

• BIOLOGICAL CONTROL PROGRAM •



1999

California Department of Food and Agriculture



BIOLOGICAL CONTROL PROGRAM

1999 SUMMARY

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INTEGRATED PEST CONTROL BRANCH**

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Cover developed by Deborah Mayhew. The line drawing of the parasite, *Entedononecremnus krauteri* Zolnerowich and Rose (Hymenoptera: Eulophidae), a natural enemy of the giant whitefly, *Aleurodicus dugesii* Cockerell, (Homoptera: Alyrodidae) was drawn by Ben Shaw.

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The Changing Face of Biological Control and the Hope for Control of the Glassy-Winged Sharpshooter

L.G. Bezark

Agriculture is a constantly changing industry in California and the rest of the world. Developments in worldwide economies, new research, and a host of social factors all contribute to these changes. New plant pests also are a critical driving force in these changes. Biological control practitioners, implementing one of the management tools for plant pests, must be prepared to respond to new pest introductions and outbreaks. As you read this year's annual report, it may become apparent that the emphasis of our program has changed to meet the ever-changing challenges that we are currently facing. Several of our longer-running programs are winding down, shifting emphasis, and being replaced by new programs.

A decade ago, the explosion of whitefly problems in California demanded a drastic focusing of resources. We have successfully established several new whitefly natural enemies since that time. Now you can see a shift in project emphasis as a result of that progress. There is a reduced emphasis on introductions and a strong focus on refining a stable level of control integrated with other management tools. Several years ago, cotton aphid took on a greater importance and the cycle began anew. Several new pest situations have recently arisen and the discipline of biological control must again respond. In this report, newly established projects on olive fruit fly, Lygus bug and a major project on pink hibiscus mealybug are outlined.

Perhaps the most dramatic emerging pest problem is now looming directly ahead in the form of the glassy-winged sharpshooter, and biological control must take its part in the eventual control strategy. This project has required significant department-wide resources. During the past year, a majority of my activities have centered on the glassy-winged sharpshooter and Pierce's disease. I have served as the Chair of two separate Task Force panels and attended numerous meetings of other task forces and committees organized by the University of California, the California Agricultural Commissioners and the private sector. Coordination of research funding and engaging in foreign exploration for natural enemies of the sharpshooter were also accomplished this past year.

Finding a solution to the glassy-winged sharpshooter/Pierce's disease pest problem is one of the top priorities for the California Department of Food and Agriculture. Secretary Lyons is committed to moving aggressively and effectively against this serious pest/disease combination that threatens so many key crops in California.

To mobilize and maximize resources, a Glassy-winged Sharpshooter/Pierce's Disease Task Force was created to ensure close communication amongst the many stakeholders and to elucidate areas of research that needed to be addressed. There was broad representation on the Task Force to encourage statewide coordination. Research funding was made available through Assembly Bill #1232 and a second task force, the Pierce's Disease Advisory Task Force was put in to place. A request for research proposals was initiated, and distributed, and a scientific panel as well as the entire Task Force screened the grant proposals. The Task Force prioritized the most critical research projects and the Secretary approved their recommendations.

Combating the glassy-winged sharpshooter problem defies a simple solution and demands a multi-pronged approach. In a very short time, the various stakeholders - CDFA,

USDA, County Agricultural Commissioners, the University of California, and the agricultural industry - have worked together to develop a strategy for fighting this problem. We are now implementing a statewide management program composed of the following four primary elements:

1. Inspection of nursery stock moving from infested counties and from other states to slow the spread of the pest.
2. Statewide survey to determine the distribution of glassy-winged sharpshooter;
3. Establishment of multi-county pest management areas to begin treatment in infested areas or to develop contingency plans to prepare for infestation.
4. Aggressive public outreach to educate growers and others about the seriousness of this pest problem.

Any actions we take must be firmly based on the best available science. Consequently, research on glassy-winged sharpshooter and Pierce's disease is another vital part of our strategy against this pest complex.

Biological control will likely play a significant role in the long term management programs. Parasitoids of the glassy-winged sharpshooter have been discovered during preliminary survey activities in California and the Southeastern United States. Initial field evaluations in Southern California indicate that one parasitoid is responsible for fairly high levels of parasitization of the fall generation of the sharpshooter. Additional field surveys are necessary to determine the extent of parasitoids that are having an impact on sharpshooter populations in the field. The introduction of additional natural enemies will be an important next step. Several exploration trips to Mexico and Southern Texas are planned in the coming years to locate parasitized egg masses and ship them to quarantine facilities for evaluation. These locations are believed to be a good source for potential sharpshooter natural enemies and a hope for eventual sustainable control.

Establishment and Culturing of Pink Hibiscus Mealybug Parasitoids in the Imperial Valley

W. J. Roltsch, D. Meyerdirk¹, R. Warkentin², R. Weddle³ and E. Andress⁴

The pink hibiscus mealybug (PHM), *Maconellicoccus hirsutus* (Homoptera: Pseudococcidae), was first detected in the town of Calexico in Imperial County, CA during August of 1999. It was found predominately on ornamental trees and shrubs including; mulberry, silk oak, hibiscus, natal plum, carob, and orchid tree. Prior to this time, PHM had become established in a number of Caribbean Islands. The USDA-APHIS (Meyerdirk and Warkentin) has imported several species of parasitoids in the family Encyrtidae through the Delaware USDA-Quarantine Laboratory. Among these species are *Anagyrus kamali* (from China and Hawaii) and *Gyranusoidea indica* (from Egypt and Pakistan).

The two parasitoids, *Anagyrus kamali* (4,200 total) and *Gyranusoidea indica* (2,000 total) were released at 16 sites in Imperial Valley from mid-September to November of 1999 to initiate a classical biological control effort against PHM. From 19 October to 2 November 1999, post release samples (approximately F₁ generation, third instar and early adult female PHM) were obtained and placed individually in gelatin capsules. Among the ten sites that were monitored, samples were obtained from mulberry (8 samples collected), pomegranate (1), silk oak (2), hibiscus (2), grape (1), natal plum (1), elm (1) and rubber tree (1). Of these PHM samples, parasitoids were obtained from mulberry, pomegranate and silk oak. In total, *Anagyrus* was detected at five of the ten monitored release sites. The average percent parasitism by *Anagyrus* at these five sites was 2.4%. We feel the initial results for *Anagyrus* were very favorable, given that our sample size would only allow detection where percent parasitism at a field site was 1 percent or greater. At two sites, masses of ovisacs were collected from the trunks of mulberry trees. Twelve female and two male parasites emerged from material collected on 19 October 1999. From material collected on 18 November, eight female and three male parasites emerged. This information suggests that *Anagyrus* reproduction was significantly skewed in favor of female's progeny at that time. Only one *Gyranusoidea* male was recovered from one PHM sample obtained from mulberry.

Rearing facilities have been established in El Centro for the PHM parasites. Two 55 foot trailers with electrical and water/sewage hookup were completed by mid-February. Ten large cabinets (5x4x2.5ft.) have been built to house the PHM culture. These cabinets provide compartmentalization and isolation of the culture, helping to prevent contamination and minimize possible contamination of the entire culture. Furthermore the cabinets facilitate the maintenance of proper humidity. The PHM culture is being maintained on potato sprouts. We intend to primarily use Japanese pumpkin and butternut squash for future production.

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

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Silverleaf whitefly, *Bemisia argentifolii*, Bellows & Perring, (Homoptera: Aleyrodidae), densities during summer and early fall months in the Mexicali Valley of Mexico are similar to those in the Imperial Valley. This region of the southern Colorado Desert basin is contiguous with the Imperial Valley. Cotton represents a large portion of the agricultural crops grown during the summer in the Mexicali Valley. In cooperation with Mexican officials, four field insectaries composed of ¼ to ½ hectare of okra were prepared in various areas of the Mexicali Valley to facilitate the regional establishment of promising exotic parasitoid species, including *Eretmocerus* nr. *emiratus* (M95107)⁵, *E. mundus* (M92014) and *Encarsia sophia* (M95107).

Each field plot was inoculated with approximately 200,000 *Eretmocerus* pupae (mixed species composition) and a similar number of *Encarsia sophia* during July 1999. Exotic species were not detected in pre-release samples (113 *Eretmocerus* specimens were examined).

Field samples were taken during early October to determine the extent of establishment of the introduced exotic species (identification based on antennal characters) within each plot. Exotic *Eretmocerus* represented a considerable proportion (18 to 50%) of *Eretmocerus* collected at each plot during this late season period. Species identification of recovered exotic material is pending. In addition to exotic *Eretmocerus* spp., *Encarsia sophia* was abundant at one site and found in low numbers in a second insectary field plot. As a result, we are hopeful that a portion of each field plot population migrated to adjacent areas to facilitate the establishment of these new exotic species in the Mexicali Valley. In release studies conducted in the Imperial Valley, we have determined that field plots such as these are capable of producing millions of parasitoids during late summer and fall.

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Introduction of an Olive Fly Parasitoid into Southern California

J. Ball, C. H. Pickett, and R. Messing¹

The olive fly, *Bactrocera oleae* (Gmelin) (Diptera: Tephritidae), a major pest of olives in Mediterranean countries, and most other areas where present, was found for the first time in California (and the U.S.) in October 1998. Delimitation surveys found that the fly was widely distributed in Southern California, ranging from San Diego in the south to Santa Barbara in the north. (Subsequently, a single fly was trapped near a commercial olive grove in Tulare County.) The size of the infestation appeared to preclude eradication and a policy of containment, to prevent spread of the organism beyond the current northern limit, was initiated. This emphasized controlling movement of olive from infested areas, establishing a survey-trapping program in “non-infested” regions of the state, and eradicating all new finds outside the southern zone. In addition to these efforts, a biological control project was started with the rationale that if a natural enemy could be established to lower the overall fly population in infested areas, natural spread might be slowed. To this end, a limited number of a known parasitoid of olive fly, *Psytalia concolor* (Szepligeti) (Hymenoptera: Braconidae), was released. *Ps. concolor*, described from olive fly in Tunisia, was established on olive fly in parts of the Mediterranean in an introduction program early in the last century. Adult parasitoids for California were obtained from the University of Hawaii where they were being reared on Mediterranean fruit fly larvae.

Parasitoids were released on September 15 and 16 in Palos Verdes (Los Angeles County) (~2000 parasites) and September 23, 1999 in Santa Barbara (Santa Barbara County) (~1200 parasites). Between 50 and 200 parasitoids were released on each tree. Olive fruit samples in Santa Barbara were collected for parasite recovery approximately one month after parasite release. Fruit averaged about 30% ripe and weighed 0.083oz. per fruit. Olive flies were recovered in the samples, but no parasites. An additional sample was collected two months later, and fruit averaged about 90% ripe and weighed 0.139 oz per fruit. The second series of samples have not yet been checked for fly or parasitoid emergence. At the time of release, fruit was generally immature, typically small and green in both counties. Under those conditions, we assumed that olive fly infestations were also light, however that did not appear to be the case at the Palos Verdes sites. The Palos Verdes infestation rate was 41.6% contrasted with 6.4% in Santa Barbara. Since no ripeness estimate or weight measurement was made in Palos Verdes, we may have underestimated the fruit maturity in that area. The differences could also reflect differences in adult fly abundance in the two areas. It seems unlikely that there was a second generation of flies produced after sampling in the rearing containers.

Table 1. Olive Fly Infestation on Olives and Parasitoid Recovery in Santa Barbara and Palos Verdes

Location	Date Sampled	Trees Sampled	Total Fruit	Ave. Fruit Weight	No. OLFF	% Infestation ^a	No. Parasitoids
Santa Barbara	10/28/99	13	1820	0.083 oz	117	6.4	0
Santa Barbara	1/19/00	13	1300	0.139 oz			
Palos Verdes	11/3/99	11	1495		622	41.6	0

^a Based on the assumption of only one fly developing in a fruit.

Although the parasitoid was not recovered in the samples, it is possible that some found hosts, but the population was too low for detection. Nevertheless, it is prudent to attempt additional releases. We plan to make additional releases of *Ps. concolor* during the fruiting season 2000-2001. To improve chances of parasite establishment, we propose a program to monitor fruit development and fly abundance, so that parasite releases can be timed to the period of greatest host availability. Although the primary object of the study is to obtain information on host availability for timing parasite releases, there are other benefits. Understanding the fruiting cycle in the areas and the dynamics of host (fruit) utilization by the fly is important in any attempt at interdiction or management.

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**Production of Two Plant Bug Parasitoids from Europe,
Peristenus digoneutis and *P. stygicus*, and Field Release in California in 1999**

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The western tarnished plant bug, *Lygus hesperus* Knight (Hemiptera: Miridae), is a major pest of several crops in California, including cotton, strawberries, seed alfalfa, safflower, and various fruit and nuts. Native to western North America and Mexico, this species appears to lack effective nymphal parasites. A partial explanation might be that, because many of the affected crops are not indigenous they may not be searched by native parasites. Alfalfa is a major host of *L. hesperus* in western United States, but is native to western Asia. It has been hypothesized that parasites attacking closely related *Lygus* infesting alfalfa in Europe may successfully attack *L. hesperus*. To this end, an effort was made to obtain two nymphal parasitoids of *Lygus* spp., *Peristenus digoneutis* Loan and *Peristenus stygicus* Loan (Hymenoptera: Braconidae), from Europe. A limited release of *P. stygicus* was made in a small alfalfa plot in Sacramento in 1998. This was detailed in the last annual report.

During 1999, additional parasite releases were made at four sites. Shipments of *Peristenus digoneutis* from Italy and *P. stygicus* from France, Italy and Spain collected by cooperators in Europe were processed through the USDA-ARS quarantine in Newark, Delaware. From these shipments, adult parasites were released in the field with some held back to establish lab colonies. In addition, a laboratory colony was established from overwintering cocoons held in the UC Berkeley quarantine facility at Albany, CA. Additional field releases were made from the lab colonies, with each geographic race kept separate. Lab colonies were kept at 24hr light and temperatures ranging between 16° and 26°C. Details on the origin of the different geographic races are listed in Table 1 and the numbers and location of releases in Table 2. Parasites were released directly in the alfalfa field or, in two cases, made inside screened cages within the field. Cage release was eventually abandoned because of difficulty in seeding the cage with *Lygus*, keeping the cage free of predators and competitors, while managing the alfalfa.

Table 1. Origin of *Peristenus* spp. released in California.

Country	Locale	Host	Host Plant
Spain	Catalognia, Navata	<i>Lygus</i> sp.	alfalfa
Italy	San Dona' di Piave	<i>Lygus</i> sp., <i>Adelphocoris</i> sp.	alfalfa
France	Herault, Lattes	<u><i>Lygus</i> sp.</u>	<u><i>Diplotaxis</i> sp.</u>

Table 2. Releases of adult parasitoids and *Lygus* nymphs exposed to parasitoids in California in 1999

Species	Origin	Released in ^a	Numbers Released		Source ^b	Dates
			Adults	Nymphs		
<i>P. stygicus</i>	France	Sacramento	162	1768	p,f ₁ ,f ₂	7/22-11/4/99
<i>P. stygicus</i>	Spain	Yolo, Kern	236	1627	p,f ₁	7/30-11/4/99
<i>P. stygicus</i>	Italy	Yolo	298	2207	f ₂ ,f ₃ ,f ₄	7/9-11/4/99
<i>P. digoneutis</i>	Italy	Sacramento	193	107	p,f ₁	9/22-11/4/99

^aCounties of release. ^bp = parent population, f = laboratory generation.

The three fields in Sacramento and Yolo Counties were sampled in early September in an attempt to recover *P. stygicus*. The *Lygus* populations were low (less than 1 per sweep) and only 200 nymphs were collected from the three fields. These were brought back to the lab and held for emergence. No parasites were recovered in this limited sample. A very limited release of *P. digoneutis* was made, as this parasite was received late in the year, and we were unable to establish a lab colony. All F₁ appeared to be male. The reason for this is unknown.

Attempts to monitor overwintering behavior: Early in the program, it was our intention to expose lab colonies to outdoor conditions in order to track winter diapause inception and termination. Unfortunately, we experienced delays setting up a weather shelter, so the first colonies were not placed outdoors until November 1, 1999 (Table 3). Approximately two months later the colonies were brought back into the lab. *Lygus* and the parasites experienced average temperatures of 9.9°C with a mean high of 16.6°C and mean low of 4.9°C, while outdoors. During that period, *Lygus* development was slow and there was excessive mortality, the original adult parasitoids had died, and no cocoons had yet been formed. Approximately two weeks after being returned to the lab, cocoons were observed in the cages and adult parasitoids began emerging about two weeks later.

Adults emerged from 50% of the cocoons. Twenty-eight days after the last adult emergence, the remaining cocoons were dissected. Most contained dead larvae or adults, however, there were several that may have contained viable resting adults. Although conditions may not have been conducive for *P. stygicus* to enter diapause, this parasite seems to tolerate extended periods of cold during development. Interestingly, only female parasitoids emerged, however, there were several males that had not emerged.

Table 3. Survival and developmental history of *P. stygicus* outdoors in late fall.

