically close relative, *Cynara scolymus* (Asteraceae), or domesticated globe artichoke, has prevented serious consideration of biological control as a control strategy for artichoke thistle.

An exotic tephritid fly, *Terellia fuscicornis* (Loew) (Diptera: Tephritidae), was first detected in California in July 1994, causing significant damage in artichoke seed fields. The fly has now been detected on artichoke thistle and/or globe artichoke over most the range of these plants in California. Large numbers of larvae have been found in individual artichoke thistle seedheads and appear to destroy a large percentage of seeds. Fortunately, the fly is not likely to be a pest to the majority of artichoke producers in California as the fly appears to attack the plant only after the artichoke heads have begun to bloom. In the context of weed control, the fly seems to be a (presumably) accidental reaquaintence of a natural enemy with its host plant. The fly is not currently being artificially spread in the state as it's pest potential has not been fully evaluated. Field observations indicated that *T. fuscicornis* also attacks milk thistle, *Silybum marianum* (L.) (Asteraceae), but its impact is unknown. Efforts are underway to evaluate the host range of *T. fuscicornis* and susceptibility of native *Cirsium spp.*

[1]USDA, ARS, Western Regional Research Center, Albany

Hydrellia pakistanae* as a Biological Control Agent of *Hydrilla verticillata

K. E. Godfrey, L. W. J. Anderson[1], C. E. Turner[2], K. Chan[2],
D. Quimayousie[3], and J. Barajas[3]

Hydrilla verticillata (L.f.) Caspary (Hydrocharitaceae), is an exotic aquatic plant that infests waterways, irrigation canals, lakes, and ponds throughout the United States. Its growth is so prolific that it can completely fill waterways with plants which restrict water flow in irrigation and drainage canals, impede navigation and public water uses, and increase sedimentation rates in flood control canals and reservoirs. The California Department of Food and Agriculture has an aggressive program aimed at eradicating *H. verticillata* (hydrilla) which is now limited to a few isolated sites in the state. In 1994, USDA-ARS, in cooperation with the Biological Control Program, began a 2-year study to investigate the use of *Hydrellia pakistanae* Deonier (Diptera: Ephydridae) a small aquatic fly that mines hydrilla leaves, to control hydrilla.

Field studies on the ability of *H. pakistanae* to establish and reduce densities of hydrilla were conducted in 1994 and 1995 in Imperial County. In September 1993, 11 sites with hydrilla were examined, and 8 were selected for inclusion in this study. Five of the sites were used as release sites, and the remaining sites, as controls (i.e., no flies released). Each site was located in an irrigation canal on the grower's side of a delivery gate in the Imperial Irrigation District. In 1994, a total of 25,920 *H. pakistanae* were released at five sites. Unfortunately, one of these sites was destroyed by grower activity shortly after the first release and was not used for the remainder of the study. In addition, one of the control sites was also destroyed during 1994, leaving four release sites and two control sites in the study for 1995. No flies were released at the sites in 1995. Instead, the sites were sampled using the same protocol as in 1994, to determine if *H. pakistanae* had successfully overwintered and begun to establish in Imperial County. No flies were recovered at any of the sites in 1995 suggesting that the flies did not survive the winter. However, at two of the release sites where the flies were abundant in 1994, there was little or no regrowth of the hydrilla in 1995 (Table 1).

In October and November 1995, all sites in Imperial County were cleaned out using a mud pump. At two of the sites where there had been little regrowth of the hydrilla in 1995, all of the material from the bottom of the site was screened, and the number of tubers recorded. For each of the remaining sites, a 0.25 m^2 sample of soil was screened and the number of tubers recorded.

The relative densities of *H. pakistanae* populations and their impact on hydrilla populations as measured by tuber production is summarized for each site in Table 1. The fly intensity rating in Table 1 refers to the abundance of *H. pakistanae* at each site in 1994. The data collected during both years of the study suggest that at those sites where *H. pakistanae* was able to increase in density and flourish, the hydrilla population was severely impacted.

Table 1. The disturbance history, fly intensity rating and number of tubers recovered at each site in Imperial County in 1995.

Site	Disturbance[a]	Fly Intensity Rating[b]	Tuber Density (No./0.25m^2)
Release Sites			
Site 1	Fertilizer burn	-	186
Site 4	N.D.	++	26
Site 5	N.D.	+	5[c]
Site 9	N.D.	++	0[c]
Control Sites			
Site 3	Fertilizer burn	-	4
Site 8	N.D.	-	32

[a]N.D. = Not disturbed
[b]Rating: - = No flies recovered in 1994; + = Damage seen, but no flies recovered in 1994; ++ = Damage seen and flies recovered in 1994
[c]Total count at site

[1]USDA, ARS, Aquatic Weed Control Research Laboratory, Davis
[2]USDA, ARS, Western Regional Research Center, Albany
[3]California Dept. of Food and Agriculture, Integrated Pest Control, El Centro

Biological Control of Gorse, *Ulex europaeus*, in California:
Introduction of *Tetranychus lintearius*

C. E. Turner[1], K. Chan[1], and M. J. Pitcairn

Gorse, *Ulex europaeus* L. (Fabaceae), is a perennial exotic shrub that infests pastures, rangeland, and wildlands in the coastal counties of California, Oregon, and Washington. It is very spiny and grows into large bushes that become impenetrable. Gorse infestations increase fire hazards, interfere with recreation, limit access to natural areas, increase control costs in rights-of-way, and remove land from grazing. In 1953, *Exapion ulicis* (Forster) (Coleoptera: Apionidae) was introduced into California as a biological control agent. *E. ulicis* is a seed-feeding weevil that infests the seed pods and is now established at all major infestations of gorse. Although the weevil attacks >50% of the seed pods, gorse plant density has not been noticeably reduced.

In 1994, the gorse spider mite, *Tetranychus lintearius* Dufour (Acari: Tetranychidae) was released in California and Oregon. This spider mite is very host specific; its only documented host in its native habitat is the genus *Ulex* (Gorse). This spider mite exists in colonies within webs that engulf the growing tips of shoots and branches. The mites feed by sucking juices from the cells in the shoots and spines. Researchers in New Zealand report that shoots heavily damaged by mite feeding usually die and less damaged shoots have reduced growth rates.

The first releases of *T. lintearius* occurred at 12 sites in four counties in 1994. The mites were released by tying an infested branch tip onto a gorse branch with plastic plant ties. In 1995, all release sites were monitored for establishment. Surveys by the authors and county biologists Anita Sauber (Marin County), Priscilla Lane (Sonoma County), and Jim Xerogeanes (Mendocino County), showed that the mite successfully overwintered and appeared to have established at all sites, except one in Sonoma County. Four additional releases of mites occurred in 1995. In Sonoma County, releases occurred at two of the three 1994 release sites with material from the mite colony at the USDA Biological Control of Weed Laboratory, Albany, one site being where the 1994 release failed to establish. In Mendocino County, Jim Xerogeanes released the mite at two new sites using material from one of the established field sites in his county. All sites will continue to be monitored for growth and spread of the mite populations.

[1]USDA, ARS, Western Regional Research Center, Albany

Weed Biological Control Workshops

B. Villegas

The Biological Control Program maintains an active weed biocontrol agent distribution program. New bioagents are introduced and established agents are distributed under this program. In 1994, releases of natural enemies were made in 41 counties from Riverside to Modoc and Siskiyou Counties. Nine natural enemies were released to aid in the control of seven weed species: Bull thistle, diffuse knapweed, gorse, hydrilla, spotted knapweed, squarrose knapweed and yellow starthistle. The current status of each project is outlined in additional reports. During 1995, the three biological control agents on yellow starthistle were distributed during twelve sessions of the distribution workshop system (Table 1). Workshops serve the purpose of training county participants about the biology of the bioagents and the management of field populations. Workshops are held at field nursery sites or at centralized locations by the Biological Control Program where county biologists observe established biocontrol agents then return to their own county and attempt to establish their own nursery sites for further distribution.

Table 1: List of workshops held in 1995 for the distribution of yellow starthistle biological control agents

WORKSHOP SUBJECT	LOCATION	DATE	COUNTY PARTICIPATION
Bangasternus orientalis	Placer	May 24, 1995	1 county
Bangasternus orientalis	Placer	May 31, 1995	4 counties
Bangasternus orientalis	Placer	June 9, 1995	1 county
Bangasternus orientalis	Siskiyou	June 13, 1995	3 counties
Eustenopus villosus	Sacramento	June 22, 1995	7 counties
Eustenopus villosus	Sacramento	June 27, 1995	4 counties
Eustenopus villosus	Sacramento	June 29, 1995	2 counties
Eustenopus villosus	Sacramento	July 3, 1995	2 counties
Eustenopus villosus	Shasta	July 13, 1995	6 counties
Urophora sirunaseva	Placer	May 31, 1995	4 counties
Urophora sirunaseva	Placer	June 9, 1995	1 county
Urophora sirunaseva	Siskiyou	June 13, 1995	3 counties

Distribution of the Bud Weevil, *Bangasternus orientalis*, in California for the Biological Control of Yellow Starthistle in 1995

B. Villegas

The bud weevil, *Bangasternus orientalis* (Capiomont) (Coleoptera: Curculionidae) was introduced from Greece into California for the biological control of yellow starthistle, *Centaurea solstitialis* L (Asteraceae). The USDA-ARS, in cooperation with the Biological Control Program and the County Agricultural Commissioners, established the first colonies of this weevil near Lincoln (Placer County), Montague (Siskiyou County) and Rumsey (Yolo County) in 1985. Additional releases were made in Loomis (Placer County), Orinda (Contra Costa County), Santa Rosa (Sonoma County), Lotus (El Dorado County), and again at the Rumsey site in Yolo County in 1986-1988. *B. orientalis* appeared to be well established at the Placer and Siskiyou County sites by 1988 when it was made available to the County Agricultural Commissioners for statewide distribution. The distribution information through 1995 is summarized in Table 1.

