# Biological Control Program
## Annual Report
## 1998

California Department of Food and Agriculture

# BIOLOGICAL CONTROL PROGRAM

# 1998 SUMMARY

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INTEGRATED PEST CONTROL BRANCH

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Cover developed by Deborah Mayhew. Photographs and sources are; clockwise from upper right: Silverleaf whitefly, *Bemisia argentifolii*, from the USDA-ARS photo gallery; musk thistle, *Carduus nutans*, photo by Don Joley; yellow starthistle, *Centaurea solstitialis*, with *Chaetorellia succinea*, photo by Baldo Villegas; *Neozygites fresenii*, a fungal pathogen of the cotton aphid, photo courtesy Dr. D. Steinkraus; *Peristenus digoneutis*, USDA-ARS photo gallery; wolf spider, photo by Bill Roltsch.

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## General Research

# Coordination, Accountability and Regulations in Biological Control

Larry Bezark

Biological control in California and the nation as a whole is an activity involving regulatory, academic, and governmental scientists. The California Department of Food and Agriculture's Biological Control Program implements biological control projects against a variety of noxious insect and weed pests throughout the state. We are not alone in this process, but rely on University and governmental partners to accomplish our goals. Practical issues such as shared quarantine space, joint development of release and post-release protocols, and staff co-ordination are jointly worked out for target pests of national concern. Continued success in the implementation of biological control in California is reliant upon a strong national presence from the United States Department of Agriculture (USDA).

The past few years have seen a dramatic re-evaluation of the federal role in biological control. There is a greater need for agencies responsible for implementing biological control to coordinate with state and university scientists as well as other federal scientists. Accountability in terms of both the soundness of biological decision making and fiscal responsibility must be addressed by all parties. Agencies responsible for developing and enforcing regulatory policies must develop a facilitated regulatory process based on streamlined regulations that are clearly written, sensible and regulate in a manner that is consistent with risk. Although many meetings regarding coordination, accountability and regulations have recently been held, councils and subcommittees created and action plans drafted and revised, much work remains to be done. The following is a summary of recent activity in this area by the USDA.

In October 1996, an invitational workshop was held with the Agricultural Research Service, the Animal and Plant Health Inspection Service (APHIS), the Cooperative States Research, Education and Extension Service, the Forest Service, State agricultural agencies, and land grant Universities. Participants recommended; 1) establishing a Department-level center to promote, facilitate, and provide leadership for biological control throughout USDA; 2) development of enhanced linkages within USDA, with customers and stakeholders including agriculture producers, and with other Federal agencies; and 3) development of a customer-driven system for measuring results, disseminating information, and interactively communicating at all levels to improve departmental policies and procedures that affect biological control regulation, research, and implementation. After the workshop, USDA created the Biological Control Coordinating Council which developed an action plan and assigned an Interagency Action Team to implement it.

It is clear that the USDA feels strongly that their biological control activities need to be coordinated with other federal and state agencies, and they have asked for customer input and have developed an action plan. However, in the nearly three years since the invitational workshop was held, the USDA is still refining action plans and renaming committees and subcommittees. How USDA does business, especially in the regulatory arena, affects how we conduct biological

control activities in California. We need to continue to provide input to USDA to ensure a practical future for biological control activities in our state.

California has had a long, successful history of implementing biological control programs dating back to 1913. Today, the California Department of Food and Agriculture, University of California, USDA and others continue to be involved, at least in some way, in the entire spectrum of biological control activities. Our department is committed to continuing our role in implementing biological control in California. This involves coordination and accountability, and an active role in determining the direction of regulations regarding biological control. We meet regularly with our partners to coordinate activities and reduce duplication of effort. We are continuing to study the non-target effects of released natural enemies, annually review several petitions for the introduction of new natural enemies, and we provide input to APHIS regarding the development of and changes to the regulations concerning biological control.

## Importation of *Peristenus stygicus* for the Biological Control of *Lygus hesperus*

C. H. Pickett, J. C. Ball, U. Kuhlmann[1], D. Coutinot[2], L. Ertle[3], K. Tilmon[4], and L. Schmidt[5]

*Lygus hesperus* Knight (Hemiptera: Miridae) has for several decades been a key pest of cotton in California. Loss in yield is typically 2-4% and can range from <1% up to 20% (P. Goodell, UCCE, KAC, Parlier, per. comm.). It is the number three ranked pest nationwide on cotton (Carter 1996). In a review of its damage to cotton in California in 1973, Stern (1973) reported that chemical control of *Lygus* cost 20 to 25 million dollars a year. Since it is typically the first early season pest, early treatments for *Lygus* on cotton often cause upsets of other serious pests such as spider mites and aphids. Additional pesticide applications to control these pests add many more millions of dollars to the *Lygus* pest problem, and could add to environmental and farm worker health problems.

*Lygus hesperus* is considered native to western United States but lacks effective nymphal parasites in California. Although egg parasites exist in California, they only provide limited natural control. An attempt at classical biological control of *L. hesperus* in 1973 resulted in poor recoveries of a nymphal parasite *Peristenus stygicus* Loan (Hymenoptera: Braconidae) imported from Europe. The parasite adult lays its eggs inside *Lygus* nymphs, generally early instars. After completing development, the larva exits its host and forms a cocoon in the duff or soil.

In the 1980's a similar attempt at classical biological control on the east coast against a close relative of *L. hesperus*, *L. lineolaris* (Palisot de Beauvois), has met with much better success. The USDA-ARS imported *Peristenus digoneutis* Loan collected from *Lygus rugulipennis* Poppius from central Europe. A recent survey showed that *P. digoneutis* is established over a wide area in northeastern United States and has reduced *L. lineolaris* to much lower levels in alfalfa than prior to importation of this natural enemy (Day 1996, Day et al. 1998). Parasitism of nymphs increased from 15% by native parasites to 50% two years later following establishment of *P. digoneutis*. *Lygus* numbers in alfalfa decreased by 75% over the same period of time. Both of the above parasites have potential for establishment, if populations are collected from areas in Europe with climate similar to California. We have expanded the geographic range of collections in Europe to include regions that closely match central California.

Foreign exploration was initiated summer 1998. Explorers with CABI Bioscience and the USDA-ARS European Biological Control Laboratory collected from alfalfa fields in southern France, Italy, and Spain. Collections were also made in New York where *P. digoneutis* has expanded its range. Parasites collected in southern France were reared to adults, cleared in quarantine at the USDA-ARS in Newark, Delaware, then shipped to Sacramento for caged field releases. High levels of parasitism under caged conditions were noted fall 1998 (>80%) when *Lygus* nymphs were exposed to parasites collected in southern France showing that this parasite readily accepts *L. hesperus* as a host. Over 1,000 attacked nymphs were placed inside field cages in a small alfalfa field next to our insectary in Sacramento. Additional releases are planned for spring 1999. In addition, over 1500 cocoons were collected in central Italy and 52 cocoons from Spain and are being held in quarantine, under refrigeration, until spring. Only small numbers of

cocoons were recovered from material collected in New York, and they too are being held under refrigeration in quarantine. Additional collections will be made in Europe summer, 1999.

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## Evaluation of Introduced Natural Enemies of the Cotton Aphid in the San Joaquin Valley

K. Godfrey, D. Ballard[1], D. Steinkraus[2], R. Yokomi[3], K. A. Casanave, and D. A. Mayhew

The cotton aphid (*Aphis gossypii* Glover) (Homoptera: Aphididae) poses a great threat to cotton production in the San Joaquin Valley. 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 yield reductions, crop value losses attributable to increased sticky cotton at harvest, and losses in profit due to the cost of additional insecticide applications. The insecticides that can be used to control other insect pests of cotton also are limited due to the impact these chemicals may have on cotton aphid population dynamics. In addition, the length of time the insecticides can keep the aphid densities low is brief due to reinvasion of the fields by alate aphids within days after treatment. Current insecticides are still effective against the cotton aphid; however, many populations in the San Joaquin Valley 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 cotton aphid management tactic, biological control, a cooperative project involving the CDFA-Biological Control Program, the USDA-Agricultural Research Service, and the University of California Cooperative Extension Service was initiated in 1996. In 1998, the University of Arkansas was added to this project. The long-term goal of this project is to construct a natural enemy complex for the cotton aphid that has more species richness than the complex that currently exists in the San Joaquin Valley. Construction of this natural enemy complex entails introducing, testing, and establishing natural enemies not currently found in California. Evaluation of the first four candidate species for inclusion in the natural enemy complex, *Aphelinus* near *paramali*, *Aphelinus gossypii* Timberlake, (Hymenoptera: Aphelinidae), *Lysiphlebia japonica* (Ashmead) (Hymenoptera: Aphidiidae), and *Neozygites fresenii* (Nowakowski) Batko (Zygomycetes: Neozygitaceae) began in 1997 and continued through 1998. Extensive field testing was conducted for *Aph.* near *paramali* (ANP) and *Aph. gossypii* (AG), but only limited testing of *L. japonica* (LJ) and *N. fresenii* (NF). The limitation on testing LJ was due to problems encountered in rearing the parasite, as there appear to be differences in host preference and acceptance among different strains of LJ. The strains currently available were collected in the field in Asia from brown citrus aphid (*Toxoptera citricida* (Kirkaldy)), so before more testing of LJ can be done, a strain of LJ collected from cotton aphid must be obtained. The limited testing of NF was due to limited quantities of the fungus being available for testing. In 1999, more extensive testing of NF will be done because of its greater availability.

During the winter of 1997 and spring of 1998, overwintering studies were conducted in plots maintained at the Shafter Research and Extension Center, Shafter. These studies began on November 10, 1997 with the placement of host plants (i.e., winter vegetables, winter weeds, and orange trees) and parasites in an overwintering plot. For the 21 orange trees, (10 spring navel orange and 11 Algerian Tanger mandarin orange) were infested with aphids, and then each tree

was covered with a sleeve cage. On December 3 and again on December 16, 1997, 10 adult ANP were added to each of 11 trees (a total of 20 ANP adults added to each tree), and 10 adult AG were added to each of another 10 trees (a total of 20 AG adults added to each tree). In addition to the sleeve cages, approximately 20,290 ANP and 15,200 AG mummies and adults were released into the overwintering plot from November 1997 through June 1998. At approximately weekly intervals, the plants in the overwintering plots were sampled, and two orange trees were scheduled to be sampled to assess aphid and parasite populations. Unfortunately, a severe windstorm in early February removed the cages from many of the trees, so the original sampling schedule had to be abandoned.

From the sampling of the overwintering plot and the citrus trees, numerous primary and secondary parasites were recovered. From the overwintering plot, 37 parasites were recovered: 1 AG, 23 *Diaeretiella*, 3 *Aphidius*, 1 *Lysiphlebus*, and 5 Charipidae (secondary parasites). Aphid mummies were numerous on the citrus trees. The balloon-shaped mummies, characteristic of aphidiids, were most numerous with a total 6,147 being present on all trees. For the cigar-shaped mummies that are characteristic of aphelinids, only 8 were recovered. From both types of mummies, 1 ANP, 383 *Lysiphlebus*, 1 *Diaeretiella*, 24 Pteromalidae (secondary parasites), and 23 Charipidae were recovered.

During the 1998 cotton season, sleeve cage and open field release studies were conducted at the Shafter Research and Extension Center to investigate the ability of ANP, AG, and NF to reduce densities of cotton aphid on cotton under field conditions. The studies were conducted from July 6 through October 8, for the insect parasites and from August 11 through September 25, for the fungus. Cage studies and open field releases were done with the insect parasites, and only cage studies were done with the fungus. At approximately weekly intervals, sleeve cages were placed on individual cotton branches along a row. Any arthropod predator found within a cage was removed. The cotton aphid density within each cage was then assessed to be sure that each cage had at least 5 adult aphids. The cages were left undisturbed for 7 days. At the end of this time, the following treatments were assigned at random to each of 20 cages: introduction of 10 ANP adults; introduction of 10 AG adults; introduction of 5 NF mummies; and controls (no natural enemies introduced). All cages were left undisturbed for 7-10 days and then were harvested to assess the aphid and natural enemy populations.

The densities of cotton aphid populations within the cotton plot began to increase in late September. For the first 8 replicates of the cage studies, the cotton aphids used were produced in laboratory culture and then released into the cages. For the remaining replicates, natural populations of the cotton aphid were used. For the parasites, 12 replicates were completed, and the results are presented in Table 1. For all replicates except replicate 4, there were no statistical differences among the treatments. In replicate 4, the cages containing AG had a higher mean density of aphids than the mean density in the control cages. The production of both parasites was very good within the cages (Table 1). This suggests that both parasites are capable of using cotton aphid on cotton for reproduction.

In the cage studies using the fungus, 5 replicates were completed, and the results are presented in Table 2. For 2 of the 5 replicates, the densities of aphids in cages with fungus

present were lower than those in the controls (Table 2). However, there were no statistical differences between the mean densities of aphids in the cages with fungus and the control cages. The mean infection rate of the fungus varied from 0-12.1% (Table 2). The low infection rate may have been due to temperatures in excess of 30°C during the first 6 hours after introduction. Temperatures above 30°C during this time period are detrimental to the germination of the primary spores and may in fact kill newly produced primary spores. The primary spores germinate to produce secondary spores that infect other aphids and result in epizootics of the fungus. Methods were employed to insure that the fungus was released under the best environmental conditions possible.

Concurrent with the sleeve cage study, open field releases were also made at Shafter Research and Extension Center. From July 21 through October 27, 1998, approximately 47,400 ANP adults and mummies and 18,265 AG adults and mummies were released in the field. The following parasites have been recovered from these releases: 6 ANP, 392 *Lysiphlebus* sp., 1 *Diaeretiella* sp., 21 Pteromalidae, and 1 Charipidae. No AG was recovered.

Table 1. The mean number of aphids, the total mummies produced, and the total number of parasites found in field cage studies conducted in cotton at the Shafter Research and Extension Center in 1998.

| | Mean No. Aphids | | | Total Mummies | | Total Parasites[a] | |
|---|---|---|---|---|---|---|---|
| Rep | ANP | AG | Control | AG | ANP | ANP | AG |
| July 15 | 65.89 | 72 | 68.9 | 12 | 9 | 2 | 6 |
| July 22 | 169.5 | 172.2 | 120 | 18 | 20 | 5 | 4 |
| July 29 | 129.9 | 144.6 | 172.9 | 62 | 9 | 8 | 20 |
| Aug 5 | 85.35 | 109.6[b] | 75.8 | 4 | 13 | 4 | 9[c] |
| Aug 12 | 72.3 | 65.6 | 72.1 | 41 | 93 | 39 | 22 |
| Aug 19 | 127.9 | 86.4 | 115.1 | 205 | 32 | 28 | 134 |
| Aug 26 | 39.3 | 31.58[d] | 16.47[d] | 72 | 3 | 1 | 18 |
| Sept. 2 | 77.2[e] | 106.6[e] | 86.6[e] | 32 | 5 | 1 | 17 |
| Sept. 9 | 212.6 | 242.1 | 136.5 | 1 | 1 | 5 | 2 |
| Sept. 16 | 556.9 | 450.9 | 596.1 | 18 | 12 | 8 | 5 |
| Sept. 23 | 542.3 | 485.3 | 600.5 | 14 | 194 | 118 | 16 |
| Sept. 30 | 764 | 626.5 | 587.7 | 0 | 0 | 7 | 15 |

[a]Number of live parasites in the cage plus the number that emerged from mummies

[b]$F = 26.91$; $df = 1,38$; $P < 0.01$

[c]Eight live AG parasites were found in the cage upon harvest.

[d]One cage in the AG treatment and three cages in the control had too many aphids to count.

[e]Two cages in the ANP treatment, two cages in the AG treatment, and one cage in the control had too many aphids to count.

Table 2. The mean number of cotton aphids within cages receiving fungus, and the mean infection rate in cages receiving fungus and in control cages in cotton at the Shafter Research and Extension Center in 1998.

| | Mean No. Aphids | Mean % Infection | |
|---|---|---|---|
| Rep | | Fungus | Control |
| Aug 12 | 50.05 | 5.53 | NS[a] |
| Aug 19 | 120.0 | 1.3 | 1 |
| Aug 26 | 21.8 | 12.1 | 21 |
| Sept. 2 | 99.9 | 1.8 | 0 |
| Sept. 9 | 208.5 | 2.2 | 0 |
| Sept. 16 | 412.8 | 0 | 0 |

[a]No sample was taken for this time period. For the other replicates, 100 aphids from the control cages were used.

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## Further Attempts to Establish *Metaphycus flavus* on the Tulip Tree Scale

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The tulip tree scale, *Toumeyella liriodendri* (Gmelin) (Homoptera: Coccidae), is native to eastern North America. A pest principally of tulip tree and certain deciduous magnolias, this species was recorded in California as early as 1942. Tulip tree scale had been under eradication in Alameda, Santa Clara, and Sonoma counties until mid 1990. After the counties ceased their eradication efforts, tulip tree scale populations resurged in several communities (the scale may be eradicated in Sonoma County, as no recent live finds have been reported).

Surveys in San Leandro, Alameda County, in 1995-96 found little evidence of natural enemies attacking tulip tree scale. Parasite emergence holes were observed in a few scale, but we were unable to rear out parasites for species determination. The parasite *Metaphycus flavus* (Compere) (Homoptera: Encyrtidae) is reported in the literature as attacking tulip tree scale. In June 1997, a strain of *M. flavus* originally from Turkey was released on infested trees in San Leandro and San Jose (Santa Clara Co.). Only four *M. flavus* were recovered in samples taken 20 days later. The limited parasitization may have been due to inappropriate host age structure at the time of release, i.e. principally early 3rd instar females and male pupae (most males had emerged as adults).

A second release was planned for early 1998, when younger hosts would be available. Approximately 460 *Metaphycus flavus*, reared on *Coccus hesperidum* at UC-Riverside, were released on eight infested trees in San Leandro and San Jose in March 1998. Parasites were released into sleeves confining selected branches on the infested trees. The scale stage at the time was mainly 2nd instar, with a few male cocoons and early 3rd instar females. The releases occurred two months earlier than in 1997 and that was reflected in the predominant host stage. A month after release, the sleeves were opened and half the enclosed branch brought back to the lab for parasite emergence.

Table 1. Tulip tree scale stages present and percentage parasitized in 1998.

| | Female Scale | | | Male Scale | | |
|---|---|---|---|---|---|---|
| | Second Instar | Early Third | Late Third | Second Instar | Third Instar | Cocoon |
| No. of scale present | 73 | 2364 | 29 | 23 | 186 | 328 |
| %Parasitized | 26 | 3 | 0 | 48 | 47 | 23 |

Assigning scale to specific instars was somewhat arbitrary and based mainly on size and color on each branch (males were distinguished as being slightly more oval than females in the early instar). Some 2nd and early 3rd instar female scale were parasitized, but most parasitization occurred to male scale. Both 2nd and 3rd instar males appeared equally attractive, with parasitization rates around 47%.

Tulip tree scale has a single generation a year so few hosts of the appropriate stages were available during our survey. In 1999, we will return to the sites to see if the parasites were able to

span the summer, however, even with establishment, *M. flavus* is unlikely to provide significant control of tulip tree scale. More effective parasites can probably be found in eastern North America where the scale is native, however funding support for maintaining a host colony for quarantine evaluation is not currently available.

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## Establishment of Introduced *Eretmocerus* Parasitoids of the Silverleaf Whitefly in Imperial Valley

W. J. Roltsch, K. A. Hoelmer [1]and G. S. Simmons[2]

Since 1994, a number of parasitoid species/strains of silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring, (Homoptera, Aleyrodidae), have been evaluated in field cages, and released in large numbers in commercial fields, refuge nursery plots and urban yards. The most promising *Eretmocerus* (Hymenoptera: Aphelinidae), for this desert region include: *E. mundus* Mercet, *Eretmocerus sp.* M96076 from Ethiopia, *E. hayati* Zolnerowich & Rose and *E. eremicus* Rose & Zolnerowich. In field studies, these and other exotic *Eretmocerus* as well as native *Eretmocerus* may all be present, but are difficult to distinguish. In this report, data are presented in terms of exotic *Eretmocerus* as a proportion of all *Eretmocerus* collected (i.e., including native *E. eremicus*). Antennal pedicel color, mesoscutal reticulation pattern and wing setal patterns in dried non-mounted specimens were used to identify exotic male specimens from native *E. eremicus*. The latter two characters were used to identify the presence of exotic female specimens. Species identification using slide mounted specimens was accomplished using recently published keys. In addition, some specimens were identified using DNA analysis (RAPD-PCR) by the USDA-APHIS Mission Plant Protection Center.

**Parasitoid population development in refuge nursery plots:** From 1994 through 1997, species of exotic parasitoids were released into long-term field plots on multiple occasions each year (Table 1). Field plots (1/2 to 1 acre) were located at the Irrigated Desert Research Station near Brawley, and at an organic farm at the south end of the county. During the warm season, the plots were planted with okra and basil. During the cool season, cole crops (esp. collard) and sunflower were present. Kenaf, roselle and eggplant were also periodically present along with adjacent plantings of cotton and spring cantaloupe. Leaf samples were taken approximately six times during each year to determine the parasitoid population densities and identities. Neither *E. tejanus* Rose & Zolnerowich nor *E. staufferi* Rose & Zolnerowich (both introduced from Texas) have been recovered following their release. During 1995, *E. melanoscutus* Zolnerowich & Rose was released in large numbers beginning in early August. Recoveries of this parasitoid were rare (Fig. 1a). Releases in 1996 began in April (Table 1). Numbers of exotic parasitoids compared to natives were high during early summer, however, the sample proportion consisting of exotic species dropped markedly by late July, indicating poor performance (population increase and persistence) during this very warm summer period (Fig 1b). During 1997, the relative performance of exotics was considerably better than in 1996 (Fig. 1c). The proportion of exotic relative to native *Eretmocerus eremicus* declined once again during late summer, however, not to the same extent.

During 1998, none of the long-term refuge plots were inoculated with exotic whitefly parasitoids. This made possible the assessment of populations released in previous years at these sites, in terms of their ability to overwinter and compete with native species of silverleaf whitefly parasitoids. Overwintering on cole crops was confirmed, albeit in low numbers. During the summer of 1998, *Eretmocerus* densities soared on okra, basil and adjacent cotton. By late August there was a greater proportion of exotic *Eretmocerus* (upwards of 80% on okra and cotton) than

native *Eretmocerus* (Fig. 1d). The determination of which exotic *Eretmocerus* species dominated is pending, however, samples collected from January to July of 1998 and submitted for genetic analysis were found to be represented by *Eretmocerus* sp. from Ethiopia and *E. mundus*.

TABLE 1. Species of *Eretmocerus* released into refuge plots during each year. None released in 1998.

| YEAR | SPECIES | USDA-APHIS ACCESSION NO. [location of origin] | TOTAL RELEASED IN 4-5 PLOTS |
|---|---|---|---|
| 1994 | *Eretmocerus tejanus* | M94003 [USA, Texas] | 28,000 |
| | *Eretmocerus staufferi* | M94002 [USA, College Sta., Texas] | 91,000 |
| 1995 | *Eretmocerus melanoscutus* | M94023 [Thailand, Sai Noi Klong Ha Roi] | 699,000 |
| 1996 | *Eretmocerus mundus* | M92014 [Spain, Murcia] | 27,600 |
| | *Eretmocerus hayati* | M95012 [Pakistan, Multan] | 995,000 |
| | *Eretmocerus emiratus* | M95104 [United Arab Emirates] | 64,000 |
| 1997 | *Eretmocerus emiratus* | M95104 [United Arab Emirates] | >200,000 |
| | *Eretmocerus sp.* | M96076 [Ethiopia] | NA at this time |

**Regional surveys**: During late summer and fall of 1998, exotic *Eretmocerus* were collected from numerous ornamental plants in several communities in Imperial Valley. In addition, leaf samples were obtained from three edges of a number of conventionally managed cotton fields during September. The fall samples of ornamental plants at 15 urban sample sites in three communities indicated that exotic *Eretmocerus* were present in 10 of 15 sites. On average, 25% of the *Eretmocerus* at the 10 locations was exotic. Also, exotic *Eretmocerus* were detected in 9 of the 23 cotton field samples. Within each cotton field collection, an average of 44% (sd +/- 10%) were female. The locations where exotic *Eretmocerus* were found were far removed from all 1998 release locations. Sample size among the 23 cotton fields varied considerably in terms of the total number of *Eretmocerus* collected for identification. Of the 23 field samples, five samples consisted of 16 to 29 *Eretmocerus* (male and females combined), while 18 samples were composed of 30 to 130 *Eretmocerus* specimens. Exotic males were easy to distinguish from native males. Exotic and native females were difficult to separate. Of the nine samples that were positive with exotic *Eretmocerus*, exotic males were found in eight samples while females were found in four samples. Assuming that the sex ratio was approximately 1:1 among all of the cotton field collections as previously indicated, exotic males are twice as likely to be identified using current sorting procedures.

**Summary:** To date, several newly released parasitoid species have established in the Imperial Valley and are capable of extensive population increase. Survey data for 1998 indicate that these species are becoming widely distributed in urban areas and relatively common in agricultural fields as well.