Strain	Colonies	No. <u>Lygus</u>	Placed Outside	Brought Indoors	1 st Cocoon	No. Cocoons	1 st Adult	No. to <u>Emerge</u>
France	3	460	11/1, 11/9	12/30	1/12	20	2/1	6♀
Italy	2	220	11/1, 11/3	12/30	1/12	7	2/1	2♀
Spain	3	430	11/1, 11/8	12/30	1/12	13	2/1	9♀

Acknowledgment:

We wish to thank Kathleen Casanave who reared the *Lygus* used in this project, Deborah Mayhew who assisted with the parasite colonies, Jim Brown who established and maintained the alfalfa plot in Sacramento, and Mark Van Horn who provided and managed the plot at UC Davis. We also thank Cliff Fong and Rodger Sanders, growers who allowed us to release in their fields.

John Andrews and Linda Schmidt of the University of California, Berkeley cleared the insects through quarantine.

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Insect Natural Enemies Mass Reared for Research and Colonization Projects

K. Casanave, J. Brown, D. A. Mayhew, L. Brace, L. Lee, E. Stallions, and L. Braddock

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 summary of the project goals. This past year, we reared natural enemies for control of cotton aphid, *Aphis gossypii*, silverleaf whitefly, *Bemisia argentifolii*, and western tarnished plant bug, *Lygus hesperus*. The USDA-ARS and USDA-APHIS provided material for starting cultures. 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.

1999 Releases of Natural Enemies by CDFA's Biological Control Program

Natural Enemy	Host	Source Population	Agency Receiving Shipments	Project Description	Stage Delivered	Total Insects Delivered
<i>Eretmocerus</i> M96076 (Ethiopia)	silverleaf whitefly	ERET-13 (DNA banding pattern)	CDFA	Culture maintenance	Pupae	
<i>Eretmocerus mundus</i> M92014	silverleaf whitefly	ERET-1 (DNA banding pattern)	CDFA/Brawley	Field release in Mexico	Pupae	447,000
<i>Eretmocerus mundus</i> M92014	silverleaf whitefly	ERET-1 (DNA banding pattern)	USDA-ARS	Whitefly control in greenhouses	Pupae	164,000
<i>Aphelinus nr paramali</i>	cotton aphid	USA Florida	USDA-ARS, Shafter/CDFA	Overwintering garden	Adults & pupae	16,300
			USDA-ARS, Shafter/CDFA	Citrus, cage study	Adults	80
			USDA-ARS, Shafter/CDFA	Melon cage/open	Adults & pupae	3,582
			USDA-ARS, Shafter/CDFA	Cotton cage/open releases	Adults & pupae	21,964
			UC Lindcove Field Station	Citrus, open release	pupae	4,750
			USDA-ARS, Shafter/CDFA	Movement of parasites, Kern Co.	Adults & pupae	28,625
<i>Aphelinus gossypii</i>	cotton aphid	S. China	USDA-ARS, Shafter/CDFA	Overwintering garden	Adults & pupae	5,500
			USDA-ARS, Shafter/CDFA	Citrus, cage study	Adults	80
			USDA-ARS, Shafter/CDFA	Melon cage/open	Adults & pupae	2,700
			USDA-ARS, Shafter/CDFA	Cotton cage/open releases	Adults & pupae	15,070
			UC Lindcove Field Station		Pupae	5,150
			USDA-ARS, Shafter/CDFA	Movement of parasites, Kern Co.	Pupae	7,630
<i>Peristenus stygicus</i>	<i>Lygus</i>	France (Herault, Lattes)	CDFA, Sacramento, North B St. facility	Cage, open release into alfalfa	Larvae/adults	1768/162
		Italy (San Donna de Piave)	Yolo Co. (UCDavis)	Cage, open release into alfalfa	Larvae/adults	2207/298
		Spain (Catalognia, Navata; nr. Figueras)	Yolo Co. (near Woodland)	Open release into alfalfa	Larvae/adults	1213/178
		Spain (Catalognia, Navata; nr. Figueras)	Kern Co. (near Maricopa)	Into alfalfa	Larvae/adults	414/58
<i>Peristenus digoneutis</i>	<i>Lygus</i>	Italy (San Donna de Piave)	CDFA, Sacramento, North B St. facility	Open release into alfalfa	Larvae/adults	107/163

Evaluation of Habitats for Introduced Parasites of the Cotton Aphid in the San Joaquin Valley

K. Godfrey, D. Ballard¹, K. Casanave, and D. A. Mayhew

The cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae), is a ubiquitous pest in the San Joaquin Valley and can take advantage of the mosaic of habitats throughout the Valley because of its broad host range. Introductions of new natural enemies that should complement the existing natural enemy complex are currently underway. Although the target crop for these releases is cotton, it would prove beneficial if the introduced natural enemies would move to habitats that are spatially and temporally adjacent to cotton along with the cotton aphid. The ability of two introduced parasites, *Aphelinus* near *paramali* and *Aph. gossypii* Timberlake (Hymenoptera: Aphelinidae), to attack cotton aphid in a variety of habitats and move with the cotton aphid was investigated.

The ability of *Aph. near paramali* and *Aph. gossypii* to move with the cotton aphid through space and time was investigated at four geographically separated sites in Kern County. Each site had at least four habitats that could support cotton aphid at some time during the year, one of which was cotton. Releases of both parasites were made at each site. Since both parasites are exotic to California, all recovered individuals came from our releases. To monitor the movement of the parasites and cotton aphid, yellow sticky cards were placed around the appropriate habitat (i.e., the habitat most likely to have cotton aphid) and changed weekly throughout the year. Directional pan trapping was also conducted, but only during the times of the year when flights of cotton aphid alates were most likely. Collections of aphids from host plants were also made when aphids could be found. These aphids were held in the laboratory under the appropriate environmental conditions to allow the development of any parasites. Adult parasites recovered from these rearings were identified and the number recorded.

The major times of cotton aphid movement were identified from the data on yellow sticky cards. The periods of movement by the aphids usually coincided with a change in the suitability of a habitat. In general, periods of movement occurred in late spring (mid-April through early May) when spring hosts were beginning to become unfavorable, and in mid-summer (July into August) when cotton was the most favorable habitat. In the middle of July, there was a major flight of alate cotton aphids at all of the trapping sites. The reason for this flight is unclear, but appears to be linked to the loss of suitability of a host plant other than cotton. The directional pan trap data indicated that at some sites, the alate aphids appear to be coming from specific directions. The introduced parasites were recovered from three of the four sites during the study. However, they were too few in number to routinely appear on yellow sticky cards. This study will continue for another year using the same four sites.

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

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

K. Godfrey, D. Ballard¹, D. Steinkraus², R. Yokomi³, K. Casanave, and D. A. Mayhew

The cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae), can attain pest status in a variety of crops in the San Joaquin Valley. Management of these populations must be done with care so as to avoid problems of insecticide resistance and/or cotton aphid population resurgence. Biological control may be one management tactic that could be used to manage aphid populations within an area of spatially or temporally adjacent crops or habitats.

In an attempt to increase the amount of biological control on cotton aphid populations, a cooperative project among USDA-Agricultural Research Service, CDFA-Biological Control Program, UC Cooperative Extension, and the University of Arkansas was initiated in 1996 and continues currently. The long-term goal of this project is to construct an exotic natural enemy complex that will complement the existing natural enemies. The new natural enemy complex will hopefully have sufficient diversity to attack and reduce densities of the cotton aphid throughout the year in most, if not all, of the habitats it occupies. During the four years of the project, four species of natural enemies have been or are currently being evaluated. These include *Lysiphlebia japonica* (Ashmead) (Hymenoptera: Aphidiidae), *Aphelinus* near *paramali* (ANP), *Aphelinus gossypii* (AG) Timberlake (Hymenoptera: Aphelinidae), and *Neozygites fresenii* (Nowakowski) Batko (Zygomycetes: Neozygitaceae). Of these natural enemies, the two aphelinids and the fungus have shown enough promise to continue evaluation of their efficacy.

During the winter of 1998 and spring of 1999, overwintering studies were conducted at the Shafter Research and Extension Center, Shafter. Releases totalling approximately 22,500 *Aph. near paramali* and 41,550 *Aph. gossypii* were made into the overwintering garden. At approximately two-week intervals, the plants in the garden were sampled. Both ANP and AG were recovered along with native parasites in the genera *Lysiphlebus* and *Aphidius*. The ANP were found from January through May, and AG, from December through April. The native parasites were found throughout the entire sampling interval. This suggests that the parasites are able to survive the winter in the San Joaquin Valley.

The ability of the three natural enemies to attack cotton aphids on melons (cantaloupes and watermelons) was also investigated. Cage studies and open field releases were conducted in a small plot at Shafter. From these studies, it does not appear that the parasites were able to successfully attack cotton aphid on melons. This is somewhat surprising because in studies conducted last year, the parasites readily attacked cotton aphid on pumpkins. It is possible that the morphology of the leaves of the cantaloupe and watermelon varieties used here have characteristics that interfere with the parasites' searching, whereas, pumpkin leaves did not. The aphids from the NF treatment have yet to be diagnosed.

During the 1999 cotton season (June 15 through October 19), sleeve cage and open field release studies were conducted at Shafter to investigate the ability of the three natural enemies to reduce densities of cotton aphid under field conditions. At approximately one-week 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 assessed to be sure there were at least 10 adult cotton aphids per leaf. The cages were then left undisturbed for seven days. At the end of this time, the following treatments were assigned at random to each of

15 cages: introduction of 20 ANP adults, introduction of 20 AG adults, introduction of 10 ANP adults and 10 AG adults, introduction of five NF cadavers, and controls (no natural enemies added). All cages were left undisturbed for seven to ten days, and then were harvested to assess the aphid and natural enemy populations.

The densities of cotton aphid within the plot began to increase in mid-September. For the first eight weeks of the cage studies, the cotton aphids were produced in laboratory culture and then released into the cages. For the remaining weeks, natural populations of cotton aphid were used. In total, 16 weekly samplings were completed, and the results are presented in Table 1 for the parasite treatments and Table 2 for the pathogen treatments. For many of the weeks, the mean density of cotton aphids in the control cages was equal to or greater than the densities in the treatments with parasites or fungus (Tables 1 and 2). In the parasite treatments, both species were able to reproduce readily on aphids in cotton as evidenced by mummies (Table 1). In those cages where both ANP and AG were present, their combined reproduction, in general, was greater than when each species was isolated (Table 1). The increase in reproduction when both species are present was probably due to the host size preference of each species of parasites. AG prefers younger instars of aphids, and older instars are preferred by ANP. The proportion of each species produced when the two species were combined was significantly different from a 1:1 ratio only for three sampling periods (Table 3). Of those three samplings, two produced more AG than ANP and one produced more ANP (Table 3).

In the treatments with NF, there were extremely low rates of infection (Table 2; note that analysis of the NF samples has been completed only for the first 12 replicates). There was no infection by the fungus in the control cages. For those treatments in which infection was found, NF was in the hyphal stage suggesting that these aphids may have been cadavers that were released rather than new infections. The methods used for releasing the fungus are currently being studied for modification.

Concurrently with the sleeve cage studies, open field releases were also made at Shafter Research and Extension Center. From June 22 through October 20, 1999, approximately 13,574 ANP adults and mummies and 7,310 AG adults and mummies were released in the cotton field. The following parasites have been recovered from these releases: seven ANP, three AG, 32 *Lysiphlebus* sp., 12 Charipidae, and two native Aphelinidae.

Table 1. The mean number of aphids and the total mummies produced in parasites and control treatments in sleeve cage studies conducted in cotton at the Shafter Research and Extension Center in 1999.

Rep	Mean No. Aphids			Control	Total Mummies		
	ANP	AG	ANP+AG		ANP	AG	ANP+AG
Jun. 24	66.0	37.3	22.2	25.67	0	0	19
Jul. 1	4.7	4.0	27.1	22.0	3	2	38
Jul. 8	8.4	5.0	6.1	5.7	8	2	6
Jul. 15	21.3	24.0	34.2	29.1	9	16	10
Jul. 22	49.9	41.1	56.5	64.8	6	40	71
Jul. 27	51.1	37.9	39.2	53.9	10	25	27
Aug. 5	62.5	35.0	134.3	65.1	7	30	106
Aug.12	62.9	64.7	106.2	120.7	16	35	68
Aug. 19	91.2	73.0	73.9	164.3	0	2	6
Aug.26	69.3	32.6	43.4	56.9	2	1	6
Sep. 2	21.8	30.7	39.6	67.8	7	2	8
Sep. 9	128.1	69.9	50.0	109.9	0	18	22
Sep. 16	489.7	293.4	369.1	454.3	7	55	25
Sep. 23	106.1	1234.3	N.S. ^a	223.42	1	1	
Sep. 30	130.9	215.3		216.9	6	10	
Oct. 7	192.8	218.3		216.0	7	3	

^aN.S. = This treatment was not set up for the final 3 weeks.

Table 2. The mean number of aphids and the percent infection by NF in the fungus and control treatments in sleeve cage studies conducted in cotton at the Shafter Research and Extension Center in 1999.

Rep	NF	Control	% Infection ^a
Jun. 24	50.8	25.7	0.55
Jul. 1	12.1	22.0	1.19
Jul. 8	9.4	5.7	0
Jul. 15	53.9	29.1	0
Jul. 22	78.0	64.8	0.41
Jul. 27	28.3	53.9	0
Aug. 5	77.9	65.1	0.41
Aug. 12	76.5	120.7	0.18
Aug. 19	195.5	164.3	0.5
Aug. 26	51.4	56.9	1.16
Sep. 2	42.9	67.8	0.51
Sep. 9	83.8	109.9	0.21
Sep. 16	318.5	454.3	
Sep. 23	135.6	223.4	
Sep. 30	157.9	216.9	
Oct. 7	118.3	216.0	

^aThere was no infection in the controls. Samples from the last four weeks are currently being processed.

Table 3. The total number of each species of parasites produced in the ANP+AG treatment in sleeve cages in cotton at Shafter Research and Extension Center in 1999.

Rep.	ANP	AG
Jun.24	9	10
Jul. 1 ^a	12	26
Jul.8	3	3
Jul. 15	5	5
Jul. 22	31	40
Jul. 27 ^a	22	5
Aug. 5	43	63
Aug. 12 ^a	23	45
Aug. 19	4	2
Aug. 26	3	3
Sep. 2	5	3
Sep. 9	9	13
Sep. 16	8	17

^aJul. 1 - $X^2 = 5.16$, $df=1$, $P < 0.05$; Jul. 27 - $X^2 = 10.7$, $df=1$, $P < 0.05$; Aug. 12 - $X^2 = 7.12$, $df=1$, $P < 0.05$.

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Evaluation of Establishment of Introduced Aphid Parasites in Citrus

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The cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae), impacts citrus through its ability to vector citrus tristeza virus. A reduction in the density of cotton aphid population will reduce the threat of citrus tristeza virus spread. Biological control is one management tactic that can be used to achieve this general reduction in cotton aphid densities. Two introduced parasites, *Aphelinus* near *paramali* and *Aphelinus gossypii* Timberlake (Hymenoptera: Aphelinidae), have been shown to reduce cotton aphid densities in other habitats such as cotton, young citrus, and winter non-crop areas. In this study, the ability of these two parasite species to establish in a mature citrus grove was investigated.

The establishment potential of *Aph.* near *paramali* and *Aph. gossypii* was investigated in a grove of Valencia oranges at the Lindcove Research and Extension Center from April 19 to October 5, 1999. Releases of the parasites were made by placing potted hibiscus plants containing cotton aphid and mummies of each of the parasites underneath the canopy of ten orange trees. These plants were left undisturbed for three weeks. After that time, the plants were replaced with new aphid and parasite-infested plants. The plants that had been in the field for three weeks were returned to the laboratory and examined for evidence of parasite reproduction.

Recoveries of both species of parasites were made from the hibiscus throughout the sampling period. Examination of the ground cover, citrus, and surrounding vegetation did not reveal either *Aph.* near *paramali* or *Aph. gossypii*. This is not surprising considering the largest density of cotton aphids within the grove was on the hibiscus plants. Therefore, it was expected that most, if not all, of the recovered parasites would be from the hibiscus plants. This study will continue next year to determine if the parasites overwintered at Lindcove Research and Extension Center.

The Valencia orange grove was provided by Lindcove Research and Extension Center

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Investigation of Population Trends of Vine Mealybug on Grapes in Kern County

J. Ball and K. Godfrey

The first record of the vine mealybug, *Planococcus ficus* (Signoret) (Homoptera: Pseudococcidae), in the San Joaquin Valley was from a vineyard in southern Kern County in August 1998. Vineyards adjacent to this vineyard were surveyed in October 1998, and four additional vineyards were identified as having infestations. Subsequently, vine mealybug (VMB) has been found as far north as southern Fresno County. Since the only information on life history and behavior of VMB in California was from studies conducted in the Coachella Valley, a limited study of VMB biology in the San Joaquin Valley was initiated as a necessary first step in developing appropriate management programs.