Bangasternus orientalis has one generation per year. Adult weevils emerge from overwintering sites from mid-April through May and find bolting yellow starthistle plants. After mating, female weevils start laying eggs on terminal leaflets and bases of young flower buds. When the female weevil lays an egg, she covers it with a mucous substance, combined with fecal particles and plant hairs. As the mucous mixture hardens, it forms a characteristic tear-shaped black protective cap. The eggs hatch within two weeks after oviposition and each larva burrows its way into the stem and up into the flower bud. There, the weevil larva feeds on the receptacle tissue as well as directly on the developing seeds. Larval development generally occurs from early May through mid to late July, followed by the pupal stage in late July and August. Adult weevils emerge through a visible hole in top of the seed head. These adult weevils presumably aestivate through the rest of the summer and fall, and overwinter in the duff and debris near the yellow starthistle plants.

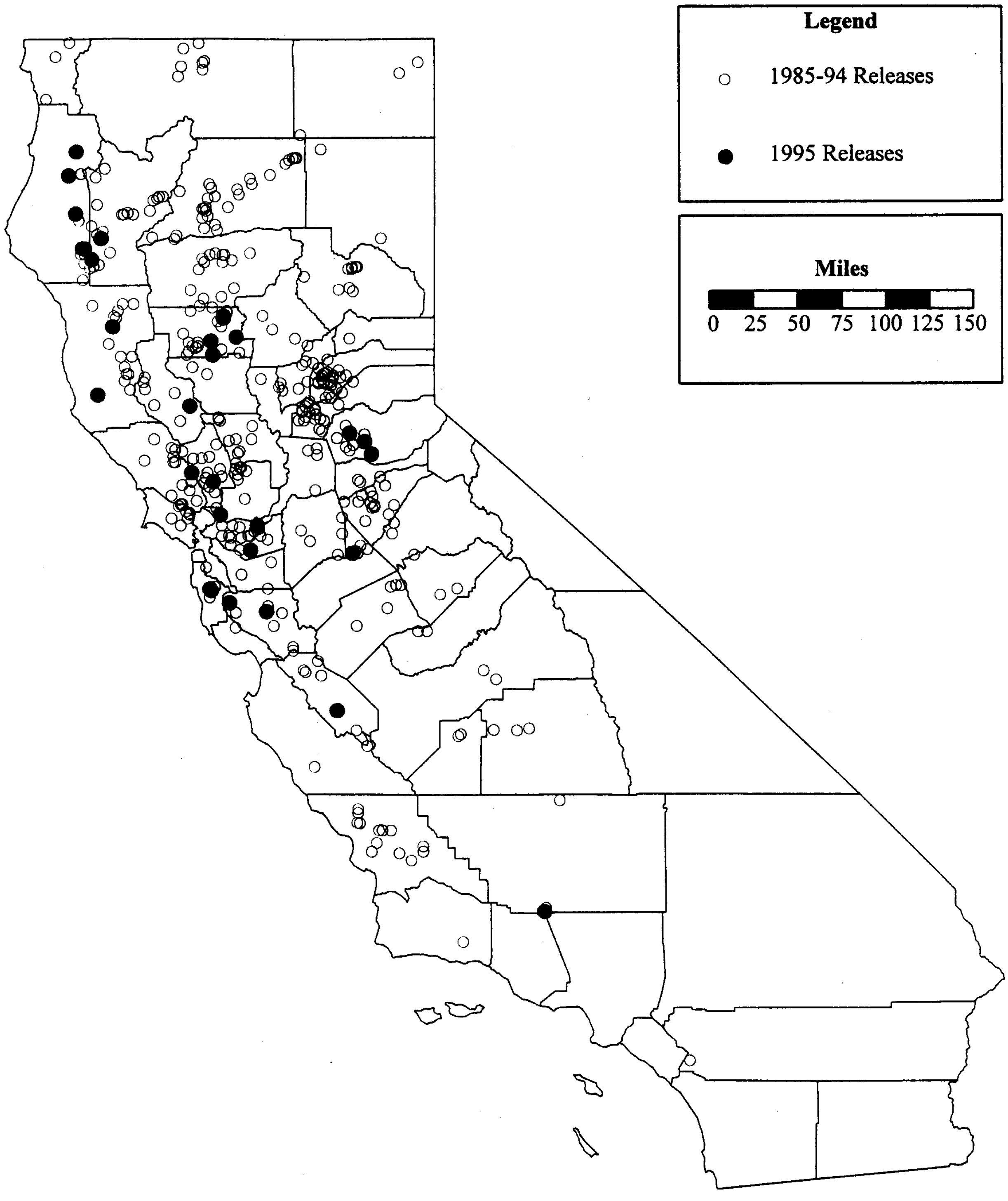
In 1995, a total of 5,414 bud weevils were released at 31 sites in 13 counties (Figure 1). Of these, some 4,322 weevils were collected by personnel from nine counties during four distribution workshops held at field collection sites near Lincoln (Placer County) and Yreka (Siskiyou County). In 1995, three counties, El Dorado, Mendocino and Trinity, made seven in-county releases of *B. orientalis* totaling 1,062 weevils. *B. orientalis* has become well established in most of the 49 counties where it was released through this distribution program.

Table 1: Releases of the bud weevil, *Bangasternus orientalis* (Capiomont) on yellow starthistle in California 1985-95

Year	1985-88	1989	1990	1991	1992	1993	1994	1995	Total
Counties	18	16	24	22	29	20	19	13	49
Individual Releases	20	23	37	64	75	74	102	31	426
Total Numbers	3809	3,929	6,382	10,552	14,055	14,999	21,621	5,414	80,761

Figure 1: Releases of the Bud Weevil in California in 1985-95

Biological Control Program, CDFA



Distribution of the Seedhead Gall Fly, *Urophora sirunaseva*, in California for the Biological Control of Yellow Starthistle

B. Villegas

The seedhead gall fly, *Urophora sirunaseva* (Hering) (Diptera: Tephritidae) was introduced from Greece into California for the biological control of yellow starthistle, *Centaurea solstitialis* L (Asteraceae). The USDA-ARS, in cooperation with the Biological Control Program and the County Agricultural Commissioners, established the first colonies of the fly in Loomis (Placer County) in 1984-1985, and near Orinda (Contra Costa), Mankas Corner (Napa County), Ukiah (Mendocino County), and Hornbrook (Siskiyou County) in 1990-1991. Populations were well established in Placer County by 1992 and the gall fly was made available for statewide distribution. The distribution information through 1995 is summarized in Table 1.

U. sirunaseva has two generations per year. In the Sacramento Valley, adult flies emerge from overwintering seedhead galls from mid-April through May while adults of the first generation emerge from late June to mid-July. Oviposition occurs on intermediate, closed head buds. After hatching, the larvae migrate to the receptacle of the yellow starthistle bud. There, gall formation around developing larvae is induced, and there is one fly larva per gall. Up to 12 galls have been found in galled seedheads. Galls induced by the fly larvae are woody with those from the overwintering generation being stronger than those induced by the first generation larvae. Buds infested with the gall flies are believed to produce less seed due to the limited amount of receptacle area for seed production.

In 1995, approximately 9,564 seedhead gall flies were released at 34 sites in 10 counties (Figure 1). Of these, some 8,864 gall flies were collected by personnel from eight counties during three distribution workshops held at field collection sites near Loomis (Placer County) and Yreka (Siskiyou County). In 1995 only Mendocino County actively collected and released the gall fly within the county, establishing five new sites with. 670 gall flies collected in Ukiah.