[1]USDA-APHIS, Phoenix Plant Protection Center, Brawley, CA
[2]USDA-APHIS-PPQ, Western Region, Brawley, CA

**Fig 1. EXOTIC ERETMOCERUS AS A PERCENTAGE OF ALL ERETMOCERUS COLLECTED FROM 3-4 REFUGE FIELD NURSERY PLOTS FROM 1995-1998, IMPERIAL VALLEY, CA**

**Fig. 1a: 1995 - Plots inoculated from summer to fall**

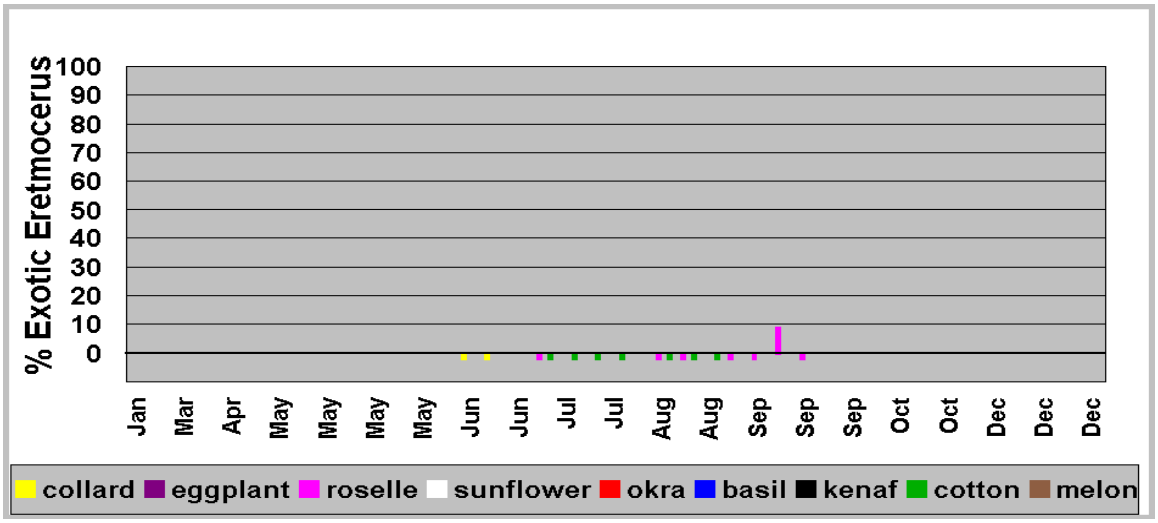



**Fig. 1b: 1996 - Plots inoculated from summer to fall**

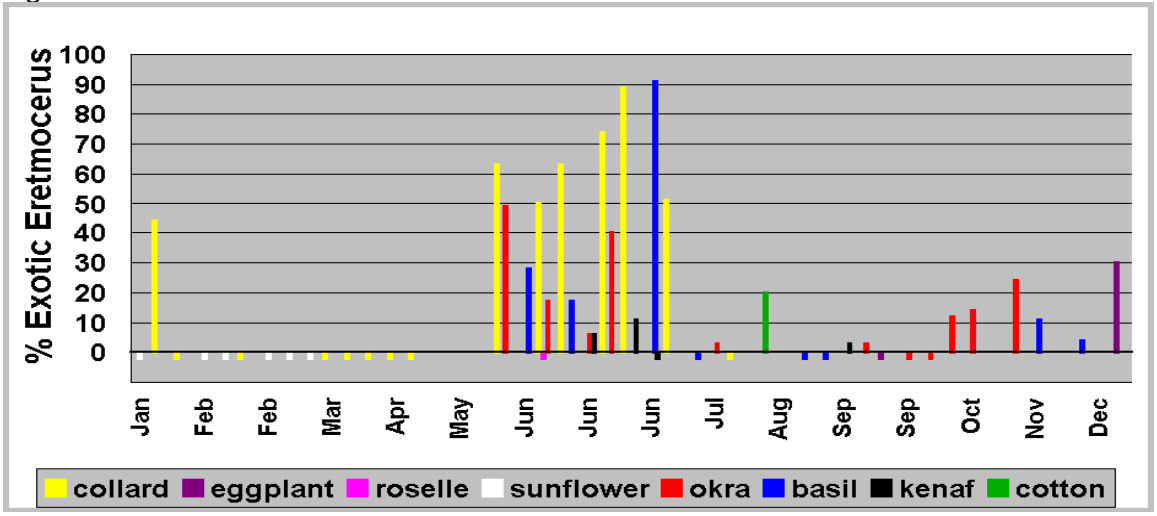


**Fig 1. EXOTIC ERETMOCERUS AS A PERCENTAGE OF ALL ERETMOCERUS COLLECTED FROM 3-4 REFUGE FIELD NURSERY PLOTS FROM 1995-1998, IMPERIAL VALLEY, CA**

CONTINUED …

**Fig. 1c: 1997 - Plots inoculated from summer to fall**

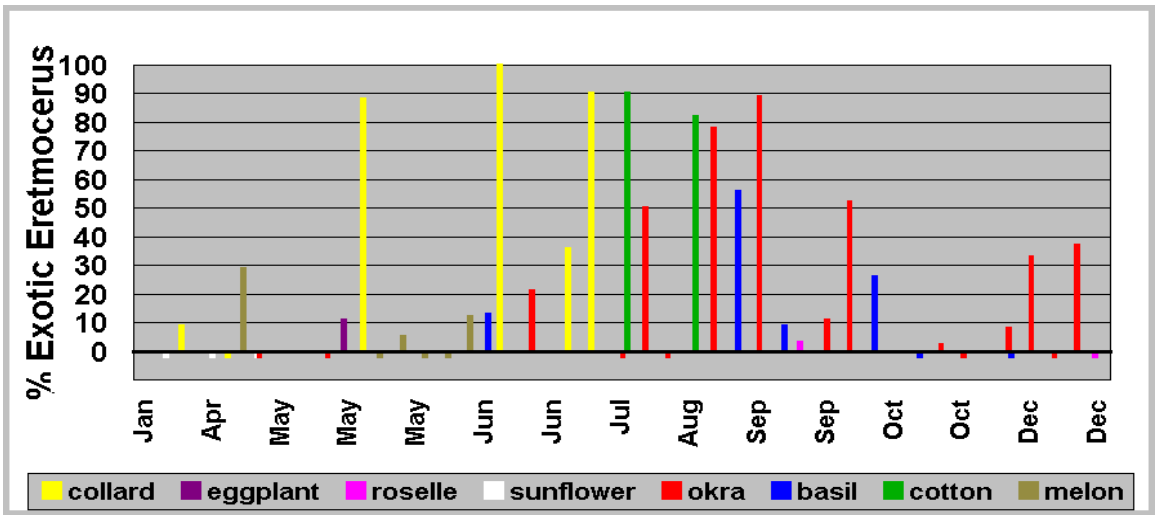
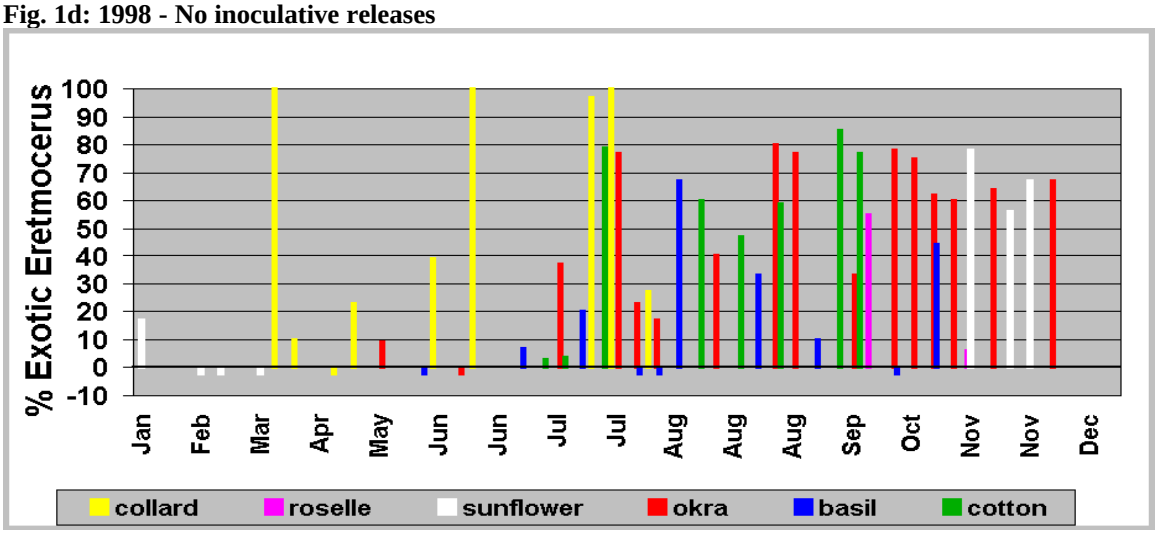



**Fig. 1d: 1998 - No inoculative releases**



**Establishment of Exotic Silverleaf Whitefly Parasitoid Species in the Genus *Encarsia* in Imperial Valley**

W. J. Roltsch, J. A. Goolsby[1], and D. Vacek[1]

Numerous species of silverleaf whitefly *Bemisia argentifolii* Bellows & Perring, (Homoptera, Aleyrodidae) parasitoids in the genera *Eretmocerus* and *Encarsia* (Hymenoptera: Aphelinidae) have been made available for release through foreign exploration. Field cage studies of bi-parental *Encarsia*, identified two populations of *E. transvena* that were most likely to become established as biological control agents in the desert southwest. One population was *E. transvena* M93003* from Spain and the second was M95107 from Multan, Pakistan. Population M93003 was mass reared and released (>150,000) in 1996. The population did not appear to build up to high densities within field refuge plots, however, this population did show promise in several home yards. It overwintered predominantly on cole crops and demonstrated moderate population increase during the spring of 1997. Population M95107 was first released (>200,000) during the summer of 1997. A remarkable population increase on whitefly infested cotton in field cages took place during the hottest period of the year. When this population was released into a refuge plot of okra, basil and nearby cotton during late June of 1997, its numbers increased dramatically. By late August, the population of M95107 was common in another refuge plot over 0.5 miles south of the original release plot. Fall surveys indicated that it could be easily found within a radius of 1.5 miles from the original field plot, based on examination of whitefly host plants in urban yards. Furthermore, based on DNA tests [(RAPD-PCR) conducted by USDA-APHIS, Mission Biological Control Center, Mission, Texas], all *E. transvena* found from July, 1997 and later in Imperial Valley were M95107. Overwintering and population increase of *Encarsia transvena* (M95107) were monitored from 1997-98. The overwintering of M95107 was recorded, albeit at low numbers. As the summer progressed, the densities increased rapidly. Whitefly and parasitoid densities were closely monitored in two home yards beginning in the spring of 1997. It is noted that this population has persisted at these sites since the summer of 1997. Densities of this parasitoid are commonly high.

Many *Encarsia* species are known as adelphoparasitoids. That is, they produce male offspring by parasitizing larval/pupal life stages of conspecific females or of other species. Although several cases of highly effective biological control by such parasitoids have been documented, concern has been expressed that species with this life history trait could negatively impact other parasitoid species of the silverleaf whitefly and impede biological control. This life history trait relative to *E. transvena* is currently being monitored by collecting field samples to determine temporal, in-field sex ratio patterns. Sex ratios strongly skewed toward male production, especially while whitefly densities are high, may indicate that the organism is doing little to control whitefly and perhaps disrupting other potentially useful species of whitefly parasitoids. Pupae are held individually within 100-cell culture trays until emergence. Figure 1a illustrates that in the one home yard site, the sex ratio is usually well over 50% females. Furthermore, field samples in the fall of 1998 were also commonly skewed toward a preponderance of females (Fig. 1b). Although it is difficult to understand the degree of impact this parasitoid is having on other parasitoids species, it is indicated that *E. transvena* reproduction is largely being allocated toward direct parasitism and mortality of the silverleaf whitefly. Data are

also being collected to identify the relationship between sex ratios, and whitefly and parasitoid densities. These data were not available at the time this report was prepared.

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[1]USDA-APHIS-PPQ, Mission Biological Control Center, Mission, TX
*Population accession code assigned by USDA-APHIS, Mission Biological Control Center

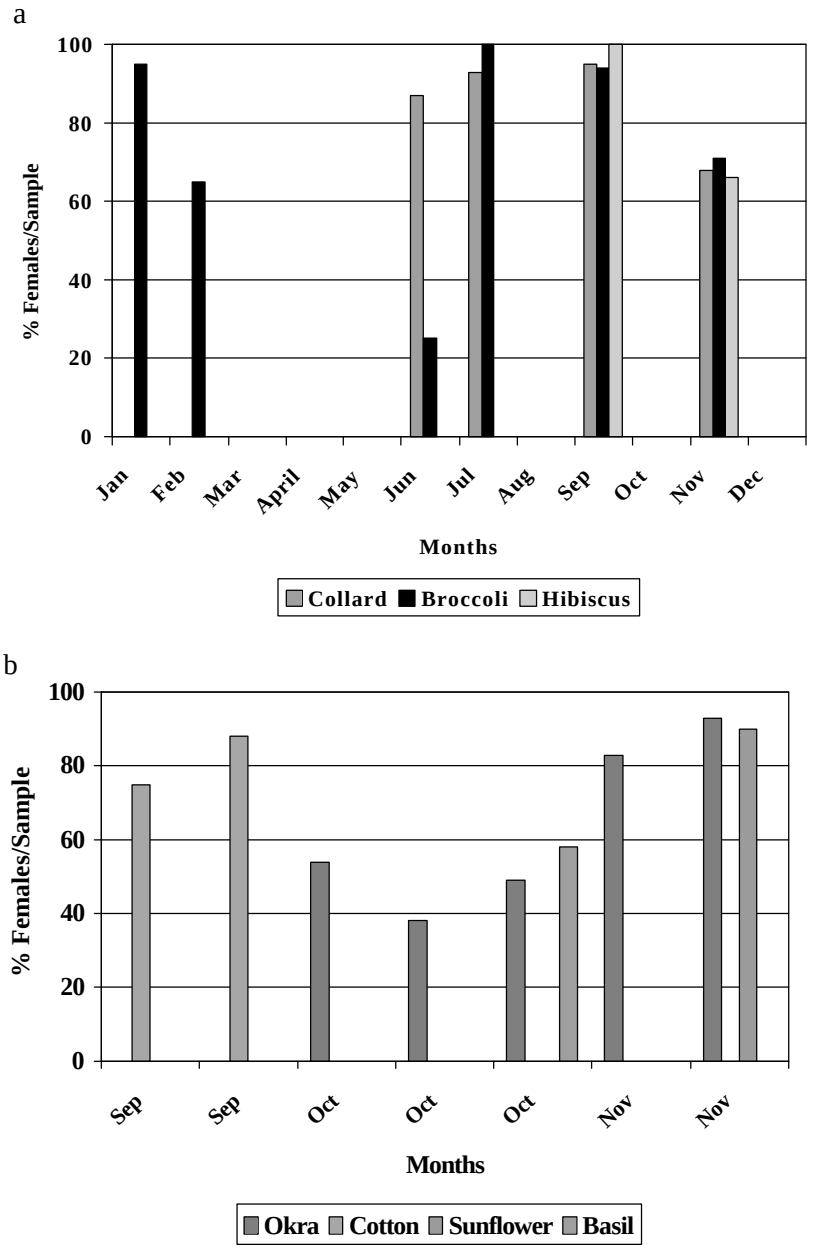


Fig. 1. Sex ratio of *Encarsia transvena* occurring on a) home garden plants, and b) natural enemy refuge field plots in Imperial Valley, CA, 1998.

## A Cooperative Effort in the Release of Silverleaf Whitefly Parasitoids in Mexicali Valley, Mexico

W. J. Roltsch, C. C. Gomez[1], L. P. Valencia[1], G. S. Simmons[2] and T. Boratynski[3]

Silverleaf whitefly, *Bemisia argentifolii,* Bellows & Perring, (Homoptera: Aleyrodidae), densities during summer and early fall months in Mexicali Valley, Mexico are similar to those in Imperial Valley. This region of the southern Colorado Desert basin is contiguous with Imperial Valley. Cotton represents a large portion of the agricultural crops grown during the summer in Mexicali Valley, exceeding 40,000 ha. In cooperation with Mexican officials, arrangements were made to release silverleaf whitefly parasitoids in two cotton fields and at three home yards.

Two hundred thousand *Eretmocerus emiratus* Zolnerowich & Rose (Hymenoptera: Aphelinidae) pupae on leaves and loose within small cups, were released on 31 July 1998 in two cotton fields approximately 10 miles east of Mexicali City and four miles south of the Mexico/USA border. In addition, 20,000 parasites were released August 20, 1998 on ornamental plants at each of two home sites in Mexicali and 60,000 at a home garden in a small community (Ejido Hermosillo) approximately 30 miles south of Mexicali City. Whitefly host plants within the home yards included hibiscus, orchid trees, mulberry trees, fig trees, roses and lantana.

Prior to the release at the two cotton fields, pre-release leaf samples were collected and placed in emergence containers. The majority of whitefly were *B. argentifolii*, however, because this was handled as a group collection (parasitoid pupae on leaves held in canisters for emergence, in contrast to collecting individual pupae) it is not certain that all parasitoid specimens emerged from *B. argentifolii* whitefly. The cotton field pre-release sample of 100 male *Eretmocerus*, yielded two exotic male *Eretmocerus* specimens (species identification is pending) and 98 native *Eretmocerus eremicus*. Four male and four female *Eretmocerus* were collected on 31 August 1998 during post release sampling. All were native *Eretmocerus eremicus*. Few specimens were obtained because a defoliant was applied to the fields prior to sampling. The pre-release and post release samples obtained at the home sites yielded very few *Eretmocerus*; 18 males and 17 females pre release and 23 males and 10 females post release. All specimens were native *Eretmocerus eremicus*.

For 1999, plans are underway to grow several ¼ ha plots of okra at two locations in Mexicali Valley for the specific purpose of creating in-field nurseries for the production of parasitoids to facilitate area-wide establishment. During June, approximately 200,000 parasites will be released into each field plot.

[1]SAGAR, Programa de Sanidad Vegetal, Mexicali, Mexico
[2]USDA-APHIS-PPQ, Western Region, Irrigated Desert Research Station, Brawley, CA
[3]USDA-APHIS PPQ, Irrigated Desert Research Station, Brawley, CA

## Fall Releases of Parasites into Citrus

C. H. Pickett, G. S. Simmons[1], J. A. Goolsby[2], and D. Overholt[3]

The silverleaf whitefly, *Bemisia argentifolii* Perring and Bellows (Homoptera: Aleyrodidae), is an increasingly important pest of cotton in the San Joaquin Valley. Field studies suggest that citrus has become an important overwintering site for this whitefly. Consequently, cotton has the highest incidence of whitefly infestations in areas of the valley with a matrix of both citrus and cotton. We report on large-scale releases of primarily *Eretmocerus emiratus* Zolnerowich and Rose, *Eretmocerus mundus* Mercet, and *Eretmocerus hayati* Zolnerowich and Rose (Hymenoptera: Aphelinidae) into three citrus groves. The study has two goals: (1) to determine if exotic parasites released into citrus during the fall will overwinter in this habitat and move into cotton the following spring; and (2) to permanently establish new populations of exotic parasites specific for the silverleaf whitefly.

Three study sites were identified, one each in Fresno, Tulare, and Kern counties. Sites consisted of citrus and cotton acreage managed by the same owner. Cotton is grown directly adjacent to the citrus, and growers have had a history of silverleaf whitefly problems. They also use the new insect growth regulators for whitefly control. We began releasing parasites in early September when whitefly nymphs were first recorded from citrus leaves. Over 100,000 parasites were released weekly at each location, and a total of 4.05 million were released in 1997 and over 10 million in fall 1998. The dispersal of the released parasites was recorded using sticky cards with identification based on the adult males since they could be readily distinguished from native *Eretmocerus* while on these traps.

The invading adult whitefly populations peaked on citrus in early September, 1997 and the egg population shortly thereafter. Although about the same number of adult whiteflies were caught on sticky cards at the Kern and Tulare County sites, far more eggs and nymphs were recorded at the former. Most of the nymphs recorded from citrus leaves at all three sites were early, not late instars. The Fresno farm never developed substantial whitefly populations in their citrus. The number of whitefly nymphs successfully developing to adults was determined by the presence of an exit hole in the exuviae. At all three sites, the number of nymphal parasites that successfully emerged to adults was only a small fraction of the number of late instar nymph, less than 1%. The maximum number of whitefly completing development (noted by whitefly exuviae) in citrus was recorded from the Kern County Site (0.016/cm$^2$ leaf), with fewer at the other two sites.

We began sampling weeds in January for the presence of whiteflies and parasites and that work is ongoing. We also began sampling cotton, but much later than anticipated, around May. Recoveries of exotic parasites in spring 1998 from weeds, sticky cards, and on cotton leaves adjacent to citrus shows that released parasites from at least one site moved into and attacked whitefly in adjacent cotton the following spring. We are continuing to sample cotton and citrus to determine which species of released parasites is dominant (80% of our releases were *E. emiratus*, and 20% *E. mundus* and *E. hayati*) and to what extent they move into the cotton at all three sites.

[1]USDA-APHIS PPMC, Brawley, CA
[2]USDA-APHIS PPQ Mission, Texas
[3] Pink Bollworm Program, CDFA, Visalia, CA.

## Oviposition Cages for Rearing *Peristenus stygicus*, a Parasite of *Lygus spp*.

J. C. Ball and C. H. Pickett

One of the most important insect pests on a variety of crops in California is the plant bug, *Lygus hesperus* Knight (Hemiptera: Miridae). Native to western North America, *L. hesperus* has few known parasites, at least in agricultural settings. In an attempt to fill this void, nymphal parasites of the genus *Peristenus* (Hymenoptera: Braconidae), attacking other species of *Lygus* in Europe, are being introduced into central California. One candidate, *P. stygicus* Loan, originating in France and passing through quarantine in Newark, Delaware, was provided to our program to attempt field establishment. The introduction program is reported elsewhere. Two *Peristenus* oviposition cages were designed to facilitate the establishment and monitoring programs for this parasite.

The first cage was designed to maximize parasitization of *Lygus* nymphs destined for field release, and was prompted by the fact that a large percentage of the *Lygus* nymphs were killed in our first attempt to obtain parasitism in the lab. Mortality may have been the result of parasite activity and/or cannibalism. Improvement in design was suggested by the experience of J. Andrews and S. Udirigiri (University of California, Berkeley). They noted that parasitism was greater when the parasites were confined closer to their host and, since *Lygus* are cannibalistic, they found that there was better survival if the nymphs were provided places to hide. In order to maintain host and parasite proximity, yet provide some refuge from predation, a standard parasite emergence cage designed by USDA scientists was used. The cage, a plastic cylinder 4¾" high with a maximum diameter of 5½", has an interior fiberglass window screen partition near its base and a top screened with 32 mesh Lumite®. The bottom is open. The cage sits over a thin layer of vermiculite in a clear plastic dish. Filter paper was used to cover the bottom screen to prevent escape of small nymphs. On top of the filter paper was placed a ¼ inch deep fiber mat composed of open hexagonal cells ~ 0.2 inches deep. The cells were to provide refuge for the nymphs. Green beans were laid on top of the mat. Sometime before parasite larvae were projected to emerge from their hosts, the filter paper was removed so that the larvae could reach the vermiculite. In addition, as *Lygus* nymphs developed into adults (not parasitized), they were removed to further reduce cannibalism.

This system was subjected to a single trial (POC-3, Table 1). Approximately 140 field collected *Lygus* nymphs were exposed for two days to 2 to 5 *P. stygicus* females. From this, 46 adult parasites emerged, indicating the cage and handling provided a fairly favorable environment for parasitization.

One of the key factors in the success of the importation effort will be the ability of the parasites to overwinter in California and emerge from diapause when *Lygus* nymphs are available. A second cage was designed to track development of the parasites in the field. Oviposition chambers were made from 50 dram, plastic, snap-cap vials in. diameter and 4½ in. height. The bottom of the vial was removed and two to four ½ in. diameter holes drilled in the side of the vial near the top. The bottom and side openings were covered with organdy. The vial was then partitioned into an upper and lower chamber. Vermiculite placed in the lower chamber was

separated from the upper chamber with fiberglass window screen. The upper chamber held the parasite adults, *Lygus* nymphs and several small green beans to serve as food for the nymphs. Five cages were set up. Three remained in the lab (DFC-1, 4, and 5 in Table 1) and two (DFC-2 and 3 in Table 1) were placed in an alfalfa field on Sept. 17, 1998. The lower chamber was buried in the soil to the upper level of the vermiculite. Parasitization occurred in the lab under 24-hr light.

Last instar parasite larvae left their host and spun cocoons in the vermiculite. Cocoons were mainly in the 1st centimeter of vermiculite and never deeper than 2 centimeters. Only 14 parasites were obtained from approximately 500 *Lygus* exposed to 12 parasites. Cannibalism was probably the most important factor in the low rate of parasitism, since the preferred host, the younger nymphs, could not hide to avoid predation by older nymphs. The adult parasites emerged in the field 41 days after their hosts were exposed to parasitization, somewhat longer than in the lab. This probably is not an expression of diapause, but rather, development slowed by the cooler ambient temperatures. Next year, when parasites are again available, this system will be refined to better reflect field conditions during parasite oviposition and improve survivorship of parasitized nymphs.

Table 1. Parasite performance and progeny survival in two types of cages.

| Cage | # Insects added to the cage | | Days Oviposition | $F_1$ Parasite Events | | | |
|---|---|---|---|---|---|---|---|
| | # Parasite ♀ | # Lygus (approx) | | 1st Cocoon (days) | 1st Emergence (days) | Emergence Span (days) | # Progeny |
| DFC-1 | 2 | 50 | 3 | 13 | 25 | 3 | 5 |
| DFC-2 | 1 | 66 | 3 | 14 | 41 | 5 | 4 |
| DFC-3 | 3 | 175 | 3 | - | - | - | 0 |
| DFC-4 | 3 | 130 | 4 | - | 26 | 3 | 4 |
| DFC-5 | 3 | 85 | - | 12 | - | - | 1 cocoon |
| POC-3 | 2-5 | 140 | 2 | 12 | 26 | 7 | 46 |

## Native Parasites of Cotton Aphid, *Aphis gossypii*, in Kern County: A Multi-Year Survey

K. Godfrey

The cotton aphid (*Aphis gossypii* Glover) (Homoptera: Aphididae) is considered the most widespread insect pest affecting cotton growers in the approximately one million acres of cotton planted annually in the San Joaquin Valley. Mid to late season increases in density of the aphid result in yield reductions, yield quality losses, and decreased profits due to the cost of additional insecticide applications for the cotton aphid. One solution to reduce pest pressure from the cotton aphid is biological control. However, prior to the introduction of exotic natural enemies as biological control agents, a survey to identify the native parasites attacking cotton aphid is required. The survey of native parasites in Kern County was initiated in the fall of 1995 and was completed in November 1998.

The native parasites attacking cotton aphid were surveyed each year at 11-16 sites in Kern County. In 1998, 11 sites were surveyed representing a variety of habitats occupied by the cotton aphid and included cotton, citrus, melons, and non-crop plants. Attempts were made to visit each site monthly and collect samples when cotton aphid host plants were present. However, due to the large amount of rain received during the late winter and spring of 1998, not all sites were accessible due to flooding and road closures. For some samples, green peach aphid (*Myzus persicae* (Sulzer)) was also collected. This aphid is found on some of the same host plants as the cotton aphid, and many of the primary parasites of cotton aphid will attack it.