It was difficult to find an undisturbed VMB population to study because of the emphasis on containing VMB and stopping or delaying its spread into other grape growing regions of the state. We eventually were allotted 12 vines (six contiguous in two adjacent rows) in an infested vineyard (Perlette variety) in the Arvin area of Kern County. These vines were left untreated for this study, but the remainder of the vineyard was treated with insecticides. The same 12 vines were followed throughout the year.

Two-minute counts of VMB were made at approximately monthly intervals between April and November on the woody parts (trunk and cordon) of each vine. Ten leaves per vine were also inspected for VMB. In addition, yellow sticky-cards were hung in the vines and a length of double-stick tape wrapped around the trunk and a cordon of each vine. These were changed every two weeks.

The greatest number of VMB recorded during timed counts was in June, then numbers dropped off considerably (Table 1). Mealybugs were not found on leaves until July. This type of data has serious drawbacks, however, since field counts are done in variable and often poor light. First instar VMB, which are the most common on leaves, are particularly difficult to see. In addition, the highly contagious distribution distorts trends (the count of 41 VMB in July was from a single leaf).

Table 1. Total number of mealybugs counted on the trunks on 12 vines during 2 minute counts and on 10 leaves.

Date	Apr. 14	May 12	Jun. 9	Jul. 7	Aug. 4	Sep. 7	Oct. 20	Nov. 29
Trunk	3	64	120	16	3	5	2	1
Leaves	0	0	0	41	11	5	4	0

The sticky trap counts probably provide a better picture of population trends through the year (Table 2). From the sticky-tape samples, VMB peaks in early July both on the trunk and cordon. Peak male VMB (the stage caught on yellow sticky-cards) flight occurred in late June, two weeks before the peak on the sticky-tapes. This further supports the trend shown in the tape counts, since most mealybugs trapped on the tapes are in the crawler stage (1st instar) and that would follow the male flight.

Table 2. Temporal distribution of vine mealybug caught on sticky-tape and yellow sticky-cards.

Date	April			May	June		July		August		Sept		Oct	Nov	
	1	14	28	12	9	23	6	7	4	18	7	29	20	10	29
Trunk	6	2	26	15	94	679	1339	857	80	31	49	76	156	87	11
Cordon	0	0	11	2	28	306	819	623	115	331	123	23	4	5	2
Y-card	0	0	0	2	17	169	38	39	51	13	27	19	8	3	1

In summary, VMB appears in relatively low numbers in spring, rising to a population peak in early July. We also know that all stages of VMB are present throughout the year and even in winter young mealybugs survive high in the vine on canes (unpublished data). It remains to be seen if this pattern holds for all varieties and practices.

Acknowledgement: We are indebted to Peggy Schrader and Marjie Bartels, UCCE, Kern County, for servicing the sticky traps.

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 increase from values 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, San Mateo, and Yolo Counties have been monitored since 1993 for ash whitefly abundance and percentage parasitism. There were four release trees per county, yielding 24 trees consisting of 14 ash, five pomegranate, and five ornamental pear trees. Sampling has been conducted once each year at all release sites (single trees). 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, statewide 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, immatures and pupae were counted. The impact of the parasitoid was also estimated on these trees. We removed 10 to 15 leaves with ash whitefly 4th instar nymphs or pupae from each tree and returned them to the laboratory. Nymphs or pupae (as many as three), were removed per infested leaf and dissected to determine parasitism. A total of 30 ash whitefly immatures were selected for dissection from each tree.

In 1999, in addition to the survey, we designed a study to measure experimentally the direct impact of *E. inaron* on ash whitefly. We want to demonstrate that the continued suppression of ash whitefly is caused by parasitism of *E. inaron*. Clip cages were used to exclude parasites from leaves inoculated with 16 to 147 ash whitefly eggs and early instars. These were paired within one meter with 'open' cages on leaves. Three sets of paired leaves were set up on ornamental pear (*Pyrus calleryana* Decaisne) and three in ash (*Fraxinus* sp.) in July 1999. About six weeks later in August, cages with leaves were removed and counted for the number of live and dead ash whitefly, parasitized nymphs and parasite exit holes. Nymphs were dissected to determine the presence of *E. inaron* larvae. Impact of *E. inaron* on ash whitefly nymphs was measured as percent change in the whitefly population. The experiment lasted from the time whiteflies were added to caged leaves until adult whiteflies started emerging, about six weeks.

Figure 1 shows the ash whitefly abundance and parasitism levels over the last 10 years. 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 individuals (eggs and nymphs) per cm². Since 1993 the population has not exceeded 0.26 individuals per cm² 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. Parasitism during the single yearly sample since that time has varied from 8.6% to 58.4%. Within year parasitism values often increased to 100% (Table 1).

The experimental study in which *E. inaron* was excluded from caged whiteflies showed a significant treatment affect (paired t-test, n= 5, p= 0.0015). The change in whitefly population when exposed to naturally occurring parasites was negative and much greater than when caged (-68% vs. +20.9%). The small increase in percentage of whiteflies that were caged could have occurred due to sampling error (missing whitefly eggs) or failure to remove all adult whiteflies. Twenty-two percent of the whitefly nymphs were parasitized by *E. inaron*, much lower than the tree average at the time (ash + ornamental pear = 80%, n= 60). Five replicates of leaves were most likely not representative of the overall activity in the tree. Additional mortality could be attributed to host feeding. Thirty-six percent of the final ash whitefly population exposed to foraging parasites were dead, while only six percent of the final whitefly population in cages were recorded as dead. The whitefly specific predator, *Clitostethus arcuatus* (Rossi) (Coleoptera: Coccinellidae) was also observed in trees. This beetle could have contributed to whitefly mortality. It has been recovered each year since we began sampling in 1994, but has been sporadic in its presence on sample trees varying from 25 to 50% of those sampled. The results from earlier field studies (Gould et al. 1992, Pickett et al. 1996), the persistent populations of *E. inaron*, significant correlation between host and parasitoid populations ($r= 0.25$, $p=0.003$; (Fig. 1), and results from exclusion cages show this parasitoid is primarily responsible for maintaining low population densities of ash whitefly since its introduction.

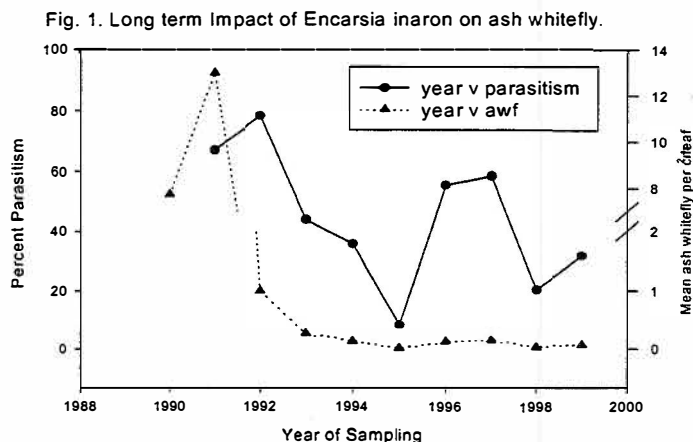


Table 1. Ash whitefly abundance on leaves, parasitism by *Encarsia inaron*, and percentage of sampled trees with *Clitostethus arcuatus* present in California.

YEAR	MEAN AWF/CM ² (RANGE), N	%PARASITISM (RANGE), N	% sites with <i>C. arcuatus</i> (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 (25)
1995	0.01 (0 – 0.10), 24	8.6 (0 – 55.0), 15	25.0 (24)
1996	0.11 (0 – 1.10), 28	55.4 (0 – 100.0), 25	37.5 (28)
1997	0.14 (0 – 0.73), 28	58.4 (0 – 100.0), 25	44.7 (28)
1998	0.04 (0 – 0.33), 28	20.6 (0 – 75.0), 21	25.0 (28)
1999	0.08 (0 – 0.36), 28	32.4 (0 – 82.0), 7	30.0 (28)

^aFrom Pickett et al. 1996

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Exotic Natural Enemies of Giant Whitefly in San Diego County

C. H. Pickett, D. Kellum¹, and M. Rose²

A new parasite of the giant whitefly, *Aleurodicus dugesii* Cockerell, (Homoptera: Aeyrodidae) was collected in Texas in 1995. *Entedononecremnus krauteri* Zolnerowich and Rose (Hymenoptera: Eulophidae) was released at numerous locations in San Diego County during 1995 and 1996. Releases were made onto a variety of ornamental plants infested with giant whitefly, including hibiscus. Large numbers of parasites built up at two of the original release sites and were used to establish populations at new sites in southern California (Table 1).

Three of the original release sites have been monitored for the progress of the released parasite, one at the San Diego Zoo, another at a home site in Carlsbad, and a third at the Carlsbad Parks & Recreation office. *Entedononecremnus krauteri* was recovered for the first time in October of 1996 at the San Diego Zoo, 12 months after the first release. The number of adults started to increase rapidly in early fall of 1997, when the giant whitefly numbers were increasing (Fig. 1). However, in 1998 they did not increase as rapidly and in 1999, they were difficult to detect. The same pattern was observed at the home site and Parks and Recreation office in Carlsbad. Giant whitefly killed one plant at the latter location, and the office decided to pull the rest. The unusually cool spring and summer temperature in San Diego County over the last two years may have retarded the parasite population growth more than the whitefly's.

A second exotic parasite was recently discovered 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. We continue to see this parasite attacking giant whitefly at the zoo.

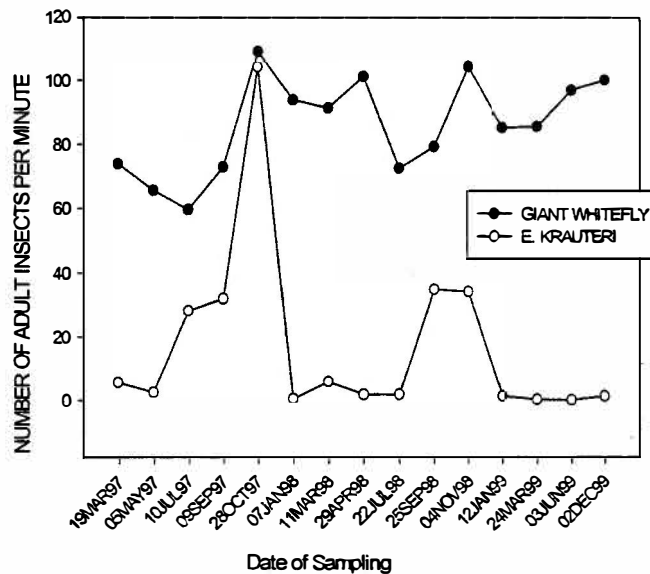
A commercially available whitefly predator, *Delphastus catalinae* (Horn) (Coleoptera: Coccinellidae), was released in Carlsbad (San Diego County) onto a single avocado tree, a citrus tree and one hibiscus plant in the fall of 1995. These beetles are known to attack other whiteflies that produce copious amounts of wax like the giant whitefly. We also released in 1997 several hundred *D. catalinae* on three avocado trees at another residence in nearby Leucadia. At the same time we started monitoring avocado trees in a paired second yard one block away to measure impact of the beetle on giant whitefly infesting avocado.

Beetles have persisted on the original avocado tree at a private residence in Carlsbad through December 1999. They have spread from the avocado to citrus and hibiscus plants at the same location. They have also been found on hibiscus in the surrounding neighborhood where we have been monitoring the infestation levels of the giant whitefly. The beetles at the Leucadia site were found within two months on the paired control tree, eliminating our ability to measure its impact on giant whitefly experimentally. At the time we made releases of beetles at the Leucadia sites, giant whitefly adults averaged between 21 and 68 individuals per minute count while at the original Carlsbad site five miles away, where beetles were released fall 1995, they averaged 0.7 to 1.2 per minute count (we did not take pre-release counts at this site). The beetles show some degree of specificity for the giant whitefly. Their numbers over this period of time were significantly correlated with that of the giant whitefly ($r=0.33$, $p=0.066$, $n=66$). However, other species of whitefly have been consistently found at one of these sites, most commonly the nesting whitefly, *Paraleyrodes minei* Iaccarino, which could also serve as a host to *D. catalinae*.

Table 1. Releases of *Endononecremnus krauteri*

Date Released	City	Address	# Released	Host
8-11-98	Encinitas	Qual Botanical Gardens	40	hibiscus
8-18-98	San Diego	Sea World	200	hibiscus
8-19-98	Encinitas	Qual 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	Qual 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
Summer 1999	San Marcos	Cassou Rd.	100	hibiscus
Summer 1999	Rancho Santa Fe	92067	60	hibiscus
Summer 1999	Escondido	Hidden Ck.	60	hibiscus
Summer 1999	Solana Beach	Solana Cir.	40	hibiscus
Summer 1999	El Cajon		100	hibiscus
Summer 1999	Encinitas	Quail Botanical Gardens	500	hibiscus

Fig. 1. Giant Whitefly and *E. krauteri* at the San Diego Zoo 1997 - 1999



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Colonization of the Tachinid Fly, *Trichopoda pennipes* for the Biological Control of the Squash Bug, *Anasa tristis*

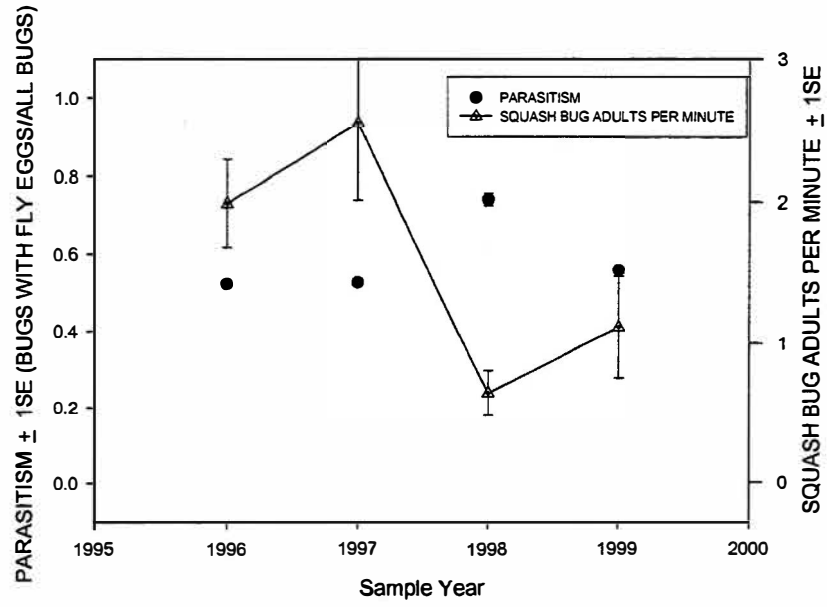
C. H. Pickett, R. Wall, and S. E. Schoenig

Efforts to establish *Trichopoda pennipes* (Fabricius) (Diptera: Tachinidae), were initiated in California in 1992. This parasite is a nymphal-adult parasite of squash bugs in the eastern United States. The project has two objectives: 1) establish flies at additional locations in central and northern California; and 2) measure the long-term impact of released *T. pennipes* on populations of the squash bug.

In 1992 and 1993, *T. pennipes* were field collected in New York and shipped to Sacramento. From 1992 to 1995, the parasite was reared under laboratory and field conditions and released at several vegetable farms in Yolo, Sacramento, and Placer Counties, including the Student Experimental Farm at the University of California, Davis. Parasites successfully persisted at these locations and have now been transferred to other locations in California. In summer 1997, we transferred 500 parasitized bugs to gardens in San Ramon (Contra Costa County). In summer 1999, approximately 300 parasitized squash bugs were shipped to and released in a commercial squash field in Fresno County by C. Summers (University of California, Davis), and 150 at an organic vegetable farm in Kern County. Neither location had previous populations of *T. pennipes*.

Parasites were recovered from the San Ramon location in 1998 and 1999. Timed counts of squash bug adults have been made over the last four years at our release sites. Sites are sampled about three times a summer. The number of adult bugs on squash plants with and without *T. pennipes* eggs on their exoskeleton have been recorded at each vegetable planting for 10 to 20 minutes. Some parasitized bugs were retained for identification of the tachinid. The squash bug density and parasitism have been averaged over the season and for all sites (Fig. 1). Changes in the squash bug population suggest it has dropped over this period of time, while parasitism (based on the presence of tachinid eggs) has remained about the same, although somewhat higher in 1998. These results show that *T. pennipes* is permanently established in some areas of California, and their presence may be correlated with changes in the squash bug population. A grower from one of our original release sites claims the squash bug is no longer a problem at his farm when growing even the more susceptible varieties of squash.

Squash Bug Population Change and Parasitism
Northern California: Seasonal Averages



Augmentative Biological Control Using Transplants

C. H. Pickett, G. S. Simmons¹, E. Lozano¹, and J. A. Goolsby²

We report on a novel approach to enhancing early season field populations of *Eretmocerus* sp. using cantaloupe transplants. Cantaloupe seedlings, prior to placement in fields, were inoculated with a highly specific whitefly parasite, an *Eretmocerus* population recently imported from Ethiopia. We want to determine whether control of whiteflies in fields receiving transplants inoculated with parasites, or “banker plants,” is more effective than control in fields receiving conventional hand releases. We also want to show that transplants with parasites can be integrated into fields using the conventional product imidacloprid (Admire[®]) at very little additional cost, or at least equal to conventional insecticide costs.