Table 1: Releases of the seedhead gall fly, *Urophora sirunaseva* on yellow starthistle in California 1984-95

Year	1984-85	1990	1991	1992	1993	1994	1995	total
Counties	2	3	3	13	30	25	10	38
Individual Releases	3	3	3	16	63	64	34	186
Total numbers	244[a]	998[ab]	756 [ab]	2,755 [b]	9,526 [b]	17,595 [b]	8,964 [b]	40,838

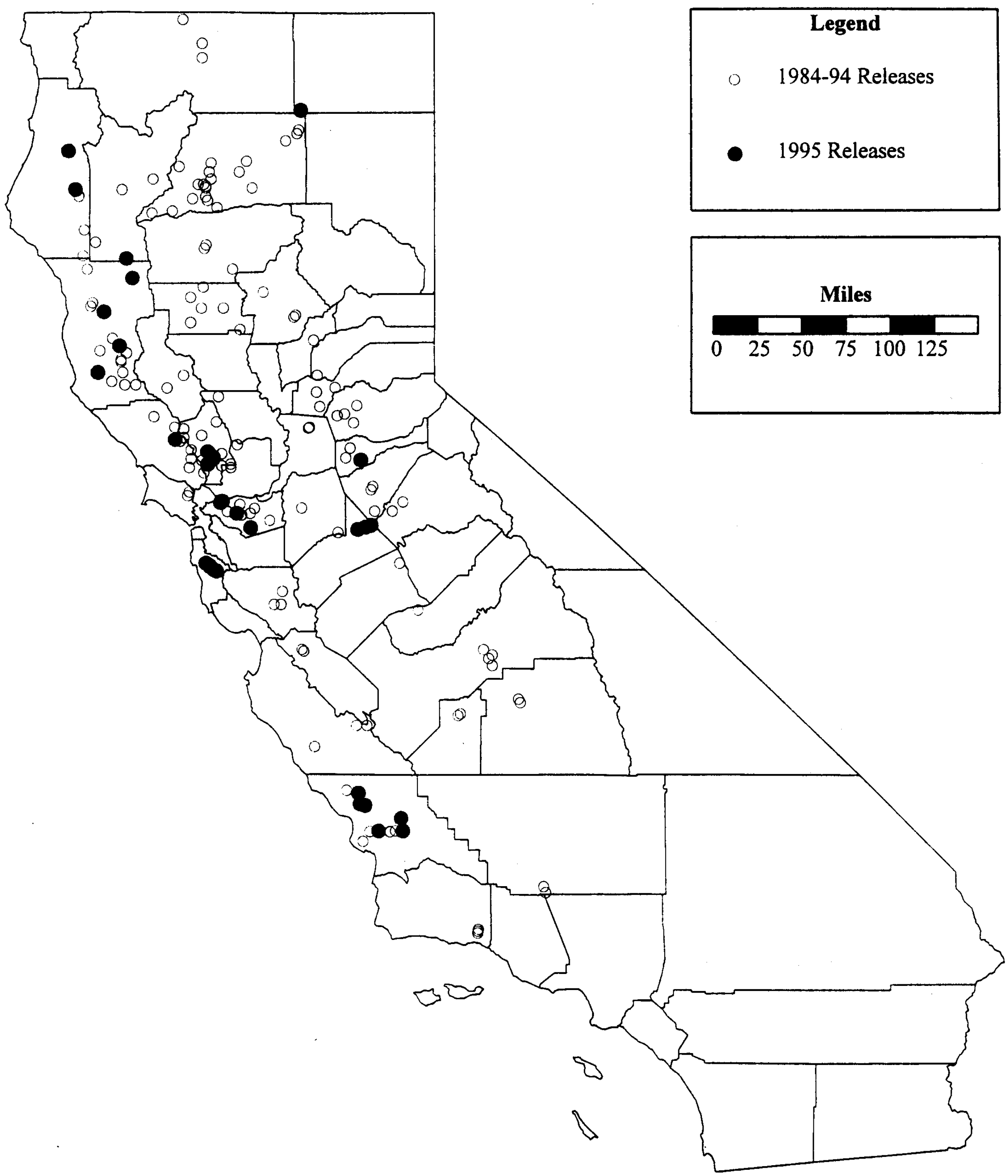
[a]colonization releases with European material.

[b]distribution releases with domestic material.

To date, *U. sirunaseva* has become widely established in California due to the efforts of agencies participating in its distributions. In areas of Siskiyou and Mendocino Counties and the Sierra Nevada foothills of central California (Nevada County through Calaveras County and Merced County), the fly has become widely distributed on its own.

Figure 1: Releases of the Gall Fly in California in 1984-95

Biological Control Program, CDFA



Distribution of the Hairy Weevil, *Eustenopus villosus*, in California for the Biological Control of Yellow Starthistle

B. Villegas

The hairy weevil, *Eustenopus villosus* (Boheman) (Coleoptera: Curculionidae) was introduced from Greece into California for the biological control of yellow starthistle, *Centaurea solstitialis* L (Asteraceae). The USDA-ARS in cooperation with the Biological Control Program and the County Agricultural Commissioners, established the first colonies of this weevil in Nevada and El Dorado Counties in 1990 and Napa, Mendocino, and Shasta Counties in 1991. Populations were so well established in El Dorado and Nevada Counties by 1992, that the hairy weevil was made available for limited distribution. The state sponsored distribution has rapidly expanded from that point. (Table 1).

Eustenopus villosus has one generation per year in the Sacramento Valley, emerging in late May, mating and ovipositing in mid June. Eggs are inserted inside closed head buds. Larvae feed on receptacle tissue reducing the number and viability of seeds. Unlike the other yellow starthistle natural enemies, the *E. villosus* adults also cause damage by direct feeding on young closed buds. This damage can be very extensive at 3rd year release sites.

In 1995, two additional sites located in Sacramento and Shasta Counties became available for collection, and a total of 23,267 hairy weevils were released at 41 counties (Table 1). Of these, 15,822 weevils were made available to 37 counties during four distribution workshops (Figure 1). Three counties, El Dorado, Mendocino and Shasta, made their own in-county releases, totaling 3,975 weevils. Seven additional releases totaling 3,470 weevils were made by Biological Control Program staff at six sites in conjunction with ongoing research on this agent.

Table 1: Releases of the hairy weevil, *Eustenopus villosus* (Boheman) on yellow starthistle in California 1990-95

Year	1990	1991	1992	1993	1994	1995	total
Counties	2	3	3	10	43	41	47
Individual Releases	2	4	3	12	85	109	105
Total Numbers	431[a]	1,014[a]	336[b]	3,150[b]	18,995[b]	23,267[b]	47, 193

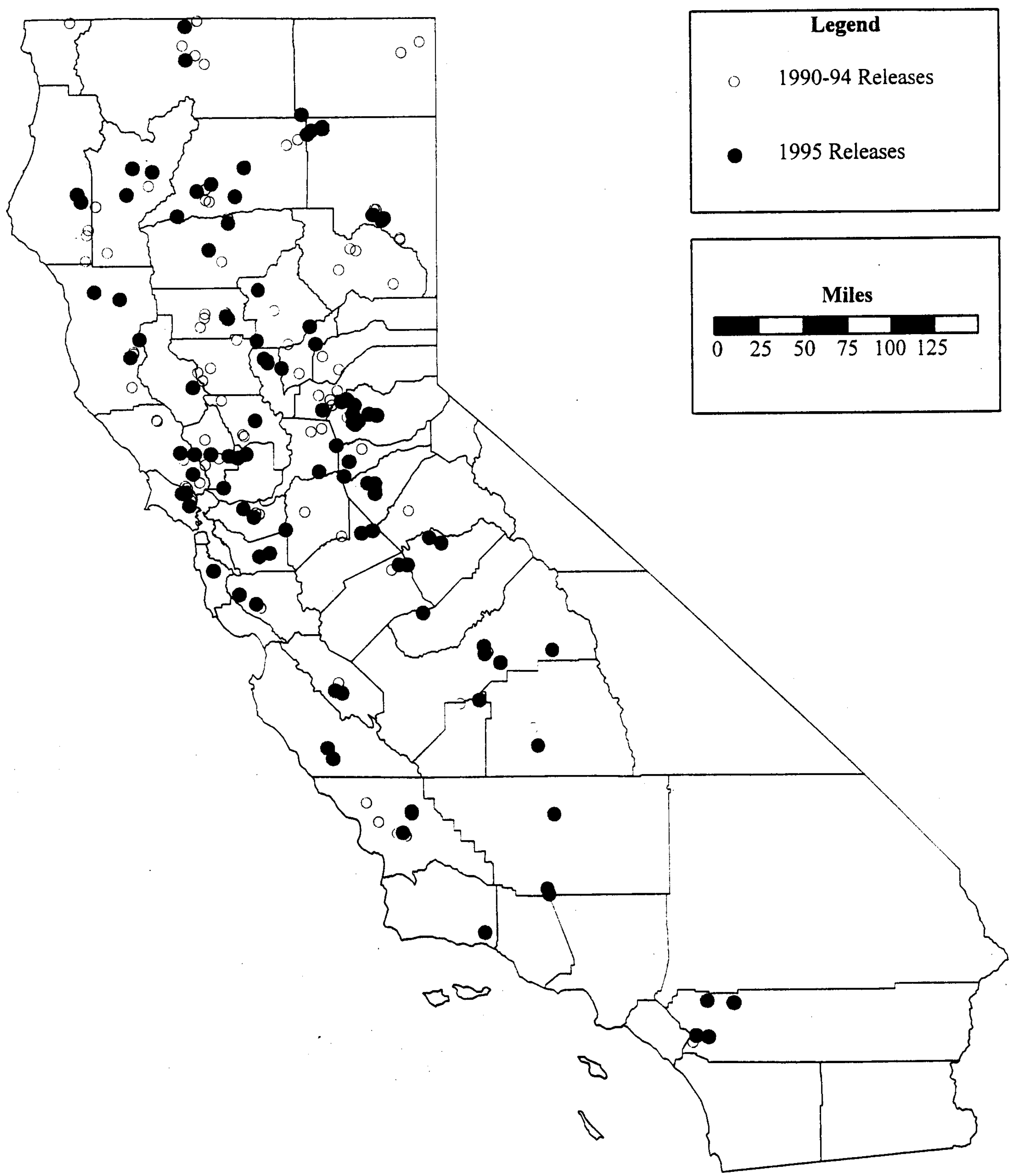
[a]colonization releases with European material.