The results of the survey conducted in 1998 are similar to the results from previous years (Table 1). However, the number of aphids found was much lower than in previous years of the survey. This reduction in aphid density was probably due to the impact of unusual weather patterns (i.e., rainier and cooler than normal) on plant, aphid, and natural enemy populations. In addition, the same genera of primary parasites and families of secondary parasites were recovered (Table 1).

The results of the multi-year survey demonstrate that the cotton aphid is present nearly year-round. The aphid appears to move from non-crop host plants in the winter and early spring to melons and cotton in the late spring and summer. In late summer and through the fall, the aphids can be found moving from senescing cotton to citrus and non-crop habitats.

Primary and secondary parasites are associated with the cotton aphid in all of the habitats that were surveyed over the years. The same parasite genera were represented each year in the survey, however, their relative abundance varied with habitat and year. Despite the presence of the parasites in all habitats, the amount of mortality that they imparted on cotton aphid populations was low due to few parasites produced and relatively large populations of aphids present. This suggests that increased species diversity is needed in the parasite complex for the cotton aphid.

Table 1. The plants sampled for cotton aphid and green peach aphid, and the total number and genera or family of primary and secondary parasites in Kern County in 1998.

| Date | Host plants sampled[a] | No. & genus of primary parasites[b] | No. & family of secondary parasites[c] |
|---|---|---|---|
| January 14 | MA | 3 Apd, 1 Dia | - |
| February 11 | MA | 2 Apd | - |
| March 9 | FD, MA | 13 Apd | - |
| April 20 | MA | 4 Lys, 16 Apd | - |
| May 20 | MA | 1 Apd | - |
| June 17 | CT | - | - |
| July 22 | CT | - | - |
| August 13 | CT | - | - |
| September 28 | CT | 1 Lys | - |
| October 14 | CT | 4 Lys | - |
| November 23 | CT, CR, MA | 14 Lys | 17 Ptr, 11 Chr |

[a]MA = cheeseweed; FD = fiddleneck; CT = cotton; CR = Citrus
[b]Apd = *Aphidius* spp.; Dia = *Diaeretiella* spp.; Lys = *Lysiphlebus*
[c]Ptr = Pteromalidae; Chr = Charipidae

## Survey of Native Fungi Attacking the Cotton Aphid, *Aphis gossypii*, in the San Joaquin Valley

K. Godfrey and D. Steinkraus[1]

The cotton aphid (*Aphis gossypii* Glover) (Homoptera: Aphididae) can take advantage of the mosaic of cropping systems and habitats that exist in the San Joaquin Valley because of its broad host range. As a result, this aphid can attain pest status in melons, citrus, and cotton. These crops occupy approximately 1.4 million acres in the valley. One tactic that shows promise for managing this insect is biological control. Studies are currently being conducted on the insect parasites attacking the cotton aphid, but little is known about its fungal pathogens in the valley. In a two-year survey conducted in 1994 and 1995 in cotton in California, no fungal pathogens were discovered in the 29,113 aphids evaluated (D. Steinkraus and J. Rosenheim, unpublished data). A second survey of the native fungal pathogens attacking cotton aphid in a variety of habitats in the San Joaquin Valley was initiated in 1997 and was continued through 1998. The results of the first year of this survey found very few fungi attacking cotton aphids. This suggests that introduction of fungi adapted to the warm dry conditions of the San Joaquin Valley could be useful.

The native fungal pathogens attacking cotton aphid were surveyed at 11 sites in Kern County during 1998. The sites represent a variety of habitats occupied by the cotton aphid and include cotton, citrus, melon, and non-crop plants. An attempt was made to visit each site monthly and collect samples when cotton aphid host plants were present. However, due to the large amount of rain received during the late winter and spring of 1998, not all sites were accessible due to flooding and road closures. For some samples, green peach aphid (*Myzus persicae* (Sulzer)) was also collected. This aphid is found on some of the same host plants as the cotton aphid, and some of the fungal pathogens that attack cotton aphid will also attack green peach aphid. All samples were returned to the laboratory and sorted. Those aphids that appeared to have symptoms of fungal disease were placed in vials in 70% ethanol. The remaining aphids were held in the laboratory with food and water for 10-14 days to allow the development of any additional fungal diseases. At the end of the rearing period, the aphids were placed in vials of 70% ethanol and sent to the Aphid Fungus Diagnostic Laboratory at the University of Arkansas for diagnosis.

Of the 1,798 aphids collected in the 1998 survey, only 90 aphids (5.0%) were infected with fungi (2/569 cotton aphids and 88/1,229 green peach aphids; Table 1). The fungal pathogens were most prevalent during the cooler and wetter parts of the year (Table 1). The most common pathogen found was *Erynia neoaphidis* with two other fungal pathogens, *Conidiobolus obscurus* and *Entomophthora planchoniana*, also being present (Table 1). For the remaining infected aphids (unknown entomophthoralean category of Table 1), only vegetative stages of the fungi were present. Therefore, these fungi could not be identified with certainty, but many were probably *Ery. neoaphidis*. The results from the 1998 survey do not differ significantly from the results of the 1997 survey, despite unique weather patterns. Thus, the complement of pathogens currently infecting cotton aphid in the San Joaquin Valley is rather small. Introductions of additional pathogens may be desirable.

Table 1. The fungal pathogens attacking cotton aphid and green peach aphid in Kern County in 1998-1999.

| Date[a] | Aphids Examined | No. of Infected Aphids[b] | | | |
|---|---|---|---|---|---|
| | | EN | CO | EP | UN |
| Cotton Aphid | | | | | |
| 3-9-98 | 3 | 1 | 0 | 0 | 0 |
| 4-20-98 | 20 | 0 | 0 | 0 | 0 |
| 8-13-98 | 91 | 0 | 0 | 0 | 0 |
| 9-28-98 | 360 | 0 | 0 | 0 | 0 |
| 10-14-98 | 82 | 0 | 0 | 0 | 0 |
| 11-23-98 | 8 | 1 | 0 | 0 | 0 |
| 1-12-99 | 5 | 0 | 0 | 0 | 0 |
| Green Peach Aphid | | | | | |
| 1-14-98 | 47 | 0 | 0 | 0 | 0 |
| 2-11-98 | 179 | 11 | 3 | 0 | 5 |
| 3-9-98 | 616 | 7 | 0 | 0 | 14 |
| 4-20-98 | 348 | 13 | 1 | 5 | 28 |
| 5-20-98 | 2 | 0 | 0 | 0 | 0 |
| 11-23-98 | 7 | 0 | 0 | 0 | 0 |
| 1-12-99 | 30 | 1 | 0 | 0 | 0 |

[a]Sites were visited in June and July; however, no cotton aphids or green peach aphids were recovered. The final visit to the sites occurred in January 1999, rather than December 1998.

[b]EN = *Erynia neoaphidis*, CO = *Conidiobolus obscurus*, EP = *Entomophthora planchoniana*, UN = unknown entomophthoralean fungus

[1]Department of Entomology, University of Arkansas, Fayetteville, Arkansas

## Evaluation of Introduced Natural Enemies of the Cotton Aphid on Pumpkins

K. Godfrey, N. Smith[1], K. A. Casanave, D. Kress, and D. A. Mayhew

Agriculture in the San Joaquin Valley is diverse and exists as a mosaic with other habitats. The cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae), can easily take advantage of this mosaic of cropping systems and habitats because it has a broad host range. As a result, this aphid can attain pest status in a number of crops, including pumpkins. To improve management of the cotton aphid in the San Joaquin Valley, enhancement of the biological control on cotton aphid populations has been suggested. It is important to determine the ability of the natural enemies used for this enhancement to recognize and use cotton aphid on a variety of host plants. Therefore, this study was conducted to determine the ability of two introduced parasites, *Aphelinus* near *paramali* and *Aphelinus gossypii* Timberlake (Hymenoptera: Aphelinidae), to recognize and use cotton aphid on pumpkins.

The ability of the two introduced parasites, *Aph.* near *paramali* and *Aph. gossypii*, to attack cotton aphids on pumpkins was investigated in a small plot in Fresno County. The plot contained five pumpkin plants each of 'Connecticut' and 'Big Max' varieties, planted in May 1998. Releases of both parasites were made at about weekly intervals into the plot once plants supported cotton aphid populations. From June 16 through October 13, 1998, approximately 12,300 *Aph.* near *paramali* and 15,410 *Aph. gossypii* mummies and adults were released into the plot. To determine the success of the parasites in using cotton aphid on pumpkin, three sets of leaf samples were taken: the first set collected on July 8, the second set on July 28, and the third set on September 23. For each set of samples, the number and type of (i.e., black, cigar-shaped indicative of aphelinid parasites, or brown, balloon-shaped indicative of aphidiid parasites) mummies were recorded. All intact mummies were held for parasite emergence.

The results of this study demonstrate that both introduced parasites will recognize and use cotton aphid on pumpkin (Table 1). For the samples taken in July, the most numerous parasites recovered were secondary parasites (Pteromalidae, Charipidae, and Encyrtidae) even though most of the mummies formed were of the aphelinid type (Table 1). In the September sample, only progeny of the introduced parasites were recovered (Table 1).

Table 1. The number and type of mummies and the number and type of parasites found in leaf samples collected in pumpkin in Fresno County in 1998.

| Sample Date | Variety | No. & Type of Mummy[a] | Parasites Emerging |
|---|---|---|---|
| July 8 | Big Max | 20 C | 10 Pteromalidae, 2 Charipidae, 1 Encyrtidae |
| | Connecticut | 5C | 2 Charipidae |
| | | 2B | 1 Lysiphlebus |
| July 28 | Big Max | 5C | 1 AG, 2 Pteromalidae, 2 Charipidae |
| | | 1B | 1 Charipidae |
| | Connecticut | 1C[b] | |
| | | 1B[b] | |
| September 23 | Big Max | 2C | 2 AG |
| | Connecticut | 2C | 1 AG, 1ANP |

[a]C = black, cigar-shaped mummy; B = brown, balloon-shaped mummy
[b]Mummy had emerged.

[1]Fresno County Department of Agriculture, Fresno, California

**Phenology of Vine Mealybug and Natural Enemies on Grapevines in the Coachella Valley**

J. C. Ball, K. Godfrey, D. Gonzalez[1] and M. Waggoner[1]

In 1994, grape growers in the Coachella Valley (Riverside County) reported that a mealybug was reducing the quality and yield of their grapes. Specimens sent to California Department of Food and Agriculture Plant Pest Diagnostic Laboratory and the US National Museum were identified as the vine mealybug, *Planococcus ficus* (Signoret). This mealybug is an economic pest of grapes in the Mediterranean region of Europe, Africa and 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. The vine mealybug has now spread into most of the vineyards in the Coachella Valley and has recently been found in several vineyards in the Central Valley. Since 1994, the Biological Control Program has been working with the University of California at Riverside to understand the biology of vine mealybug in the desert vineyards of the Coachella Valley and assess natural enemy impact. This report covers the work accomplished in 1998.

Populations of vine mealybug and parasites were monitored on 12 vines in an organic vineyard. These vines were preselected for having a readily observable mealybug infestation and were followed throughout the year. Populations were sampled using three types of traps, first set out in late April. Yellow sticky cards (3" x 5") were hung under the canopy of each vine. Also on each vine, a single width of double-sided tape was wrapped around an arm of the trunk, a cordon, and the previous years cane on one side of the vine. Both types of sticky traps were replaced every two weeks. On the opposite side of each vine, 2" wide strips of bubble wrap (~ 23 bubbles per inch$^2$) were wrapped around an arm, cordon and year old cane, and held in place with duct tape. These were meant to provide a sheltered environment, at least from larger predators, where vine mealybug reproduction, mortality, and movement could be monitored. Three wraps were placed on each part of the vine in order to provide 6, 12, and 18-week monitoring intervals. When removed, the “six-week” wraps were replaced with another wrap to provide for the next 6-week interval. The wraps, along with any mealybugs underneath adhering to the vine, were placed in snap-cap vials and brought to the lab for counting.

Population development was similar to that found in previous years. Fairly large densities of vine mealybug occurred on the trunk early in June, peaked at 6.7 per cm of sticky tape in mid July, and then, abruptly declined through late August, when the study was terminated. The population peaked in early June on cordons and mid June on canes. Under the “bubble-wrap”, mealybugs were heaviest on trunk and cordon during the first 6 week period, between April 22 and June 3, and on canes during the second period between June 3 and July 15.

Adults of two exotic, primary parasitoids, *Anagyrus pseudococci* Grlt. and *Leptomastidea abnormis* (Grlt.) (Hymenoptera: Encyrtidae), released by UCR between March 18 and June 10, 1998, were first picked up on the yellow sticky cards June 1998. The releases had been made a minimum of 13 rows from vines used in this study. Counts for both species peaked between July 16 to July 31. *L. abnormis* was trapped at 8 of the 12 vines but most individuals (95%) were caught on only two vines, whereas *A. pseudococci* were trapped at all vines. The hyperparasite,

*Chartocerus* sp. (Hymenoptera: Signiphoridae), was also taken on the yellow card traps. It peaked on August 14, two weeks after the peak flight of the two primary parasites, which would be expected from a hyperparasite whose development must follow that of the primary parasite. However, on the sticky tape traps, *Chartocerus* peaked on July 31, coinciding with the peak of primary parasites on yellow cards. The reason for this anomaly is uncertain, but since the *Chartocerus* caught on sticky tapes are derived mainly from mummies trapped underneath the tape, events timed by the two types of traps may not be comparable.

The most abundant predators captured on the yellow cards were spiders, followed by brown lacewings. Big-eyed bugs, pirate bugs, and green lacewings were also relatively common. Brown lacewing adults peaked in mid July, but remained relatively common through late August. Spiders were most abundant in August.

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## Host Feeding by *Eretmocerus* spp. on Citrus

C. H. Pickett, J. A. Brown, H. Kumar, and D. A. Mayhew

Field monitoring for large scale releases of parasites of silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring (Homoptera: Aleyrodidae), revealed high amounts of nymphal mortality and only a small number of whitefly eggs developing to adults. A greenhouse study was initiated to measure the amount of nymphal mortality that may be attributed to parasite host feeding. Two groups of three to five-year old citrus saplings (Washington navels) were exposed to 2000 adult silverleaf whitefly in organdy covered cages for 7 to 10 days, after which whitefly adults were removed by vacuum. Up to 30 leaves with whitefly eggs were tagged on each of 6 potted plants. Half of each tagged leaf was removed by cutting with scissors along the midvein; the number of whitefly eggs on each removed half leaf was counted with the aid of a dissecting microscope. Following leaf removal, three of the potted plants were removed to a second greenhouse and placed in an organdy-covered cage and then exposed to 1,000 to 2,000 adult parasites (*Eretmocerus emiratus* or *E.* nr. *emiratus*, M96076). Two to three weeks later, when parasite development was visible, the second half of each leaf was removed. The number of early (instars 1-2) and late (instars 3 – pupa) whiteflies, parasitized whitefly, dead whitefly nymphs, and whitefly exit holes were recorded with the aid of a dissecting scope. Survivorship was measured as a proportion: the number of nymphs and whitefly exit holes counted 3 to 5 weeks later on the second half of each leaf divided by the number of nymphs and eggs counted during the first sampling (first half leaf removed) (we assumed a random distribution of eggs across leaves). Dead nymphs were also measured as a proportion: the number of dead early and late instar nymphs recorded from the second sampling divided by the number of eggs and early instar nymphs recorded from the first sampling. The experiment was repeated three times in 1998, once in May, September and November. Survivorship of whitefly from egg to adult per leaf area (per $cm^2$) was about 3 times greater, on average (range), in cages lacking parasites: 0.76 (0.28 – 1.0) vs. 0.21 (0.1 – 0.33). The number of dead nymphs recorded from plants exposed to parasites was almost three times greater: 0.43 (0.06 – 0.66) vs. 0.16 (0.02 – 0.3). The difference between the proportion of dead nymphs measured in the two treatments should reflect the amount of host feeding, i.e. 16% of the nymphs died from natural causes (i.e. inability to develop on citrus tissue), the remainder from host feeding (27%).

**Screening and Utilization of Perennial Arid Landscape Plants for Silverleaf Whitefly, *Bemisia argentifolii,* Natural Enemy Refuges**

W. J. Roltsch, C. H. Pickett, and J. A. Brown

Perennial plant species native to the desert southwest, along with other low maintenance landscape plants, are being screened as potential refuge plants for parasitoids of the silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring (Homoptera: Aleyrodidae). Once established, perennial plant systems could provide a stable habitat and source for whitefly parasitoids. Such plants must support a high proportion of parasitized whitefly, and survive high summer temperatures and occasional winter frosts. In addition, they must grow in relatively high alkaline soils and over a broad range of soil moisture conditions, and have few special management needs. The present information represents an update to last years report. It also identifies our initial experiences in taking several of the most promising plants and setting up a large-scale field plot. Results to date are preliminary [see table].

Over 30 perennial plant species have been screened in small field plots to determine their potential as natural enemy refuge plants. Several landscape plant species have been of interest. Because of a favorable whitefly/parasitoid relationship, lavatera (*Lavatera thuringiaca*) has been closely monitored for three years at demonstration and evaluation plots at an organic farm and the USDA field station in Imperial Valley. Initial establishment of this plant was strong, however, a large percentage (>50%) of the plants have died each year during high summer temperatures. Silverleaf whiteflies on rue are commonly parasitized by either *Eretmocerus* or *Encarsia* (Hymenoptera: Aphelinidae). *Encarsia* species appear to be found more frequently on this plant species than on other plants that have been monitored. Interestingly, native *Hibiscus californica* has shown some potential as a summer/fall refuge plant species; however, it died after three years. It is native to relatively wet areas of central California, and the environmental conditions in Imperial Valley are most likely incompatible. Purple potato vine (*Lycianthes rantonnetii*) harbors considerable numbers of whitefly that are commonly parasitized, however, its compatibility with Imperial Valley's climate and soil characteristics remains in question after two years of monitoring. Much of the foliage is lost and many branches die during late summer. Growth in the fall has been relatively strong. Blue hibiscus (*Alyogyne huegelii*) has been monitored for one year. It is native to Australia and is becoming a popular ornamental in southern California. An initial assessment of this plant suggests that it is suited for climatic and soil conditions in Imperial Valley, retains its leaves through the winter, is moderately attractive to whiteflies much of the year and supports high levels of parasitism.

Plant candidates were also selected by inspecting plants at botanical gardens in California and Arizona. To date, chuparosa (*Justicia californicus*), yellow bells (*Tecoma stans stans*) and *Ruellia peninsularis* show promise as parasitoid, perennial refuge plants. Chuparosa is a dense, unstructured shrub growing to 4 ft in height. *Tecoma stans stans* is a large shrub growing to a height of 12 feet, possibly being of some value as a windbreak as well. *Ruellia peninsularis* is a small to medium size shrub growing to 5 feet in height. Whitefly densities on these plants are typically low, however they can accumulate when migrating whitefly densities are high. These plants are predominantly late summer and fall whitefly host plants; therefore they are not yearlong

providers of whitefly parasitoids. Compared to *T. stans* and *R. peninsularis*, chuparosa is a whitefly host over a greater portion of the year and is the most likely candidate to have some role in carrying parasite populations through the winter, albeit in low densities. Whitefly predation on these plants due to predators is typically very high (e.g. *Geocoris* spp. and *Orius* spp.).

Most perennial plant species require 18 months or more before they are well established. Therefore, they must be planted for a lengthy period of time before they can be sampled and evaluated. No whitefly host plants have been found that can be recommended for use outside of managed field areas due to water and soil limitations. All of these plants can make attractive yard plants.

**Large scale plantings:** During 1998, five plant species were selected, propagated and transplanted in October into a 275 m long, two bed hedgerow on an organic farm in southern Imperial Co. Before planting, the two beds and their furrows were covered with ground fabric for weed control. Plants included, yellow bells, chuparosa, rue, blue hibiscus and wild buckwheat (*Eriogonum fasciculatum*). Wild buckwheat is not a whitefly host plant, however it does harbor various predators including *Geocoris* spp. Over 30% of the yellow bells were killed due to frost damage. Our observations indicate that it is best to transplant blue hibiscus and wild buckwheat in the fall because these plants grow extensively during winter conditions, establishing well at that time. In contrast, it is best to transplant yellow bells and chuparosa during early spring (February), because these plants do not grow during the winter (following fall transplanting) and are very susceptible to frost damage at that time. Rue does best when transplanted while the plants are very young (approx. 4 in. tall) and rapidly growing.

PERENNIAL PLANT EVALUATION LIST [March 1999]

| FAMILY | SPECIES | COMMON NAME | Plant growth potential | Affinity of Whiteflies to Plant | Affinity of Parasitoids to Plant | | Years Observed |
|---|---|---|---|---|---|---|---|
| **Acanthaceae** | *Justicia californica* | chuparosa | *** | ** | *** | [a] | 3 |
| | *Justicia carnea* | Brazilian plume | * | | | | |
| | *Justicia ovata* | red justicia | * | *** | *** | [a] | 3 |
| | *Justicia spicegera* | Mexican honeysuckle | * | *** | ** | [a] | 3 |
| | *Ruellia californica* | | **** | * | *** | [a] | 3 |
| | *Ruellia peninsularis* | | *** | * | *** | [a] | 3 |
| **Bignoniaceae** | *Tecoma stans stans* | yellow bells | *** | ** | *** | [a] | 2 |
| **Asteraceae** | *Echinacea purpurea* | purple coneflower | * | | | | 2 |
| | *Rudbeckia hirta gloriosa* | gloriosa daisy | * | ** | *** | | 2 |
| **Curcurbitaceae** | *Curcurbita foetidissima* | wild gourd | * | | | | 2 |
| | *Curcurbita palmata* | coyote melon | * | | | | 2 |
| **Euphorbiaceae** | *Euphorbia xantii* | spurge | * | ** | ** | [a,b] | 3 |
| **Malvaceae** | *Alyogyne huegelii* | blue hibiscus | * * * | *** | **** | | 1.5 |
| | *Anisodontea [tara's choice]* | Tara's mallow | * | * | *** | [a] | 3 |
| | *Hibiscus californica* | California hibiscus | ** | ** | *** | [a] | 3 |
| | *Hibiscus rosa-sinensis* | Chinese hibiscus | ** | *** | **** | [a,b] | 3 |
| | *Lavatera bicolor* | tree mallow | ** | * | *** | | 3 |
| | *Lavatera thuringiaca* | lavatera | * | *** | *** | [a] | 3 |
| | Althaea rosea | hollyhock[2] | ** | *** | *** | [a] | 3 |
| **Rutaceae** | *Ruta graveolens* | rue | *** | *** | *** | [a,b] | 3 |
| **Solanaceae** | Datura discolor [1] | jimsonweed | * | | | | 3 |
| | Datura meteloides | jimsonweed | ** | * | ** | [a] | 3 |
| | Lycianthes rantonnei | purple potato vine | ** | **** | *** | | |
| | *Nicotiana glauca* | tree tobacco | **** | * | *** | [a,b] | 1 |
| | *Nicotiana trigonophylla* | tobacco bush | ** | * | *** | [a] | 1 |
| **Verbenaceae** | *Verbena peruviana* | St. Paul's verbena | * | | | | 1 |

Performance rating: poor *, fair **, good ***, excellent ****.

1=annual species of *Datura*, 2=generally an annual species that re-seeds itself.

Letters in brackets signify whether *Eretmocerus* [a], *Encarsia* [b], or both are common on each plant type.

**The Use of a Summer Refuge Nursery System for Establishing Parasitoids of the Silverleaf Whitefly, *Bemisia argentifolii***

W. J. Roltsch and G. S. Simmons[1]

The silverleaf whitefly, *Bemisia argentifolii*, Bellows & Perring, (Homoptera: Aleyrodidae), has been a primary pest of numerous crops in the Imperial Valley since 1991. Native species of parasitoids (*Eretmocerus eremicus* Rose & Zolnerowich, *Encarsia luteola* Howard, and *E. meritoria* Gahan (Hymenoptera: Aphelinidae)) and predators have been unable to achieve sufficient biological control of this pest. An intense effort has been made to establish the most promising of numerous exotic *Eretmocerus* and *Encarsia* parasitoid species that have been made available through foreign exploration by USDA-ARS and others.

In-field summer refuge nursery production plots have been employed for two years for producing large numbers of exotic parasitoids. These half-acre field plots are used from mid-summer through fall in order to facilitate the regional establishment of newly introduced whitefly natural enemies. Each site is composed of one-quarter acre of okra and one-quarter acre of basil that were planted in March. Second through fourth instar whitefly nymphs are characteristically present on these plantings by early June. During late May through June, each field plot is inoculated with approximately 200,000 of one or a combination of exotic parasitoid species. The number of parasitoids emerging from these plants is very high by late August. Releases in 1998 included *Eretmocerus emiratus* Zolnerowich & Rose, and *Eretmocerus sp*. M96076* from Ethiopia. Based on sample data that consisted of counts of parasitoid pupae per leaf, number of leaves per plant and plants per 1200 row feet (approx. ¼ acre), it was estimated that on 28 August 1998 over one million *Eretmocerus* parasitoids were emerging on a daily basis from the okra plants alone. Furthermore, it was determined that 50% of these were exotic. Because of small leaf size and complex overall plant structure, the estimation of absolute densities of parasitoids on basil was not done. Typically, whitefly densities on basil are much lower than on okra in Imperial Valley, however, percent parasitism is very high. During 1998, emerging parasitoid densities were sufficient to nearly eliminate the whitefly, however, whitefly recruitment from adjacent areas continued to provide some hosts within the plots. At one site, approximately 4,000 *Encarsia transvena* (Timberlake) reared on potted collard plants were released. By 28 August, there were over 84,000 adult wasps emerging per day on okra. These production values are very comparable to those calculated during 1997 within a planting of okra and basil.

For two successive years, this method has demonstrated its usefulness for propagating large numbers of exotic parasitoids, from late summer through fall, to facilitate regional establishment of promising newly introduced parasitoids of the silverleaf whitefly. To be successful, it is important to have a well-maintained plot of vigorously growing okra and basil. Weedy plots or otherwise unthrifty plants are of little value.