The use of ‘banker plants’ was tested for two consecutive field seasons (1998-1999) at an organic farm in the Imperial Valley. Three treatments; banker plants, hand-releases of parasites, and a no-release control, were assigned to 1/3 ac (1998) and ½ ac (1999) plots using a randomized complete block design with four replicates. Monitoring consisted of randomly selecting 40 leaves each bearing early to late instar nymphs, from each treatment plot. In both years the addition of parasites significantly reduced the whitefly nymphal population over the parasite control plot. However, no consistent, significant differences were detected across both years when comparing insect population means between the two release strategies. Parasitism means in the transplant plots were consistently higher than in the hand release plots in 1999, but about the same in 1998. Plots were smaller (1/3 ac) and much closer to each other in 1998, promoting cross contamination among treatments. Whitefly numbers were generally lowest in the transplant plots in 1998, but mixed in 1999. These results suggest that the transplant treatment had a measurable advantage over hand releases, in terms of higher parasitism, but was not great enough to cause consistent, measurable differences in whitefly densities.

We were also successful in augmenting the parasite population in one-acre plots in a conventional field using the conventional product imidacloprid (Admire[®]) for silverleaf whitefly control in 1999. The conventional field used in 1998 never developed a whitefly population, and we were too late in getting our plants into two additional conventional fields in 1999 and many of the transplants died in their transfer. Parasitism levels in the 1999 conventional field were several fold greater than in the control; maximums were 27% versus 5%, respectively. Outside of our plot, the conventional field essentially lacked parasitism. This difference was reflected in the silverleaf whitefly population: a maximum of four whiteflies per cm² in the control plot and 1.2 in the treated plots. Furthermore, these results show that the parasites on the transplants not only survived through the imidacloprid treatment but actually reproduced a new generation. This finding is supported by data from the other two conventional fields treated with imidacloprid. The number of parasitized whitefly on transplants five weeks after placement in these fields was approximately the same as on plants used in the transplanting, but held back and grown under controlled conditions at the USDA field station.

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Tracking the Impact of Released Parasites Using Sentinel Plants

C. H. Pickett, J. Brown, and B. Abel¹

The establishment and impact of parasites of silverleaf whitefly, *Bemisia argentifolii*, Bellows & Perring, (Homoptera: Aleyrodidae) has been difficult to track. The whiteflies attack a broad range of annual and perennial plants in a wide range of habitats that include both urban and agricultural settings. Sentinel plants have been used to monitor parasitism of other pests when they were difficult to locate in the environment (Marston, N. 1980. Sampling parasitoids of soybean insect pests, *In* Kogan & Herzog (Eds.), Sampling Methods in Soybean Entomology). Sentinel plants allow for sampling in a variety of habitats, independent of host plant type, without concern for impacts from insecticides, and independent of the host plant density. We developed a sentinel plant sampling system to measure the change in parasite species composition and the change in magnitude of parasite oviposition by introduced exotics, over and above that attributable by natives.

We started using hibiscus cuttings as sentinel plants in 1998 but switched to cotton plants in 1999 due to contamination problems. Plants were grown from seed in pots that were isolated in individual cages. After two weeks of exposure to adult whiteflies, one foot tall plants were placed outdoors at 30 protected sites in the southern San Joaquin Valley, two plants per site. After one week of exposure to extant parasites, plants were placed back into individual cages. These were placed indoors for two weeks allowing for additional incubation of insects. Leaves were then stripped from plants allowing for adult whitefly and parasite emergence under controlled conditions. These individuals were counted and recorded for each of four monthly sampling events during the latter part of 1998 and 1999.

Most of the problems encountered during our first two years using sentinel plants were overcome. We eliminated contamination of sentinel plants and were able to produce a cotton sentinel plant that could withstand field conditions. This method is apparently sensitive to low populations of parasites. We picked up native parasites during the first run in May 1998 at 6 of 26 sites. The regional whitefly population in the San Joaquin Valley was exceptionally low in 1999. Parasites weren't recorded until September when sentinel plants from 10 of 29 sites captured parasites; four of these captured exotic *Eretmocerus*. Exotic *Eretmocerus* made up 11% of all captured species when including *Encarsia* spp., and 80% when excluding them. One third of the *Encarsia* were *Enc. inaron*, introduced for control of the ash whitefly, *Siphoninus phillyreae* (Haliday). All of the remaining parasites were *En. pergandiella*, except for one *En. meritoria*. Exotic *Eretmocerus* made up 0.16% of emerged adult insects (parasites + silverleaf whitefly) and natives made up 0.04%.

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Fall Releases of Whitefly Parasites into Citrus

C. H. Pickett, E. Lozano¹, and D. Overholt²

The silverleaf whitefly (*Bemisia argentifolii*) Bellows & Perring, (Homoptera: Aleyrodidae) was an increasingly important pest of cotton in the San Joaquin Valley from 1994 through 1997 when this project was initiated. Field studies suggested that citrus had become an important overwintering site for this whitefly. In areas of the Valley with a matrix of both cotton and citrus, cotton has a much higher incidence of whitefly infestations than other farming patterns. We report on large-scale releases of *Eretmocerus emiratus* (M95104), *Eretmocerus* nr. *emiratus* (M96076, Ethiopia), *E. mundus* (M92014), and secondarily *E. hayati*, and trace numbers of *Encarsia transvena* (M95107) (a possible contaminant) into four citrus groves. The study had two goals: (1) to determine if exotic parasites released into citrus during the fall would overwinter in this habitat then move into cotton the following spring; and (2) to permanently establish new populations of exotic parasites specific for the silverleaf whitefly.

Three study sites were identified initially, one each in Fresno, Tulare, and Kern Counties. A fourth was added because one of the original growers stopped farming cotton (Kern Co.). Sites consisted of 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. We began releasing parasites in early August or September 1997, 1998, and 1999 when migrating 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, over 10 million in fall 1998, and 3.1 million in 1999. 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*.

Whitefly migration into orchards was ongoing when we began sampling in August 1997, but didn't commence until late September in 1998 and 1999. The delay in cotton maturity, as a result of a cool spring, undoubtedly played a role in forestalling the emigration of whiteflies from this preferred host plant. Parasitism of silverleaf whitefly on citrus was often quite high. However the number of nymphs parasitized per leaf was low, usually much less than 1 per cm².

Survey of local weeds showed that the released parasites are capable of surviving during winter months on a number of weedy plants common to citrus orchards. We also found parasitized whiteflies on weeds in spring and summer when few if any whiteflies were recorded from citrus.

Exotic parasites have been recorded from three of four cotton fields adjacent to orchards, one year after releases. The one exception to date has been the most recently added release site in the southern end of Kern County. No whiteflies were recorded from cotton this last summer, thus no whitefly hosts were available for parasitism. Parasitism levels at the Tulare and Fresno county farms reflect mixed populations of native and exotics. All recovered and identified parasites at the original Kern County site have been exotic; therefore, the parasitism levels resulted entirely from our releases. Parasitism was recorded early in the season on weeds and citrus at all sites showing that these parasites can rapidly find and attack silverleaf whitefly present at very low numbers. All samples were taken within 100 yards of the orchard.

The use of citrus, adjacent to cotton, as a release site appears to have been a good choice. Although low numbers of whiteflies have been found overwintering in citrus, most of these were found parasitized. These low numbers, multiplied by the large number of perennial trees in the area, greatly increase the likelihood of spread and permanent establishment of these parasites. The weedy plants in the area add to this establishment effort.

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Survivorship of Silverleaf Whitefly Overwintering in Citrus in the San Joaquin Valley

C. H. Pickett and D. Overholt¹

The survivorship of silverleaf whitefly *Bemisia argentifolii* Bellows & Perring, (Homoptera: Aleyrodidae) has been monitored in citrus throughout the year as part of an effort to establish new, exotic parasites of this pest. Four study sites have been used; one each in Fresno, Tulare Counties, and two in Kern County. 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. The whitefly population on citrus leaves was monitored every one to four weeks. Leaves were removed from trees, shipped to our laboratory, and examined for the number of parasitized pupae, whitefly eggs, early instar nymphs, and late instar nymphs using a dissecting microscope.

We summarized whitefly density counts using a multicohort stage frequency analysis method to measure the survivorship of silverleaf whitefly (Manly, B. 1990. Stage-Structured Populations, Sampling, Analysis and Simulation). We wanted to measure the proportion of eggs laid on citrus leaves in fall that successfully developed to adults the following spring. Few whiteflies reproduce in citrus during summer months. Large numbers of adults, however, migrate into orchards shortly after or during cotton defoliation, i.e., September - November. Two estimates were used to measure the likelihood of eggs maturing to adults over this period of time when whiteflies were continuously present in the orchards: survivorship from egg to adult, and from egg to late nymph, the former being a far more conservative measurement (successful adult maturation was measured by the presence of an exit hole in the late stage exuviae, which can fall off within two weeks of adult eclosion, i.e. some were likely missing). The actual value is probably somewhere in between. On average from 1997 to 1998, 0.10% to 4.95% of eggs survived to adults. The following winter fewer whiteflies survived, 0.06% to 0.48%, almost an entire order of magnitude less. Summer and fall, 1998 were cooler than the previous year, which may explain part of the drop in survivorship. The peak in egg production came almost two months later in 1998 (November vs. September), increasing the exposure of eggs to lower temperatures. Also, the cumulative number of eggs oviposited was about half in 1998 than in 1997: 12.5 vs. 7.6 per cm² leaf (not including the new site in Kern County). Another trend is the drop in egg survivorship as one moves from the southern end of the San Joaquin Valley to the central region. This was not true for other instars suggesting egg survivorship is more sensitive to lower temperatures than nymphal stages.

The delay in cotton maturity, as a result of the cool spring, undoubtedly played a role in forestalling the migration of whiteflies from this preferred host plant. Survivorship data shows that anywhere from 49% to 100% late instar whiteflies die before maturing to adults. Most likely many young parasites never fully develop because they die within these hosts. Although we have found a high percentage of parasitized nymphs (see Pickett et al. this volume), the number of parasitized nymphs surviving until early spring has been only a small fraction of the initial number of whitefly eggs. Under optimal conditions, i.e. inside a heated greenhouse, we have found up to 72% of nymphs dying in the absence of any predation or parasitism. Citrus appears to be a poor host for silverleaf whitefly.

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

W. J. Roltsch, E. R. Address¹, K. A. Hoelmer², and G. S. Simmons³

Several exotic *Eretmocerus* and *Encarsia* species/strains have been evaluated in field cages, and released in large numbers in commercial fields, refuge nursery plots and urban yards. The most promising *Eretmocerus* for this desert region include *E. emiratus* Zolnerowich & Rose, *E. nr. emiratus* from Ethiopia and *E. mundus* Mercet. *Encarsia sophia* (= *Encarsia transvena*) from Multan, Pakistan appears promising as well. Identification to species was accomplished using recently published keys and by DNA analysis (RAPD-PCR) by the USDA-APHIS, Mission Biological Control Center, Mission, TX. This report is a summary of parasitoid population development in long-term refuge nursery plots from 1994-1999, and a survey of the current status on a region-wide basis.

From 1994 through 1997, exotic parasitoids were released into long-term refuge field plots on multiple occasions each year. Plots (1/2 to 1 acre) were located at the Imperial Valley Research Center near Brawley, CA, and at an organic farm at the south end of Imperial County. During the warm season, the plots consisted of okra and basil. During the cool season, cole crops (esp. collard) and sunflower were present. Kenaf, roselle and eggplant were also periodically present (1994-1996) along with adjacent plantings of cotton and spring cantaloupe. Leaf samples were taken approximately six times during each year to determine the status of parasitoid population increase and persistence. Neither *E. tejanus*, nor *E. stauferi* (i.e., *Eretmocerus* spp. from Texas) have been recovered following their release in 1994. During 1995, *E. melanoscutus* was released in large numbers but recoveries of this parasitoid were rare (Fig. 1a). Releases of *E. mundus*, *E. hayati* and *E. emiratus* began in April of 1996. Numbers of exotic parasitoids compared to natives were high during early summer; however, the proportion of the sample 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, *E. emiratus* and *E. nr. emiratus* were released. The relative performance of exotics was considerably better than in 1996 (Fig. 1c). The proportion of exotic *Eretmocerus* 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 and 1999, *Eretmocerus* densities soared on okra, basil and adjacent cotton (Figs. 1d & e). By late August, there was a greater proportion of exotic *Eretmocerus* (upwards of 80% on okra and cotton) than native *Eretmocerus*. The order of dominance of exotic *Eretmocerus* species is *E. nr. emiratus*, *E. emiratus* and *E. mundus*. *Encarsia sophia* has reached high densities during the summer and fall of 1998 and 1999 in several of the refuge field plots as well.

Regional surveys of the Imperial Valley occurred 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 of 1998 and 1999. The fall samples of ornamental plants at 15 urban sample sites in three communities indicate that exotic *Eretmocerus* were present in 10 of 15 sites. On average, 25% of the *Eretmocerus* at the 10 locations was

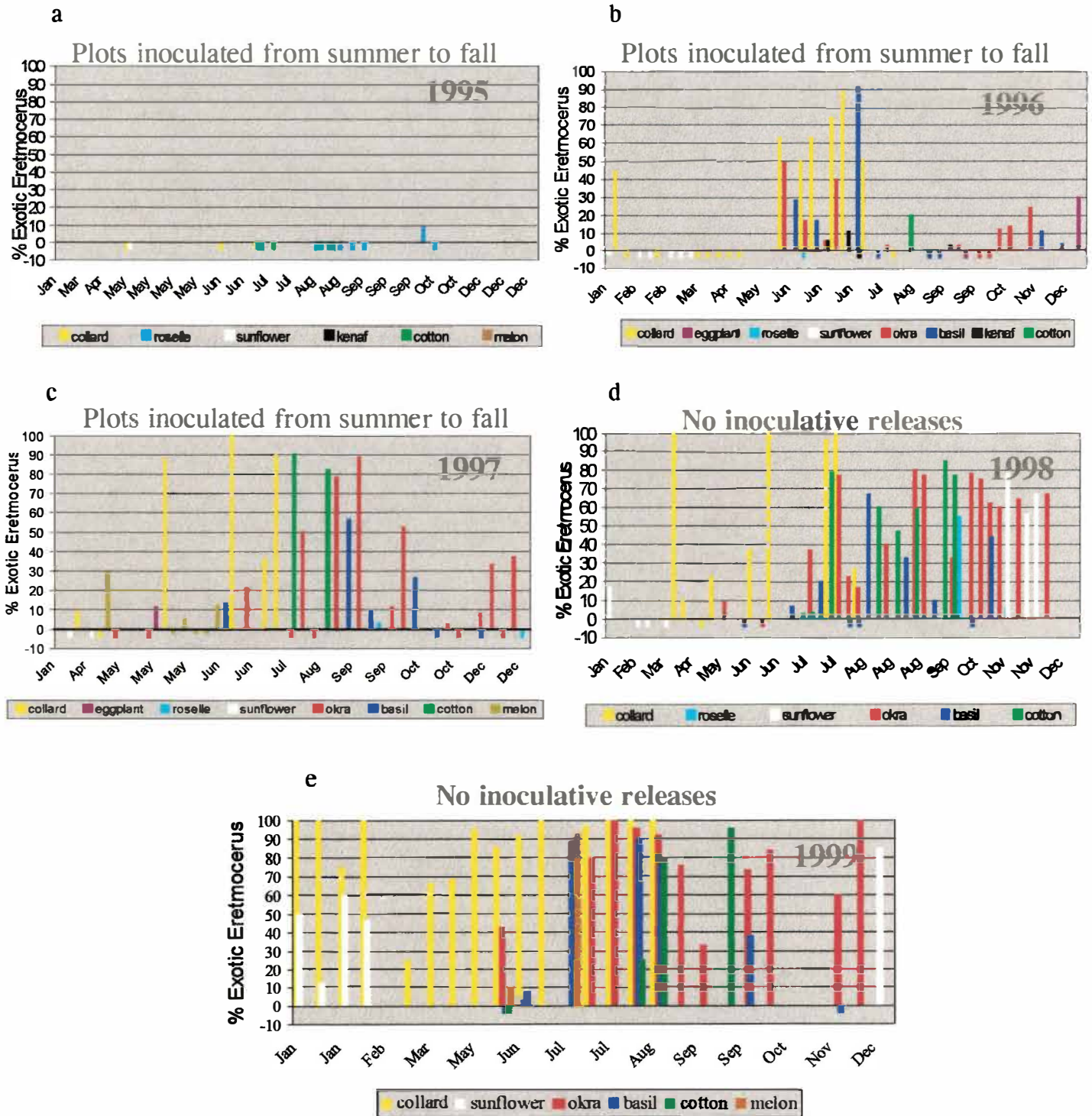
exotic. Among cotton fields, exotic *Eretmocerus* were detected in nine of the 23 fields (i.e., 39%) sampled in the fall of 1998 and in 31 of 42 fields (i.e., 74%) sampled in the fall of 1999. In fields where exotics were detected, 6% of the *Eretmocerus* were exotics in 1998 and 23% were exotic in 1999. Similarly, an increase in *Encarsia sophia* was noted as well from 1998 to 1999. *Encarsia sophia* was detected in only one of 23 cotton fields (i.e., 4%) in 1998 and in 27 of 42 cotton fields (64%) in 1999.