[b]distribution releases with domestic material.

To date, the hairy weevil has become well established at the seven sites where it was released through 1992 (Nevada, El Dorado, Mendocino, Napa, Shasta, Placer and Sacramento Counties) as well as six 1993 release sites (Butte, Contra Costa, Glenn, Placer, Shasta, and Siskiyou Counties). Preliminary surveys of most of the 1994 and 1995 release sites indicate that the hairy weevil establishes readily in most areas of California but prefers the hotter interior parts of the State. The weevil appears not to have established well in coastal areas of California that are affected by fog and persistent cool temperatures.

Figure 1: Releases of the Hairy Weevil in California in 1990-95

Biological Control Program, CDFA



A New Seedling Pathogen of Yellow Starthistle, *Centaurea solstitialis*

D. M. Woods

An endemic soilborne plant pathogen has been found in California causing seedling mortality of yellow starthistle, *Centaurea solstitialis* L. (Asteraceae). Field observations of yellow starthistle infestations had suggested that a significant number of yellow starthistle seedlings die during the winter prior to becoming rosettes. The impact of seedling diseases on yellow starthistle has never been documented.

Several species of fungi were isolated from collapsing seedlings, with one isolate of fungus from the genus *Ascochyta* proving to be the most effective pathogen so far. This fungus killed over 90% of seedlings by 28 days after inoculation (Table. 1). Experiments performed in growth chambers demonstrate that temperature may be a critical factor in the ability of the host to avoid or recover from infection. Seedlings inoculated and maintained at relatively cold temperatures were usually killed by the disease, but seedlings maintained at warm temperatures generally survived. (Table 1)

Table 1. Effect of Temperature on Yellow Starthistle Infected with *Ascochyta* Seedling Disease

	Day-Night Temperature		
	12°C-2°C	15°C-5°C	24°C-12°C
Percentage of Seedlings Surviving 28 Days after Inoculation with *Ascochyta*	7%	15%	90%

Additional plants have been tested for susceptibility to the *Ascochyta* pathogen. Diffuse knapweed, *Centaurea diffusa* Lamarck (Asteraceae), and spotted knapweed, *Centaurea maculosa* Lamarck (Asteraceae), are both weedy relatives of yellow starthistle and are susceptible to the fungus, but less so than yellow starthistle (Fig. 1). Several agronomic crops are also being evaluated for susceptibility to the yellow starthistle pathogen. The majority of crop species tested to date are not susceptible. Four California cultivars of safflower, and one cultivar each of sunflower, onion, tomato, and radish have been tested without a single plant death from *Ascochyta*. Lettuce and shasta daisy have, however, proven susceptible in at least one trial. The fungus appears to have potential as a biological control agent as it seems to be fairly host specific, while causing significant mortality to weedy *Centaurea* species, particularly, yellow starthistle.

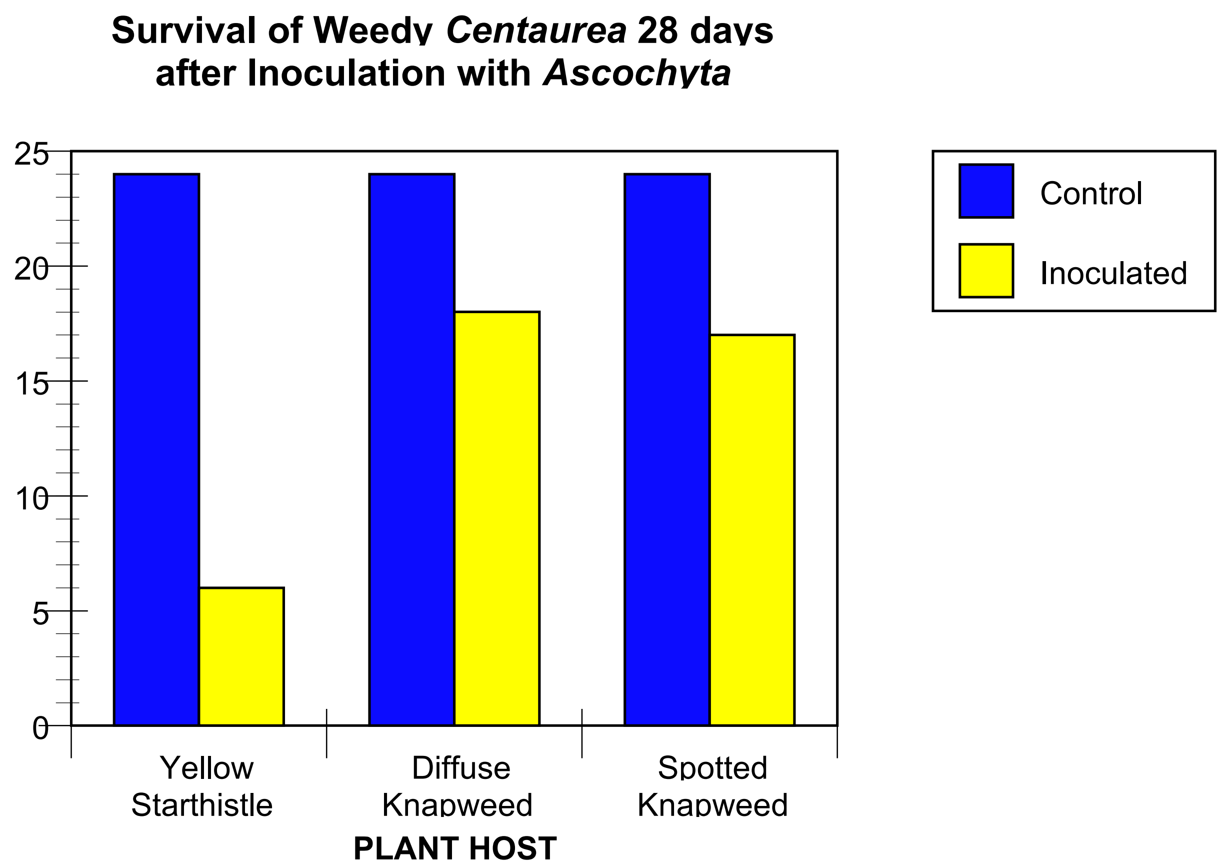


FIG. 1. Effect of *Ascochyta* on seedlings of yellow starthistle, diffuse and spotted knapweed. Twenty-four seedlings were inoculated and grown under a 15°C/5°C day/night regime.

Individual Impact of Three Biocontrol Agents on Yellow Starthistle, *Centaurea solstitialis* in California

M. J. Pitcairn, D. B. Joley, D. M. Woods, and C. E. Turner[1]

Yellow starthistle, *Centaurea solstitialis* L. (Asteraceae), is one of California's worst weeds. This exotic annual was first discovered in the 1860's in Alameda County and now infests more than 8 million acres statewide. Yellow starthistle is poisonous to horses causing brain lesions in affected animals. The spines produced on seed heads of mature plants irritate the mouthparts of grazing animals and may also cause a condition called "pink eye" in cattle. Yellow starthistle can produce dense stands which reduce productivity of infested rangeland.

In an attempt to reduce yellow starthistle abundance statewide, federal, state, and county personnel have introduced five exotic insects into California. Four are now established; three are widespread: *Bangasternus orientalis* (Capiomont) (Coleoptera: Curculionidae), the bud weevil: *Urophora sirunaseva* (Hering) (Diptera: Tephritidae), the gall fly; and *Eustenopus villosus* (Boheman) (Coleoptera: Curculionidae), the hairy weevil. The impact of each of these biocontrol agents on yellow starthistle in California is unknown, so studies were initiated in 1992 to examine seed destruction and infestation rate.