[1]USDA-APHIS-PPQ, Western Region, Brawley, CA

*Population accession code assigned by USDA-APHIS, Mission Biological Control Center

## Augmentative Biological Control Using Transplants

C. H. Pickett, G. S. Simmons[1], and J. A. Goolsby[2]

Early season augmentative releases of *Eretmocerus* species (Hymenoptera: Aphelinidae) for control of silverleaf whitefly infesting spring planted melons in Imperial Valley can eliminate the need for late season applications of pyrethroids and other broad spectrum insecticides. This approach can enhance the regional population of highly effective whitefly parasites important to summer and fall field and vegetable crops. It may also delay buildup of resistance in whiteflies to insecticides by reducing their usage. However, like other augmentative releases of natural enemies, use of *Eretmocerus* spp. is currently expensive, possibly exceeding their short-term economic benefit. We report on a novel approach to enhancing early season field populations of *Eretmocerus* spp. using cantaloupe transplants. Prior to placement in fields, cantaloupe seedlings are inoculated with a highly specific whitefly parasite, *Eretmocerus emiratus* Zolnerowich and Rose, recently imported from the United Arab Emirates. We wanted to determine whether control of whiteflies in fields receiving transplants inoculated with parasites, or "banker plants," is more effective than in fields receiving conventional hand releases. We hypothesized that parasites on transplants would be a more efficient means of introducing parasites because they would immediately be distributed throughout the entire field and have food readily available to them. Hand released parasites must first search for widely dispersed, low density prey, before parasitizing them. We also wanted to show that transplants with parasites can be integrated into imidacloprid treated fields at very little additional cost, or at least equal to conventional insecticide costs.

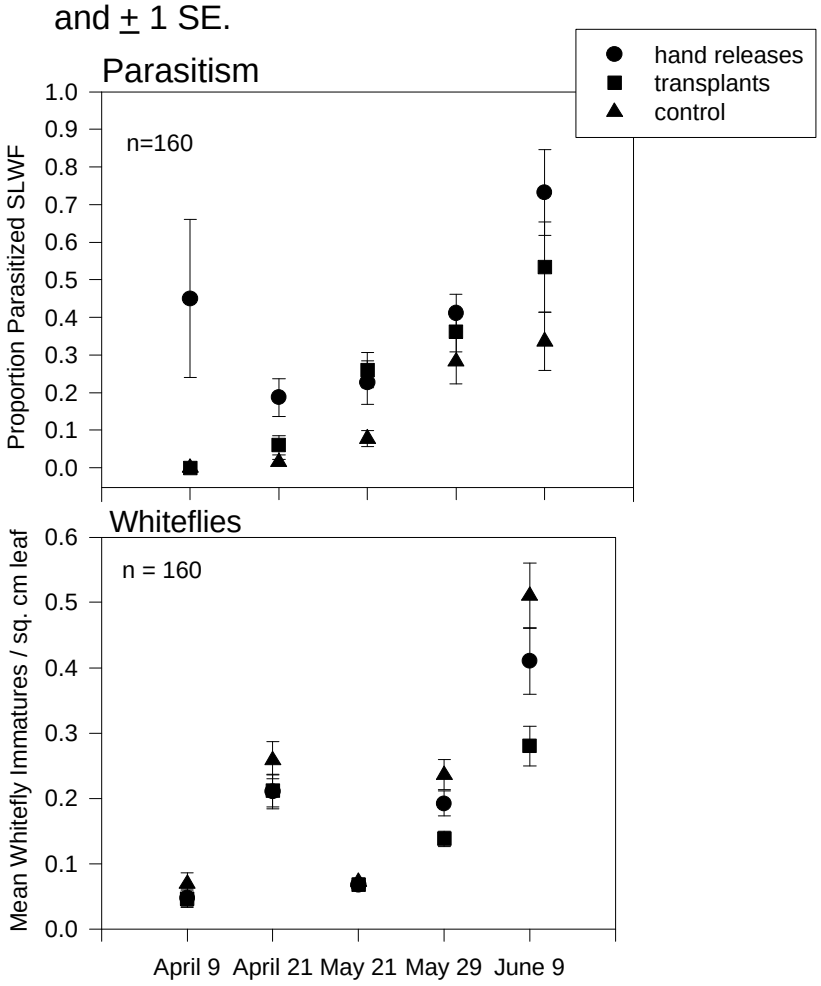
We completed our first field season spring of 1998. Parasites were released into two commercial farms of cantaloupe in the Imperial Valley. The first was an organic operation, where we compared the effect of banker plants (transplants with parasites) against plots receiving hand-releases of parasites, and a no-release control. Treatments were assigned to 1/3 ac plots using a randomized complete block design with 4 replicates. The second site was a conventional operation that uses imidacloprid; there we compared whitefly densities in 2 pairs of 1 acre plots with and without the addition of banker plants.

We succeeded in getting parasites onto banker plants and into fields at both the organic and conventional fields. Banker plants represented about 10% of the total plants in the fields. However, we ended up releasing far fewer parasites using banker plants than we had planned; about 6400 to 7800 parasites per acre at the organic farm and approximately 24,000 per acre at the conventional farm. We hand released ca. 9,000 parasites per acre at the organic farm. This is much lower than our target of 40,000, the number found to give good control of whiteflies using conventional hand releases. Nevertheless, we measured significant differences in whitefly nymphal populations between the different treatment plots at the organic site. The lowest nymphal populations on the last two sample dates were recorded from the transplant plots, with increasing number in the hand release, and control plots (Fig. 1). On 29 May 1998 banker plant plots averaged 0.13 nymphs/$cm^2$ followed by hand release plots at 0.18 nymphs/$cm^2$, and control plots, 0.23 nymphs/$cm^2$; and on 9 June 1998 bankers plant plots averaged 0.28 nymphs/$cm^2$ followed by hand release plots at 0.41 nymphs/$cm^2$, and control

plots 0.51 nymphs/cm$^2$. Whitefly populations were too low in the conventional field receiving an imidacloprid treatment to measure parasitism.

[1]USDA-APHIS PPMC, Brawley, CA
[2]USDA-APHIS PPQ Mission, Texas

Fig. 1. Organic melons, Imperial Valley, 1998. Effect of different release methods. Shown are means and ± 1 SE.



## Exotic Natural Enemies of Giant Whitefly in San Diego County

C. H. Pickett, D. Kellum[1], and M. Rose[2]

A new parasite of the giant whitefly, *Aleurodicus dugesii* Cockerell (Homoptera: Aleyrodidae), was collected in Texas in 1995. *Entedononecremnus krauteri* Zolnerowich and Rose (Hymenoptera: Eulophidae) was released at twelve locations in San Diego County during October 1995. Releases were made onto a variety of ornamental plants infested with giant whitefly, including hibiscus. Additional shipments were made from Texas in June 1996 and released onto hibiscus at two locations in San Diego County. Large numbers of parasites have built up at two of the original release sites and were used to establish populations at new sites in Los Angeles and Santa Barbara counties summer 1997. The number of giant whitefly adults at one of the original 1995 release sites have started to decline. Eleven thousand parasites were moved to new and former release sites in San Diego during the summer of 1998 (Table 1) using parasites collected at the Zoo. A new exotic parasite was discovered in 1998 associated with giant whitefly at the San Diego Zoo. *Encarsia hispida* (identified by J. Heraty, UC Riverside and G. Evans, University of Florida) attacks the early instars of giant whitefly nymphs, complementing the impact of *Entedononecremnus krauteri* which attacks the later instars.

A commercially available whitefly predator, *Delphastus catalinae* (Horn) (Coleoptera: Coccinellidae), was released in San Diego County onto a single avocado, a citrus tree and hibiscus plant fall 1995. These beetles are known to attack other whiteflies that produce copious amounts of wax like the giant whitefly. Beetles have persisted on the avocado tree through January 1999. They have spread from the avocado to other trees at the same location, a private residence in Carlsbad, and have been recovered from citrus and hibiscus plants. They have also spread onto hibiscus in the surrounding neighborhood where we have been monitoring the infestation levels of the giant whitefly. We released several hundred *D. catalinae* on three avocado trees in one residence and are monitoring avocado trees in a second yard one block away to measure impact of the beetle on giant whitefly infesting avocado.

Table 1. Releases of *Entedononecremnus krauteri* 1998

| Date Released | City | Address | # Released | Host |
|---|---|---|---|---|
| 8-11-98 | Encinitas | Quail Botanical Gardens | 40 | hibiscus |
| 8-18-98 | San Diego | Sea World | 200 | hibiscus |
| 8-19-98 | Encinitas | Quail Botanical Gardens | 100 | hibiscus |
| 8-20-98 | | Del Dios Hwy | 150 | hibiscus |
| 9-10-98 | San Diego | Sea World | 400 | hibiscus |
| 9-23-98 | Chula Vista | Fredericka Manor | 400 | hibiscus |
| 10-6-98 | Santa Barbara (Co.) | | 500 | hibiscus |
| 10-12-98 | San Diego | Sea World | 400 | hibiscus |
| 10-10-98 | Encinitas | Quail Botanical Gardens | 300 | hibiscus |
| 10-15-98 | Chula Vista | Fredericka Manor | 300 | hibiscus |
| 10-24-98 | San Marcos | Cassou Rd. | 200 | hibiscus |
| 10-26-98 | Vista | Kellyn Ln. | 200 | hibiscus |
| 10-26-98 | El Cajon | Trucksess | 200 | hibiscus |
| 10-26-98 | Oceanside | PEP BOYS | 300 | hibiscus |
| 10-30-98 | Rancho Bernardo | Crest Way | 100 | hibiscus |

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[2]Department of Entomology, Montana State University, Bozeman, MT

## Insect Natural Enemies Mass Reared for Research and Colonization Projects

J. A. Brown, K. A. Casanave, and C. H. Pickett

Each year one or more insect natural enemies are mass reared for a variety of projects conducted by the Biological Control Program or other state and federal agencies. These research or colonization projects may not be reported elsewhere in our annual summary. Below we list these projects, the agency primarily involved in the work, and a description of the project goals. This past year, we reared natural enemies for control of silverleaf whitefly, *Bemisia argentifolii* Perring and Bellows (Homoptera: Aleyrodidae), and cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae), and made the first field cage releases of a *Lygus* parasite, *Peristenus stygicus* Loan (Hymenoptera: Braconidae). In the case of *P. stygicus*, *Lygus* nymphs were parasitized in the lab and then released into the field. Adult *Peristenus* spp. were collected by D. Coutinot (USDA-ARS, France) and cleared through quarantine by L. Ertle (USDA-ARS, Newark, Delaware).

The DNA "banding patterns" reported below for whitefly parasites are from a PCR fingerprinting technique developed by the USDA-APHIS, PPQ, Plant Protection Center at Mission, Texas. The patterns are considered unique to strains or species of parasites that have not been described or identified using traditional morphological techniques.

1998 Releases of Natural Enemies by CDFA's Biological Control Program

| Natural Enemy | Host | DNA banding pattern | Agency Receiving Shipments | Project description | Stage delivered | Total insects delivered |
|---|---|---|---|---|---|---|
| *Eretmocerus* M95104 (United Arab Emirates) | silverleaf whitefly | ERET-12 | CDFA, Imperial Co. | field, urban releases | pupae | 1,020,000 |
| | | ERET-12 | USDA-APHIS-PPQ, Imperial Co. | augmentation studies in Imperial County | pupae | 463,800 |
| | | ERET-12 | USDA-APHIS-PPQ, Mission, Texas | cultures | pupae | 500 |
| | | ERET-12 | USDA-ARS, Shafter | control whiteflies in greenhouse | pupae | 8,000 |
| | | ERET-12 | USDA-APHIS PPQ, Phoenix, AZ | field releases | adults & pupae | 437,000 |
| | | ERET-12 | CDFA, San Joaquin Valley | releases into citrus | pupae | 1,093,464 |
| *Aphelinus nr paramali* | cotton aphid | --- | USDA-ARS, Shafter/CDFA | open release | adults | 24,776 |
| | | | USDA-ARS, Shafter/CDFA | open release | pupae | 43,692 |
| | | | USDA-ARS, Shafter/CDFA | cage studies | adults | 2,950 |
| | | | Sanger, CA, Pumpkin | open release | adults | 5,500 |
| *Aphelinus gossypii* | cotton aphid | --- | USDA-ARS, Shafter/CDFA | open release | pupae | 8,787 |
| | | | USDA-ARS, Shafter/CDFA | cage study | adults | 2,950 |
| | | | USDA-ARS, Shafter/CDFA | open release | adult | 24,125 |
| | | | Sanger, CA pumpkin | open releases | adults | 8,102 |
| | | | Sanger, CA pumpkin | open releases | pupae | 7,308 |
| *Peristenus stygicus* | Lygus | --- | CDFA, Sacramento, North B St. facility | cage, releases | larvae | 1,150 |

## Long Term Evaluation of the Ash Whitefly Parasitoid, *Encarsia inaron*

C. H. Pickett and R. Wall

The ash whitefly, *Siphoninus phillyreae* (Haliday) (Homoptera: Aleyrodidae), invaded southern California in 1988. Populations rapidly spread throughout the state, infesting ornamental street trees commonly planted by city governments. Clouds of adult whiteflies in urban centers were common. The importation of a single species of wasp, *Encarsia inaron* (Walker) (Hymenoptera: Aphelinidae), reduced their populations to levels difficult to detect. Despite its dramatic success, there is some question as to the stability and long term behavior of the host-parasitoid relationship. The balance in a host-parasitoid system is achieved through the density dependent response of the parasitoid to increase in host abundance. Theoretical population models have shown that if there is a delay in the response by the parasitoid to the build-up of its host, the host density will be forced to very low values, then gradually increase with time until the system has stabilized. The ash whitefly densities may therefore stabilize at greater densities than currently observed. Secondly, the impact of local climate may disrupt the stability of this system. Extreme climatic conditions such as a prolonged cold winter, may allow the host to reproduce unaffected by parasitoid-induced mortality for several generations. The ash whitefly would have wider fluctuations in abundance than those where mortality is consistent.

We designed a study to characterize the long-term population dynamics of ash whitefly and *E. inaron* among several geographic and climatic areas of central and northern California. Original release trees in Contra Costa, El Dorado, Madera, Sacramento, Shasta, and Yolo counties have been monitored since 1993 for ash whitefly abundance and percentage parasitism. At four release trees per county, we sampled 24 trees consisting of 14 ash, 5 pomegranate, and 5 ornamental pear trees. All sites were visited once over a two week period beginning in mid-July. We chose this time since it corresponded with the peak number of ash whitefly recorded in our earlier, state-wide study. The abundance of whiteflies was recorded from 30 leaves selected arbitrarily within arms reach from the lower canopy of each tree. These were examined under a microscope and all ash whitefly eggs, nymphs and pupae were counted. The impact of the parasitoid was estimated by removing 10 to 15 leaves with ash whitefly $4^{th}$ instar nymphs or pupae from each tree and returning them to the laboratory. As many as 3 nymphs or pupae were removed per infested leaf and dissected to determine parasitism. A total of 30 ash whitefly immatures were dissected from each tree.

Figure 1 shows the ash whitefly abundance and parasitism levels. Data from the first three years, 1990 – 1992, were collected as part of a statewide parasitoid release/establishment effort (Pickett et al. 1996) and thereafter as part of a long-term study. Populations of ash whitefly peaked in 1991 at 13 per $cm^2$. Since 1993 the population has not exceeded 0.26 individuals per $cm^2$ leaf, 50 times below the highest value recorded in the absence of *E. inaron*. Seasonally averaged parasitism peaked in 1992, just two years after most parasites were released. The single yearly sample since that time has varied from 8.6% to 58.4% parasitism. Values often reached 100% at some sites (Table 1). Scientists at the University of California, Riverside also released a whitefly specific predator, *Clitostethus arcuatus* (Rossi) (Coleoptera: Coccinellidae), about the same time at a number of locations in northern California outside of our release sites. It has been

recovered each year since we began sampling in 1994 at our own sites, but has been sporadic in its presence on sample trees varying from 25 to 44.7% of those sampled. Generalist predators, and even the specialized ones like *C. arcuatus*, most likely have little impact on low densities of ash whitefly. The results from earlier field studies (Gould et al. 1992, Pickett et al. 1996), the persistent populations of *E. inaron*, and a significant correlation between host and parasitoid populations (r= 0.24, p=0.005; Fig. 1) indicate this parasitoid is primarily responsible for maintaining low population densities of ash whitefly since its introduction.

Fig. 1. Densities of ash whitefly and percent parasitism by Encarsia inaron in California.

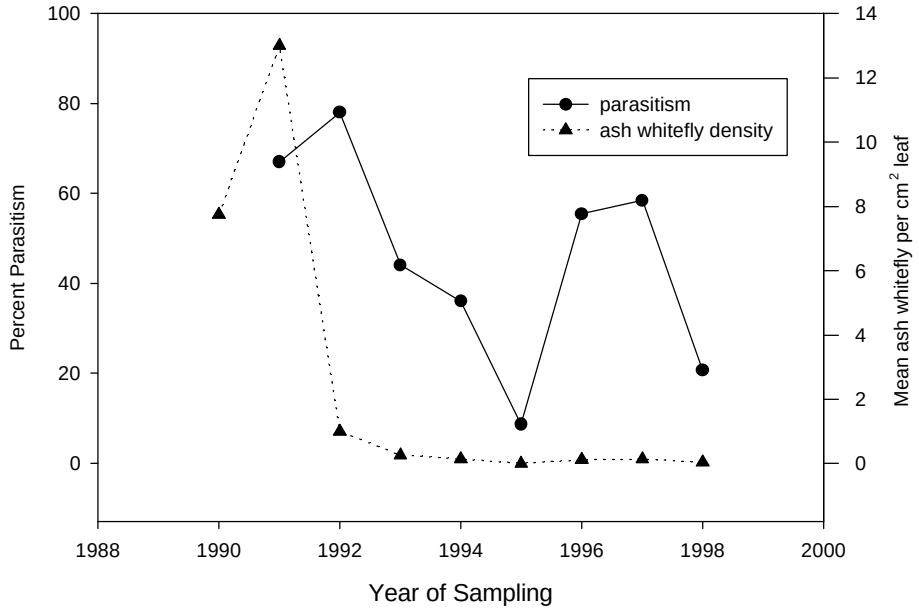

Table 1. Ash whitefly abundance on leaves, parasitism by *Encarsia inaron*, and percentage of sampled trees with *Clitostethus arcuatus* present in California.

| YEAR | MEAN AWF/CM$^2$ (RANGE), N | %PARASITISM (RANGE), N | % sites with *C. arcuatus* (n) |
|---|---|---|---|
| 1990 [a] | 7.74 (0 – 24), 54 | ---- | --- |
| 1991 [a] | 13.06 (0 – 84), 243 | 67.0 (not available), 77 | --- |
| 1992 [a] | 1.04 (0 – 24), 300 | 78.0 (not available), 53 | --- |
| 1993 | 0.26 (0 – 1.85), 25 | 44.5 (0 – 96.7), 21 | --- |
| 1994 | 0.14 (0 – 0.98), 25 | 36.0 ( 0 – 84.2), 23 | 37.5 (8) |
| 1995 | 0.01 (0 – 0.10), 24 | 8.6 (0 – 55.0), 15 | 25.0 (12) |
| 1996 | 0.11 ( 0 – 1.10), 28 | 55.4 (0 – 100.0), 25 | 37.5 (8) |
| 1997 | 0.14 (0 – 0.73), 28 | 58.4 (0 – 100.0), 25 | 44.7 (16) |
| 1998 | 0.04 ( 0 – 0.33), 28 | 20.6 (0 – 75.0), 21 | 25.0 (16) |

[a]From Pickett et al. 1996

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## Multi-Year Survey of Parasites of Native Gracillariidae: A Possible Source of Parasites for the Citrus Leafminer

K. Godfrey, J. Heraty[1], N. Smith[2], and D. Haines[3]

Citrus leafminer (*Phyllocnistis citrella* Stainton) (Lepidoptera: Gracillariidae) is a small lepidopterous pest that is poised to invade California citrus. Currently, citrus leafminer can be found in Florida, Alabama, Louisiana, Texas, Mexico, and southward through Central America. Within a year of its detection in Florida, native parasites belonging to the genera *Pnigalio*, *Sympiesis*, *Zagrammosoma*, *Closterocerus*, *Horismenus*, and *Elasmus* (Hymenoptera: Eulophidae) were found attacking citrus leafminer. These native parasites moved from their native hosts (other leafmining insects including Lepidoptera and Diptera) to the citrus leafminer. In California, there are representatives of each of these genera attacking various native leafminers. It is possible that once the citrus leafminer enters California some of these native parasites will move from their native hosts to citrus leafminer just as their counterparts did in Florida. Therefore, a survey initiated in 1996 was continued through 1998 to identify the parasites attacking native gracillariids in Fresno and Tulare counties.

The native parasites attacking gracillariid leafminers were surveyed at four sites, two in Fresno County and two in Tulare County. Each site had a stand of oak located near a citrus grove and a stream or waterway. Samples were collected from spring until late fall. During 1998, the monthly sampling began on April 7, 1998 and continued until November 3, 1998. In Fresno County, blue oak (*Quercus douglasi*) and interior live oak (*Q. wislizenii*) were sampled at both sites. In Tulare County, blue oak was sampled at one site, and interior live oak, at the other site. At each site on each sampling date, leaves containing mines were collected. The leaves were then held individually at 25°C for the emergence of the parasite or leafminer adult.

In all three years of the survey, parasites and gracillariid adults were recovered. The results are summarized in Table 1. For all 3 years of the survey, only a few parasites and adult gracillariids were recovered. For those parasites that have been identified, most belong to the family Eulophidae (*Euderus*, *Zagrammosoma*, *Sympiesis*, and *Chrysonotomyia*), primary parasites of leafminers. The remaining parasites represent the families Encyrtidae (*Paraleurocerus*) and Pteromalidae. Identifications of the remaining parasites and gracillariids are pending. The results of this survey demonstrate that two genera of parasites known to attack citrus leafminer in Florida are present in Fresno and Tulare Counties. This suggests that if citrus leafminer is found in northern California, parasites are already present that may attack it and be of some use in its management.

Table 1. The number of parasites and gracillariid adults recovered from sampling and the host plants sampled at sites in Fresno and Tulare counties from 1996 through 1998.

| Site | Sample Date | Host Plant | No. of Parasites[a] | No. of Gracillariidae[b] |
|---|---|---|---|---|
| 1996 | | | | |
| Fresno 1 | June 4 | Blue Oak | 1 *Euderus* sp. | - |
| Fresno 1 | June 4 | Interior Live Oak | 3 *Euderus* sp. | - |
| Fresno 1 | June 4 | Blue Oak | 3 *Zagrammosoma* | - |
| Fresno 2 | May 7 | Blue Oak | 1 *Sympiesis* | - |
| Fresno 2 | July 10 | Blue Oak | 1 Pteromalidae | - |
| Tulare 2 | November 11 | Interior Live Oak | 1 *Chrysonotomyia* | - |
| 1997 | | | | |
| Fresno 1 | May 15 | Blue Oak | - | 1 |
| Fresno 2 | November 17 | Blue Oak | 1[b] | - |
| Tulare 2 | May 15 | Interior Live Oak | - | 1 |
| Tulare 2 | November 17 | Interior Live Oak | - | 1 |
| 1998 | | | | |
| Fresno 1 | June 24 | Blue Oak | - | 1 |
| Fresno 1 | October 1 | Blue Oak | 2[b] | - |
| Fresno 2 | June 24 | Blue Oak | 3[b] | - |
| Tulare 2 | June 24 | Interior Live Oak | 5 *Paraleurocerus* | - |
| Tulare 2 | August 18 | Interior Live Oak | - | 2 |

[a]Only genera are given for those parasites that have been identified. The entries for which species are known are as follows: *Zagrammosoma americanum* Girault, and *Sympiesis* near *marylandensis*.
[b]Identifications yet to be determined.

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## Releases of Two Species of *Galerucella* Leaf Beetles for the Biological Control of Purple Loosestrife in California

B. Villegas, D. B. Joley, L. Bezark and E. Coombs

Purple loosestrife, *Lythrum salicariae* L. (Lythraceae), an invasive weed of wetlands in the northern U.S., is limited to relatively small acreages in California. In 1996-1997, the Biological Control Program released *Hylobius transversovittatus* Goeze (Coleoptera: Curculionidae), a root boring weevil, and *Nanophyes marmoratus* (Goeze) (Coleoptera: Curculionidae), a flower-bud weevil, against this weed in Butte and Shasta Counties, but no recoveries have been made.

In 1998, two leaf-feeding beetles, *Galerucella calmariensis* L., and *G. pusilla* (Dufft.) (Coleoptera: Chrysomelidae) were approved for release in California. Approval for their introduction had been withheld in California due to concern that adult beetles could feed on crape myrtle foliage. It took two summers of additional greenhouse and field studies in Oregon to clarify the insect-host relationship and in spring 1998, the beetles were approved for release in California.

In 1998, a total of 7,500 beetles (both species) were obtained on three different dates and released at 16 sites in five counties in California (Table 1). The first lot, containing about 2,000 beetles, was obtained from Eric Coombs on May 14, and the beetles were released at ten sites in Butte, Nevada, Shasta, and Siskiyou Counties. The second lot, containing about 1,000 beetles, was released on July 7, by personnel from the United States Fish and Wildlife Service at the Tulelake National Wildlife Refuge in Siskiyou County. The last lot, containing approximately 4,500 beetles, was released on July 17, at eight sites in Butte, Nevada, and San Joaquin Counties. All beetles were obtained from the Baskett Slough National Wildlife Refuge, Salem, Oregon.

Table 1: Releases of the two *Galerucella* leaf feeding beetles on purple loosestrife in California

| County | Nearest City | Sites | Releases | Number Released | 1998 Recovery Notes |
|---|---|---|---|---|---|
| Butte | Oroville | 7 | 7 | 2,600 | Yes, 3 sites (First Generation) |
| Nevada | Grass Valley | 2 | 5 | 1,900 | Yes, 2 sites (First Generation) |
| San Joaquin | Lodi | 1 | 1 | 1,000 | No |
| Shasta | Fall River Mills | 3 | 3 | 600 | No |
| Siskiyou | Tulelake | 3 | 3 | 1,400 | No |

All release sites were surveyed shortly after release for evidence of establishment by the beetles. Egg masses, larvae and leaf feeding damage consistent with *G. calmariensis* and *G. pusilla* were noticed at three sites in Butte County and two sites in Nevada County where releases occurred in May 1998. Subsequent visits to the same five sites revealed first generation (F1) adults present until mid July, but larvae attributable to F1 adults could not be confirmed at all five sites. Egg and larval stages attributable to the beetles released in July were not confirmed. Additional releases and monitoring will continue in spring 1999.