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Fig. 1. Exotic *Eretmocerus* as a Percentage of All *Eretmocerus* Collected From 3-4 Refuge Field Plots From 1995 – 1999 in Imperial Valley, CA



[Bars less than zero represent zero percent; i.e., no exotic *Eretmocerus* in sample]

Releases of *Nosema*-Free *Larinus curtus* for the Biological Control of Yellow Starthistle in California in 1992-1999

B. Villegas, M. J. Pitcairn, and E. Coombs¹

The flower weevil, *Larinus curtus* (Hochhut) (Coleoptera: Curculionidae) was introduced from Greece into California, Idaho, Oregon, and Washington in 1992-1994 by the United States Department of Agriculture, Agriculture Research Service, for the biological control of yellow starthistle, *Centaurea solstitialis* L. (Asteraceae). This weevil oviposits inside open flowers of yellow starthistle and the larvae feed directly on the seeds. A single larva can consume most of the seeds in the seedheads of yellow starthistle.

In California the initial five releases were made in Amador, Placer, Sonoma, Sutter, and Yolo Counties. Although the weevil is currently considered to be established at all five of these sites (Table 1), it was only recovered at low levels at three of the five sites for many years. One possible explanation for the poor establishment was thought to be an infection by the protozoa, *Nosema* sp. discovered in the gut of weevils collected from the Sutter County site as well as other release sites in Oregon and Washington. At other release sites where *Nosema* sp. was not detected, *L. curtus* populations seem to have established well and built up in large population densities. In order to determine if “*Nosema*-free” weevils could get established faster in California, collections of *L. curtus* were made at a site near The Dalles in northern Oregon that appears to be free of the protozoan disease in July 1997-1999. To insure that these weevils were free from *Nosema*, about 20-30 weevils from each of the Oregon collections were killed and examined for *Nosema* by Dr. Bud Thomas of Consulting Diagnostic Service, a certified insect pathologist, formerly with the Insect Pathology Laboratory at the University of California at Berkeley. All the 1997-1999 release sites were preselected in order to match as much as possible the habitat where the weevils were collected in northern Oregon. Consequently the bulk of the releases were made in northern California in dense stands of yellow starthistle.

Table 1 lists all the releases that were made in California between 1992-1999 (both the initial releases as well as the ‘*Nosema* -free’ weevils) as well as recovery information for each of the release sites. At two of the initial release sites, located in Amador and Sutter Counties, *L. curtus* is well established and the weevils can be found well beyond the release area. In June 1999 the flower weevil was found established over a large area of Amador County just north from where it was initially released in 1992. This initial release site was severely disturbed by cattle shortly after the release was made and, it was thought that the site had been destroyed. A survey of the area in July 1999 showed that the weevil had moved out of the release area and had become well established at yellow starthistle infested areas as far as 12 miles from the original release site. The movement of the weevil was mainly northward both towards the east and west. Very little movement of the weevil was recorded south of the release site. Two samples of the weevils, consisting of over 60 specimens each, were made in June and July and sent to Dr. Bud Thomas to check for the presence of *Nosema* in the gut of the weevils. Since samples were found negative for *Nosema*, one redistribution release was made at the Sacramento/Amador County line.

A total of 23 releases were made in 15 counties in California in 1997-1999 with flower weevils collected in northern Oregon. Since it is very difficult to survey for this weevil during the first year after release, emphasis has been placed in surveying sites released during 1997-98.

To date, *L. curtus* has been slow in establishing measurable populations. One major problem that was noticed during the 1997-1998 releases was the tendency of the weevils to immediately fly away after being released on the yellow starthistle flowers. During the 1999 season, the release method was adjusted to reduce the chance of the weevils flying away from the release site. Table 1: Releases and Recoveries of the flower weevil, *Larinus curtus* (Hochhut) in California, 1992-1999

County	Nearest City	Released	Year	Source	Status
<u>1992-1994 Releases</u>					
Amador	Ione	200	1993	Greece	Established
Placer	Auburn	200	1994	Greece	Established
Sonoma	Kenwood	200	1994	Greece	Established
Sutter	Sutter Buttes	270	1992	Greece	Established
Yolo	Woodland	400	1993-4	Greece	Established
Total Releases		1,270			
<u>1997-1999 Releases</u>					
Contra Costa	Concord	600 500	1999	Oregon	Not surveyed
Glenn	Black Butte	200	1998	Oregon	Light Recovery
Kern	Lebec	200	1998	Oregon	Not surveyed
Kern	Tehachapi #1	200	1998	Oregon	Not surveyed
Kern	Tehachapi #2	200	1999	Oregon	Not surveyed
Lassen	Pittville	200	1998	Oregon	Light Recovery
Monterey	Hunter-Leggit #1	400	1999	Oregon	No recovery
Monterey	Hunter-Leggit #2	400	1999	Oregon	No recovery
Monterey	Jolon	200	1998	Oregon	Light Recovery
Placer	Granite Bay	180	1997	Oregon	Established
Plumas	Quincy	225	1998	Oregon	Light Recovery
San Benito	Hollister	200	1998	Oregon	Not surveyed
Santa Clara	San Jose #1	158	1997	Oregon	Light Recovery
Santa Clara	San Jose #2	200	1998	Oregon	Light Recovery
Shasta	Fall River Mills	200	1998	Oregon	Light Recovery
Shasta	Oak Run	200	1998	Oregon	Light Recovery
Shasta	Round Mountain	200	1998	Oregon	No Recovery
Siskiyou	Hornbrook	200	1998	Oregon	Light Recovery
Siskiyou	Weed	200	1998	Oregon	Light Recovery
Tehama	Payne Creek	200	1998	Oregon	Light Recovery
Trinity	Lewiston Lake	500	1999	Oregon	Not surveyed
Tulare	Lemon Cove	300	1999	Oregon	Not surveyed
Tulare	Porterville	200	1998	Oregon	Light Recovery
Total Releases		5,763			
<u>1999 - Redistributions</u>					
Amador	Rancho Murrieta	300	1999	Amador	Not surveyed

¹Oregon Department of Agriculture, Salem Oregon

Biological Control of Purple Starthistle: Release of Knapweed Insects

D. M. Woods, M. J. Pitcairn, D. A. Mayhew, L. Braddock, B. Villegas and E. Coombs¹

Purple starthistle, *Centaurea calcitrapa* L. (Asteraceae), is a weed of regional importance in coastal California especially for the San Francisco Bay area. Although several pathogens and insects are present in portions of its native range such as Turkey (Woods personal observation), the weed has not been deemed important enough to warrant the expense of a classical biological control introduction. Fortunately, a few insects were tested on purple starthistle as part of their pre-release evaluation for use on spotted and diffuse knapweed, and demonstrated acceptance of purple starthistle as a host. We are attempting to establish some of these insects on field populations of 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.

Three knapweed natural enemies, *Terellia virens* (Loew) (Diptera: Tephritidae), *Larinus minutus* Gyllenhal (Coleoptera: Curculonidae) and *Bangasternus fausti* (Reitter) (Coleoptera: Curculonidae) were selected based on laboratory acceptance of purple starthistle as a host. All of these insects are currently established in Oregon on spotted and/or diffuse knapweed. Two of these species, *T. virens* and *L. minutus* were collected field released in California during 1998. During 1999, *L. minutus* as well as *B. fausti* were collected in Oregon for release on additional infestations of purple starthistle. Four hundred adult weevils were released in each location. The seedhead fly, *T. virens*, was not plentiful enough in 1999 to warrant additional experimental introductions.

Ten plants were collected from each site in October 1999, after the plants had fully matured, for evaluation of insect attack. All the seedheads (up to 50) on each plant were dissected and examined for evidence of insects. The apparent colonization of purple starthistle by *L. minutus* in two locations in 1998 was particularly encouraging. Unfortunately, none of the 1999 samples have been as rewarding. No evidence of any of the biological control agents was detected during 1999, including resampling of sites that previously had evidence. *Lasioderma haemorrhoidale* (Illiger) (Coleoptera: Anobiidae), an accidentally introduced stored product pest, was found on a few of the plants in several locations throughout the state in both 1998 and 1999. It is still too early to judge the success of the releases of biological control agents but we hope to establish something on this weed.

Table 1. Release of knapweed biological control insects on purple starthistle in California

	1998		1999	
	Released	Recoveries	Released	Recoveries
Contra Costa				
Rheem Valley	-	-	<i>B. fausti, L. minutus</i>	none
Marin				
Center Rd	<i>Larinus minutus</i>	none	-	none
Lucas Valley	<i>Larinus minutus</i>	none	-	none
Stafford	-	-	<i>B. fausti, L. minutus</i>	Not complete
Napa				
American Canyon	<i>Larinus minutus</i>	none	-	none
Gordon Valley	<i>Larinus minutus</i>	1%	-	none
Henry Rd	-	-	<i>Bangasternus fausti</i>	none
Wooden Valley	-	-	<i>Bangasternus fausti</i>	none
Solano				
Blue Ridge	<i>Larinus minutus</i>	1%	-	none
Lagoon Valley	<i>Terrelia virens</i>	none	-	none
Red Top	-	-	<i>Bangasternus fausti</i>	Not complete
Sonoma				
Chileno Valley	-	-	<i>B. fausti, L. minutus</i>	none
Lakeville	<i>Larinus minutus</i>	none	-	Not complete
Shiloh	-	-	<i>Bangasternus fausti</i>	Not complete

¹Oregon Department of Agriculture, Salem Oregon

Two-Year Development of Biological Control Agents On Squarrose Knapweed, *Centaurea squarrosa*

D. M. Woods, V. Popescu, B. Villegas and E. Coombs¹

Squarrose knapweed, *Centaurea squarrosa* Willd (Asteraceae), is one of the few knapweeds with large established populations in California. Unfortunately, a classical biological control program has never been initiated on squarrose knapweed. It is, however, closely related to both diffuse and spotted knapweed, which have been targets of classical programs. 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. We have initiated efforts to field test available knapweed biological control insects on established populations of squarrose knapweed and determine the potential for impacting the larger infestations in California.

In at least one site, it appears that some of the insects have successfully established. We released two natural enemy species in a large infestation near Pittville in Lassen County. The weevils, *Bangasternus fausti* (Reitter) (Coleoptera: Curculionidae), and *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae) were field collected in Oregon then released in July 1998 on a large infestation of squarrose knapweed near Pittville. The release site for *B. fausti* was approximately 50 meters south of the *L. minutus* release site within the same infestation. During a field evaluation on October 8, 1998, we found adult weevils of both *B. fausti* and *L. minutus* as well as damage on squarrose knapweed consistent with their attack. Field samples were collected from both sites and evaluated in the laboratory. One year later, on September 8, 1999, adults of both species were again noticed at the site and field samples collected. Field samples consisted of ten plants that were collected along a transect established across the release site. All the seedheads were removed, and subsamples (>300 heads/site) were selected for evaluation. The results are shown in Table 1.

Both of the weevil species as well as the 'UV knapweed seed head fly' *Urophora quadrifasciata* Meigen (Diptera: Tephritidae), seem to be established. *Urophora quadrifasciata* was released in very small numbers at the same time as the weevils but may also have migrated to the site previous to the release as it is a strong flier. The relatively low numbers that we detect suggest that it will not likely become a major factor in controlling the weed. The weevil *B. fausti* has been shown to establish low populations on squarrose knapweed in Utah and at an additional site in California. The population levels reported here, although low, are actually quite encouraging for a weed biological control agent less than 24 months from release. Adult weevils did emerge from the samples collected in 1999, confirming that *B. fausti* has completed two generations on squarrose knapweed at this site and is likely established. Adult weevils of *L. minutus* also emerged this year confirming that it also colonized well at this site. Attack rates have dramatically increased around the actual release site. Over 88% of the seedheads were attacked by *L. minutus* near the release site, with most of the attacked seedheads being completely destroyed. *Larinus minutus* is also spreading out from the release site as adults were found at least 50 meters away. Based on these preliminary findings, efforts will be continued to distribute these weevils to other squarrose knapweed sites in the state.

Table 1. End of season status of biological control insects released early summer 1998 on squarrose knapweed near Pittville California. Number indicates the percentage of seedheads attacked by an individual insect species.

Biocontrol Agent	<i>B. fausti</i> site		<i>L. minutus</i> site	
	1998	1999	1998	1999
<i>U. quadrifasciata</i>	1.3%	1.0%	4.6%	2.1%
<i>Bangasternus fausti</i>	0.3%	1.9%	0.7%	0%
<i>Larinus minutus</i>	1.0%	6.7%	11.3%	88.5%

¹Oregon Department of Agriculture, Salem Oregon

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

B. Villegas, D. B. Joley, and E. Coombs¹

Purple loosestrife, *Lythrum salicariae* L. (Lythraceae), is an exotic, invasive weed of wetlands in the northern U.S. and Canada. It is native to Europe, Asia, and northern Africa and was introduced into the United States prior to 1800 because of its herbal qualities and pretty flowers. Once established, the plant produces millions of small seeds that can be spread by wind, transported by wildlife, and carried long distances by water currents. In California, purple loosestrife has been reported in a number of locations throughout the state with the largest infestations located in Butte, Kern, Shasta and Siskiyou Counties.

In 1996 the Biological Control Program initiated a biological control program directed towards the largest infestations of purple loosestrife in California. Two species of weevils, *Hylobius transversovittatus* Goeze (Coleoptera: Curculionidae), a root boring weevil, and *Nanophyes marmoratus* (Goeze) (Coleoptera: Curculionidae), a flower-bud weevil, were released in Butte and Shasta Counties during 1996-1997. To date no recoveries have been made of these two weevils.

In 1998 two leaf-feeding beetles, *Galerucella californiensis* 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. Over a two-year period, greenhouse and field studies by collaborators at the Oregon Department of Agriculture and Oregon State University clarified the insect-host relationship and in spring 1998, the leaf beetles were approved for release in California.

Approximately 7,500 beetles (both species) were obtained on three different dates and released at 16 sites in five counties in California (Table 1) during 1998. All the beetles were collected at Baskett Slough approximately 10 miles west of Salem, Oregon during May and July. In 1999, spring collections of the beetles could not be made due to the unusual wet weather in the Salem area of Oregon. The only collection of the beetles took place in mid July with approximately 2,000 *Galerucella* leaf-feeding beetles collected at a site in Salem, Oregon. These beetles were released at two sites in Shasta and Kern Counties.

In 1998 all the release sites were monitored for colonization by the leaf beetles. First generation leaf feeding damage, eggs, larvae, and adults of *Galerucella californiensis* and *G. pusilla* were noticed at five release sites in Butte, Nevada, and Shasta Counties from May through mid July. After mid July, the adult beetles at all release sites disappeared and could not be detected until spring 1999 when they were found at only a few of the original release sites. It appears from the 1998 releases that the best time to release the leaf feeding beetles in California is in the spring months using overwintering field collected adults since summer collected adults disappear after being released.

Table 1: Releases of the two *Galerucella* leaf feeding beetles on purple loosestrife in California during 1998-1999

County	Nearest City	Year	Sites	Releases	Number Released	Recovery Notes
Butte	Oroville	1998	7	7	2,600	Weak recovery
Kern	Onyx	1999	1	2	1,200	Not surveyed
Nevada	Grass Valley	1998	2	5	1,900	Weak recovery; main site sprayed out by owner
San Joaquin	Lodi	1998	1	1	1,000	No recovery
Shasta	Fall River Mills	1998	3	3	600	Weak recovery
		1999	1	1	800	Not surveyed
Siskiyou	Tulelake	1998	3	3	1,400	No recovery

Releases of the Bull Thistle Gall Fly, *Urophora stylata*, for the Biological Control of Bull Thistle in California

B. Villegas and E. Coombs¹

Bull thistle, *Cirsium vulgare* (Savi) (Asteraceae), is a biennial weed native to Europe, western Asia, and North Africa. It was introduced into the western United States about 1900. In California, it is widespread in disturbed as well as natural areas although it is associated with a high degree of disturbance, such as overgrazed rangeland, pastures and woodland clearings.

The bull thistle gall fly, *Urophora stylata* (Fabricius) (Diptera: Tephritidae), is a host specific biological control agent of bull thistle in its native range. The gall fly has one generation per year, adult flies emerging from overwintering seedhead galls from late May through early July and living for several weeks. The flies lay their eggs in 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 on which the larvae feed. A multi-chambered gall forms on the receptacle with individual larvae occupying separate chambers of the gall. 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 first North America releases of *U. stylata* were in Canada in 1973 from Germany and Switzerland and then into the United States in 1983. It was first released in California in 1993 from established populations in northwestern Oregon. The 1993-1995 releases did not appear to establish so a second establishment effort was initiated in 1997. Table 1 lists the sites where the gall flies were released since 1993 along with notes on establishment.