Study sites were selected where each agent had increased to high densities in the absence of the other two (some sites were invaded by one or more of the other agents, but their numbers were very low during these studies). Field plots consisted of four transects along which rods were placed at 40 positions using a restricted randomization procedure. The plant located closest to each rod was selected for monitoring (total = 160 plants). The selected plants were examined weekly during the flowering season. All seed heads on a plant were bagged shortly after flowering using cloth bags to retain seeds and agents infesting the seed heads. Upon death of the host plant, it was collected by cutting the stem at ground level. Yellow starthistle plant density was estimated at harvest. Seed heads were examined individually in the laboratory for presence, number, and life stage of the agent, diameter and weight of seed head, and number and viability of seeds. All remaining plant materials were oven-dried and weighed. Buds and branch tips were scored for damage from adult feeding or immature death. The field portion of these studies has been completed for *B. orientalis and E. villosus* (Table 1). The field work for *U. sirunaseva* will be completed in 1996. Processing of plant and seed head samples in the laboratory is now underway.

Table 1. List of impact studies of individual yellow starthistle bioagents in California

Biocontrol agent	Site	No. years	Location
Bangasternus orientalis	Site 1	3 years	Placer County
Urophora sirunaseva	Site 1	2 years	Mendocino County
	Site 2	1 year	Solano County
Eustenopus villosus	Site 1	2 years	Placer County
	Site 2	2 years	Napa County

[1]USDA, ARS, Western Regional Research Center, Albany

Biological Control of Yellow Starthistle with Multiple Biocontrol Agents

D. B. Joley, M. J. Pitcairn, D. M. Woods, and C. E. Turner[1]

Yellow starthistle, *Centaurea solstitialis* L. (Asteraceae), infests more that 8 million acres in California. It is considered to be California's worst rangeland weed. Studies with individual biocontrol agents on yellow starthistle are completed or are underway to evaluate their impact on seed production and biomass. However, no information is available on the combined impact of all agents acting together. Studies to evaluate the combined impact were initiated in 1995. These studies include looking at impact on seed production and long term changes in yellow starthistle plant populations and species constituency. Sites are being selected to represent a diversity of climatic and geographic ranges of yellow starthistle in California. This will allow us to monitor population buildup and interactions of the agents in different habitats.

Three sites have been selected and the agents released in the following areas to date: Central Valley (Yolo County); Sierra Nevada foothills (Placer County); Coast Range (Sonoma County) (Table 1). *Bangasternus orientalis* (Capiomont) (Coleoptera: Curculionidae), was obtained from field sites in California and released at the Yolo and Sonoma County sites, but was well established already at the Placer County site. *Urophora sirunaseva* (Hering) (Diptera: Tephritidae) was collected in California and released at all three sites. *Eustenopus villosus* (Boheman) (Coleoptera: Curculionidae), was collected in California and released at all three sites. *Chaetorellia australis* (Hering) (Diptera: Tephritidae) is not established yet in California; it has not been released at the three sites. *Larinus curtus* Hochhut (Coleoptera: Curculionidae) was directly released from European shipments.

Table 1. Month and year of bioagent release at three sites.

LOCATION	BIOAGENTS RELEASED			
	Bangasternus orientalis	*Urophora sirunaseva*	*Eustenopus villosus*	*Larinus curtus*
Yolo County[a]	May 1993 May 1994	May 1993 May 1994	June 1993 June 1994	June 1993 July 1994
Placer County	Established previously	July 1993	July 1993	July 1994
Sonoma County	June 1994	June 1994	July 1994	July 1994

[a]Site burned October 1993

In 1995, field plots were set up at the three study sites, and one year of data has been collected on seed production, biocontrol agent infestation rate, adult plant (and seedhead) density, and plant community cover. Each field plot measures 30 x 30 meters within which 100 quadrats (20 x 20 cm) are placed in a systematic grid. Each small quadrat is nested in the middle of a 1 x 1 meter buffer quadrat. Data collected from each of the smaller quadrats include seedling and adult yellow starthistle plant (and seedhead) density and plant community cover estimates. Preliminary data on adult and seedling yellow starthistle plant densities (number per square meter) at the three sites are shown in Table 2. Mature plants were counted in October 1995; seedlings were counted in January 1996, instead of the normal time in December, due to the lateness of rains in 1995.

Table 2. Average densities (number per square meter) of seedling and adult yellow starthistle plants in permanent field plots at three sites in northern California in 1995.

LOCATION	ADULT PLANTS	SEEDLINGS
Interior Valley[a] Yolo County	1095	975
Sierra Foothills Placer County	651	332
Coast Range Sonoma County	897	241

[a]Site re-established after fire destroyed the first one in 1993

Seed production and biocontrol agent infestation levels in seedheads will be monitored to establish baseline data at these three sites for two years (1995 and 1996), soon after the release of bioagents. At each site, two transects, each 20 m long were placed alongside two edges of the plot (total = 4 transects). Rods were placed at 40 positions (restricted randomization procedure) along each transect, and the nearest adult plant to the rod was selected. All seedheads on each plant are to be bagged shortly after flowering with cloth bags to retain seeds and agents infesting the seedheads. Plants will be harvested after they die and taken to the laboratory for processing. Seedheads will be examined individually for presence, number, and life stage of all agents, size of seedhead (diameter and weight), and the number and viability of seeds. All remaining plant materials will be oven-dried and weighed. Buds and branch tips will be scored for damage from adult agent feeding or immature death. Processing of 1995 samples in the laboratory is currently underway.

[1]USDA, ARS, Western Regional Research Center, Albany

Status of *Larinus curtus*, a Biological Control Agent on Yellow Starthistle

C. E. Turner[1], M. J. Pitcairn, and B. Villegas

The flower weevil, *Larinus curtus* (Hochhut) (Coleoptera: Curculionidae) is the fifth natural enemy introduced into California by the USDA, ARS, for the biological control of yellow starthistle, *Centaurea solstitialis* L. (Asteraceae). Adult flower weevils deposit their eggs in open flowers and the larvae feed on the developing seeds. According to reports from the USDA, ARS, European Biological Control Laboratory, *L. curtus* larvae may destroy as much as 96% of the developing seeds in a flower head.

Attempts to establish this agent began in 1992 when several shipments of adult weevils were received from Greece. As many as 2-3 shipments were received each year from 1992-1995 and releases were made in California, Oregon, Washington, and Idaho. Upon receipt, a small sample of adults from each shipment was dissected and examined for parasites and diseases. This was done to ensure that the stock of adults released into the field were disease-free. Disease and internal parasites can compromise the ability of these agents to reproduce, reducing their impact on yellow starthistle abundance. For the shipments of *L. curtus*, the following pattern emerged: weevils in the first shipment of each year were found to be disease-free; weevils in all later shipments were found to be infested with *Nosema* sp., an internal protozoan infesting the digestive tract. Only adult weevils from the early disease-free shipments were field released in the western United States.

Field observations at release sites showed that establishment success of *L. curtus* was poor and that for those populations that did establish, the population build-up was slow. These observations plus the pattern of *Nosema* infection among shipments, suggested that the weevils in the early shipments may have also been infested, but the infection was not detected. As of 1995, *L. curtus* had established in California at only one of the 1992 release sites and none of the later release sites. Ten adult weevils were collected from this site and examined for *Nosema*: four were found infected. Cooperators in Washington, Oregon, and Idaho were contacted, and *L. curtus* adults were sent from their established populations. The following results were obtained:

Location	Number Adults	Number Infected
Peck, Idaho	6	1
Dworshak Dam, Idaho	9	0
Clover Creek, Oregon	24	4
Washington State	10	0

It appears that *Nosema* is present in at least three of five populations of *L. curtus* in the western United States. The impact of this pathogen on the ability of *L. curtus* to reduce yellow starthistle seed production is unknown. Contact has been made with Joseph Maddox, University of Illinois, an insect pathologist, who has agreed to identify the species of pathogen. Samples of adult weevils will be sent to him in 1996. No collections or distribution of *L. curtus* will occur until more is learned about the epidemiology of this disease.

Transmission of this pathogen occurs through feces, and studies were performed to examine the potential spread from *L. curtus* adults to *Eustenopus villosus* (Boheman)

(Coleoptera: Curculionidae), another weevil bioagent on yellow starthistle. In one study, healthy *E. villosus* adults were caged for one week with infected *L. curtus* adults on yellow starthistle flower heads. After allowing for incubation of the *Nosema*, the *E. villosus* adults were dissected, and all were found free of the pathogen. In the second study, 15 adult *E. villosus* were fed yellow starthistle flower buds painted with a *Nosema* suspension from infected *L. curtus*. After allowing for incubation, all adults were dissected and found to have no evidence of infection. These preliminary data suggest that this *Nosema* sp. does not easily spread to *E. villosus* and that the infection of *L. curtus* may not pose a threat to the impact on other weevil agents on yellow starthistle.

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Pollination of Yellow Starthistle in California

D. B. Joley, D. M. Maddox[1], D. M. Supkoff[2], and A. Mayfield[1]

Yellow starthistle, *Centaurea solstitialis* L. (Asteraceae), is an annual Eurasian plant that has become one of California's worst weeds. Despite its importance in California and other western states, little ecological information on pollination and seed production is known. Yellow starthistle was previously considered to be an obligate out-crosser, although selfing is known in other *Centaurea*. The ability to produce viable seeds without cross-pollen would significantly enhance the potential for single yellow starthistle plants to colonize remote areas. Therefore, the pollination biology in yellow starthistle, and in particular, the role of cross-pollination, was examined using field and greenhouse studies during 1983 through 1989. The following synopsis is from a paper accepted for publication in 1995[3].