[1]Oregon Department of Agriculture, Salem, Oregon

## Release of Knapweed Biological Control Agents on Purple Starthistle

D. M. Woods, B. Villegas and E. Coombs[1]

There are currently no approved classical biological control agents specifically selected for purple starthistle, *Centaurea calcitrapa* L. (Asteraceae). A few insects that were tested on purple starthistle as part of their pre-release evaluation for use on spotted and diffuse knapweed, however, were shown to accept it. We are attempting to establish some of these insects on purple starthistle in California. In spite of their acceptance of purple starthistle in laboratory testing, we cannot be sure that the insects will establish in the field or have an impact. The timing of their life cycles may be significantly different in the field and not coincide with purple starthistle development.

Two knapweed natural enemies were collected in Oregon during July for field release on July 17, 1998, in California. *Terellia virens* (Loew) (Diptera: Tephritidae), a small seedhead fly was available in small numbers, so only one release of 200 adults was made. The knapweed weevil, *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae) was collected in large numbers and released in Solano (1 release), Napa (2 releases), Marin (2 releases), and Sonoma (1 release) Counties. Four hundred adult weevils were released in each location.

On September 29, ten plants were collected from each site for evaluation of insect attack. Laboratory evaluation of a portion of the samples has been completed and at one site in Napa County, one adult *L. minutus* developed fully in one of the seedheads, and single adults were found in four heads at a site in Solano County. More sites may show evidence of infestation when all the samples are completed. Successful establishment will not be known until the natural enemies can maintain a population over two winters. Additional sites will receive releases in 1999.

[1]Oregon Department of Agriculture, Salem Oregon

**Release of Knapweed Biological Control Agents on Squarrose Knapweed, *Centaurea squarrosa***

D. M. Woods, B. Villegas and E. Coombs[1]

Squarrose knapweed, *Centaurea squarrosa* Willd (Asteraceae), has not been a direct target for classical biological control. It is, however, closely related to both diffuse and spotted knapweeds which have been direct targets. Some, but not all, of the biological control insects approved for release on spotted or diffuse knapweed were tested on squarrose knapweed during the pre-release host-testing phase. Since both California and Utah have substantial populations of this noxious weed, and the weed is a substantial threat to other states, we have initiated efforts to test whether available knapweed biological control insects can establish and impact the large infestations of squarrose knapweed in California.

The knapweed gall flies, *Urophora quadrifasciata* Meigen and *Urophora affinis* Frauenfield (Diptera: Tephritidae) appear to have immigrated into California from Oregon and are established on squarrose knapweed in at least one site. We have attempted releases of three other natural enemies within the state. In 1996, *Bangasternus fausti* (Reitter) (Coleoptera: Curculionidae) and *Cyphocleonus achates* (Fahraeus) (Coleoptera: Curculionidae) were released at a large infestation near Hawkinsville in Siskiyou County. During 1997, additional collections of *B. fausti* as well as *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae) were released at several sites within the same infestation. During 1998, releases of *B. fausti*, *L. minutus*, *Sphenoptera jugoslavica* Obenberger (Coleoptera: Buprestidae), *U. quadrifasciata* and *U. affinis* were made at a site in Lassen County near Pittville. Adult insects were collected in Oregon then field released in July. During field examinations on October 8, adult weevils of both *B. fausti* and *L. minutus* as well as damage consistent with attack were found. Field samples were collected from both sites and evaluated in the laboratory.

A total of 1,505 heads from the Hawkinsville site were dissected and rated for insect attack (Table 1). This site is extremely arid and has a short growing season. The gall fly *U. quadrifasciata* is well established at this site but has yet to increase to population levels that could significantly impact seed production. The other gall fly, *U. affinis*, remained at low levels this year. Very few galls have been found in the heads, but adults have been successfully reared from mass collected heads. Eggs of the weevil, *B. fausti* were not found in the field at this site, but the weevil is clearly established. Infestation levels reported in Table 1 are based primarily on detection of pupal chambers, but two adult weevils were found in mature heads this year. Damage consistent with attack by *L. minutus* was found for the second year in only 2 heads, but no larva or adults were found. Infestation rates generally were low for all agents suggesting squarrose knapweed may be a poor host for these insect species.

Infestation rates for the Pittville site (Table 1) were based on 604 seedheads collected near the release sites of *L. minutus* and *B. fausti*. Since these samples do not represent over-wintering capability we cannot say that the weevils are successfully established at this location yet. During field evaluations on October 8, 1998, newly emerged adults of both species could be found indicating that the life cycle could be completed at this site. Additionally, eggs on *B. fausti* were

common near the release site. Adults and pupal chambers of both weevils were found in laboratory processed samples. This site is cooler and better timed with the source site in Oregon for the insects. Additional releases are planned in Northern California at similar sites to establish these weevils on squarrose knapweed.

Table 1. Biological control insects released on squarrose knapweed in California

| Biocontrol Agent | Year Released | Status October 1996 | Status October 1997 | Status October 1998 |
|---|---|---|---|---|
| Hawkinsville | | | | |
| *U. quadrifasciata* | immigrated by 1990 | 1.4% | 25.9% | 12.8% |
| *U. affinis* | immigrated by 1995 | <0.1% | 3.7% | 0.1% |
| *Cyphocleonus achates* | 1995 | No recoveries | No recoveries | No recoveries |
| *Bangasternus fausti* | 1996 and 1997 | No recoveries | 2.8% | 0.5% |
| *Larinus minutus* | 1997 | NA | 0.1% | 0.1% |
| Pittville | | | | |
| *U. quadrifasciata* | 1998 | NA | NA | 2.9% |
| *U. affinis* | 1998 | NA | NA | No recoveries |
| *Bangasternus fausti* | 1998 | NA | NA | 0.5% |
| *Larinus minutus* | 1998 | NA | NA | 8.7% |
| *S. jugoslavica* | 1998 | NA | NA | No recoveries |

[1]Oregon Department of Agriculture, Salem Oregon

## Releases of the Bull Thistle Gall Fly, *Urophora stylata* on Bull Thistle in California

B. Villegas and E. Coombs[1]

Bull thistle, *Cirsium vulgare* (Savi) (Asteraceae), is a widespread exotic biennial weed that is associated with a high degree of disturbance, such as overgrazed permanent pasture and woodland clearings. The bull thistle gall fly, *Urophora stylata* (Fabricius) (Diptera: Tephritidae), is a host specific biological control agent introduced from Europe for the biological control of bull thistle. The gall fly has one generation per year. Adult flies emerge from overwintering seedhead galls from late May through early July and may live for several weeks. The flies lay their eggs on top of developing flower buds and the eggs hatch after about one week. After hatching, the larvae migrate to the receptacle where they induce gall tissue formation. Multi-chambered galls form on the receptacle of the seedhead with individual larvae occupying separate chambers. Each gall may contain up to 20 larvae. Seedheads infested with the gall flies produce less seed due to the limited amount of receptacle area for seed production.

The bull thistle gall fly was released in California in 1993-1995 at ten sites in El Dorado, Marin, Mendocino, Modoc, San Luis Obispo, Shasta, and Tulare Counties by the USDA-ARS, Biological Control of Weeds Laboratory, University of California (Riverside), and the US Fish and Wildlife Service (Modoc County). With the exception of two sites in Marin County, none of the gall flies became established. In 1997, the Biological Control Program started a second series of introductions in order to determine the type of habitats that the gall fly prefers for establishment in California. Bull thistle seedheads infested with the gall fly were mass collected from central Oregon and transported to Sacramento for subsequent rearing in sleeve cages. The infested seedheads were kept refrigerated until release sites containing the appropriate seedhead stages could be secured. In 1997, the emphasis was placed on securing coastal sites influenced by fog. These types of sites were given higher priority as previous releases of the flies at higher elevation areas as those in the Sierra Nevada and at hot dry sites such as those found in central northern California failed to establish. In 1998, site selection was expanded to include coastal as well as sites in central California not influenced by marine weather. Multiple sites in the same general area were favored in order to try different release techniques.

In 1997, a total of 1,210 flies were released in Humboldt and Marin Counties in late July and early August. Approximately 660 flies were released at two sites in Humboldt and 550 flies were released at two sites in Marin County (Table 1). In 1998, releases of the gall flies were made using two different techniques. The first technique involved separating 150 infested bull thistle seedheads, placing them in10 lb. empty orange bags, and shipping them to cooperators in Humboldt, Marin, San Joaquin, and Tulare Counties for deployment at one site in each of the four counties. The bags containing the infested seedheads were hung near a bull thistle infestation and the flies were allowed to emerge from the seedheads and fly to the nearby host. The second release method involved direct release of the adult flies emerging in sleeve cages in laboratory conditions. These flies were either shipped overnight to cooperators or transported to release sites by Biological Control Program personnel. In 1998, a total of 2,235 flies were released at nine sites in Humboldt, Marin, San Joaquin, and Tulare Counties. It is unknown how many flies emerged

from the orange bags containing the 150 infested bull thistle seedheads but based on laboratory tests it is estimated that at least 600 flies emerged.

All 1997 and 1998 release sites were surveyed for establishment of the bull thistle gall fly during the fall and winter. Recoveries were made at all sites where bags of infested seedheads were deployed. Recoveries were much better at moderately to heavily infested sites. The best recoveries took place at the two 1997 release sites in Marin County. At these sites the flies were recovered at least 200 yards from the actual point of release. In Humboldt County, recoveries at the 1997 release sites were low, but this might have been due to an active thistle control program by the property owners. Recoveries of the bull thistle gall flies were made at all but two of the 1998 release sites. These two sites were severely impacted by weed control strategies and by cattle gaining access to the pasture where the release took place.

It is still too early to make conclusions regarding the suitability of release sites as each site needs to surveyed for at least two years following release in order to insure survival of the flies as well as population growth and dispersal from the original sites. However, based the evaluations of the 1997 release sites, the fly is able to establish in northern California especially along coastal areas influenced by marine climates.

Table 1: Releases of the Bull Thistle Gall Fly, *Urophora stylata* Fabricius, in California in 1997-98.

| County | Nearest City | Year | # Flies | Release Method | Recovery? |
|---|---|---|---|---|---|
| Humboldt | Eureka #1 | 1997 | 330 | Adult flies | Weak recovery (1998) |
| | Eureka #2 | 1997 | 300 | Adult flies | Weak recovery (1998) |
| | Eureka #3 | 1998 | 600 | 150 seedheads | Recovered |
| | Eureka #4 | 1998 | 200 | Adult flies | Weak recovery |
| | Eureka #5 | 1998 | 100 | Adult flies | No recovery |
| | Blue Lake | 1998 | 250 | Adult flies | Recovered |
| Marin | Tomales #1a | 1995 | 234 | Adult flies | Weak Recovery (1996) |
| | Walker Creek #1a | 1995 | 300 | Adult flies | No recovery (1996) |
| | Tomales #1b | 1997 | 39 | Adult flies | Recovered (1997 & 98) |
| | Walker Creek #1b | 1997 | 250 | Adult flies | Recovered (1997 & 98) |
| | Tomales #2 | 1998 | 600 | 150 seedheads | Recovered |
| | Tomales #3 | 1998 | 300 | Adults | Recovered |
| | Tomales #4 | 1998 | 300 | Adults | Recovered |
| | Tomales #5 | 1998 | 130 | Adults | No recovery |
| San Joaquin | Stockton #1 | 1998 | 600 | 150 Seedheads | Recovered |
| | Stockton #2 | 1998 | 300 | Adults | Recovered |
| | Stockton #3 | 1998 | 300 | Adults | Recovered |
| Tulare | Success Valley #1 | 1998 | 600 | 150 Seedheads | Weak recovered |
| | Success Valley #2 | 1998 | 330 | Adults | Recovered |

[1]Oregon Department of Agriculture, Salem, Oregon

## Releases of *Nosema*-Free *Larinus curtus* for the Biological Control of Yellow Starthistle

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

The flower weevil, *Larinus curtus* (Hochhut) (Coleoptera: Curculionidae) was introduced from Greece into California in 1991 by the USDA, ARS, for the biological control of yellow starthistle, *Centaurea solstitialis* L. (Asteraceae). During 1991-1994, the weevil was released in Sutter, Amador, Yolo, Sonoma, and Placer Counties (Figure 1) as well as sites in Oregon and Washington with material shipped directly from Greece. Only in Sutter County is the weevil well established. In the other counties, it exists at very low population levels or failed to establish. The poor establishment in California may be due to infection by the protozoa, *Nosema* sp., which was discovered in the gut of weevils collected from Sutter County as well as other release sites in Oregon and Idaho. In contrast, *Nosema* sp. was not detected at sites in Oregon and Washington where *L. curtus* had built up large population densities.

In high numbers, *L. curtus* has potential to significantly impact yellow starthistle seed production and would be a valuable addition to the guild of insects introduced to control this weed in California. Thus, efforts to establish *Nosema*-free populations in California were initiated. Collections of weevils occurred in July 1997 and 1998 at a site near The Dalles in northern Oregon. Weevils from this site had been shown to be *Nosema*-free during earlier surveys. A small sample of weevils (20-30 weevils) from the 1997 and 1998 Oregon collections were examined by Dr. Bud Thomas of Consulting Diagnostic Service, a certified insect pathologist, to insure that these weevils had remained free of *Nosema*. All weevils from both collections were without detectable levels of *Nosema* sp.

In 1997, releases totaling 338 weevils were made at two sites in Placer and Santa Clara Counties. In 1998, a total of 3,025 weevils were made at 15 sites located in eleven counties in California (Figure 1; Table 1). Most of the sites chosen were in mountainous areas in order to try to match the same type of habitats where the weevils were collected in northern Oregon.

The 1997 release sites and most of the 1998 release sites were surveyed for establishment during 1998. At the 1997 Placer County release site, rapid population buildup of the flower weevils appears to have taken place. Exit holes, pupal cells and damage consistent with that of *L. curtus* was noted in seedheads beyond a 50 meter radius from the release point. Unfortunately, at the Santa Clara County 1997 release site, weevil damage was very low. Low recovery levels were also noted for all 1998 release sites surveyed. It should be noted that seedhead damage by *Eustenopus villosus* at several of the 1998 release sites made assessment of *L. curtus* damage difficult. All the sites will be surveyed in 1999 during the summer activity period (*L. curtus* adults) and again in the fall to determine a better way to monitor for this biological control agent.

[1]Oregon Department of Agriculture, Salem, Oregon

Table 1: Releases and Recoveries of *Larinus curtus* in California, 1997-1998.

| County | Nearest City | Number Released | Date | Status |
|---|---|---|---|---|
| Glenn | Black Butte | 200 | 7/16/98 | Light Recovery |
| Kern | Lebec | 200 | 7/16/98 | Not surveyed |
| Kern | Tehachapi | 200 | 7/17/98 | Not surveyed |
| Lassen | Pittville | 200 | 7/16/98 | No Recovery |
| Monterey | Jolon | 200 | 7/16/98 | Light Recovery |
| Placer | Granite Bay | 200 | 7/15/98 | Recovered |
| Plumas | Quincy | 180 | 7/20/98 | Not surveyed |
| San Benito | Hollister | 225 | 7/16/98 | Not surveyed |
| Santa Clara | San Jose | 200 | 7/16/98 | Light Recovery |
| Santa Clara | San Jose | 158 | 7/21/98 | Light Recovery |
| Shasta | Oak Run | 200 | 7/16/98 | Light Recovery |
| Shasta | Fall River Mills | 200 | 7/16/98 | Light Recovery |
| Shasta | Round Mountain | 200 | 7/16/98 | No Recovery |
| Siskiyou | Hornbrook | 200 | 7/16/98 | Light Recovery |
| Siskiyou | Weed | 200 | 7/16/98 | Light Recovery |
| Tehama | Payne Creek | 200 | 7/16/98 | No Recovery |
| Tulare | Porterville | 200 | 7/16/98 | No Recovery |
| | | Total =3,363 | | |

## Figure 1: Releases of Larinus curtus weevils in California in 1992-1998

## Biological Control Program, CDFA

## Weed Biological Control Workshops for 1998

B. Villegas

The Biological Control Program maintains a distribution program for weed biological control agents through the active participation of biologists from county agriculture departments and other State and federal agencies. The distribution program operates primarily through a series of workshops conducted at field nursery sites or at centralized locations by the Program. These workshops are designed to train the participants in the identification, collection and release of newly established biological control agents. Based on the training at the workshop, the county biologists field collect available biological control agents, then return to their own county and attempt to establish their own nursery sites for further distribution.

The original intention of this program was to assist in technology transfer from CDFA to the county biologists in order to expedite the distribution of newly introduced biological control agents throughout the State. Beginning in 1997, personnel from other public agencies have been invited to participate in the distribution program. In 1998, biologists and weed control personnel from California State Parks and U.S. Department of Interior participated in the workshops.

Seven workshops were held during 1998 for the distribution of biological control agents (Table 1). All workshops were devoted to the distribution of the hairy weevil, *Eustenopus villosus* (Boheman). *Bangasternus orientalis* (Capiomont) and *Urophora sirunaseva* (Hering) are already widely established.

Table 1: Workshops held in 1998 for the Distribution of Yellow Starthistle Biological Control Agents

| WORKSHOP | LOCATION | DATE |
|---|---|---|
| Tulare County | Lindsay, CA | June 16, 1998 |
| Sacramento County | Folsom, CA | June 22, 1998 |
| Sacramento County | Folsom, CA | June 23, 1998 |
| Sacramento County | Folsom, CA | June 24, 1998 |
| Sacramento County | Folsom, CA | July 1, 1998 |
| Shasta County | Redding, CA | June 25, 1998 |
| Shasta County | Redding, CA | July 1, 1998 |

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

B. Villegas

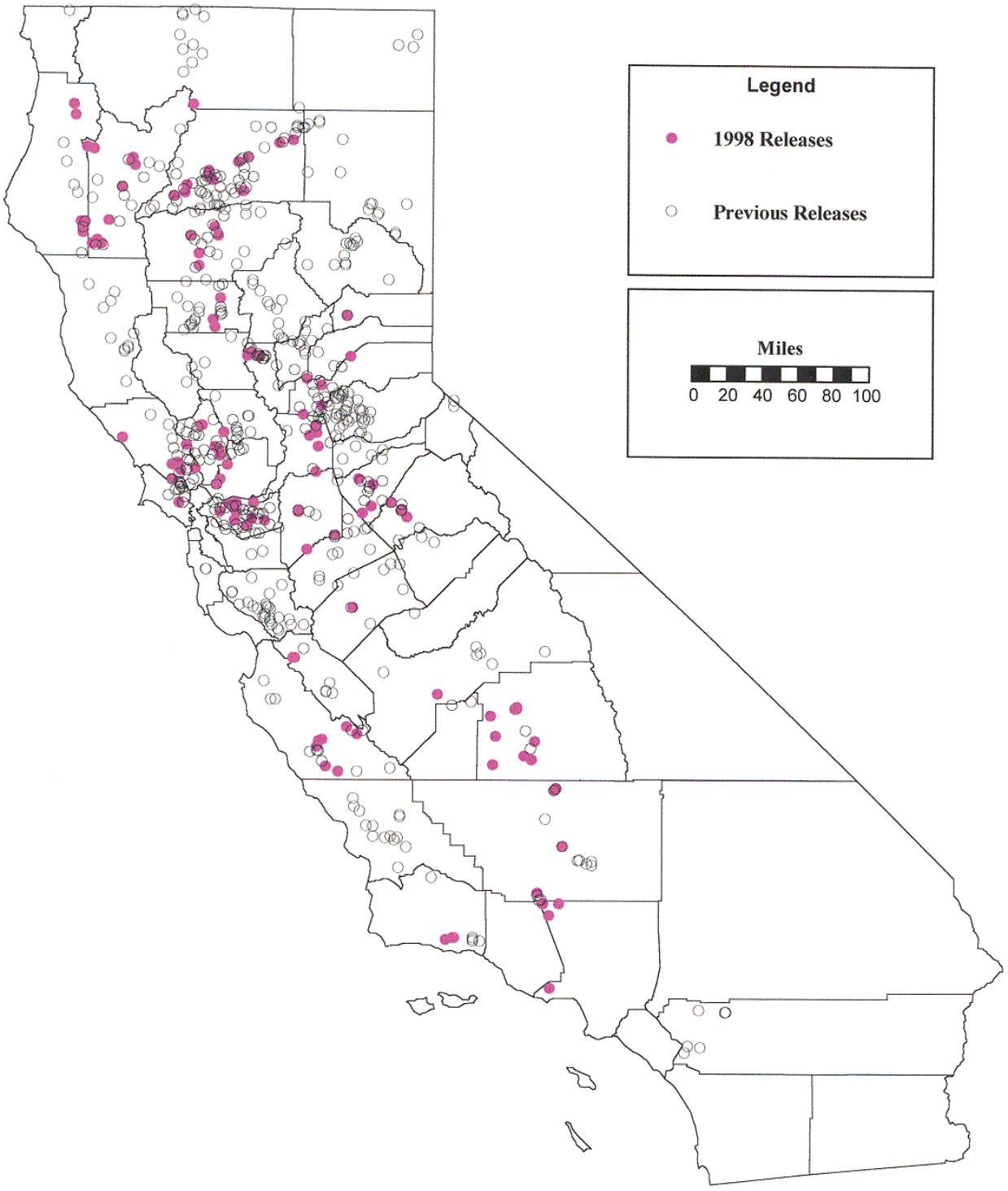
The hairy weevil, *Eustenopus villosus* (Boheman) (Coleoptera: Curculionidae), was introduced from Greece for the biological control of yellow starthistle, *Centaurea solstitialis* L. (Asteraceae). The USDA-ARS, in cooperation with the Biological Control Program and the County Agricultural Commissioners, established the first colonies 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 in 1992 that two small collections were made at one of the sites and moved to two lower elevation sites around Folsom Lake in Sacramento and Placer counties. The following year the Nevada and El Dorado County sites served as sources of weevils for 12 releases (ten counties). From 1994 through 1998, this distribution program rapidly expanded with some 206,372 hairy weevils released at over 650 sites in 49 counties in California (Figure 1).

In 1998, distribution workshops were held in Tulare, Sacramento, and Shasta. From these sites a total of 18,621 hairy weevils were collected and released at 59 locations in 14 counties. Research projects dealing with integrated control methods of yellow starthistle took place in Merced, Monterey, Sacramento, and San Benito Counties. Some 28,673 weevils were released at these four research sites.

Within county distributions increased during 1998 with more counties having productive nursery sites. Twelve counties (Glenn, Monterey, Napa, Placer, Sacramento, Shasta, Plumas, Sonoma, Sutter, Tehama, Trinity and Shasta) made their own in-county releases totaling 17,970 weevils from one or more county nursery sites. Other counties having county nursery sites that are available for in-county release collections include Amador, Butte, Calaveras, El Dorado, Fresno, Marin, Merced, Nevada, San Mateo, Sierra, and Siskiyou Counties. Of the 49 counties that have received releases, at least half have at least one nursery site that could be used as a nursery site for in-county redistributions.

**Figure 1: Releases of Eustenopus villosus Weevils in California in 1990-98**

**Biological Control Program, CDFA**



**Detection of the Rust Disease, *Puccinia carduorum*, on Musk Thistle in California**

D. M. Woods, M. J. Pitcairn, W. L. Bruckart[1], and D. G. Luster[1]

*Puccinia carduorum* Jacky, originally collected in Turkey in 1978, was introduced into the eastern United States as a potential biological control agent for musk thistle, *Carduus nutans* L. (Asteraceae). The rust underwent extensive host specificity testing by USDA-ARS in quarantine facilities at Frederick, Maryland. The rust was field released in Montgomery County, Virginia, from 1987-90, in a series of field experiments. The pathogen has been spreading across the United States on musk thistle since these original releases. By the summer of 1992, it had spread westward as far as 580 km from the release site, and during 1997 was found in Oklahoma.

On September 22, 1998, we detected rusted musk thistle plants on the shoulder of Mt. Shasta in Northern California. The majority of the plants at the site had matured, desiccated and lost most of their leaves, so infestation levels of the rust could not be assessed. A few plants still had green leaves and stems, and these were heavily infected with the rust. The rust was identified as *Puccinia carduorum*. There are previous records of a strain of *Puccinia carduorum* occurring on *Carduus tenuiflorus*, slenderflower thistle in California. However, laboratory testing of the *C. tenuiflorus* biotype has shown that it is incapable of infecting musk thistle. A region of DNA sequence identity in the ITS2 of *P. carduorum* permits us to distinguish the musk thistle biotype from the morphologically similar slenderflower thistle strain of *P. carduorum*. Teliospores of *P. carduorum* collected from musk thistle plants at the Mt. Shasta site were found to contain the same ITS2 sequence as that from the Turkish isolate originally released in Virginia. Thus it appears that the exotic musk thistle rust isolate has traveled unaided across the continent.

[1]USDA,ARS, Foreign Disease-Weed Science Research, Ft. Detrick, Maryland

## Sicilian Starthistle and Tocalote as Potential Hosts to Yellow Starthistle Biological Control Insects

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

Biological control of weeds has an excellent record for safety, particularly in recent years. Extensive host specificity testing prior to release is designed to delineate the potential host range of prospective biological control agents. Consequently, the field host range is not expected to be a surprise. Although not all plants are tested in advance, sufficient species are tested to anticipate a logical host range. For most biological control agents, this is limited to a few closely related species. Post release monitoring can be used to confirm the anticipated host range and the continued safety to other plant species.

Sicilian starthistle, *Centaurea sulphurea* Willd., and Tocalote (=Napa thistle), *Centaurea melitensis* L., are two exotic weeds that might be expected to be hosts to yellow starthistle biological control agents. Both are closely related to yellow starthistle, are similar in appearance and share a portion of their range. Consequently, if the yellow starthistle biological control agents have the potential to attack these closely related species we may expect some level of control. None of the yellow starthistle insects were tested on either Sicilian starthistle or tocalote during pre-release host testing. Establishment on these weeds, however, may actually be advantageous as these weeds are not likely to become targets of direct biological control introductions.