The 1997-1999 releases were made from bull thistle seedheads containing over-wintering mature gall fly larvae collected in northwestern Oregon. The infested seedheads were transported to Sacramento and kept refrigerated until release sites with bull thistle in the appropriate seedhead stages could be secured. Coastal sites influenced by fog as well as inland sites not influenced by marine weather were secured in as many different areas of central and northern California as possible. Multiple sites in the same general area were favored in order to also try different release techniques. Each year direct release of adult flies reared in sleeve cages in Sacramento were made at some of the sites. These flies were either shipped overnight to cooperators or transported to release sites by BC Program personnel. At most sites, however, releases of the flies were made by putting infested seedheads inside empty #10 orange bags or bags made of netting material. The bags containing the infested seedheads were hung near bull thistle infestations and the flies were allowed to emerge from the seedheads. In 1999, it is estimated that about 6,100 flies were released at 20 sites in Amador, Glenn, Humboldt, Lake, Lassen, Plumas, San Benito, San Luis Obispo, Sierra, Siskiyou and Trinity Counties (Table 1). We are not certain how many flies emerged from the bags containing infested seedheads, but based on emergence records in Sacramento, we estimate that at least 300 flies emerged from each lot of 150 seedheads.

During 1999, most release sites were surveyed for establishment by examining heads for galls during the fall and winter months. The presence of galls in a seedhead can be determined in the field nondestructively by squeezing the seedhead. Uninfested heads are soft and depress with gentle pressure, while infested heads have a hardened gall that is detectable under firm pressure. Establishment of the gall fly was noted at three of the 1993-1995 release sites in San Luis Obispo

and Marin Counties. Two of the sites in Marin County had received additional gall fly releases in 1997, but the San Luis Obispo site had not. This site was thought to have been destroyed by overgrazing and was not checked until this year. Estimates of the infestation rate were performed at several release sites. All the seedheads, including immature ones on 15 plants along a transect, were counted and tallied whether they contained fly galls or not. The infestation rate of the bull thistle seedheads at the 1993-1995 sites ranged from 13.2% to 70.2% for the Marin County sites and was 47% for San Luis Obispo County site #1. Unfortunately, most release sites were severely overgrazed or disturbed and could not be sampled. Two 1998 release sites in Marin County (Tamales #4 and Tamales #5) showed considerable differences in the infestation rate (5.1% to 51.7%). Monitoring of all sites will be attempted in 2000.

Table 2: Infestation of bull thistle seedheads by *Urophora stylata*, in Marin and San Luis Obispo Counties - Fall 1999

County	Site ID	Year of Release	# Plants	# Seedheads	% Infestation
Marin	Tamales #1	1995 & 1997	15	218	53 (70.2%)
Marin	Tamales #2	1995 & 1997	15	436	58 (13.3%)
Marin	Tamales #4	1998	15	391	20 (5.1%)
Marin	Tamales #5	1998	15	259	134 (51.7%)
San Luis Obispo	SLO #1	1995	20	284	133 (47%)

¹Oregon Department of Agriculture, Salem, Oregon

Table 2. Releases of *Urophora stylata* in California for the biological control of bull thistle, *Cirsium vulgare*.

Region	County/City	Year	#Flies	Release Method	Recovery?
Coastal					
	San Luis Obispo/#1	1994	348	Adults	Recovered (99)
	Marin/ Tomales #1	1995	234	Adults	Recovered (96)
	Marin/ Tomales #2	1995	39	Adults	No recovery (96)
	San Luis Obispo/ San Simeon	1995	290	Adults	Not surveyed
	Humboldt/ Eureka #1	1997	410	Adults	Recovered (99)
	Humboldt/ Eureka #2	1997	450	Adults	Recovered (99)
	Marin/ Tomales #1	1997	300	Adults	Recovered (97-99)
	Marin/ Tomales #2	1997	250	Adults	Recovered (96-99)
	Humboldt/ Blue Lake #1	1998	250	Adults	Recovered (99)
	Humboldt/ Eureka #3	1998	600	Seedheads	Recovered (99)
	Humboldt/ Eureka #4	1998	125	Adults	Site destroyed (98 & 99)
	Marin/ Tomales #3	1998	600	Seedheads	Recovered (98)
	Marin/ Tomales #4	1998	300	Adults	Recovered (98-99)
	Marin/ Tomales #5	1998	300	Adults	Recovered (98-99)
	Marin/ Tomales #6	1998	130	Adults	No recovery (98-99)
	Humboldt/ Arcata	1999	300	Seedheads	Recovered (99)
	Humboldt/ Blue Lake #2	1999	300	Seedheads	Recovered (99)
	Humboldt/ Fortuna	1999	300	Seedheads	No recovery (99)
	San Luis Obispo/ Montana de Oro	1999	300	Seedheads	Recovered (99)
	San Luis Obispo/#2	1999	300	Seedheads	No recovery (99)
Inland					
	El Dorado/ Georgetown	1993	227	Adults	Recovered (93); Disappeared (94)
	Modoc/ Alturas	1994	50	Seedheads	No recovery
	Shasta/ Redding	1994	197	Adults	Recovered (94); Disappeared
	Tulare/ Lamont Meadow	1994	50	Seedheads	No recovery
	Mendocino/ Ukiah	1995	280	Adults	No recovery (1996)
	Mendocino/ Willits	1995	50	Adults	Recovered (1996); Disappeared
	San Joaquin/ Stockton #1	1998	600	Seedheads	Recovered (98); site burnt (99)
	San Joaquin/ Stockton #2	1998	300	Adults	Recovered (98); site burnt (99)
	San Joaquin/ Stockton #3	1998	300	Adults	Recovered (98); site burnt (99)
	Tulare/ Success Valley #1	1998	330	Adults	Recovered (98 & 99)
	Tulare/ Success Valley #2	1998	600	Seedheads	Recovered (98 & 99)
	Amador/ Ione #1	1999	100	Adults	No recovery (99)
	Amador/ Ione #2	1999	300	Adults	Recovered (99)
	Glenn/ Orland	1999	300	Seedheads	Recovered (99)
	Glenn/ USFS	1999	300	Seedheads	Not surveyed
	Glenn/ Willows	1999	300	Seedheads	No recovery (99)
	Lake/ Cobb	1999	300	Seedheads	Not surveyed
	Lassen/ Janesville	1999	300	Seedheads	No recovery (99)
	Lassen/ Johnsonville	1999	300	Seedheads	No recovery (99)
	Plumas/ Quincy	1999	300	Seedheads	Recovered (99)
	San Benito/ Paicines	1999	600	Adults	Recovered (99)
	Sierra/ Bordertown, NV	1999	300	Seedheads	Recovered (99)
	Siskiyou/ Gazelle	1999	300	Seedheads	Not surveyed
	Siskiyou/ Montague	1999	300	Seedheads	Not surveyed
	Trinity/ Lewiston	1999	300	Seedheads	Not surveyed
	Trinity/ Trinity Alps	1999	300	Seedheads	No recovery (99)

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

B. Villegas, T Lenigar¹ and D. Haines²

The hairy weevil, *Eustenopus villosus* (Boheman) (Coleoptera: Curculionidae), was introduced from Greece in 1990 for the biological control of yellow starthistle, *Centaurea solstitialis* L. (Asteraceae). This weevil has one generation per year. The adults overwinter in the duff and litter at the base of previous-year stands of yellow starthistle and they emerge to feed on young, closed buds as temperatures warm up in May and June. Unlike other yellow starthistle natural enemies, this damage can be very extensive and readily noticeable. The weevils mate on top of the buds and the females lay individual eggs inside feeding holes on the sides of mature buds from late May through July depending in the area in California. After oviposition, the feeding hole is filled with a distinctive black plug that protects the egg from dehydration and potential predators. The larvae feed on receptacle tissue and on developing seeds inside the seedheads. A single larva is capable of consuming most of the seeds in seedheads of yellow starthistle.

The USDA-ARS, in cooperation with the Biological Control Program and the County Agricultural Commissioners, established the first colonies of the hairy weevil in Nevada, El Dorado, Mendocino, Napa and Shasta Counties in 1990-1991. By 1992, populations of the weevil were so well established at the El Dorado site that two small collections were made and moved to two lower elevation sites in Sacramento and Placer Counties. The Nevada and El Dorado County sites served as sources of weevils for 12 releases (ten counties) in 1993, and for most of the 84 releases made in 43 counties during 1994. As other release sites became available for mass collection of the hairy weevils, biological control training redistribution workshops were scheduled in central and northern California. To date, some 263,130 hairy weevils have been released at over 900 different sites in 49 counties in California (Table 1; Figure 1).

In 1999, personnel from 18 different counties attended workshops in Tulare, Sacramento and Shasta and released some 18,528 hairy weevils at 72 sites. An additional lot of 2,600 weevils were collected from the Tulare County workshop site and released at 12 sites as part of an Integrated Pest Management component being implemented at the Fort Hunter Liggett Military Base by the United States Department of Defense, University of California and the California Department of Food and Agriculture. This weed management plan is hoped to be a model for the control of exotic weeds over wide areas.

The hairy weevil has become widely established in California. Table 1 lists a summary of weevil establishment per county based on over 200 sites monitored at all 49 counties where the hairy weevils were released. Table 1 also displays a listing of counties with sites capable of sustaining in-county redistributions. Seventeen of the 26 counties listed have already made in-county movements of the weevils. In 1999, 11 counties (Glenn, Merced, Monterey, Napa, Plumas, Sacramento, San Benito, Shasta, Tehama, Trinity and Tulare) made 128 in-county releases totaling 36,630 weevils from their own hairy weevil nursery sites. Since two counties, Shasta and Tulare Counties, made most of the in-county redistribution releases, following is a short description from each county's redistribution effort:

Shasta County

Prior to the summer of 1999, the local newspaper in Redding, CA ran a feature article on the biological control of yellow starthistle. The Shasta County Department of Agriculture listed their phone number in the article for those landowners interested in receiving the hairy weevil. The article specified that the landowner should have a large track of land with plenty of starthistle that would not be disturbed by mowing, grazing, or pesticide application. The article generated a huge response, and a list was developed of qualified landowners. The distribution was prioritized by two basic criteria; if a release had previously been made in that section of the map, and how large the track of land. First priority was given to landowners with large pieces of land in sections of the map that had not had previous releases. Extra staff was hired to collect the weevils. The weevils were collected several times a week in order to keep the insects fresh and viable, and from mid June until mid July 1999, landowners would come into the Shasta County Agricultural Commissioner's Office to pick up the weevils and draw a map of the release site. A total of 57 releases totaling 11,600 insects were released during the 1999 season.

Tulare County

In 1999 Tulare County released 13,200 hairy weevils at 63 in-county release sites and provided the Fresno County Department of Agriculture with over 2,600 weevils to release north of the Tulare-Fresno County line. Tulare County's ability to collect and release these and other biological control agents was greatly enhanced in 1999 by the involvement of the Tulare County Summer Youth Work Program. Two high school students were trained and supervised by Tulare County Department of Agriculture's Staff Biologist and Nursery Inspector Dennis Haines. This meant that after early morning nursery survey work was completed, weevils could be field collected, sorted in the laboratory, and scheduled for release by the Staff Biologist the following day. This freed up the Staff Biologist to concentrate on greatly expanding the distribution of *Eustenopus* in Tulare County. Releases were prioritized as follows:

- 1) requests from ranchers and growers;
- 2) new areas with extensive populations of YST; and
- 3) expanding existing release areas.

Grower/Rancher participation was solicited through the media and a joint field-day hosted by the Tulare County Agricultural Commissioner's Office, UC Cooperative Extension, and the Tulare County Weed Management Task Force. This is the fifth year that Tulare County has participated in the Summer Youth Work Program, but the first time the youth were used extensively in biological control. The success of the 1999 effort will insure to do this again during the 2000 field season.

¹ Shasta County Department on Agriculture, Redding California

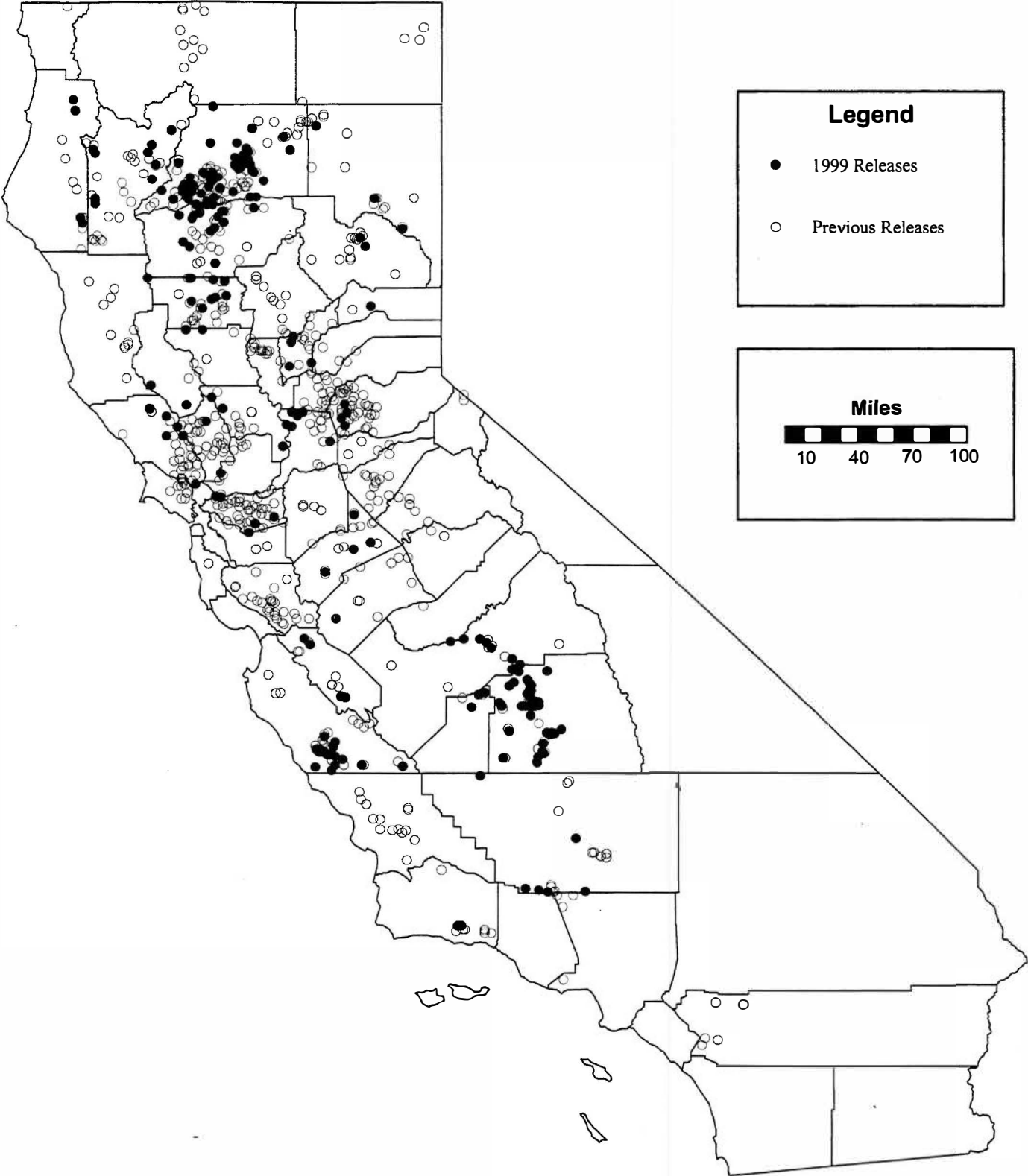
² Tulare County Department on Agriculture, Visalia California

Table 1. Releases of the hairy weevil, *Eustenopus villosus* in California, 1990-1999.