Field studies were carried out in which individual flower heads were excised or bagged at pre-determined phenological stages of flowering to investigate when pollination and seed development occur. Flower heads excised in mid bloom (F-2M - when most of the florets were open) produced few viable seeds, mostly along the periphery, but there were no seeds in heads excised before this stage (Table 1).

Table 1. Mean number of total viable (plumed and non-plumed) seeds/excised flower head

Phenological Stage[a]	Total viable seeds	
	Excised Heads	Bagged Heads
BU-4	0	0.3
F-1	0	0.3
F-2E	0	1.4
F-2M	1.6	16.2
F-2L	7.3	40.9
SD	16.4	44.7
M	40.1	44.4

[a]BU-4 = late bud; F-1 = flower 1 (no florets open); F-2E = flower 2 early (peripheral florets open); F-2M = flower 2 mid (most florets open); F-2L = flower 2 late (all florets open); SD = seed development (florets oxidized, bracts green); M = mature (oxidized florets still intact, bracts brown).

Closer examination of flower heads during anthesis showed that the first florets to open along the periphery were sterile, a characteristic known in other *Centaurea*. Fertile florets and, consequently seeds, developed from the periphery to the center of the receptacle, as in other Asteraceae. Non-plumed seeds developed first along the periphery and plumed seeds developed later in the central portion. For bagged heads, there was a low mean number of viable seeds in heads bagged at the BU-4 through F-2E stages (most heads had zero seeds). Usually there was evidence of insects (frass, etc) in bagged heads with the seeds, so we suspect that inadvertent cross-pollination had occurred. The number of seeds in heads increased significantly when bagged at the F-2M stage and increased to near maximum levels at the F-2L stage.

Greenhouse studies carried out with plants that were maintained fairly insect-free with insecticides provided more conclusive evidence that yellow starthistle is mainly dependent on cross-pollination for seed production. Some flower heads at the F-2M stage were manipulated manually to simulate self- or cross-pollination treatments, while others were bagged at the late bud stage to prevent cross-pollination. The mean numbers of viable seeds per head ranged from 9.1 for cross-pollination, 0.02 for selfing and 0.08 for the bagged control.

In one of the greenhouse studies, one of 30 insecticide-treated plants produced several viable seeds in bagged seedheads. Progeny grown from these seeds appeared to inherit the selfing trait, but unequally, as only one of the progeny was found to be significantly self-fertile.

Because cross-pollination was shown to be important, potential pollinators were surveyed. Insects visiting two or more flower heads of yellow starthistle were collected during July and August at one location in California, and identified. A total of seventeen species in eleven families and five orders were collected (Table 2). Of those species, nine were Hymenoptera, with the European honeybee being the most common by far.

Table 2. Insects[a] visiting yellow starthistle flower heads at Loomis, California

Order	Family	Insect
Hymenoptera	Apidae	*Apis mellifera* Linn.
		Bombus pennsylvanicus sonorus Say
		Bombus californicus Smith
	Anthophoridae	*Xenoglossodes pomonae* (Cockerell)
		Xenoglossodes sp.
		Anthophora (Anthophora), *urbana urbana* Cresson
	Megachilidae	*Megachile* (Eutricharaea) *rotundata* (Fabricius)
	Halictidae	*Halictus* sp.
	Pompilidae	*Aporinellus yucatanensis* Cameron
Lepidoptera	Papilionidae	*Battus philenor hirsuta* (Skiu.)
	Nymphalidae	*Vanessa virginiensis* (Drury)
		Junonia coenia (Hbn.)
	Noctuidae	*Heliothodes joaquin* Mcd.
Hemiptera	Pentatomidae	*Chlorochroa ligata*
Orthoptera	Acrididae	*Dissosteira pictipennis*
		Melanoplus sp.
Coleoptera	Meloidae	*Epicauta fallax* Horn

[a]Identified by biosystematists with the California Department of Food and Agriculture

[1]USDA, ARS, Western Regional Research Center, Albany (Retired)
[2]California Department of Pesticide Regulation, Sacramento
[3]Maddox, D.M., D.B. Joley, D.M. Supkoff, and A. Mayfield. 1996. Pollination Biology of Yellow Starthistle, *Centaurea solstitialis* L. in California. Canadian Journal of Botany 74:262-267.

Contracted Research

Title: Search for and Obtain Natural Enemies of Russian Thistle, *Salsola australis* in Europe and Asia

Principle Investigators: L. Knutson, R. Sobhian, G. Campobasso, and J. Kashefi
Address: USDA, ARS European Biological Control Laboratory, Montpellier, France

Russian thistle, *Salsola australis* R. Brown (Chenopodiaceae), is a very disruptive exotic pest plant in California. Economic damage results, in part, from the tumbling nature of plants as they pile up against fences and buildings, clog irrigation canals, or become a road hazard. Also, a substantial amount of damage occurs on agricultural land. Russian thistle is a good host to the beet leafhopper which is the primary vector of curly top virus, an extremely serious gemini virus infecting several hundred varieties of ornamentals and commercial crops including sugar beets, tomatoes, melons, cucumbers, peppers, squash, spinach, and beans. The seriousness of the problem has resulted in annual abatement efforts by the California Department of Food and Agriculture's Integrated Pest Control Branch. In addition to the beet leafhopper, three plant pests, Say's stink bug, *Chlorochroa sayi* Stal, and two *Lygus* spp. (especially *Lygus hesperus* Knight), use Russian thistle as an alternate host. Recent observations of *Lygus* and stink bug activity show that Russian thistle is an important host on which they increase in density and disseminate to commercial vegetable and field crops in late spring and summer. Reports from growers indicate that as much as 60% of tomatoes and peppers may be damaged by stink bug feeding in fields near foothill and riparian areas where Russian thistle is common.

Biological control efforts on Russian thistle began in the 1970's when two coleophorid moths were introduced into California. *Coleophora parthenica* Meyrick (Lepidoptera: Coleophoridae) feeds internally within the stems, but causes little damage to the plant. Thus, despite it being abundant in the western San Joaquin Valley, seed production by Russian thistle is not substantially reduced. The second agent, *Coleophora klimeschiella* Toll (Lepidoptera: Coleophoridae), feeds externally on the foliage, but is heavily parasitized by native parasitic wasps which prevent it from building high enough population densities to cause substantial damage to the host plant. Further effort to search for additional natural enemies was halted, in part, because travel in Russia was severely restricted. Recent changes in the political structure of the Soviet Union now allow access to previously unexplored regions of Asia so the effort to search for new biological control agents has been renewed.

In 1995, the Biological Control Program awarded a research contract to the European Biological Control Laboratory to search for natural enemies of Russian thistle in Europe and Asia. Trips were made by USDA, ARS, scientists to Turkey, Uzbekistan, Turkmenistan, Kazakstan, China, Greece, Italy and France. The following new natural enemies have been collected and recommended for further examination:

Turkey
Lixus salsolae Becker (Coleoptera: Curculionidae)
Piesma salsolae (Becker) (Hemiptera: Piesmatidae)
Stephaniola sp. (Diptera: Cecidomyiidae)
Aceria n. sp. (Acari: Eriophyidae)
Uromyces salsolae Reich (Uredinales: Pucciniaceae)
Smut disease (not yet identified)

Uzbekistan
Desertovelum stackelbergi (Diptera: Cecidomyiidae)

Turkmenistan
No specific natural enemies found

Kazakstan
Gymnancella sfakesella Chretien (Lepidoptera: Pyralidae)

China
Stem boring larvae (not yet identified)

Greece
Three species of Lepidoptera (not yet identified)
An eriophyid mite (not yet identified)
A fungal disease (not yet identified)

Italy
Uromyces salsolae Reich (Uredinales: Pucciniaceae)

France
Piesma salsolae (Becker) (Hemiptera: Piesmatidae)
Gymnancella canella Hubner (Lepidoptera: Phycitidae)

Contracted Research

Title: Biotypes of Russian Thistle, in California Determined With Molecular Markers

Principal Investigator: Frederick J. Ryan[1] and Debra R. Ayres[2]
Address: USDA, ARS Aquatic Weed Laboratory, Davis, California

Russian thistle or tumbleweed [*Salsola australis* R. Brown. (Chenopodiaceae)] is widely distributed throughout North America including California. The plant is believed to be a native of Eurasia and first appeared in the United States in the 1870's and in California in the 1890's. The source of the original infestation is not known, nor whether there was more than one introduction. This work was undertaken to determine the population structure of *S. australis* in California using allozymes and Random Amplified Polymorphic DNA (RAPD) assays. Plants from California were compared to accessions from Europe and Asia using the same molecular markers.