Field evaluation was initiated at two sites to investigate the potential of tocalote and Sicilian starthistle to serve as hosts to yellow starthistle biological control agents. The tocalote site is near Lindsey in Tulare County, and the Sicilian site is near Folsom in Sacramento County. Both sites are near infestations of yellow starthistle with established biological control insects. *Eustenopus villosus* (Boheman) is the only biological control insect established at the Lindsay site where yellow starthistle and tocalote grow somewhat intermixed. Four insects, *E. villosus*, *Bangasternus orientalis* (Capiomont), *Urophora sirunaseva* (Hering) and *Chaetorellia succinea* (Costa) are established on yellow starthistle near the Sicilian starthistle site in Folsom. On June 2, 1998, *E. villosus* weevils were noticed climbing on the tocalote in Lindsay in high numbers. Thirty complete tocalote plants were field collected on June 16, then examined in the laboratory for evidence of attack by *E. villosus*. An additional 55 plants were selected for seed destruction evaluations. A single mature flower head that had just completed pollination but had not shed florets or seed was selected on each plant. Small cotton bags were used to enclose the heads, confining all developing seeds as well as insects developing within them. At the end of the summer, the bags were collected for laboratory evaluation. Monitoring of Sicilian starthistle began on May 19. Forty flower heads were bagged each week for nine weeks, collected 3 weeks after the last bagging date and evaluated for evidence of attack. All four yellow starthistle insects were observed on or around Sicilian starthistle at this plot.

*Lasioderma haemorrhoidale* (Illiger) (Coleoptera: Anobiidae), an accidentally introduced stored product pest, has been found on yellow starthistle throughout the state. Adults are present on yellow starthistle with the earliest flowers in the spring. Larvae and adults seem to feed

extensively on the mature seeds and seedhead material. The beetle is described here for the first time occurring on both *C. melitensis* and *C. sulphurea*.

Adult *E. villosus* weevils feed on developing buds of yellow starthistle. This has an important effect on seed production, as these buds die and no longer contribute to seed production. Apparently, *E. villosus* can have a similar effect on young buds of tocalote. On the 30 complete plants collected June 16, there were 133 dead buds, 81% (108) of which had clear evidence of feeding damage. That left 113 buds capable of seed production. There were 54 full size buds with green bracts. These would be similar to buds that *E. villosus* selects for oviposition in yellow starthistle. Of these, 61% (33) had evidence of attack by adult weevils. Evidence entailed either an oviposition wound or dark hard callus tissue developing inside the seedhead at the point of oviposition or feeding. A single dead larva was found in one head. There were 59 straw-colored, mature seedheads that had shed seed. Only 20% (12) of these had evidence of attack. There were no heads with pupal cells or adult emergence.

The bagged seedheads averaged 7.51mm in external diameter and produced an average of 21.2 seeds per head. Oviposition or feeding wounds were found in 62% of these heads, much like the rate observed on the non-bagged heads in the June 16 samples. Some insect development was supported in 5.4% of the heads (=3 heads, 2 with dead larvae and 1 with a dead pupae). Heads that had oviposition wounds produced only 76% of the seed that non-attacked seedheads did (Table 1). For the three heads where a larva tried to develop, seed destruction increased 16%. Again, there were no heads with pupal cells or adult emergence. The anobiid, *L. haemorrhoidale*, infested only 2 heads, but these produced 37% of the average number of seeds produced by the clean heads. Tocalote does not seem to be a good host for either *L. haemorrhoidale* or *E. villosus*. The presence of *E. villosus* on tocalote in the field and the relatively high oviposition rate provides evidence that *E. villosus* finds this an attractive host. The inability to support insect development, however, suggests that this host is unsuitable.

Sicilian starthistle has very large seedheads and very large seeds, but from a distance looks much like yellow starthistle. Although several biological control agents were reasonably common in the area, they do not seem to find Sicilian a satisfactory host. Only 3% of the heads were fed or oviposited on by *E. villosus*. The feeding or oviposition wounds and resultant callus tissue resulted in seed reduction of 70%. Two heads late in the year supported development to a pupal stage. The pupae both died, but seed production in these heads was reduced 74%. Sicilian starthistle does not seem to be either an attractive host or an acceptable one for *E. villosus*. The anobiid, *L. haemorrhoidale,* however, was fairly successful on this host, infesting 19% of the heads (primarily early season) and causing a 72% seed reduction in those heads. *C. succinea* attacked only 2% of the heads, but did reach maturity in 5 of the 6 heads. Multiple adult flies were reared from 3 of these heads. This aggregation tendency by *C. succinea* is common in yellow starthistle and increases the level of seed destruction. The large seedheads of Sicilian starthistle (average = 13.2 mm) can easily support multiple larvae. A large proportion of the seed (75%) was destroyed by *C. succinea*.

In general, results from the pre-release host specificity testing were supported by the field evaluations. Two insects, *B. orientalis*, and *U. sirunaseva*, although locally common, gave no evidence of attack on Sicilian starthistle. Neither insect was present near the tocalote site. The hairy weevil, *E. villosus*, did find both hosts somewhat attractive but was unable to complete development on either species. *C. succinea* is an accidentally introduced natural enemy and did not undergo host specificity testing. Consequently it is not surprising that *C. succinea* completed development on Sicilian starthistle in comparison to agents that receive full screening to eliminate the broader host range candidates. Sicilian starthistle is not a preferred host for *C. succinea* as it's attack rate and successful development is at a much lower rate than in nearby yellow starthistle. *L. haemorrhoidale* is less specific in its feeding habit as evidenced by successful development on both weeds. Finally, both *C. sulphurea* and *C. melitensis* flower and mature earlier than yellow starthistle, so may escape peak emergence of the bioagents.

Table 1. Summary of yellow starthistle insect interactions *on C. sulphurea* and *C. melitensis*

| | Sicilian | Tocalote |
|---|---|---|
| Average diameter | 13.2 mm | 7.51mm |
| Average Seeds/head | 21.8 | 21.2 |
| Seed reduction due to; | | |
| *E. villosus* feeding or oviposition | 30% | 24% |
| *E. villosus* – larva/pupa | 74% | 40% |
| *L. haemorrhoidale* | 72% | 63% |
| *C. succinea* | 75% | - |
| Number of Seedheads | | |
| No damage | 261 (77%) | 21 (38%) |
| *E. villosus* feeding or oviposition | 10 (3%) | 34 (62%) |
| *E. villosus* – larva/pupa | 2 (1%) | 3 (5%) |
| *L. haemorrhoidale* | 64 (19%) | 2 (4%) |
| *C. succinea* | 6 (2%) | - |

**Increases in New Biological Control Agents on Diffuse Knapweed in Trinity County Offer Hope for the Eventual Control of this Weed**

D. B. Joley and D. M. Woods

Diffuse knapweed, *Centaurea diffusa* Lamarck (Asteraceae), occurs in California as single plants or in small patches, and is under eradication in most areas of the state except in Trinity County. The Biological Control Program has had an ongoing project to release available biocontrol agents on this weed in Trinity County since 1976.

Six biocontrol agents are established on diffuse knapweed in Trinity County *Urophora affinis* Frauenfield (Diptera: Tephritidae), first released in 1976, and two immigrants from Canada, , *Urophora quadrifasciata* (Meigen) (Diptera: Tephritidae), and the pathogen, *Puccinia jaceae* (Otth.) (Uredinales), appear to have minimal impact. The root beetle, *Sphenoptera jugoslavica* Obenberger (Coleoptera: Buprestidae), released in 1980, is well established, but also does not appear to significantly weaken plants. More recent introductions, *Bangasternus fausti* (Reitter) (Coleoptera: Curculionidae), released in 1994 and 1995, and *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae), released in 1995 and 1996, are well established and are beginning to disperse away from the original release sites.

In 1998, seedhead samples were collected from sites away from the original release sites to determine natural dispersal of the biocontrol agents (Table 1). These sites are distributed along two roads (Miller and Lemonade Spring) that run north and south, extending from near the top of a ridge down to the South Fork Trinity River. The primary monitoring site is located between the lower and upper Miller Road sites. Lemonade Spring Road runs parallel to Miller Road, approximately 0.5 mi. to the east. *S. jugoslavica* has extended its range from the 1980 release near the river, but does not appear to have made it to the area we designated as "Windy Hill" ridge area. *B. fausti* was first released at the primary monitoring site in 1994-95, then released at the lower Miller Road site in 1996, two miles away, but the level of infestation has not increased to that observed at the primary monitoring site. *L. minutus* was released at three sites along Miller Road (bottom, lower, and upper) and is increasing. Adults were observed at the primary monitoring site by 1997. Although it is too soon to determine which insect will dominate the area, *B. fausti* appears to be currently increasing at a higher rate. Nevertheless, *L. minutus* may play an important role in seed destruction as it appears to be the more mobile of the two weevils. Given the movement and buildup of the two weevils, it may not be necessary to move them to new sites. Nevertheless, the "Windy Hill" ridge area is currently undergoing a dramatic increase in knapweed density, and since it is easily accessible to the general public there is greater risk of accidental seed spread to new areas of the state. Therefore, adults of *B. fausti*, *L. minutus*, and *S. jugoslavica* will be moved to this site in 1999.

Four years of data on plant density and seed head attack (1995 through 1998) have been collected from the primary monitoring site. Several insect species are now present at this site and were evaluated, but primary focus was on the two weevils. Seedhead samples were collected in mid-autumn and the percentage infestation consisted of harvesting all or parts of 10 plants, removing and combining all seedheads, then dissecting and scoring a subsample of heads

(minimum 100) for insects or empty pupal cells under a microscope. Due to the similarity in damage caused by the two weevils, only total number of heads attacked are reported. Density estimates of reproductive knapweed plants were also made at the same time by counting all reproductive plants within a 0.25 $m^2$ frame placed at 15 contiguous locations along two permanent, parallel transects.

The percentage of heads attacked by the weevils at this site has increased significantly over the four years of monitoring (Table 2). The increase in the rate of attack in 1998 by both weevils was even more impressive than in 1997, but knapweed plant density has also increased over the same time period. Nevertheless, the continuing rapid and sustained increase in attack rates by the two weevils at this site provides some reason for optimism that enough seed destruction may yet occur to cause a decrease in knapweed density. The reason(s) for the recent sustained increases in plant density are unknown, but weather factors are suspected.

Table 1. Percentages of attack of seedheads by the weevils, *Bangasternus fausti* and *Larinus minutus* in 1998 at the primary monitoring site and at sites various distances away from the monitoring site.

| | Percent Attack | | | |
|---|---|---|---|---|
| Location | *S. jugoslavica* | *B. fausti* | *L. minutus* | Total Weevils[1] |
| "Windy Hill" Ridge | 0 | 0 | <1 | <1 |
| Miller Spring Road | | | | |
| Head | 0 | 1 | 0 | 1 |
| Upper | 20 | 0 | 32 | 35 |
| Primary Monitoring Site | 90 | 74 | 4 | 81 |
| Lower | 70 | 27 | 37 | 65 |
| Bottom | 100 | 11 | 49 | 63 |
| Lemonade Spring Road | | | | |
| Upper | 50 | <1 | 6 | 7 |
| Lower | 90 | 5 | 11 | 18 |

[1]Total weevils percentage is slightly elevated above that for the combined total of both weevils due to inability to always differentiate between *Bangasternus* and *Larinus* induced damage.

Table 2. Combined percentages of attack of seedheads by combined weevils *Bangasternus fausti*, released in 1994 and 1995, and *Larinus minutus*, released in 1995 and 1996, and density of reproductive diffuse knapweed plants at the primary monitoring site.

| Year | Weevil Attacked Heads (%) | Plants/$m^2$ (No.) |
|---|---|---|
| 1995 | 8 | 30 |
| 1996 | 23 | 39 |
| 1997 | 43 | 63 |
| 1998 | 81 | 75 |

**Seed Destruction by *Larinus minutus* in Spotted Knapweed,**

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

Spotted knapweed, *Centaurea maculosa* Lamarck (Asteraceae), is an invasive weed in much of the western United States including California. Several natural enemies have been imported into North America as biological controls of spotted knapweed. Five of these natural enemies have been intentionally released in California. Unfortunately, there is very little information about the impact these insects may have on spotted knapweed. In 1998, we initiated a field study to determine the amount of seed destruction attributable to the seed-feeding insects at a spotted knapweed infestation in the Big Bend area of Shasta County. One of the insects, *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae), established quickly at the site and appears to have a significant impact within infested seedheads. Adult weevils lay eggs in developing flower heads and the larvae consume most of the contents of the seedhead prior to pupation.

The impact of *L. minutus* on seed production was monitored at two sites approximately 1/4 mile apart along the Pit River. The east site received releases of *L. minutus* in 1995, and the west site in 1997. Maturing flower heads of spotted knapweed were enclosed with cotton bags when the florets had begun to oxidize following pollination to contain both the seeds and any bioagents that develop in the heads. Heads were bagged at two-week intervals over a five-week period, with no more than one flower head bagged on any one plant. Bags were left on the plants to mature for at least two weeks prior to removal of the seedhead from the plant. Seedheads were evaluated in the laboratory for evidence of insect attack and number of viable seed.

The potential seed production of spotted knapweed at this site was determined by collecting flowerheads in full bloom at each bagging date and counting the number of complete flowers in each seedhead. Complete flowers are distinct from the showy, sterile flowers that rim the flowerhead and act as pollinator attractants. Counting the number of complete flowers in a flowerhead provides an estimate of the potential for the plant to produce seed at a given site. While this number varied over the season, the average was just over 30 flowers per head (Table 1). Moisture, pollinators, attack by bioagents *Urophora affinis, Urophora quadrifasciata, Terellia virens,* and other factors combined at this site to reduce the potential seed production to 10.77 seeds per head for heads without attack by *L. minutus*. *L. minutus* further reduced seed production per head an additional 71 % to 3.09 seeds per head (Table 2). If populations of the weevil can be maintained at high levels over a large area, the long term impact could be substantial. The infestation rates at the two sites was significantly different, but was likely due to releases at the hill occurring two years later than those at the plot area. Hopefully, when both sites reach a maximum infestation rate, the impact on seed production will translate into reduced plant numbers.

TABLE 1. Flower and seed production of spotted knapweed in the presence of *L. minutus*. Values represent averages from the two sites.

| | | July 15, 1998 | July 30, 1998 | August 17, 1998 | Average |
|---|---|---|---|---|---|
| Complete flowers per seedhead (# evaluated) | Unbagged Flowerheads | 33.0 (19) | 26.9 (20) | 31.39 (33) | 30.57 |
| Seeds per seedhead without *L. minutus* | Bagged Seedheads | 8.37 | 13.73 | 9.40 | 10.77 |
| Seeds per seedhead with *L. minutus* | Bagged Seedheads | 2.15 | 3.85 | 3.81 | 3.09 |
| % Seedheads infested with *L. minutus* | Bagged Seedheads | 61% | 42% | 42% | 49% |

TABLE 2. Impact of *Larinus minutus* on spotted knapweed at two sites in Shasta County in 1998

| | West Site - Hill | East Site - Plot | Total |
|---|---|---|---|
| Seeds per seedhead without *L. minutus* | 12.38 | 5.53 | 10.77 |
| Seeds per seedhead with *L. minutus* | 4.80 | 2.62 | 3.09 |
| % Seedheads infested with *L. minutus* | 21% | 76% | 49% |
| Year released | 1997 | 1995 | --- |

**Field Studies to Examine Growth Habit and Population Resurgence of Scotch Thistle in Northern California**

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

Scotch thistle, *Onopordum acanthium* L. (Asteraceae), is a noxious, exotic weed in the western United States. Field infestations in California are aggressively treated with herbicides with the goal of eradication. Unfortunately, eradication will be difficult, due to the large number of infestations and the long-lived nature of the soil seed bank (20+ years by most estimates). Long-term control of Scotch thistle may eventually include the use of imported natural enemies and effort by the USDA-ARS is underway to evaluate several insects that have been cleared and released in Australia. In autumn 1996, we initiated field studies in northeastern California anticipating that biological control agents would be available in the next few years.

Two field sites were selected in Modoc County for research, one at the Modoc National Wildlife Refuge (U.S. Fish and Wildlife Service), near Alturas and the other at the Ash Creek State Wildlife Area (CA Department of Fish and Game) near Bieber. Scotch thistle infestations at both sites have experienced, several years of herbicide treatments and physical controls, so now consist of small patches or scattered plants. Refuge managers set aside a small area where treatment was to be withheld and plant density purposely allowed to resurge. The interim between the curtailment of spraying and release and impact of anticipated biocontrol agents was viewed as a unique opportunity to study Scotch thistle resurgence and to determine plant growth habit (annual, biennial, etc) in California.

In 1998, it became apparent that *Lixus cardui* Olivier (Coleoptera: Curculionidae), one of the most promising (damaging) insects, failed quarantine tests at the USDA–ARS quarantine laboratory at Albany and would not be released in the United States because adults fed and oviposited on, and larvae developed on, native *Cirsium* species. A second insect, *Trichosirocalus* n. sp. (Coleoptera: Curculionidae), a crown-mining weevil, is currently being evaluated in host specificity tests at Albany. Additional natural enemies identified by Australia may also be evaluated for potential use in the United States, however, additional foreign exploration will likely be necessary to find the right mix of species to control Scotch thistle in the United States.

Considering the likelihood of additional delays before safe and effective biological control agents are available for release, we decided in 1998 to curtail the portion of our studies involving the resurgence of Scotch thistle to avoid jeopardizing the progress made over the years to reduce or eliminate this weed at both sites. Therefore, once a plant began to bolt it was recorded as reproductive, then uprooted before it flowered and set seed. We also discontinued monitoring the seed bank after 1997. Therefore, the study is currently being focused on survivorship and growth habit of Scotch thistle in northeastern California.

**Scotch thistle seed bank:** Soil cores were collected at the two field sites in autumn 1996 and 1997 to examine Scotch thistle seed within the soil profile. Ten 1 $m^2$ grids, were located three meters apart, along each of ten transects (N=100 grids). Two cores (3cm diameter by 10cm depth) were collected at each grid. Each core was separated into two 5 cm (upper and lower)

portions and the two corresponding portions from each grid were combined and placed in separate, labeled zip-lock bags. The soil cores were dry sieved to extract Scotch thistle seeds. Only two seeds total were recovered in 1996 (both in upper core portions) at the Modoc site (= ca 28 seeds/$m^2$); no seeds were recovered at the Ash Creek site. In 1997, a total of 8 seeds were recovered at Modoc and 5 seeds were recovered at Ash Creek. Long-term chemical control of Scotch thistle at these sites appears to have reduced the seed bank greatly.

**Opportunistic herbivore survey:** Roots and seed heads of several flowering plants growing outside of the field plot were dissected in the field during October 1997, but there was no apparent damage by insects or diseases.

**Scotch thistle growth habit:** In 1997, all Scotch thistle plants within 300 one square meter quadrats (10 transects with 30 contiguous quadrats per transect) were identified with flagged wires at the Modoc and Ash Creek sites. The sites were visited in May, June, August, and October and all new seedlings (unmarked) were flagged and are identified as a cohort. On return visits flagged plants were inspected and recorded as dead, rosette, or bolting.

Modoc site #1

Only two Scotch thistle rosettes were found within the quadrats at the Modoc site on 16, October 1996. Both of these plants flowered and shed seeds during summer 1997. A total of 80 new plants were identified during the 8 May 1997 visit (Table 1). Although most of the 1997 seedlings emerged before 8 May 1997, seedling emergence continued over an extended period of time with a significant number (41) emerging during the warm summer months. Only 69 of the 121 plants emerging in 1997 remained alive on 23 June 1998. Of those, 41 had bolted, 20 died, and 8 remained as rosettes on 8 October 1998.

Plant growth habit varied both within and between cohorts. For example, the time required from emergence to flowering for the May cohort ranged from 14 plants bolting within 12 months to 2 plants remaining as rosettes for at least 17 months. Growth habit presumably will have significant effects on biocontrol agent impact. Plants flowering in less than one year from emergence are typically smaller and produce fewer seed heads, and may be more vulnerable to destruction by biological control agents than those continuing to develop vegetatively and flowering later.

Table 1 Scotch thistle growth habit; Modoc National Wildlife Reserve site

| Month of 1997 Cohort | Initial # of Plants | # Bolted In 1997 | # Bolted in 1998 | # Died 1997 | # Died 1998 | # Remaining Oct 1998 |
|---|---|---|---|---|---|---|
| May[1] | 80 | 0 | 35 | 15 | 28 | 2 |
| June[2] | 21 | 0 | 3 | 8 | 7 | 3 |
| August[3] | 19 | 0 | 3 | 1 | 13 | 2 |
| October[4] | 1 | 0 | 0 | 0 | 0 | 1 |

[1]May = emerged between 16 October 1996 – 8 May 1997
[2]June = emerged between 8 May – 26 June 1997
[3]August = emerged between 26 June – 6 August 1997
[4]October = emerged between 6 August – 3 October 1997

During 1998, cohorts of new Scotch thistle plants were flagged on 23 June (12), 14 July (9), 19 August (341), and 8 October (208). None of these plants bolted during 1998. Factors responsible for the large infusion of new seedlings July through October were thought to be late summer rains, warm temperatures, and fresh seeds in the soil seed bank that dispersed from the two large plants during 1997.

Ash Creek site #1.

On 15 October 1996, no Scotch thistle rosettes were identified at the Ash Creek site. During 1997, a total of 59 plants were marked (Table 2). Seedling emergence occurred over an extended period of time, as at the Modoc site. Although most plants (32) emerged between 15 October 1996 and 8 May 1997, a significant number (23) emerged during the summer months. None of the plants flowered during 1997.

During June 1997, this field plot was treated with herbicide, eliminating this site from further study. Nevertheless, we were able to determine fate of most of the plants during follow-up visits. Forty-three of the plants marked in 1997 were alive on 23 June 1998. Of those, 39 had bolted. Two of the remaining four rosettes were killed by the spray treatment, whereas the other two remained alive on 6 August.

Table 2. Scotch thistle growth habit; Ash Creek Wildlife Area site #1

| Month of 1997 Cohort | Initial # of Plant | # Bolted in 1997 | # Bolted in 1998 | # Died 1997 | # Died 1998 | # Remaining Oct 1998 |
|---|---|---|---|---|---|---|
| May[1] | 32 | 0 | 23 | 4 | 4 | 1 |
| June[2] | 18 | 0 | 11 | 2 | 5 | 0 |
| August[3] | 5 | 0 | 4 | 1 | 0 | 0 |
| October[4] | 4 | 0 | 1 | 0 | 2 | 1 |

[1]May = emerged between 15 October 1996 – 8 May 1997
[2]June = emerged between 8 May – 26 June 1997
[3]August = emerged between 26 June – 6 August 1997
[4]October = emerged between 6 August – 2 October 1997

**Ash Creek site #2**

A second site at Ash Creek was set up on 22 June 1998. Fifteen contiguous $1m^2$ quadrats were established along each of six parallel transects, arranged over a 12m by 15m area (total = 90 quadrats). During setup of the field plot, we removed all bolting and mature rosette plants of Scotch thistle within and adjacent to the plot, leaving young rosettes and seedlings. A total of 246 young rosettes and an additional 30 seedlings (cotyledons present) were marked. Of the rosettes, two bolted during the summer. No other plants bolted during 1998. Monitoring of this and the previous sites will continue for several years.

## Biological Control of Musk Thistle at Mt. Shasta

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

Musk thistle, *Carduus nutans* L., (Asteraceae) is a widespread, noxious exotic weed occurring in the United States and Canada. The largest infestation in California occurs in pine plantations on the western slope of Mt. Shasta, near Mt. Shasta City at an elevation between 4000 and 5000 feet. The thistles at Mt. Shasta grow predominantly on berms of soil remaining after the brush was bulldozed in the 1960's and later to make way for tree plantings.

The seedhead weevil, *Rhinocyllus conicus* (Froelich) (Coleoptera: Curculionidae), was released on musk thistle in the Mt. Shasta area beginning in November 1974. By 1981, *R. conicus* had spread throughout the primary infestation and had increased to very high numbers. Studies were carried out at Mt. Shasta from 1979 through 1991 to monitor thistle density and impact of *R. conicus.* Musk thistle in three monitoring plots declined to less than 25% of its former abundance (Joley et al., unpublished data). However, significant thistle stands remain, especially in areas where there is little competing vegetation. These stands have tended to maintain themselves at relatively high levels during most years, and are a source of seed for both stand maintenance and initiating satellite infestations.

Given the continuing concern about the dispersal of seed from the area, and the possibility that new biocontrol agents would be released in the near future, we resumed evaluation of musk thistle stands at Mt. Shasta to determine if new biocontrol agents were warranted. Four more insects and a pathogen are being considered for biocontrol of musk thistle: *Trichosirocalus horridus* (Panzer) [Coleoptera: Curculionidae], *Urophora solstitialis* L. [Diptera: Tephritidae], *Cheilosia corydon* Harris [Diptera: Syrphidae], *Psylliodes chalcomera* Ill. [Coleoptera: Chrysomelidae], and *Puccinia carduorum* (Jacky) [Uredinales: Pucciniaceae]. Uncertainty remains, however, whether any of the new insects will be released in California because of questions concerning their safety (host-specificity).

In 1995, the United States Forest Service re-issued a permit for CDFA to carry out biocontrol work on musk thistle for an additional ten year period. During spring 1995, we set up two long-term field sites with comparatively stable, moderately dense stands of musk thistle within the pine plantations. New field sites were needed because sites monitored during 1979-1991 were no longer useable. At each monitoring site, fifty 1-$m^2$ quadrats were distributed systematically along two parallel transects (25 per transect) on top of the berm (areas covered by shrubs were skipped). Reproductive musk thistle plants were counted within each quadrat in autumn 1995-1998. Also, mature flower heads were counted and plant heights measured in 1995, 1997, and 1998 within some or all quadrats.

Adult plant height as well as plant and seed head abundance varied from year to year (Table 1). The cause(s) for these variations are not clearly apparent, but weather is suspected as a major contributor. Both the 1996-97 and 1997-1998 winters were wet and relatively mild. The latter winter was a major El Niño event, with very mild, wet conditions which continued until mid July. These conditions were ideal for development of very large, multi-stemmed plants which

produced many heads. The height of the musk thistle plants during 1998 was higher than we have ever seen since observations began in 1979.