County	1999		1990-1999		Redistributions Possible or done	Establishment Rating
	Releases	#Weevils	Releases	#Weevils		
Alameda	0	0	2	500	NO	Low
Alpine	0	0	2	199	NO	Low
Amador	0	0	5	1,200	YES	Medium-High
Butte	0	0	11	2,470	YES	Medium-High
Calaveras	0	0	16	3,197	YES	Medium-High
Colusa	0	0	3	800	NO	Medium
Contra Costa	5	2,300	41	17,107	NO	Medium
Del Norte	0	0	2	500	NO	Medium
El Dorado	4	863	42	9,661	1992-97	Medium-High
Fresno	8	3,000	13	4,050	NO	Medium
Glenn	16	3,200	39	10,820	1996-99	Medium-High
Humboldt	4	800	20	4,125	NO	Medium
Imperial						
Inyo						
Kern	6	1,155	25	4,755	NO	Medium
Kings	3	600	5	1,010	NO	Low
Lake	2	500	4	1,720	NO	Low-Medium
Lassen	3	1,000	15	3,100	NO	Low-Medium
Los Angeles	0	0	5	2,367	NO	Low
Madera	0	0	1	254	NO	Medium
Marin	0	0	12	3,235	YES	Medium
Mariposa	0	0	5	1,065	NO	Medium-High
Mendocino	0	0	11	2,084	1994-95	Medium
Merced	1	300	11	22,663	1998-99	Medium-High
Modoc	0	0	6	1,350	NO	Medium
Mono						
Monterey	24	5,500	46	15,065	1998-99	Medium-High
Napa	4	1,050	35	10,439	1998-99	Medium-High
Nevada	0	0	2	400	1992-96	High
Orange						
Placer	0	0	20	3,515	1996-98	Medium-High
Plumas	2	510	19	4,210	1997; 1999	Medium
Riverside	0	0	7	700	NO	Low
Sacramento	7	1,400	20	6,916	1995-99	Medium-High
San Benito	4	690	19	4,710	1999	Medium-High
San Bernardino						
San Diego						
San Francisco						
San Joaquin	0	0	7	1,550	NO	Low
San Luis Obispo	0	0	21	9,765	YES	Low-Medium
San Mateo	0	0	5	915	YES	Medium-High
Santa Barbara	5	1,300	15	6,850	NO	Low
Santa Clara	0	0	27	5,902	NO	Low
Santa Cruz						
Shasta	57	11,600	120	25,975	1995-99	Medium-High
Sierra	1	200	3	500	YES	Medium
Siskiyou	0	0	10	2,100	NO	Medium
Solano	0	0	5	1,700	NO	Low-Med.
Sonoma	6	1,250	31	8,716	1998	Medium
Stanislaus	4	800	15	2,950	NO	Low
Sutter	0	0	22	5,280	1997-98	Medium-High
Tehama	17	2,740	41	9,320	1998-99	Medium-High
Trinity	12	1,850	40	8,400	1998-99	Medium-High
Tulare	63	13,200	80	18,875	1998-99	Medium-High
Tuolumne	0	0	10	2,387	NO	Low
Ventura						
Yolo	0	0	10	4,408	YES	Medium-High
Yuba	4	950	16	3,350	YES	Medium
TOTAL:	262	56,758	942	263,130		

Figure 1: Releases of the Hairy Weevil in California in 1990-1999

Biological Control Program, CDFA



Preliminary Evaluation of Musk Thistle Rust, *Puccinia carduorum*, in California

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

The musk thistle rust, *Puccinia carduorum* Jacky, originally collected in Turkey in 1978, was introduced into the eastern United States as a potential biological control agent for musk thistle, *Carduus nutans* L. (Asteraceae). The rust was field released in Montgomery County, Virginia, from 1987-90, and has been spreading across the United States on musk thistle since these original releases. On September 22, 1998, we detected rusted musk thistle plants on the shoulder of Mt. Shasta in Northern California. On August 12, 1999 we found the rust near Mogul in the state of Nevada. Thus, with the musk thistle rust in both California and Nevada, it seems likely that the disease is well established throughout the western United States. It appears that the exotic musk thistle rust isolate of *Puccinia carduorum* has traveled unaided across the continent.

We initiated preliminary evaluation studies of the rust at the Mt Shasta infestation this year. On May 26, 1999, musk thistle rosettes were selected in the field, their rosette diameters measured and rated for rust infection. Plants were again evaluated on September 28 to compare the change over the season for both rusted and non-rusted plants. The two groups of plants were roughly matched for size and age on May 26. Rusted plants listed in Table 1 had rust pustules on 25% of the surface of at least three leaves during the early season evaluation.

Rusted plants proved less likely to bolt over the season than the clean plants. However, those that bolted were taller and produced more heads per plant than clean plants. It is possible that the rust creates enough stress on the rosettes that smaller plants no longer have the reserves to complete bolting. In spite of relatively small degree of visible damage to the plants, the rust disease seems to have important effects on the seasonal development of musk thistle. Studies will continue for several years and will emphasize early growth stages of the plant, including transitions between development stages.

	Status on May 26 1999	
	No Rust	Rust
Number of plants	27	19
Average rosette area (cm ²) on May 26	317	337
Average bolted height on Sept 28 (cm)	33.9	48.2
% of plants bolting by Sept 28	81.4	68.4
Number of heads/bolted plant on Sept 28	3.18	6.38

Impact of Seedling Pathogens on Yellow Starthistle in California

M. J. Pitcairn, D. M. Woods, D. B. Joley, D. G. Fogle¹, 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. The life cycle of yellow starthistle begins with seed germination following the onset of winter rains in November. It grows as a rosette through winter, bolts in April and begins to flower in late June or early July. Seeds are highly germinable and most germinate once wetted. Field studies have shown that extremely high densities of seedlings are present in early winter but, by early spring, densities have dropped by 50-75%. Surveys of yellow starthistle seedlings in Solano County identified at least three naturally occurring seedling pathogens: *Ascochyta* n. sp., *Colletotrichum gloeosporioides*, and *Sclerotinia minor*. All three appeared 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 and spring of 1997-98. Plants in 80 10 cm x 10 cm plots were followed approximately weekly from onset of germination (early November 1997) to flowering (July 1, 1998). The results (summarized in 1998 Annual Report) showed that yellow starthistle seedlings emerged in high numbers immediately following the first rain in early November (peak abundance approximately ten days following first rainfall) then experienced a steady decline in density. Field observations of disease symptoms and laboratory cultures of pathogens suggested that *S. minor* was the predominant pathogen during that study. The fungus, *C. gloeosporioides* was also detected throughout the season, usually on individual plants. *Ascochyta* n. sp., noted in previous years, was not observed during that study. In addition to the occurrence of disease, many seedlings appeared to have been fed upon by small rodents, snails, and slugs.

Encouraged by these findings, the study in Solano County was repeated in 1998-99. Additional study plots were established in the Sierra Nevada foothills east of Auburn (Placer County) and in the Coast Range east of Santa Rosa (Sonoma County) to measure the impact of local endemic seedling pathogens in other geographic regions of California. Sample areas at the Placer and Sonoma County sites were established using the same procedure as at Solano County except that half of the number of sample areas were established. The 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 with 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 two sub-quadrats randomly selected among the four. Thus, there were two sample areas per quadrat and a total of 40 sample areas for the plot. Monitoring consisted of identifying and counting all yellow starthistle seedlings by developmental stage. Seedlings were grouped into five developmental stages: cotyledon, 1-leaf, 2-leaves, 3-leaves, and 4 or more-leaves. To document activity of seedling pathogens, representative samples of diseased seedlings were removed and cultured in the laboratory. Monitoring began October 5, 1998, 10 days after the first rain event, and continued weekly until late April 1999, when all surviving seedlings had grown four or more leaves. After April, monitoring continued every four weeks until July 8, 1999. Sample areas were examined again on September 15, and all surviving plants were counted. For each plant, height and the number of flower heads per plant was determined.

The first rain of the winter of 1998 occurred in early October and was light (approx. 0.25 in.) then was followed by a dry period. No seedlings were observed at the Solano County site in

early October. In contrast, seedling counts in Placer County were 37.5 plants per square meter, however, all plants died within four weeks (Figure 1). The next rainfall event was late October and seedling recruitment began at all three sites. The pattern of seedling emergence and mortality was similar at all three sites: high germination and seedling recruitment in early November followed by steady decline in density through December and into spring. Germination at the Solano County site was lower and more protracted than the previous year. The crop of new seedlings was rapidly attacked by *C. gloeosporioides* and an unidentified species of *Alternaria*. The fungus, *S. minor*, was not detected but this was a relatively dry year and dense seedling stands did not develop. Large populations of the European grey garden slug, *Deroceras reticulatum*, completely consumed the remaining seedlings in the sample areas. New seedlings emerged over the next two months associated with rainfall, but were quickly consumed by the slugs. No plants survived to flower and produce seed at this study site in 1999. The Placer and Sonoma sites followed the pattern seen in the first year at the Solano site with rapid but continual decline in seedling numbers. The fungus, *S. minor*, was detected once at the Placer County site but not at the Sonoma County site. The primary cause of mortality at these sites appeared to be *C. gloeosporioides*. Seedlings that survived to the four-leaf stage usually survived to bolt and flower. However, only 26% of the initial seedlings survived to the early bolting stage (July 1) and only 19.4% were observed on September 15, 1999, to contribute new seed.

It appears that yellow starthistle seedling mortality can be very high. Despite the huge number of seeds produced annually, less than 20% survived to reproduce in 1998 and 1999. 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. Disease 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. At the Solano County site in 1997-98 and at Placer County in 1998-99, *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 a 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 at all three study sites and believe that it can have a significant impact on yellow starthistle. The complete elimination of yellow starthistle plants at the Solano County site in 1998-99 by the European grey garden slug was unexpected and may be an anomaly. The high rainfall in 1998 resulted in a heavy canopy of adult yellow starthistle plants. Once dead, the plants didn't break down and effectively shaded the ground and may have provided an ideal habitat for the slugs to proliferate. European grey garden slugs were also observed at the Placer and Sonoma County sites but their impact appeared to be substantially less. These two sites were more open and did not have a thick overstory of dead adult yellow starthistle plants. The open canopy may have limited the feeding activity of the slugs. This study will be continued in 1999-00 at all three sites.

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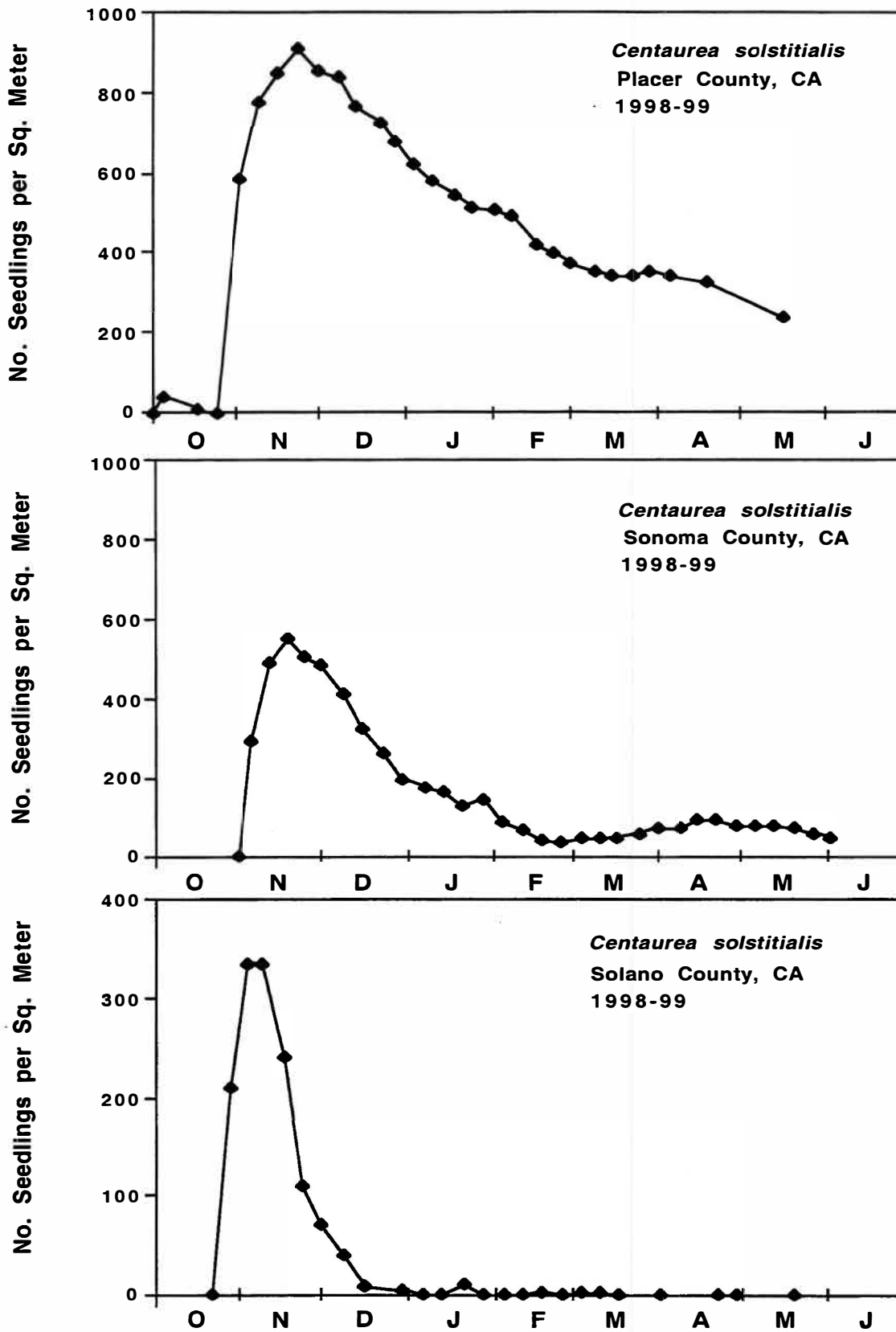


Figure 1. Yellow starthistle density from October, 1998, through June, 1999, at three sites in California.

Five-Year Population Buildup and Combined Impact of Biological Control Insects on Yellow Starthistle

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

Six exotic insect species have been introduced for biological control of yellow starthistle in the western United States. Five species are established in California; three species, *Bangasternus orientalis* (Capiomont) (Coleoptera: Curculionidae), *Urophora sirunaseva* (Hering) (Diptera: Tephritidae), and *Eustenopus villosus* (Boheman) (Coleoptera: Curculionidae), are widespread. The two other species, *Chaetorellia australis* Hering (Diptera: Tephritidae) and *Larinus curtus* Hochhut (Coleoptera: Curculionidae) are abundant in the Pacific Northwest but are limited to isolated populations in California. In addition, the seedhead fly, *Chaetorellia succinea* (Costa) (Diptera: Tephritidae), an accidental introduction into western North America, 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 individual impact of each insect species 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 noxious weed in California. A study was initiated in 1993 to evaluate the population buildup, combined impact, and interaction of all available biological control insects on yellow starthistle. Field sites were established in Yolo, Placer, and Sonoma Counties to represent three different climatic regions where yellow starthistle occurs in abundance. Four insects (*B. orientalis*, *U. sirunaseva*, *E. villosus*, and *L. curtus*) were released at each site in 1993 and 1994 and long-term monitoring of the weed and insect populations was initiated. 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 1300 ft elevation in the Sierra Nevada foothills east of Auburn; the Sonoma County site is at 1200 ft elevation in the Coast Range foothills southeast of Santa Rosa. Various aspects of the weed-insect interaction are being monitored including canopy cover estimates of yellow starthistle and competing species, yellow starthistle seedling recruitment, adult plant density, seedhead numbers, seed production, and insect infestation rates. Preliminary results from 1995-99 are presented in Table 1.

Five years after the first releases, we have evidence that attack by these biological control agents have reduced seed production by yellow starthistle. The weevil, *E. villosus*, has become the most abundant insect at all three sites. In addition to seed destruction by larvae, adult *E. villosus* feed on and kill young developing buds. The loss of early buds produces a structural change and result in plants dominated by stems. Instead of flowers born on the tips of stems, new flowers are produced on short stems arising from the leaf axils along the main stems. Population densities of *E. villosus* increased steadily from 1995-1998 but, interestingly, declined in 1999 at all three sites. This decline may have been due to the unusually cool spring temperatures, which delayed growth and flower production by yellow starthistle until later in summer. Because *E. villosus* oviposition activity usually occurs from June-August, delayed in flower production may have been out-of-phase with *E. villosus* oviposition and resulted in a lower attack rate.

The infestation rates of *B. orientalis*, *U. sirunaseva*, and *L. curtus*, have been less than 25% and have not shown a steady upward trend. The false peacock fly, *C. succinea*, was first recovered in 1996 at the Yolo County site and in 1998 at the Placer and Sonoma County sites. Population densities have steadily increased each year and infestations rates in 1999 ranged from 8-23% 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). Unfortunately, the lower attack rate by *E. villosus* in 1999 resulted in a significant increase in seeds per head and total seed production (seeds/m²).

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. Interestingly, despite the increase in plant density and seed production observed in 1998, seeds per head and total seed production (seeds/m²) declined in 1999. The continuing increase in *C. succinea* may have offset the decline in *E. villosus* attack and continued the decline in seed production at this site.

The density of biological control agents at the Placer County site built up quickly but showed little change from 1995-1997. Insect attack rate increased in 1998 but declined in 1999. *Eustenopus villosus* is the most abundant insect, infesting 58% of the seedheads in 1999; while the other biological control agents occurred at rates 0-9%. 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 the increase in *C. succinea* continue over the next few years and result in additional seed loss.