Plants were collected in 13 sites in the Central Valley and its eastern hills and in four sites in Owens Valley. Dried plant material was furnished by Dr. Lloyd Knutson, USDA, ARS, Montpellier, France, from several sites in France and Turkey. For some of the fresh material, tissue was extracted in buffer and allozyme profiles were determined for aspartate aminotransferase and 6-phosphogluconate dehydrogenase. Preliminary screening indicated that these enzymes were polymorphic in *S. australis*. For other samples from the same sites, DNA was extracted and RAPD assays were run. Screening was carried out after preliminary work had identified five informative primers. DNA was extracted from dried material from France and Turkey and used in RAPD assays.

A limited number of patterns of fixed variation were detected using allozymes; these were described as Biotypes A through E. The frequencies of the biotypes in small samples throughout the state are shown in Table 1. Biotypes A and B together comprise 89% of the samples examined. Results from the RAPD assays to date confirm this pattern; the RAPD assays result in assignment of phenotypes into a limited number of groups, consonant with those assigned from isozymes. These results suggest that *S. australis* has fixed heterozygosity, perhaps indicating that the plant is tetraploid. Samples from *S. australis* from France appeared identical to Biotype A from California, judged from the RAPD assay, while material from Turkey was distinguishably different from California Biotype A.

Subsequent work will include estimating the frequencies of the various biotypes by large samples (N=100) from several localities. In addition, sampling sites from southern California will be included in the work.

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[1]USDA, ARS, Aquatic Weed Laboratory, Davis. Present address: USDA, ARS, Postharvest Quality Laboratory, Fresno, CA
[2]Evolution and Ecology Graduate Program, University of California, Davis

Table 1. Frequencies of biotypes of *S. australis* in California.

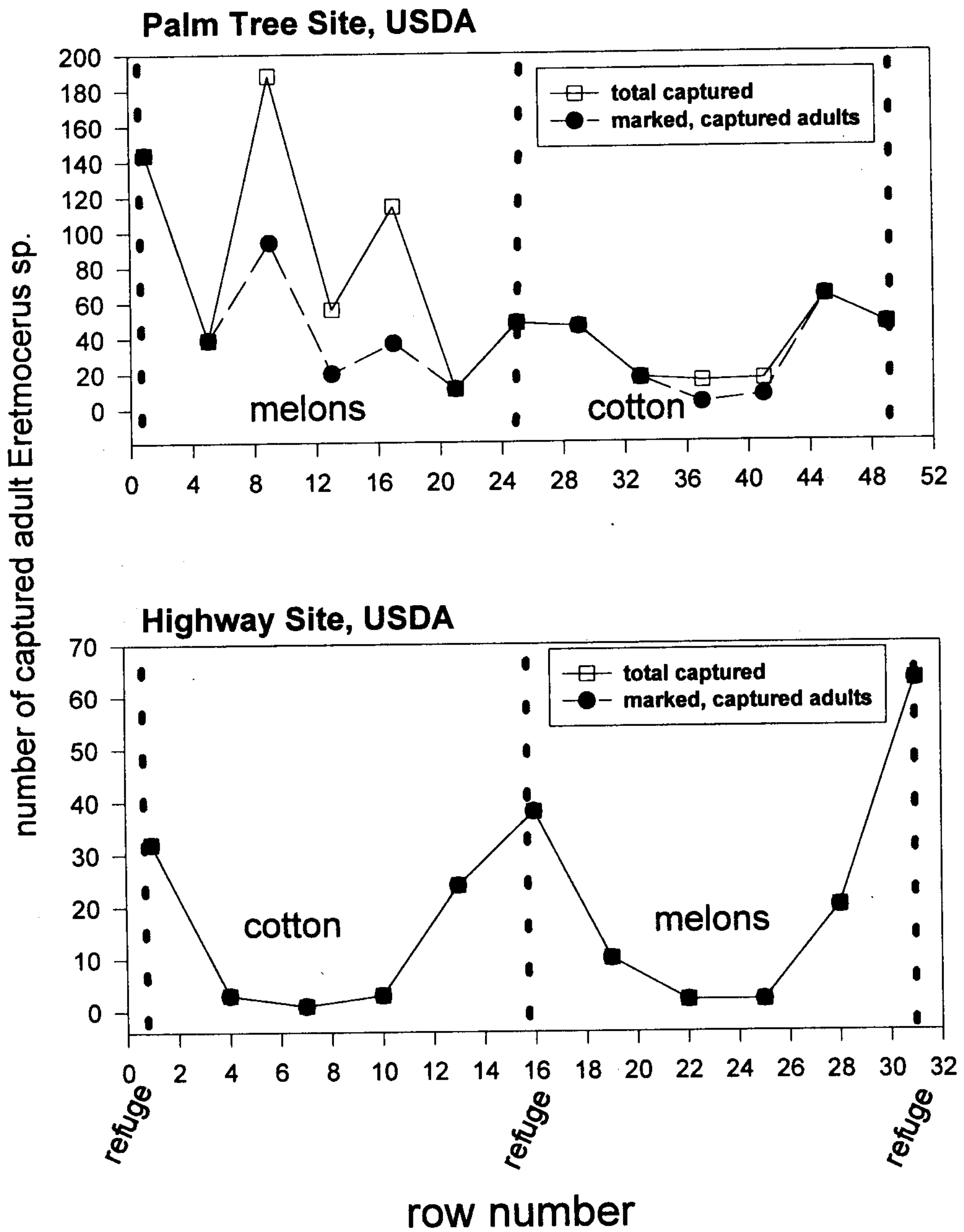
Site	N	Biotype: A	B	C	D	E
Hollister	11	0	100	0	0	0
Livermore						
Site 1	5	0	100	0	0	0
Site 2	6	82	18	0	0	0
Fresno	8	100	0	0	0	0
Santa Nella	10	30	70	0	0	0
Lathrop	11	100	0	0	0	0
Davis						
Site 1	11	100	0	0	0	0
Site 2	11	0	100	0	0	0
Marysville						
Site 1	6	0	68	32	0	0
Site 2	6	0	100	0	0	0
Yuba Foothills	12	100	0	0	0	0
Rescue	5	100	0	0	0	0
Cameron Park						
Site 1	6	0	0	0	0	100
Site2	6	55	0	35	10	0
Owens Valley:						
Alta Vista	5	80	0	20	0	0
Big Pine	5	80	0	20	0	0
Laws	5	100	0	0	0	0
Chidago Canyon	5	100	0	0	0	0
TOTAL	134	55.2	33.6	4.5	0.7	4.5

Contracted Research

Title: Marking Studies to Measure the Movement of Native Aphelinids from Refugia to Adjacent Crops Infested with Silverleaf Whitefly

Principle Investigator: A. Corbett
Address: Department of Entomology, University of California, Davis, California

Although refuge plants at silverleaf whitefly, *Bemisia argentifolii*, Bellows & Perring (Homoptera: Aleyrodidae), study sites in Imperial Valley produce parasitoids during the entire year, their contribution to the parasitoid population in adjacent crops has been difficult to measure. Parasitoids may also be emigrating from distant overwintering sites. The movement of parasitoids from refugia to an adjacent crop was measured using the naturally occurring element rubidium. This element was applied to refuge plants *in situ* which in turn were fed on by silverleaf whitefly. When parasitoids in the refuge feed on these whiteflies, their level of rubidium is greatly increased over that which occurred naturally. Refugia within two plots at the USDA, ARS, field station in Brawley were treated approximately every two weeks from late March to May 1995. Yellow sticky cards were placed in the refuge strips, and at increasing distances across neighboring melon and cotton plots in order to capture adult parasitoids. Results show a gradient of marked individuals from the strips, which one would expect if the parasitoids had dispersed into fields. At the Palm Tree study site, marked *Eretmocerus* adults varied from 22% to 100% of those captured (Fig. 1). At the Highway site all of the captured individuals came from the refugia. At the Palm Tree site, a large number of parasitoids in the center of the field originated from outside sources, and the total captured was higher than the total captured in the refuge. One major difference between the Palm Tree and Highway study sites was the degree of plant cover. The melons at the former site were much larger and covered a greater portion of the ground. Very few *Encarsia* were picked up on yellow sticky cards and their rubidium content has yet to be analyzed.



Palm Tree Site, USDA
total captured
marked, captured adults
melons
cotton
Highway Site, USDA
total captured
marked, captured adults
cotton
melons
refuge
refuge
refuge
number of captured adult Eretmocerus sp.
row number

Contracted Research

Title: An Illustrated Key to Nominal North American Species of *Eretmocerus* and to Species Reared from *Bemisia* in the U.S.A.

Principle Investigator: M. Rose & G. Zolnerowich
Address: Department of Entomology, Texas A&M University, College Station, Texas

The purpose of this project is to produce a color and line drawing brochure to educate users about the history, biology, application in biological control, systematic study, and taxonomy of *Eretmocerus* (Hymenoptera: Aphelinidae), one of the most important taxa attacking homopteran pests in the Aleyrodidae. Further, keys to the North American species of *Eretmocerus* and other parasite genera that attack whitefly will be provided. These keys will be numerically associated with color photographs and line drawings of significant characters.