Table 1. Musk thistle populations (plants/$m^2$), number of seed heads, and average plant height at two sites near Mt. Shasta, California.

| | 1995 | | 1996 | | 1997 | | 1998 | |
|---|---|---|---|---|---|---|---|---|
| | East | West | East | West | East | West | East | West |
| Plants/$m^2$ | 5.5 | 26.8 | 19.2 | 12.5 | 3.5 | 3.3 | 7.2 | 9.5 |
| Heads/$m^2$ | 42.4 | 126.0 | - | - | 36.6 | 61.0 | 99.7 | 22.5 |
| Heads/plant | 7.8 | 4.7 | - | - | 10.5 | 18.2 | 13.9 | 12.8 |
| Ave. height | 72.2 | 64.7 | - | - | 80.3 | 104.7 | 159.1 | 157.0 |

An assessment of *R. conicus* infestation rate was performed in 1997 and 1998. In 1997, 22 random plants at the east site were harvested (including upper roots). Stems and roots were dissected and examined in the field to determine if other (fortuitous) natural enemies were present. Seed heads were transported to the laboratory. In 1998, 16 random plants at the west site were selected and all mature heads harvested. In the laboratory, seed heads were dissected and the number of *R. conicus* pupal cells was recorded per head.

Both stems and roots collected in 1997 were relatively free of insects or damage from insects. There was no evidence of any significant natural enemy besides *R. conicus* at either site. Weevil-infested seed heads (containing at least one pupal chamber) had an average of 20.6 chambers per head in 1997 (range = 1-125) and 26.0 in 1998 (range = 1-109) which does not appear to be greatly different. However, in 1998, an estimated 60% of mature heads escaped attack (no larval chambers) by *R. conicus* compared to 33% in 1997. Although seed production was not determined, the much greater percentage of heads escaping attack in 1998 suggested that many more viable seeds were produced in 1998 than in 1997. The effect of this increased seed production is currently unknown, but thistle abundance is expected to increase for the next few years.

## Propagation of Asteraceous Plants for Host Range Testing of Weed Biological Control Agents

K. A. Casanave and L. Brace

One of the goals of the Biological Control Program is to assist in the introduction of insects and diseases on exotic weeds in California. For this, the Biological Control Program collects and maintains seed of native and agricultural plants for use by cooperators in host range testing of potential biological control agents. In addition, the Program propagates some native and agricultural plants which are shipped to cooperators for immediate use in host range tests.

In 1998, 16 species/varieties of Asteraceous plants were propagated by the Biological Control Program for the USDA-ARS Biological Control of Weeds Quarantine, Albany, CA. The plants were used for host range testing of *Lixus cardui* Olivier and *Lixus* sp., potential bioagents on Scotch thistle and *Ceratapion basicorne* (Illiger) and *Chaetorellia succinea* (Costa), potential bioagents on yellow starthistle.

In addition to the plants provided by the Biological Control Program, seeds of several native thistles were provided by the Program. These thistles were propagated by the USDA at their Albany facility.

| **Weeds** | | **Native Plants** | | **Commercial Crop Plants** | |
|---|---|---|---|---|---|
| **Species/ Variety** | **Number Grown** | **Species/ Variety** | **Number Grown** | **Species/ Variety** | **Number Grown** |
| Scotch Thistle | 63 | *Cirsium fontinale var. obispoense* | 3 | Artichoke "Imperial Star" | 11 |
| Spotted Knapweed | 16 | *Cirsium undulatum* | 1 | Cardoon | 17 |
| Squarrose Knapweed | 26 | *Cirsium loncholepis* | 2 | Safflower 4440 | 64 |
| Diffuse Knapweed | 27 | *Cirsium rhothophilum* | 8 | Safflower 880L | 16 |
| Musk Thistle | 12 | *Cirsium andersonii* | 1 | | |
| Yellow starthistle | 16 | *Cirsium brevistylum* | 24 | | |
| Italian thistle | 24 | *Cirsium scariosum* | 22 | | |
| Slenderflower thistle | 20 | *Cirsium douglasii* | 24 | | |
| Bull thistle | 24 | | | | |
| Distaff thistle | 17 | | | | |
| Musk thistle | 18 | | | | |
| Milk thistle | 1 | | | | |

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

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

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

Preliminary evaluations of the impact of individual biological control insects on seed production in California suggest that no single agent will be the dramatic silver bullet in reducing yellow starthistle abundance. Rather, a combination of the current, and possibly, future natural enemies may be necessary to control this plant. A study was initiated in 1993 to evaluate the population buildup, combined impact, and interaction of all available biological control insects on yellow starthistle. Field sites were established in Yolo, Placer, and Sonoma Counties to represent three different climates where yellow starthistle occurs in abundance. Four insects (*B. orientalis*, *U. sirunaseva*, *E. villosus*, and *L. curtus*) were released at each site in 1993 and 1994 and long-term monitoring of the weed and insect populations was initiated. The fifth insect, *C. succinea*, invaded these sites on its own between 1996-1998. The Yolo County site is open Sacramento Valley rangeland located west of Woodland; the Placer County site is at 1500 ft elevation in the Sierra Nevada foothills east of Auburn; the Sonoma County site is in the Coast Range foothills southeast of Santa Rosa. Various aspects of the weed-insect interaction are being monitored annually including plant cover estimates of yellow starthistle and competing species, yellow starthistle seedling emergence, adult plant density, seedhead numbers, seed production, and insect infestation rates. Preliminary results from 1995-98 are presented in Table 1.

Four years after the first releases, we have evidence that these biological control agents are having an impact on yellow starthistle seed production that may translate into a decline in mature plant populations. The weevil, *E. villosus*, has become the most abundant insect at all three sites infesting 47-79% of the flower heads. In addition, adult *E. villosus* feed on young developing buds. This feeding kills the buds and produces a structural change in the plants which remain dominated by stems. Instead of flowers born on the tips of stems, the early flowers are killed as developing buds. Later, the plant produces new flowers on short stems which arise from the leaf axils along the main stems. The other three insects, *B. orientalis*, *U. sirunaseva*, and *L. curtus* have increased more slowly and have remained at infestation rates less than 25%. The seed head fly, *C. succinea*, was first recovered in 1996 at the Yolo County site and in 1998 at the Placer and Sonoma County Sites. Infestations rates in 1998 ranged from 1-10% of the seed heads among sites.

The Sonoma County site has had the most dramatic changes in both insect populations and yellow starthistle seed production. The rapid increase of *E. villosus* resulted in a steady decline in the number of flowers per plant and the number of seeds per head. The percentage of mature heads infested by at least one biological control insect increased from 22% in 1995 to 83% in 1998. In addition, there has been a concurrent decrease in seed production (13,839 to 3,802 seed per sq. m) and seedling density (897 to 234 seedlings per sq. m). If this trend continues, we anticipate a significant decline in adult plant density in subsequent years.

The Yolo County site was the first location in California to be confirmed with established populations of all five natural enemies. Significant declines in adult plant and seed densities occurred from 1995-1997. However, an increase in plant density and seed production was observed in 1998, presumably due to the unusually high rainfall which extended into early summer. Still, the population densities of *E. villosus* and *C. succinea* have increased steadily over the last three years and may increase to densities high enough to cause a sustained decline in plant abundance.

The density of bioagents at the Placer County site built up quickly but showed little change from 1995-1997. However, a significant increase in insect densities was observed in 1998. *E. villosus* is the most abundant insect, infesting 79% of the seedheads in 1998; the other insects occurring at rates 0-12%. There has been little change in plant density and flower production at this site, but there has been a steady decline in seed production. We hope to see an increase in *C. succinea* over the next few years that will complement the impact of the other insects.

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

Table 1. Status of Yellow starthistle and its natural enemies at three multiagent research sites

**Placer County**

| **Plant** | | **95** | **96** | **97** | **98** | **99** |
|---|---|---|---|---|---|---|
| Seedlings/square meter | | - | 651 | 669 | 883 | 666 |
| Adult plants/square m | | 332 | 83 | 108 | 151 | |
| Heads/ square meter | | 679 | 280 | 438 | 378 | |
| Seed/head | | 8.2 | 18.0 | 16.2 | 6.7 | |
| Seeds/square meter | | 5,568 | 5,040 | 7,096 | 2,533 | |
| **Insect & release year** | | | | | | |
| *B. orientalis* | 93 | 6.7% | 0.6% | 1.6% | 12.0% | |
| *U. sirunaseva* | 93 | 4.7% | 5.0% | 8.7% | 7.4% | |
| *E. villosus* | 93 | 51.6% | 50.9% | 54.8% | 79% | |
| *L. curtus* | 94 | 0 | 0 | 0.2% | 0% | |
| *C. succinea* | - | 0 | 0 | 0 | 3% | |
| Heads w/ 1 or more sp | | 58% | 60% | 60% | 83% | |

**Yolo County**

| **Plant** | | **95** | **96** | **97** | **98** | **99** |
|---|---|---|---|---|---|---|
| Seedlings/square meter | | - | 1095 | 1928 | 1076 | 642 |
| Adult plants/square m | | 975 | 322 | 180 | 422 | |
| Heads/ square meter | | 1181 | 369 | 343 | 830 | |
| Seed/head | | 24 | 27 | 13 | 15 | |
| Seeds/square meter | | 28,344 | 9,963 | 4,459 | 12,450 | |
| **Insect & release year** | | | | | | |
| *B. orientalis* | 91 | 5% | 3% | 7% | 4% | |
| *U. sirunaseva* | 93 | 13% | 20% | 12% | 17% | |
| *E. villosus* | 93 | 5% | 16% | 24% | 47% | |
| *L. curtus* | 94 | 0 | 0 | 0.2% | 0% | |
| *C. succinea* | 96 | 0 | 2% | 7% | 10% | |
| Heads w/ 1 or more sp | | 20% | 36% | 38% | 60% | |

**Sonoma County**

| **Plant** | | **95** | **96** | **97** | **98** | **99** |
|---|---|---|---|---|---|---|
| Seedlings/square meter | | - | 897 | 822 | 624 | 234 |
| Adult plants/square m | | 241 | 233 | 222 | 231 | |
| Heads/ square meter | | 547 | 442 | 508 | 486 | |
| Seed/head | | 25.3 | 14.9 | 8.0 | 7.8 | |
| Seeds/square meter | | 13,839 | 6,586 | 4,064 | 3,802 | |
| **Insect & release year** | | | | | | |
| *B. orientalis* | 94 | 5.4% | 9.5% | 4.2% | 12.4% | |
| *U. sirunaseva* | 94 | 4.8% | 16.3% | 19.7% | 22.7% | |
| *E. villosus* | 94 | 12.9% | 37.3% | 73.9% | 72.7% | |
| *L. curtus* | 94 | 0 | 0 | 0.7% | 0.5% | |
| *C. succinea* | - | 0 | 0 | 0 | 1.0% | |
| Heads w/ 1 or more sp | | 22% | 56% | 80% | 83% | |

## Impact of Seedling Pathogens on Yellow Starthistle in Central California

M. J. Pitcairn, D. M. Woods, D. B. Joley, D. G. Fogle[1], and V. Popescu

Yellow starthistle, *Centaurea solstitialis* L., is an exotic annual weed that is widespread throughout California. Adult plant populations can reach high densities (200-800 plants per square meter) and produce over one million seeds per acre. Its life cycle begins with the onset of winter rains as seeds are highly germinable and most germinate once wetted. Life table studies have shown that extremely high densities of seedlings are present in early winter but, by early spring, densities can drop by 50-75%. Surveys of yellow starthistle seedlings at one site in Solano County have identified at least three naturally occurring seedling pathogens: *Ascochyta* n. sp., *Colletotrichum gloeosporioides*, and *Sclerotinia minor*. All three appear to cause locally high rates of mortality. To quantify their impact, a study was initiated at a field site in Solano County during the winter of 1997-98. A 8 x 20 meter plot was divided into ten 4 x 4 meter subplots and two 0.5 x 0.5 meter quadrats were randomly located within each subplot. Each quadrat was divided into four 0.25 x 0.25 meter sub-quadrats and one 10 x 10 cm sample area was randomly located within each sub-quadrat. Thus, there were four sample areas per quadrat and a total of 80 sample areas for the plot. Monitoring consisted of identifying and counting all yellow starthistle seedlings by stage. Seedlings were grouped into 5 developmental stages by the number of true leaves produced: cotyledon, 1-leaf, 2-leaf, 3-leaf, and 4 or more-leaf. To document activity of seedling pathogens, representative samples of diseased seedlings were removed and cultured in the laboratory. Monitoring began November 21, 10 days after the first rain event and continued weekly until March 29, 1998 when all surviving seedlings had grown four or more leaves. After March, monitoring continued every four weeks until July 1, 1998. Sample areas were examined again on September 15, and all surviving plants were counted. For each plant, the number of flower heads was determined, then harvested by cutting the stem at the soil, dried and weighed.

Yellow starthistle seedlings germinated in high numbers immediately following the first rain on November 10, 1997 (Figure A). Peak abundance occurred on November 20, ten days later. On this date, seedlings occurred in 79 of 80 sample areas with densities from 0 to 8,400 seedlings per square meter (average density was 1,812 seedlings per square meter). The one sample area without seedlings on this date was occupied on December 31, 1997, thus all sample areas received at least one seedling. Seedling mortality began early and most died by the end of December. The rate of decline was highest in late November and early December (32 seedlings per day). After mid December, the rate of decline was substantially lower (4 seedlings per day). Rainfall was frequent during November and early December, but a dry period occurred in the second half of December. Rainfall was frequent again from early January through February then sporadic thereafter. A second cohort of yellow starthistle seedlings occurred in January possibly due to the re-initiation of rainfall and warmer temperatures. By December 31, 17 of 80 sample areas were without yellow starthistle seedlings. Germination by the second cohort reduced this number to 10 of 80 sample areas without seedlings. Still, the rate of mortality for the second cohort was similar to the first and most died soon thereafter. On April 29, yellow starthistle was absent in 21 of 80 sample areas; average density was 565 seedlings per square meter, a decline of 71% from peak abundance.

Seedling mortality was greatest during the cotyledon stage (Figure B). Peak numbers of 1-leaf stage was 61% lower than observed for peak numbers of cotyledon stage. Declines in peak numbers between 1-leaf and 2-leaf stages and 2-leaf and 3-leaf stages were 22% and 24%, respectively. Peak abundance of the 1-leaf stage was three weeks following peak cotyledon abundance. The time between peak abundance for the subsequent stages was two weeks. This suggests that yellow starthistle added one new leaf every two to three weeks during this study.

Field observations of disease symptoms and laboratory cultures of pathogens suggest that *S. minor* was the predominant pathogen during this study. Mycelial growth could be seen emerging from infected leaves, stems, and petioles following heavy rains. During drier periods, the infected tissue collapsed and became slimy prior to complete death. Patches of diseased plants encompassed irregular areas up to 0.5 meter in diameter. The fungus, *C. gloeosporioides* was also detected throughout the season, usually on individual plants. *Ascochyta* n. sp. was not observed during this study. In addition to occurrence of disease, many seedlings appeared to have been fed upon. The cotyledons and leaves of many plants were chewed away or removed. Suspected organisms are small rodents, snails and slugs.

It appears that yellow starthistle seedling mortality can be very high. Despite the huge number of seeds produced annually, less than 20% survive to reproduce. Most mortality occurred prior to bolting and appears to limit mature plant density. While seedling mortality is an annual event of yellow starthistle, particularly in dense stands, the presence of any single disease was sporadic or somewhat localized. This mortality occurred either on isolated plants or as large patches of dead plants, but has not been noticed because of the large numbers of plants that remain. During the winter of 1997/1998, *S. minor* was particularly devastating in high density settings and where skeletons of previous years starthistle plants provided shading. Aerial mycelia were common and fairly easy to detect. *S. minor* has an extremely broad host range that includes many broad-leaved plant families including major crops such as lettuce. Although the pathogen might potentially have some use as an externally applied bioherbicide for yellow starthistle seedlings under moist conditions, the lack of host specificity may limit its usefulness. The fungus, *C. gloeosporioides*, has been used as both a classical and commercial biological control product, but we have not yet refined the degree of host specialization for our isolate. The field symptoms of this fungus were most commonly detected as single plants that appear wilted or yellowed. Occasionally, small patches of dead seedlings surrounded by symptomatic plants were detected. We have found this disease in several California counties and believe that it can have a significant impact on yellow starthistle.

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[1]Plant Pest Diagnostics Branch, CDFA, Sacramento, CA

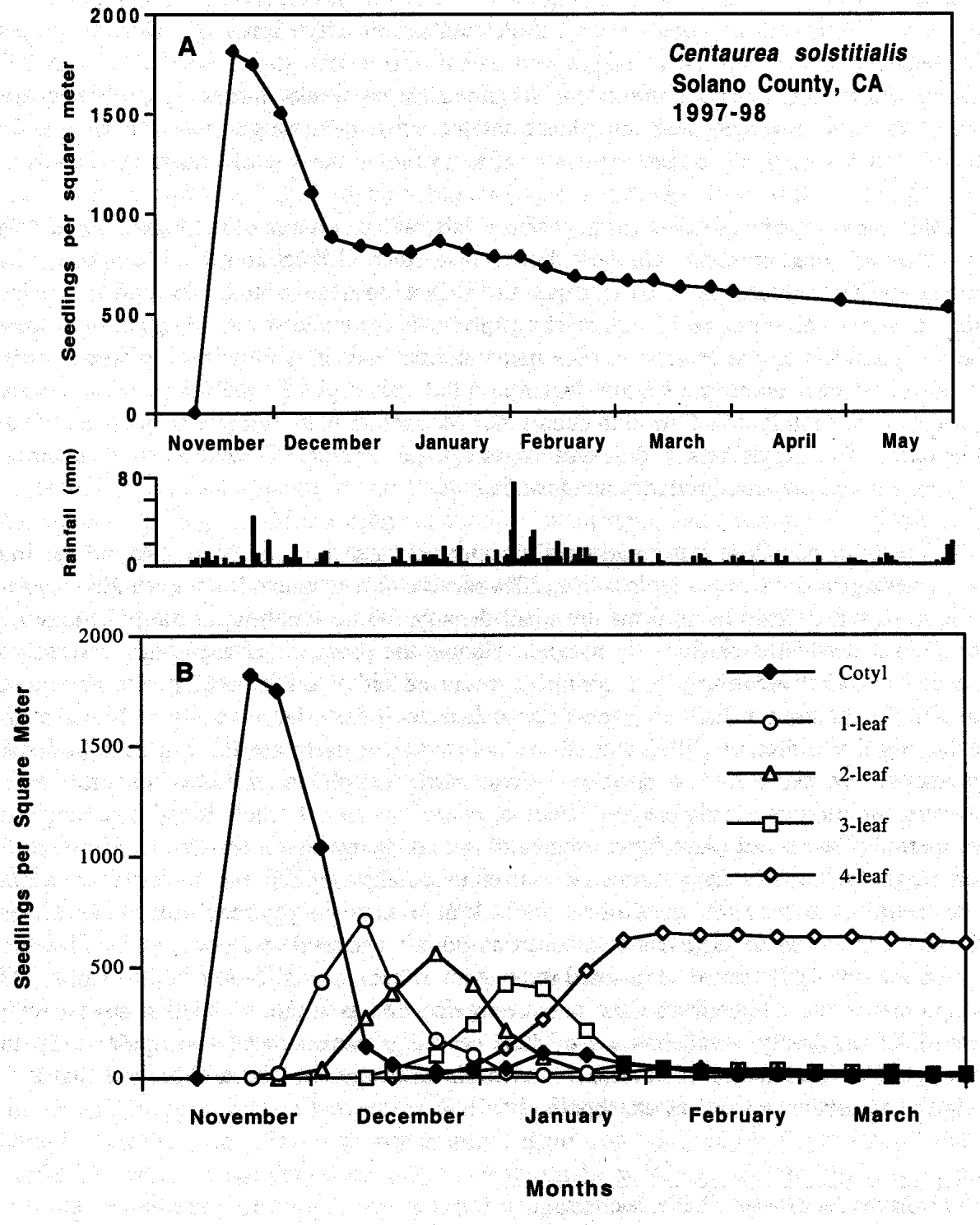


**Figure 1. A:** Yellow starthistle density from November, 1997, through May, 1998, in Solano County, CA; daily rainfall from November, 1997, through May, 1998.
**B:** Abundance of yellow starthistle seedlings by stage from November, 1997, through March, 1998.

## Integrating Chemical and Biological Control Methods for Control of Yellow Starthistle

M. J. Pitcairn, J. M. DiTomaso[1], and J. Fox

Yellow starthistle, *Centaurea solstitialis* L. (Asteraceae), is an exotic weed that has become one of California's worst pests. Several control methods have been developed to manage this weed, including mowing, timed grazing by sheep, goats, and cattle, competitive planting of grasses and clovers, burning, pre- and post-emergent herbicides, and biological control. Few, if any, of these methods have proven successful when used as the sole control method. Even those that provide excellent within-season results fail to produce significant reductions unless continuous control efforts for at least three years significantly deplete the soil seedbank.

A limited number of herbicides are registered for use against yellow starthistle in California. Most effectively kill yellow starthistle plants and provide good, within-season control. There are some concerns, however, with the continuous use of these herbicides. For example, clopyralid (Transline©) damages many annual legume species, which are important components of rangelands, pastures, and wildlands. Furthermore, a Washington population of yellow starthistle developed resistance to repeated use of picloram (Tordon©), and this population was also cross-resistant to clopyralid, which has a similar mode of action. Thus, the potential exists for the development of herbicide resistance if used year after year. Integrating or alternating other control methods into a management strategy may minimize the development of herbicide resistance.

The USDA, Agricultural Research Service's Exotic and Invasive Weed Management Research Unit in Albany and the CDFA's Biological Control Program are currently pursuing biological control of yellow starthistle in California. To date, six exotic insects have been established and three have become widespread: *Bangasternus orientalis* (Coleoptera: Curculionidae), *Urophora sirunaseva* (Diptera: Tephritidae), and *Eustenopus villosus* (Coleoptera: Curculionidae). A fourth insect, *Chaetorellia succinea* (Diptera: Tephritidae), was accidentally introduced in Oregon in 1994 and has since spread throughout most of California. All four insects attack the flower heads of yellow starthistle.

Control methods such as burning, mowing, grazing, and tillage can damage insect populations and are not compatible with the use of biological control insects. By comparison, clopyralid applications in late winter (February-March) which kill young seedlings do not coincide with summer insect activity and appear unlikely to directly injure the biological control agents. We hypothesize that combining clopyralid applications with insect bioagents may provide for more effective long-term control of yellow starthistle. Annual clopyralid applications (2-3 years) will reduce plant density and the seed bank. The attack of biocontrol insects on escaped plants in subsequent years should slow the rate of re-infestation by impacting the few flower heads available. Previous studies have shown that yellow starthistle can resurge from 5% to 80% of pre-treatment densities within two years following fire or herbicide treatments. It is hoped that seed destruction by established biological control agents can retard resurgence to 4-6 years and thereby reduce the need for continuous herbicide treatments and lower the economic costs required for effective long-term management of yellow starthistle.

To investigate this possibility, a study was initiated in 1997 one year prior to herbicide treatments. The study site was located in a 20 acre area along the east shore of Lake Natoma, Sacramento County. The site is part of the Folsom Lake State Recreation Area owned by the U.S. Department of Interior, Bureau of Reclamation and is managed by the California Department of Parks and Recreation. The site was chosen because all four biological control agents are established there and all managing partners agreed to cooperate on the project.

In 1997, we evaluated the pre-treatment infestation rate of and seed destruction from the four biological control insects known to occur at this site. On August 4, approximately 100 senesced flower heads in each of three stages of development were individually enclosed in a cloth bag to collect all seeds and insects. The three developmental stages were 1) dried, brown flowers with brown bracts, 2) faded yellow flowers with brown bracts, and 3) fading yellow flowers with green bracts. These stages represented early, mid, and late July activity periods for the insects and coincided with peak flower production. The results are shown in Table 1. Two of the biological control agents, *B. orientalis* and *U. sirunaseva*, appear to have caused negligible damage to seed production. Even though *B. orientalis* eggs were present on early July flower heads, no larval damage was found at all three sample dates. *U. sirunaseva* occurred at levels too low (0-6%) to have much impact. The most damaging insects were *E. villosus* and *C. succinea*. Oviposition or adult feeding damage by the weevil was found on 93% of the seedheads and larvae were present in 47% of the early July flower heads. The number of infested flower heads was reduced in mid July and early August samples. In contrast, the occurrence of *C. succinea* increased from early July to early August samples where 38% of the seedheads were attacked. The complimentary attack of *E. villosus* and *C. succinea* resulted in significant reductions in seed production from early July through early August. Uninfested flower heads produced an average of 31.3 seeds/head. The average seed number in the presence of bioagents was 7.5 seeds/head resulting in an estimated reduction of 76% of the potential seed production.

In 1998, four plots (replicates) were established within the research site. Each plot (25 x 40 m) was divided into two 25 x 20 m subplots; one subplot received a herbicide treatment, the other was left untreated. The size of subplots is large enough to prevent rapid invasion of yellow starthistle from adjacent areas and only the central 20 x 15 m was be used for data collection. Clopyralid treatments were made using a commercial field sprayer. The lowest labeled rate of clopyralid is 1.5 oz ae/A. We used a rate of 0.5 oz ae/A clopyralid to ensure an adequate number of yellow starthistle escapes. The goal of the herbicide treatment is to substantially reduce the seed bank and reduce the abundance of yellow starthistle to 5-10% of untreated subplots. Herbicide applications will be repeated in March 1999 and 2000 if required to achieve this goal. Subplots will be monitored for several years to document resurgence of yellow starthistle following herbicide applications. Control plots without biocontrol agents were not used in the design of this experiment as they would require a level of intervention (e.g. repeated insecticide treatments or large scale enclosures) that would significantly impact yellow starthistle seed production. Yellow starthistle requires full sunlight and any cage covering would slow growth rates and consequently seed production by the plant. Furthermore, yellow starthistle is an obligate outcrosser that depends on insect pollinators for effective seed set. Thus, potential seed production in the absence of biocontrol agents was estimated by determining the average seed

production in uninfested flower heads and multiplying this value by the total head number per unit area.

The first clopyralid application occurred March 6, 1998. Prior to treatment, seedling densities were determined by counting total seedlings in a 20 cm diameter ring tossed within each plot (10 replicates/plot). Yellow starthistle seedling densities ranged from 45-177 plants/$m^2$ and did not significantly differ between subplots within each plot. On July 6, 1998, vegetative cover of mature yellow starthistle populations was estimated by determining the presence or absence of yellow starthistle at one-foot intervals along randomly placed 50 ft line transects (6 transects/plot). Similar measurements will be repeated annually. After the first year of clopyralid treatment, yellow starthistle cover averaged 96% in untreated and 26% in clopyralid treated plots.

An augmentative release of *E. villosus* adults was also investigated in 1998. Each subplot was divided in half and adult *E. villosus* weevils were released into one half-subplot. The weevils were released on June 26 and distributed by hand throughout the half-subplot. Approximately 500 weevils were released per half-subplot (1,000 weevils per plot, 4,000 total for the site) which is a release rate of one weevil per square meter. The objective was to determine if supplemental releases can increase the impact of *E. villosus* on yellow starthistle seed production. This weevil was chosen because previous studies have indicated that it had a significant impact on yellow starthistle seed production. In addition, adults of this species are reluctant to fly and move slowly away from release sites.

The impact of the biological control insects was estimated by sampling senesced yellow starthistle flower heads in different times and stages of development. Heads with recently senesced flowers (faded flowers with green bracts) were enclosed with cloth bags on July 6 and two types of flower heads (faded flowers and green bracts, and faded flowers and brown bracts) were bagged on August 5. The July 6 flower heads represented the early season flower heads, the August 5 heads with brown bracts represented the mid season flower heads, and the August 5 heads with green bracts represented late season flower heads. For each sample, 30 flower heads from randomly selected plants were bagged in each half-subplot (60 bags/subplot, 120 bags/plot). After three weeks the bagged flower heads were collected, taken to the laboratory and dissected. Filled pappus and non-pappus seeds were counted in each head. Species, number, and life stage of the biocontrol insects occurring in each head were also determined. In addition to the flower head samples, the density of reproducing yellow starthistle plants was estimated by harvesting randomly located square meter quadrats on August 5. All plants in each sample were counted, and height and flower head number were measured.

The results of the supplemental releases of *E. villosus* were evaluated by comparing the attack rate (number of feeding and oviposition wounds per flower head) between half-subplots for each treatment (Table 2). While the attack rate during early season was higher than observed during mid- or late seasons, there was no significant increase in attack in the half-subplots receiving additional weevils. Thus, all data were summarized by subplot and treatment analysis was performed using subplots as replicates.

As observed in 1997, both *B. orientalis* and *U. sirunaseva* appeared to have little impact on seed production (Table 3). Even though *B. orientalis* eggs were present on early July flower heads, no larval damage was found from all three sample dates. *U. sirunaseva* occurred at levels (0-8%) too low to have much impact. The attack rate of *E. villosus* and *C. succinea* was lower than observed in 1997 but still appeared to have a significant impact on seed production. Oviposition or adult feeding damage by the weevil was found on 43-52% of the early season flower heads; later heads were attacked at a lower rate, especially heads in the untreated subplot. Interestingly, the infestation rate was higher in the herbicide treated subplots than in the untreated subplots. Most of the escaped plants in the herbicide plots germinated late, after the February herbicide treatment. As a result, plants in the treated subplots matured and flowered later than those in the untreated subplots. Also, escaped plants in the treated plots were larger that those in the untreated subplots. The difference in attack rate may be due to larger plants being more desirable and the later developing plants supporting flower heads in developmental stages preferred by the weevil later into the summer. In contrast, *C. succinea* attack ranged from 18-72% and was highest among the mid-season flower heads. The combined attack of *E. villosus* and *C. succinea* again resulted in significant reductions in yellow starthistle seed production. Uninfested flower heads produced an average of 9.7-31.5 seeds/head among subplots. The average seed number in the presence of bioagents was 3.8-15.5 seeds/head resulting in an estimated reduction of 13-64% in potential seed production.

Differences between 1997 and 1998 may have been due, in part, to differences in rainfall. In 1997, the Sacramento Valley experienced a significant spring drought which resulted in a thin stand of yellow starthistle (<25% cover). In contrast, 1998 had an extremely wet spring and the level of yellow starthistle infestation increased dramatically (96% cover). Interestingly, the number of seeds per flower head and the rate of insect attack were lower in 1998 than observed in 1997 (compare Tables 1 and 3). This was likely due to the higher number of flower heads produced in 1998 which may have, at times, exceeded the ability of pollinators and biocontrol insects to utilize the standing crop of flower heads. Although the clopyralid treatment reduced the density of yellow starthistle by 97% (Table 4), this resulted in only 75% reduction in vegetative cover and 84% reduction in seedhead numbers per area. Thus, individual surviving plants were larger and produced more seedheads than plants in untreated subplots. In this study, clopyralid alone was estimated to reduce seed production by 80% (Table 4). The presence of the biological control agents provided an additional 51% reduction in seed numbers (averaged over all treatments and seasons). Thus the total combined reduction in seed number was 90%. In contrast, the biological control agents alone in the untreated subplots reduced seed production by only 33%. These initial results are in agreement with our hypothesis that a combination of biological control agents and clopyralid would provide enhanced, and perhaps synergistic, control of yellow starthistle seed production. However, more years of observations, especially during years of normal rainfall will be required to further support these initial findings.

[1]Weed Research and Information Center, University of California, Davis, CA

**Table 1.** Occurrence (% of flower heads attacked) and seed destruction by four biological control agents on yellow starthistle at a field site near Folsom, CA, in 1997. N=98-101 per sample.

| Insect species | Evidence of Attack | Percentage of heads | | |
|---|---|---|---|---|
| | | Early July | Mid July | Late July |
| *Bangasternus orientalis* | eggs | 32 | 2 | 1 |
| | larvae | 0 | 0 | 0 |
| *Urophora sirunaseva* | galls | 8 | 2 | 0 |
| *Eustenopus villosus* | feeding/oviposition | 93 | 59 | 38 |
| | larvae | 47 | 14 | 2 |
| *Chaetorellia succinea* | larvae | 9 | 11 | 38 |
| None | Uninfested seedheads | 4 | 28 | 35 |
| Mean no. seeds/head[1] (% reduction from potential) | | 9.5 (70) | 6.9 (78) | 6.1 (81) |

[1]Average number of seeds produced in an uninfested seedhead (potential seed production without insects) was 31.3.

**Table 2.** Attack rate (number of feeding and oviposition wounds per flower head) by *Eustenopus villosus* on yellow starthistle near Folsom, CA, in 1998. N=29-30 flower heads per sample, values represent average of four samples.

| Insect species | Treatment | Number of Attacks per Head | | | | | |
|---|---|---|---|---|---|---|---|
| | | Early-season | | Mid-season | | Late-season | |
| | | w/ suppl. releases | no suppl. releases | w/ suppl. releases | no suppl. releases | w/ suppl. releases | no suppl. releases |
| *Eustenopus villosus* | Herbicide | 0.61 | 0.60 | 0.40 | 0.57 | 0.45 | 0.48 |
| *Eustenopus villosus* | No Herbicide | 0.69 | 0.60 | 0.21 | 0.20 | 0.14 | 0.08 |

**Table 3.** Occurrence (% of flower heads attacked) and seed destruction by four biological control agents on yellow starthistle near Folsom, CA, in 1998. N=229-239 flower heads per season and treatment. Shaded areas indicate statistically significant difference (P<0.05) between clopyralid treated and untreated plots for each seasonal analysis.

| Insect species | Cause of damage | Percentage of heads | | | | | |
|---|---|---|---|---|---|---|---|
| | | Early-season | | Mid-season | | Late-season | |
| | | Herbicide treated | Untreated | Herbicide treated | Untreated | Herbicide treated | Untreated |
| *Bangasternus orientalis* | eggs | 28 | 19 | 11 | 5 | 5 | 1 |
| | larvae | 0 | 0 | 1 | 1 | 0 | 0 |
| *Urophora sirunaseva* | galls | 1 | 2 | 13 | 4 | 21 | 3 |
| *Eustenopus villosus* | feeding/eggs | 43 | 52 | 39 | 16 | 36 | 10 |
| | larvae | 7 | 27 | 1 | 6 | 1 | 0 |
| *Chaetorellia succinea* | larvae | 56 | 27 | 72 | 70 | 39 | 18 |
| None | Uninfested seedheads | 21 | 27 | 13 | 19 | 33 | 74 |
| Average number of seeds per uninfested head | | 31.5 | 24.2 | 14.1 | 9.7 | 20.0 | 17.9 |
| Mean no. seeds/head (% reduction from potential) | | 14.0 (56) | 15.4 (36) | 5.1 (64) | 3.8 (61) | 13.6 (32) | 15.5 (13) |

**Table 4.** Effect of biological control insects and a clopyralid application on yellow starthistle density, flower head and seed production. Values in parentheses represent percent reduction compared to untreated plots.

| Treatment | Plants/$m^2$ | Seedheads/$m^2$ | Potential seed prod./$m^2$ without biocontrol agents | Estimated seed prod./$m^2$ with biocontrol agents |
|---|---|---|---|---|
| Clopyralid | 3 (97) | 139 (84) | 3,039 (80) | 1,515 (85) |
| Untreated | 116 | 893 | 15,419 | 10,329 |

**Endemic Natural Enemy Fauna of Yellow Starthistle and Purple Starthistle in Central California**

M. J. Pitcairn, D. M. Woods, E. F. Van Mantgem, and R. K. Wall

Yellow starthistle, *Centaurea solstitialis* L. (Asteraceae), and purple starthistle, *Centaurea calcitrapa* L. (Asteraceae), are invasive weeds of Eurasian origin. Yellow starthistle is an annual plant and was first reported in 1869 in Alameda County. It has now become widespread, occupying over 8 million acres statewide. Purple starthistle is a biennial and was first reported in 1887 in Solano County. It occurs primarily in rangeland near the coast and is particularly troublesome in Solano, Napa, Marin, and Sonoma Counties. Yellow starthistle is the target of a intensive biological control program by CDFA and the USDA-ARS. A total of five insects have become established due to this effort. While there is currently no overseas effort to obtain biological control agents for purple starthistle, the Biological Control Program has performed experimental releases of insects approved for other exotic *Centaurea* spp. (spotted and diffuse knapweeds). In 1998, a survey to assess the occurrence of endemic arthropod and pathogen fauna of yellow starthistle and purple starthistle was initiated. It is hoped that the data will allow comparison with the natural enemy fauna in Europe and Asia, and identify open niches that could be exploited by introduced biological control agents. Some exotic natural enemies of weeds have been introduced accidentally (e.g. *Agonopterix alstroemeriana* (Clerck) on poison hemlock). This survey will also serve to show if identified potential biological control agents are present prior to their release.

The fauna of yellow starthistle was surveyed at five sites that formed a transect across central California from the coast to the Sierra Nevada foothills. Three of the sites are located in the foothills of the Sierra Nevada Mountains in Sacramento and Placer Counties. One site is located in the Sacramento Valley in Solano County and one site is located in Marin County. All five sites were heavily infested with yellow starthistle infestations ranging from 5 to over 100 acres in size. The fauna of purple starthistle was surveyed at one site in Marin County and three sites in Napa County. All four sites were heavily infested with purple starthistle and infestations ranged from 5 to 20 acres in size.

All sites were visited for 1-2 hours every two weeks from bolting stage in May through onset of senescence in August. For arthropods, only individuals actually landing on the plant were collected; individuals flying around the plant were not collected. Most arthropods were collected by hand-picking or with a net. Rosette and stem leaves were removed and examined under a microscope for mites, thrips, and aphids. Internal feeders were surveyed by removing 100 adult plants with roots from each site. All stems and roots were split and examined under a microscope for feeding damage and evidence of disease.

The survey yielded over 2,500 collections. Of these, over 2,000 were insects, representing 12 orders. The remaining collections consisted of non-insect arthropods. All specimens are currently being identified by specialists. Preliminary observations suggest that the majority of species collected are incidental and that only a few species appear to feed directly on yellow starthistle or purple starthistle. The most common taxa feeding on yellow starthistle were aphids,

cicadellids, fulgorids, mirids, and thrips. Larvae of the native butterfly, *Vanessa cardui* (Linni),were commonly observed (but not abundant) feeding on the foliage. Larvae of the exotic beetle, *Lasioderma haemorrhoidale* (Ill.), were common in seedheads. Other taxa observed feeding directly on yellow starthistle included spider mites on rosette and stem leaves and the European gray garden slug, *Deroceras reticulatum* (Muller). Of these taxa, only the gray garden slug, which feeds on young seedlings in winter, appeared to have a significant impact on yellow starthistle. No root feeders were found on yellow starthistle, however, a stem mining mordellid was collected at 4 of 5 sites. No evidence of plant disease could be detected in mature plants collected at the end of the season. Pathogens may have been present at earlier stages which resulted in mortality and were not sampled.

The most common taxa feeding on purple starthistle were aphids, cicadellids, mirids, and thrips. Larvae of *V. cardui* were observed on the foliage and *L. haemorrhoidale* larvae were common in the seedheads. Other taxa included spider mites on rosette and stem leaves. Purple starthistle seedlings were not monitored for damage. The natural enemy fauna of purple starthistle was similar to that observed for yellow starthistle except that a coleopteran larva was found feeding in the roots of purple starthistle. None of these taxa appeared to have a significant impact on purple starthistle growth or reproduction. Only one plant pathogen was detected on mature purple starthistle plants in the field. Charcoal rot caused by the fungus, *Macrophomina phaseoli*, was present in several roots and lower stems at one site. Infested plants were not noticeably different from uninfected plants.

It appears that the endemic phytophagous fauna of yellow starthistle and purple starthistle will not interfere with introductions of additional biological control agents in California. The resident fauna is sparse and comprised of generalists. This is in contrast with faunal surveys in Europe and Asia where at least 42 species of herbivores have been reported to attack yellow starthistle in its native habitat. Many of these species attack the flower head but all parts of the plant are exploited. Introduction of natural enemies that attack the rosette and stem of yellow starthistle in California would complement the flower head insects that are currently established.

## Survey of *Chaetorellia* Seedhead Flies on Commercial and Non-Commercial Safflower in California

B. Villegas, D. A. Mayhew, and J. Balciunas

A state-wide survey of seedhead infesting insects in commercial and non-commercial safflower was initiated in 1997. During 1997, 30 *Chaetorellia succinea* (Costa) flies were reared from safflower seedheads collected from a non-commercial safflower field near Red Bluff in Tehama County (Table 1) prompting concerns that *C. succinea* might become a pest of safflower in California. This seedhead fly was accidentally introduced in 1991 during efforts to established *Chaetorellia australis* Hering for the biological control of yellow starthistle, *Centaurea solstitialis* L. A small safflower survey in 1997 had emphasized safflower fields in Napa, Butte, Glenn, Tehama, and Shasta Counties because they were close to yellow starthistle that was infested with *C. succinea* thus insuring that the safflower had the opportunity to be attacked by this fly. A total of 6 flies emerged from a sample of 300 seedheads taken on July 31, 1997 from a non-commercial safflower field in Tehama County. An additional sample consisting of 3,217 seedheads was collected on August 19, 1997 from which 24 additional flies emerged. *Chaetorellia* flies emerged from yellow starthistle sampled near each of the 1997 safflower samplings.

In 1998, a total of 45 safflower fields in 20 counties were sampled (Table 1). The samples were taken from 42 commercial fields and three non-commercial fields. Samples contained a total of 17,299 seedheads from 1,386 plants. Flies emerged only from two of the safflower samples. On August 13, 1998 one fly emerged from a Contra Costa County commercial field sample containing 283 seedheads obtained from 10 plants. The USDA-ARS Biological Control Laboratory resampled the infested field on August 26, 1998 and collected 2,191 seedheads from 250 plants. No flies emerged from this sample. Some 73 flies emerged in the 1998 sample from a non-commercial safflower field located about 0.5 mile from the safflower field found infested in 1997 near Red Bluff, Tehama County. This field was planted with the same seed lot planted in 1997 as the field belongs to the same ranch. The sample contained 1,522 seedheads from 232 plants.

Yellow starthistle was again sampled near the safflower fields whenever possible. Emergence of flies occurred in 32 of the 1998 samples of yellow starthistle. No *Chaetorellia* flies emerged from safflower at any of these sites. At some sites flies did not emerge from either yellow starthistle or safflower. Based on the lack of fly emergence from 45 commercial safflower fields and five non-commercial safflower fields, it appears that *C. succinea* is not a threat to commercial safflower in California. However, it is possible that some varieties of safflower like that planted in the two non-commercial fields in Tehama County may be more susceptible to *Chaetorellia* attack than other varieties. The 103 flies reared from the three safflower samples collected at the Tehama County ranch represent approximately a 2% infestation of the 5,039 seedheads collected. On the other hand, several pests, such as the safflower seedhead moth and spider mites, were commonly found in the safflower samples.

Table 1: Results of the 1997 and 1998 Safflower Surveys for Seedhead Flies

| County | Nearest City | Collection date | Safflower Type | # Plants Collected | # Heads Collected | Emergence from Safflower | Emergence from Yellow Starthistle |
|---|---|---|---|---|---|---|---|
| 1997 | | | | | | | |
| Butte | Honcut | 7/23/97 | Commercial | unknown | 300 | No | Yes |
| Glenn | Willows | 7/31/97 | Commercial | unknown | 300 | No | Yes |
| Napa | Yountville | 7/21/97 | Non-Commercial | unknown | 300 | No | Yes |
| Shasta | Cottonwood | 7/31/97 | Commercial | unknown | 300 | No | Yes |
| Tehama | Red Bluff | 7/31/97 | Non-Commercial | unknown | 300 | 6 *Chaetorellia* flies | Yes |
| Tehama | Red Bluff | 8/19/97 | Non-Commercial | unknown | 3,217 | 24 *Chaetorellia* flies | Yes |
| | | | 1997 Total | | 4,717 | | |
| 1998 | | | | | | | |
| Butte | Chico | 8/4/98 | Commercial | 30 | 300 | No | Yes |
| Colusa | Grimes | 8/4/98 | Commercial | 50 | 287 | No | Yes |
| Colusa | Princeton | 8/4/98 | Commercial | 15 | 316 | No | Yes |
| Colusa | Sycamore | 8/4/98 | Commercial | 29 | 302 | No | Yes |
| Contra Costa | Brentwood | 8/13/98 | Commercial | 50 | 306 | No | Yes |
| Contra Costa | Discovery Bay | 8/13/98 | Commercial | 10 | 285 | No | Yes |
| Contra Costa | Werner | 8/13/98 | Commercial | 10 | 283 | *1 Chaetorellia fly* | Yes |
| Contra Costa | Werner | 8/26/98 | Commercial | 250 | 2,191 | No | No sample |
| Fresno | Firebaugh | 7/31/98 | Commercial | 18 | 276 | No | No sample |
| Fresno | Firebaugh | 7/31/98 | Commercial | 11 | 633 | No | No sample |
| Glenn | Afton #1 | 8/4/98 | Commercial | 26 | 309 | No | Yes |
| Glenn | Afton #2 | 8/4/98 | Commercial | 24 | 339 | No | Yes |
| Kern | Corcoran #1 | 7/30/98 | Commercial | 25 | 338 | No | No sample |
| Kern | Delano | 7/30/98 | Commercial | 22 | 369 | No | No sample |
| Kern | Wasco | 7/30/98 | Commercial | 10 | 376 | No | No sample |
| Kings | Armona | 7/30/98 | Commercial | 11 | 339 | No | No sample |
| Kings | Corcoran #2 | 7/30/98 | Commercial | 35 | 378 | No | No sample |
| Kings | Leemore | 7/30/98 | Commercial | 17 | 360 | No | No sample |
| Merced | Gustine | 8/13/98 | Commercial | 50 | 254 | No | Yes |
| Merced | Gustine #1 | 8/13/98 | Non-Commercial | 110 | 290 | No | Yes |
| Merced | Gustine #2 | 8/13/98 | Non-Commercial | 50 | 262 | No | Yes |
| Monterey | Priest Valley | 8/14/98 | Commercial | 10 | 300 | No | No |
| Sacramento | Elverta | 8/6/98 | Commercial | 26 | 293 | No | Yes |
| Sacramento | Rio Linda #1 | 8/7/98 | Commercial | 28 | 297 | No | Yes |
| Sacramento | Rio Linda #2 | 8/7/98 | Commercial | 17 | 293 | No | Yes |
| San Joaquin | Stockton #1 | 8/11/98 | Commercial | 17 | 297 | No | Yes |
| San Joaquin | Stockton #2 | 8/11/98 | Commercial | 48 | 335 | No | Yes |
| San Joaquin | Stockton #3 | 8/11/98 | Commercial | 24 | 303 | No | Yes |
| San Luis Obispo | Paso Robles #1 | 8/13/98 | Commercial | 10 | 84 | No | No |
| San Luis Obispo | Paso Robles #2 | 8/13/98 | Commercial | 10 | 33 | No | No |
| Santa Clara | San Jose #1 | 8/13/98 | Commercial | 30 | 324 | No | Yes |
| Santa Clara | San Jose #2 | 8/13/98 | Commercial | 10 | 343 | No | Yes |
| Shasta | Cottonwood | 8/18/98 | Commercial | 25 | 400 | No | Yes |
| Solano | Davis | 7/31/98 | Commercial | 25 | 292 | No | Yes |
| Solano | Vacaville #1 | 8/4/98 | Commercial | 28 | 298 | No | Yes |
| Solano | Vacaville #2 | 8/7/98 | Commercial | 19 | 300 | No | Yes |
| Sutter | Kirkville | 8/6/98 | Commercial | 10 | 362 | No | Yes |
| Sutter | Tudor #1 | 8/6/98 | Commercial | 17 | 304 | No | Yes |
| Sutter | Tudor #2 | 8/6/98 | Commercial | 16 | 295 | No | Yes |
| Tehama | Red Bluff | 8/7/98 | Non-Commercial | 232 | 1,522 | *73 Chaetorellia flies* | No sample |
| Tulare | Angiola | 7/30/98 | Commercial | 40 | 270 | No | No sample |
| Yolo | Knights Landing | 8/4/98 | Commercial | 23 | 331 | No | Yes |
| Yolo | Woodland #1 | 7/17/98 | Commercial | 59 | 338 | No | Yes |
| Yolo | Woodland #2 | 7/31/98 | Commercial | 38 | 290 | No | Yes |
| Yuba | Arboga #1 | 8/12/98 | Commercial | 11 | 304 | No | Yes |
| Yuba | Arboga #2 | 8/12/98 | Commercial | 10 | 298 | No | Yes |
| | | | 1998 Total | 1,636 | 17,299 | | |

## Figure 1: Location of Safflower Sampling Fields in California

### Biological Control Program, CDFA

## Survey of *Chaetorellia* Seedhead Flies on *Cirsium* Thistles in Close Proximity to Yellow Starthistle in California

B. Villegas, D. A. Mayhew, F. Hrusa, and J. Balciunas

A survey of native *Cirsium* thistles was initiated in 1998 over concerns that two biological control agents, *Chaetorellia succinea* (Costa) and *C. australis* Hering, introduced for the biological control of yellow starthistle, *Centaurea solstitialis* L. might also attack native thistles in California. During this first year of a multi-year survey, attempts were made to evaluate *Cirsium* thistles within close proximity to yellow starthistle populations known to support one or both species of *Chaetorellia*. A collection permit for a number of native thistles was obtained from the California Department of Fish and Game, and botanical specimens were collected from all thistle populations evaluated. These were deposited with the Botany Laboratory of the California Department of Food and Agriculture's Plant Pest Diagnostics Center. All insects reared from the thistles sampled were pinned and labeled and stored for subsequent taxonomic identification. Voucher specimens will be deposited at the Entomology Museum of the California Department of Food and Agriculture, Bohart Museum of Entomology at the University of California at Davis, and the USDA Systematic Entomology Laboratory.

Seedheads of *Cirsium* species as well as seedheads from nearby yellow starthistle were collected. The seedheads from each host collection were kept separate and transferred to emergence containers in the laboratory. All plant samples were collected at the stage between flowering and seed dissemination. In the laboratory the emergence containers were monitored and any emerging insects were collected, recorded, and stored in entomological collection trays for subsequent identification.

In 1998, a total of six native *Cirsium* species, including three varieties of *Cirsium occidentale* three weedy *Cirsium* species, and were sampled for the presence of the seedheads flies, *C. succinea* and *C. australis* (Table 1). No *Chaetorellia* seedhead flies of either species were reared from any of the thistles collected. Native phytophagous insects were found in most of the thistles sampled. The only non-native insect reared from a number of thistles was *Rhinocyllus conicus* (Froelich), (Coleoptera: Curculionidae), a seedhead weevil introduced into California for the biological control of musk thistle (*Carduus nutans* L.), milk thistle [*Silybum marianum* (L.)], and Italian thistle (*Carduus pycnocephalus* L. ).

Table 1: *Cirsium* Thistles Sampled during 1998 for *Chaetorellia* and other seedhead insects.

| SCIENTIFIC NAME | N/I* | COMMON NAME | COUNTY SITES | EMERGENCE NOTI |
|---|---|---|---|---|
| *Cirsium andersonii* (A. Gray) Petr. | N | Red-stem thistle | Placer & Nevada | No *Chaetorellia* flies |
| *Cirsium arvense* (L.) Scop. | I | Canada thistle | Modoc & Plumas | No *Chaetorellia* flies |
| *Cirsium brevistylum* Cronq. | N | Indian thistle | Humboldt | No *Chaetorellia* flies |
| *Cirsium canovirens* Rydb. | N | Grey-Green thistle | Nevada | No *Chaetorellia* flies |
| *Cirsium cymosum* (Greene) J. T. Howell | N | Peregrine thistle | Siskiyou, Modoc & Lassen | No *Chaetorellia* flies |
| *Cirsium douglasii* DC. var. breweri (A. Gray) D. J. Keil & C. Turner | N | Swamp thistle | Humboldt & Nevada | No *Chaetorellia* flies |
| *Cirsium occidentale* (Nutt.)Jeps. var. *californicum* (A. Gray) D. J. Keil & C. Turner | N | Sierra thistle | Los Angeles | No Sample taken |
| *Cirsium occidentale* (Nutt.)Jeps. var. *candidissimum* (E. Greene) J.F. MacBr. | N | Snowy thistle | Trinity, Siskiyou, Lassen, Plumas | No *Chaetorellia* flies |
| *Cirsium occidentale* (Nutt.)Jeps. var. *venustum* (Greene) Jeps. | N | Venus thistle | Humboldt | No *Chaetorellia* flies |
| *Cirsium ochrocentrum* A. Gray | I | Yellow-spine thistle | Modoc | No *Chaetorellia* flies |
| *Cirsium vulgare* (Savi) Ten. | I | Bull thistle | Humboldt & Siskiyou | No *Chaetorellia* flies |

*N = Native: I = Introduced
** YST = Yellow starthistle