All color photos, scanning electron micrographs and line drawings (53 in all) for this brochure have been completed and have been encoded at five levels of resolution on compact disc. The accompanying text and keys have also been completed. The text and figures are now being configured by a graphic artist in Texas A&M's Office of Information Technology. The brochure will be printed as soon as the new species names of four *Eretmocerus* reared from *Bemisia* collected in the United States are accepted as part of a recent journal submission.

Contracted Research

Title: Survey of the Natural Enemies of Certain Homopterous Pest Insects in Florida for Possible Introduction into California for Biological Control

Principle Investigator: Harold W. Browning
Address: University of Florida, IFAS, Citrus Research and Education Center

The three goals associated with this contract included; a) surveying Florida populations of target homopterans for natural enemies with potential for introduction into California, b) collecting, processing and documenting samples of selected natural enemies for shipment to California and, c) providing support for attempts at establishing natural enemies of in California.

Surveys for natural enemies of target pests were carried out during April-December, 1995 according to established protocols. Pest populations demonstrating evidence of parasites or predators were retrieved and processed in the laboratory. Life stages of associated natural enemies were isolated with hosts for completion of their life cycles, yielding adult specimens for identification. Critical point drying techniques were used for specimen processing. Surveys included Nesting whitefly, *Paraleyrodes minei* Iaccarino, Green shield scale, *Pulvinaria psidii* Maskell, Pyriform scale, *Protopulvinaria pyriformis* (Cockerell), and tuliptree scale, *Toumeyella liriodendri* (Gmelin), and related insects on fruit trees, ornamental shrubs, and native vegetation in central Florida. In addition, travel to southern China in September of 1995 allowed for survey for natural enemies of some target pests in this region. A relational database is being used to organize and store collection data from surveys in Florida. Dr. Greg Evans, a taxonomist specializing in parasitic hymenoptera, has been engaged in evaluating parasite material resulting from surveys.

Attempts to introduce the aleyrodid parasite, *Encarsia variegata* Howard, into California were fostered during this reporting period when several shipments of the parasitoid were made. Field collections of parasitized whitefly were returned to the laboratory in Florida where parasitoid pupae were isolated for packaging and shipment. Overnight carriers were used to convey insulated containers with numbers of parasite pupae, ranging between 200 and 500 per shipment.

A culture of the coccinellid, *Azya orbigera* Mulsant, a soft scale feeder which attacks *Coccus viridis* (Green), was established during fall of 1995, in Florida. Immatures and adults of this predator feed on soft scale insects, and cultures are maintained on *C. viridis* and on hemispherical scale, *Saissetia coffeae* (Walker) in the laboratory. Preliminary screening of the beetles for the presence of pathogens is underway, with plans for shipment of laboratory-reared beetles to California slated for spring of 1996. No endoparasites or other natural enemies have been observed in associated with the *Azya* culture.

Prior to shipment to California, individuals of *Encarsia variegata* were held for emergence. After adult parasites emerged, they were fed and mated and shipped to California, where subsequent field colonization occurred in preselected sites in San Diego County. Schedules for sequential parasite shipments were coordinated with field colonization efforts and seasonal availability of the whitefly host populations.

Contracted Research

Title: Development of DNA-Based Assays for Identifying Parasitoids of Sweetpotato Whitefly

Principle Investigator: Bruce C. Campbell
Address: USDA, ARS, Western Regional Research Center, Albany, California

The research outlined in the contract with CDFA is to identify regions of genetic variability among aphelinids (Hymenoptera) used in a biocontrol program against *Bemisia tabaci* Biotype B (=silverleaf whitefly) (Homoptera: Aleyrodidae), species complex. The aphelinids are in the genus *Encarsia* and *Eretmocerus*. This past year our focus was on *Eretmocerus*. Our mission was to identify *Eretmocerus* taxa using a genetically based assay. We tested seven parasitoid taxa provided by Charles Pickett, CDFA-Sacramento, with the following accession numbers: *Eretmocerus* spp. M92019, M94001, M94002, M94003 and M94019, M94120 and M95012. Initial cultures were reared by the USDA-APHIS-PPQ in Mission, Texas.

We successfully amplified by polymerase chain reaction (PCR) the ITS2 and 28S D2 of each of the above taxa. We cloned these genetic regions for all taxa. These clones are maintained in a stock library at our laboratory in Albany, CA. Full nucleotide sequences (both top and bottom strands) of these cloned genetic regions have been completed. Comparisons of these sequences among taxa have identified genetic differences that could be used to differentiate the taxa.

Sequences of >600 base-pairs of the 28S rDNA-D2 among taxa show that there are >40 informative site differences between *Encarsia formosa* Gahan and the various accessions of *Eretmocerus*. Among *Eretmocerus*, there was considerable difference (>20 informative sites) between Old World and New World accessions. Differences between New World 94-001, 002, and 003, were negligible.

The 28S-D2 region described above is a highly conserved region of genetic variation. Therefore we also completed sequencing the more variable ITS2s region for the provided aphelinid taxa. The size of this region varied among taxa with a range of about 440 bases for 94001, 94002 and 94003 to 462 bases for 92019 and for *Encarsia*, 569 bases. The size differences reflect sections of nucleotides that are present in some taxa and absent in others. There were vastly higher numbers of differences in overall nucleotides than were seen among taxa in the 28S-D2 region. These differences were exploitable for the development of an assay to identify these taxa, especially *Encarsia* and Old and New World *Eretmocerus*. There were few base differences in ITS2s among New World 94001, 94002 and 94003. However, there were enough to differentiate these taxa using an assay that combined PCR and digestion of the amplified DNA with Hha I. Using this assay this upcoming year, we plan to identify *Eretmocerus* which have become established in field releases in California.

In summation, the level of difference between Old World and New World *Eretmocerus* are vast. Based on ITS2 sequences *Eretmocerus* 94001, 94002, and 94003 appear to be a closely related complex. Old World *Eretmocerus* are genetically distinct enough from the New World

Eretmocerus examined thus far to perhaps warrant membership in a separate subgenus. These genetic differences are in concordance with a recently discovered physical antennal character that differentiates Old and New World *Eretmocerus*.

Additional *Eretmocerus* taxa are being procured from Charles Pickett and added to the analysis in order to build a genealogical framework for this group of parasitoids. Such information is useful for selecting lineages of parasitoids that may be the most efficient parasitoids of *Bemisia* whiteflies. By building a large database of aphelinid nucleotide sequences, it will be possible to assay whitefly nymphs (native and exotic) by a simple PCR + RFLP analysis to identify the parasitoid. This could be operational for any whiteflies collected in California in future outbreaks or for assessing biocontrol programs. In addition to enlarging the database of aphelinid nucleotide sequences, we will also attempt to develop a radiological assay that might identify parasitoids at the egg, or early nymphal stages.

Also, based on nucleotide sequences of more conserved regions, an inferred phylogeny for the Chalcidoidea (Hymenoptera) has been constructed. Cloning and nucleotide sequencing of a region of the 28S and 18S rRNA genes indicates that lineages of chalcidoids diverged during the Late Cretaceous concurrently with radiation of major whitefly lineages. The ability to parasitize whiteflies evolved a number of different times among chalcidoids. The phylogeny also suggests that Aphelinidae is paraphyletic and, as such, does not comprise a coherent assemblage of taxa. Lastly, genetic distances based on numbers of nucleotide differences between taxa may be a reliable approach to systematic classification of these wasps.

Contracted Research

Title: Curation and Archiving of Biological Control Vouchers and Development of Identification Expertise

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The Bohart Museum of Entomology at the University of California, Davis, houses one of the largest wasp collections in the country. Among its personnel are world renowned systematists in parasitic Hymenoptera. This insect group is a major component of the natural enemies used in natural and manipulated biological control programs. Currently, the Department of Food and Agriculture does not have expertise in the identification of parasitic hymenoptera. And, housing their specimens in the Department of Food & Agriculture collection would severely limit their availability to the scientific community.

The museum provides a permanent archive for voucher specimens from research projects, with an accompanying database that allows for the tracking of information based on taxonomic name, specimen, research program, study site, project name, personnel or date of collection. These vouchers are permanently scored and provide a physical confirmation of the true species identity of the organisms studied in a project. Vouchers are archived for university, state, national and international studies. Information on these specimens is maintained in a relational database, which is routinely updated, and is available to the research community.

Additions of specimens to the voucher database for the current year were from the Biological Control of Yellow Starthistle, *Centaurea solstitialis* L. A total of 278 specimens in 9 different taxa were mounted, identified, and added to the voucher collection. These specimens were reared from yellow starthistle seedheads collected two miles southeast of Merlin, Oregon. One surprise was the large number of specimens of a species of *Mesopolobus* (Pteromalidae) collected. This genus was not recorded from any of the yellow starthistle seedheads collected in the California rearings, but predominated in the collections from Oregon. Species of *Mesopolobus* are commonly taken from composite seedheads. There were also two Eulophidae, *Aprostocetus* sp. and *Pronotalia* sp., which were unrecorded from California. *Pronotalia* is known from Tephritidae in seedheads, and species of *Aprostocetus* are encountered when rearing almost anything.