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

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

Placer County						
Plant	95	96	97	98	99	00
Seedlings/square meter	-	651	669	883	666	842
Adult plants/square m	332	83	108	151	54	
Heads/ square meter	679	280	438	378	256	
Seed/head	8.2	18.0	16.2	6.7	10.6	
Seeds/square meter	5,568	5,040	7,096	2,533	2,730	
<u>Insect & release year</u>						
<i>B. orientalis</i>	93	6.7%	0.6%	1.6%	12.0%	9.4%
<i>U. sirunaseva</i>	93	4.7%	5.0%	8.7%	7.4%	6.0%
<i>E. villosus</i>	93	51.6%	50.9%	54.8%	79%	58.4%
<i>L. curtus</i>	94	0	0	0.2%	0%	0%
<i>C. succinea</i>	-	0	0	3%	8%	
Heads w/ 1 or more sp	58%	60%	60%	83%	73.8%	
Yolo County						
Plant	95	96	97	98	99	00
Seedlings/square meter	-	1,095	1,928	1,076	642	992
Adult plants/square m	975	322	180	422	72	
Heads/ square meter	1,181	369	343	830	249	
Seed/head	23	26	13	18	17	
Seeds/square meter	27,163	9,594	4,459	14,691	4,275	
<u>Insect & release year</u>						
<i>B. orientalis</i>	91	5%	2%	4%	3%	4%
<i>U. sirunaseva</i>	93	13%	18%	17%	13%	11%
<i>E. villosus</i>	93	5%	19%	23%	50%	24%
<i>L. curtus</i>	94	0	0	0.2%	0%	0%
<i>C. succinea</i>	96	0	2%	8%	12%	23%
Heads w/ 1 or more sp	19%	33%	31%	57%	36%	
Sonoma County						
Plant	95	96	97	98	99	00
Seedlings/square meter	-	897	822	624	234	1,020
Adult plants/square m	241	233	222	231	64	
Heads/ square meter	547	442	508	486	414	
Seed/head	25.3	14.9	8.0	7.8	15.1	
Seeds/square meter	13,839	6,586	4,064	3,802	6,232	
<u>Insect & release year</u>						
<i>B. orientalis</i>	94	5.4%	9.5%	4.2%	12.4%	12.9%
<i>U. sirunaseva</i>	94	4.8%	16.3%	19.7%	22.7%	22.2%
<i>E. villosus</i>	94	12.9%	37.3%	73.9%	72.7%	65.5%
<i>L. curtus</i>	94	0	0	0.7%	0.5%	0%
<i>C. succinea</i>	-	0	0	1.0%	12.9%	
Heads w/ 1 or more sp	22%	56%	80%	83%	78%	

Integrating Chemical and Biological Control Methods for Control of Yellow Starthistle

M. J. Pitcairn, J. M. DiTomaso¹, and V. Popescu

Yellow starthistle, *Centaurea solstitialis* L. (Asteraceae), is an exotic noxious 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 over the long term unless the the soil seedbank is depleted.

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 concerns, however, that continuous use of these herbicides may result in a loss of effectiveness due to herbicide resistance. Integrating or alternating several control methods into a management strategy may prevent or delay the development of herbicide resistance. Biological control may be a valuable component of this management strategy as all of the yellow starthistle insects attack the flower heads and reduce seed production. Application of the herbicide, clopyralid, occurs in late winter (February-March) on young seedlings and does not coincide with summer insect activity. We hypothesize that combining clopyralid applications with attack by exotic insects may provide for more effective long-term control of yellow starthistle which may slow the rate of re-infestation by impacting the few flower heads available. Previous studies have shown that yellow starthistle seed bank 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 four to six 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 hypothesis, a field study was initiated in 1997. The study site was located in a 20-acre area along the east shore of Lake Natoma, Sacramento County and 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 four exotic insect species (*Bangasternus orientalis* (Coleoptera: Curculionidae), *Urophora sirunaseva* (Diptera: Tephritidae), *Eustenopus villosus* (Coleoptera: Curculionidae) and *Chaetorellia succinea* (Diptera: Tephritidae) are established there and all managing partners agreed to cooperate on the project. The pre-study infestation rate and seed destruction from the four exotic insects present at the site was estimated in 1997 by bagging senesced flower heads of different stages of development. The results showed that *B. orientalis* and *U. sirunaseva* occurred in low abundance and appeared to have caused negligible damage to seed production. 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. In contrast, *C. succinea* increased from early July to early August when 38% of the seedheads were attacked.

In 1998, four plots (replicates) were established within the field site. Each plot (25m x 40m) was divided into two 25m x 20m 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 with only the central 20 x 15 m being used for data collection. Pretreatment estimates of seedling density showed no differences between subplots. Clopyralid treatments were made by hand using a commercial field sprayer. The lowest labeled rate of clopyralid is 1.5 oz ae/A. We used a single application of clopyralid at a rate of 0.5 oz ae/A to ensure an adequate number of yellow starthistle escapes. The goal of the herbicide treatment was to substantially reduce but not eliminate yellow starthistle seed production in the treated subplots. The field site will be monitored for several years to document resurgence of yellow starthistle. Control plots without biocontrol agents are not being used 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 lower growth rates and reduce seed production by the plant. Furthermore, yellow starthistle is an obligate outcrosser and requires insect pollinators for effective seed set.

Following the herbicide application, the treated subplots were dominated by grasses while the untreated subplots were dominated by yellow starthistle. Vegetative cover of mature yellow starthistle populations (early July) averaged 96% in untreated subplots and 26% in the clopyralid treated subplots. The attack rate and impact of the biological control insects between the treated and untreated plots were estimated by bagging senesced flower heads at different times and in different stages of development. The results in 1998 indicated that *E. villosus* and *C. succinea* were again the only two insects to significantly impact seed production. Oviposition or adult feeding damage by *E. villosus* was found in 43-52% of the early season flower heads; later heads were attacked at a lower rate, especially heads in the untreated subplot. In contrast, *C. succinea* attack ranged from 18-72% and was highest among the mid-season flower heads.

Monitoring of the field site continued in 1999. Plant density was monitored near peak flowering (July 26) by removing plants and heads within a square meter at three locations within each subplot. All plants and flower heads were counted then dried and weighed. The amount of cover due to yellow starthistle was estimated using point locations along three transects in each subplot. A total of 50 points was observed at one foot intervals along each transect. The impact of the exotic insect attack was estimated using two sampling methods. One method consisted of enclosing flower heads with recently senesced flowers (faded flowers with green bracts) with cloth bags. The first of these samples occurred on July 26 and represented the early season flower heads; the second sample occurred on August 20 and represented the mid/late season flower heads. The length of the flowering period was attenuated in 1999 and few new flowers occurred after the August 20 sample. For each sample, 30 flower heads were bagged in each subplot (60 bags per plot, 240 bags per date). After three to four weeks, all bagged flower heads were removed to the laboratory and dissected. Filled pappus and non-pappus seeds in each head were counted. Species, number, and life stage of the insects occurring in each head were also determined. The second method to estimate the impact of the exotic insects was to remove five plants from each subplot at the end of the flowering period (September 10). The advantage of this method was that it provided an estimate of insect attack for the whole season, not just on two dates. All of the mature flower heads on a plant were dissected and examined for insect attack. The damage caused by each of the four exotic insects is different enough that species identification is possible. For each attack head, the insect causing the damage was recorded and the number of pupal chambers counted.

The results show that while plant density declined in the untreated subplots, it was unchanged in the treated subplots (Table 1). The large decline in plant abundance may have been due to the infrequent rainfall in October and November, 1998, which resulted in an unusually high mortality of the emerging seedlings. All other parameters (head density, biomass, cover) also declined in 1999. Comparison of the treated and untreated subplots shows that plant abundance in the treated subplots was approximately half of that observed in the untreated subplots.

The impact of the four exotic insects was similar to what was observed last year. Both *B. orientalis* (0-11%) and *U. sirunaseva* (0-13%) occurred at low numbers (Table 2). Even though *B. orientalis* eggs were present on the July 26 bagged heads, no larval damage was found on either sample dates. The attack rate of *E. villosus* in the early season samples was higher than observed in 1998 but was substantially lower in the August 20 samples. Attack by *C. succinea* increased from early season to mid/late season. There appears to be little difference in attack rate between the treated and untreated plots for all insects and both sample dates.

The end-of-year plant samples (unbagged) showed yet another view of the attack by the exotic insects. Not all flower buds mature to produce flowers and seed. Approximately 54% of the immature flower buds in the treated subplots and 64% in the untreated subplots die before flowering (Table 3). Death was primarily due to feeding by *E. villosus* adults which resulted in 79% and 89% of bud death in the treated and untreated subplots, respectively. Flower production among the 40 plants sampled ranged from 7-62 mature flower heads per plant (n=444 heads in the treated subplots; n=367 heads in the untreated subplots). Flower head attack by *E. villosus* and *C. succinea* were similar (*E. villosus*: 36% and 47% for treated and untreated subplots, respectively; *C. succinea*: 44% and 27% for treated and untreated subplots, respectively). Also, the proportion of heads not attacked by any exotic insect was nearly identical (24% and 26% for treated and untreated subplots, respectively). These results show that attack of yellow starthistle by the four exotic insects was similar in the treated and untreated subplots indicating that these insects do not avoid the treated areas of low plant density. Monitoring of these sites will continue in 2000.

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Table 1. Yellow starthistle plant density, head density, biomass and % cover at full bloom (mid July) in treated and untreated plots in 1998-99.

		1998	1999
Plants/m ² -	treated	3.0	3.0
	untreated	116.0	6.6
Heads/m ² -	treated	184.0	31.1
	untreated	892.8	69.8
Biomass(g/ m ²)	treated	77.3	8.6
	untreated	680.6	21.8
%Cover	treated	26.3	6.2
	untreated	96.2	14.5

Table 2. Mean attack rate (%) and seed destruction by four exotic insects among bagged yellow starthistle flower heads near Folsom, CA, in 1999.

Insect species	Cause of damage	Percentage of Heads			
		Early-season		Mid-season	
		Herbicide treated N=119	Untreated N=110	Herbicide treated N=117	Untreated N=114
<i>B. orientalis</i>	eggs	6	11	0	0
	larvae	0	0	0	0
<i>U. sirunaseva</i>	galls	0	2	4	13
<i>E. villosus</i>	Feeding/oviposition	74	69	10	8
	larvae	20	13	4	2
<i>C. succinea</i>	larvae	28	23	37	44
None	No damage	11	15	54	42

Table 3. Mean attack rate on unbagged yellow starthistle (no. heads per plant) by four exotic insects. Plants were collected at the end of the flowering period on September 10, 1999 near Folsom, CA.

Stage	Treated				Untreated			
	No. per plant	Number Attacked (%)			No. per plant	Number Attacked (%)		
		<i>E. villosus</i>	<i>U. sirunaseva</i>	<i>C. succinea</i>		<i>E. villosus</i>	<i>U. sirunaseva</i>	<i>C. succinea</i>
Buds 1&2	22.8	18.1 (79)			32.0	29.1 (91)		
Bud 3&4	2.8	2.1 (76)			1.3	0.5 (38)		
Flowers	22.2	7.9 (36)	0.8 (4)	9.9 (44)	18.4	8.7 (47)	1.7 (9)	4.9 (27)
Total Hds	47.8	28.1 (59)			51.7	38.3 (74)		

Survey of *Chaetorellia* Seedhead Flies on *Cirsium* Thistles in Close Proximity to *Centaurea* spp. in California

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

A survey of native *Cirsium* thistles was initiated in 1998 and continued during 1999 to address the question that two biological control agents, *Chaetorellia succinea* (Costa) and *Ch. australis* Hering, introduced for the biological control of yellow starthistle, might also attack California native thistles. During this survey, efforts were made to evaluate *Cirsium* thistles naturally occurring within close proximity of known host plants of the *Chaetorellia* seedhead flies. The known hosts are yellow starthistle, *Centaurea solstitialis* L., bachelor button, *Centaurea cyanus* L. and tocalote, *Centaurea melitensis* L. Botanical specimens were collected from all thistle populations evaluated and deposited with the Herbarium (CDA) of the California Department of Food and Agriculture's Plant Pest Diagnostics Center. Collection permits for rare or endangered native thistles were obtained from the California Department of Fish and Game.

The seedheads from each host collection were kept separate and transferred to emergence containers in the laboratory. The *Cirsium* thistle samples collected included unopened flower buds and parts of the peduncles in order to insure a complete collection of the seedhead insects. This is a change in the seedhead sampling protocol done during 1998, which emphasized sampling mature seedheads between flowering and seed dissemination. In the laboratory the emergence containers were monitored and any emerged insects were collected, pinned, labeled, recorded, and stored in entomological collection trays for subsequent identification.

In 1998, a total of 12 native *Cirsium* species, two exotic weedy thistle species, and four varieties of *Cirsium occidentale* were sampled for attack by the seedheads flies, *Ch. succinea* and *Ch. 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 and these will be reported later when the survey is completed. The only non-native insect reared from a number of thistles was *Rhynocyllus conicus* (Frolich), a seedhead weevil introduced into California for the biological control of musk thistle, *Carduus nutans* L., milk thistle, *Silybum marianum* (L.) Gaertn., and Italian thistle, *Carduus pycnocephalus* L.

Table 1: *Cirsium* Thistles sampled during 1998-1999 for *Chaetorellia* seedhead flies

Scientific Name	Common name	N/I*	Year	Samples	County	Emergence Notes
<i>C. andersonii</i> (A. Gray) Petrak	red stemmed thistle	N	1998	2	Nevada	No <i>Chaetorellia</i> flies
<i>C. arvense</i> (L.) Scop.	Canada thistle	I	1998	2	Modoc, Plumas	No <i>Chaetorellia</i> flies
<i>C. brevistylum</i> Cronq.	clustered thistle	N	1998	1	Humboldt	No <i>Chaetorellia</i> flies
<i>C. canovirens</i> Rydb.	gray-green thistle	N	1998:1999	3	Nevada	No <i>Chaetorellia</i> flies
<i>C. crassicaule</i> (Greene) Jepson	slough thistle	N	1998	1	Kern	No <i>Chaetorellia</i> flies
<i>C. cymosum</i> (Greene) J. T. Howell	peregrine thistle	N	1998:1999	5	Modoc, Siskiyou	No <i>Chaetorellia</i> flies
<i>C. douglassi</i> DC	Douglas' thistle	N	1998	3	Humboldt; Nevada	No <i>Chaetorellia</i> flies
<i>C. edule</i> Nutt.		N	1998	1	Oregon	No <i>Chaetorellia</i> flies
<i>C. loncholepis</i> Petrak	bog thistle (La Graciosa thistle)	N	1999	1	San Luis Obispo	No <i>Chaetorellia</i> flies
<i>C. occidentale</i> var. <i>californicum</i> (A. Gray) Keil & Turner	Sierra thistle	N	1998	1	Los Angeles	No <i>Chaetorellia</i> flies
<i>C. occidentale</i> var. <i>candidissimum</i> (Greene) J.F. Macbr.	snowy thistle	N	1998:1999	6	Modoc, Mono, Plumas, Shasta, Siskiyou, Trinity	No <i>Chaetorellia</i> flies
<i>C. occidentale</i> var. <i>occidentale</i> (Nutt.) Jepson	cobwebby thistle	N	1999	1	San Luis Obispo	No <i>Chaetorellia</i> flies
<i>C. occidentale</i> var. <i>venustum</i> (Greene) Jepson	Venus thistle	N	1999	7	Humboldt, Kern, Fresno, San Benito, San Luis Obispo, Santa Barbara	No <i>Chaetorellia</i> flies
<i>C. ochrocentrum</i> A. Gray	yellow-spined thistle	N	1998	1	Modoc	No <i>Chaetorellia</i> flies
<i>C. scariosum</i> Nutt.	elk thistle	N	1998:1999	2	Plumas	No <i>Chaetorellia</i> flies
<i>C. undulatum</i> (Nutt.) Spreng.	wavyleaf thistle	N	1999	1	Oregon	No <i>Chaetorellia</i> flies
<i>C. vulgare</i> (Savi) Ten.	bull Thistle	I	1998:1999	6	Humboldt; Siskiyou; San Luis Obispo; Marin; Oregon	No <i>Chaetorellia</i> flies

*N= Native thistle; I= Introduced weedy thistle

Seed Destruction in Sicilian Starthistle by Yellow Starthistle Biological Control Insects

D. M. Woods and V. Popescu

Sicilian starthistle, *Centaurea sulphurea* Willd., is an exotic weed closely related to yellow starthistle. It can be locally troublesome but is not particularly invasive. In fact, although it has been present at a site near Folsom for several decades, it has spread relatively little. It has however, formed a fairly dense, nearly monotypic stand that is often mistaken for an infestation of yellow starthistle. Nearby stands of yellow starthistle have been heavily targeted for biological control and currently support high populations of introduced biological control insects. We have conducted a two-year study to monitor the natural infestation of Sicilian starthistle by yellow starthistle biological control insects and evaluate their impact on seed destruction. This report is limited to data collected in 1999, the second year of the study.

Many species of herbivorous insects become active prior to the full receptiveness of their host. Sicilian starthistle plants begin to bolt earlier in the year than yellow starthistle and may be attractive to yellow starthistle biological control agents that have emerged before their normal host is ready. Three natural enemies, *Eustenopus villosus* (Boheman), *Bangasternus orientalis* (Capiomont), and *Urophora sirunaseva* (Hering) are established on yellow starthistle near the Sicilian starthistle site in Folsom. All three yellow starthistle insects were observed on or around Sicilian starthistle at this site. Additionally, *Lasioderma haemorrhoidale* (Illiger) (Coleoptera: Anobiidae), an accidentally introduced stored product pest, and *Chaetorellia succinea* (Costa) an accidentally introduced yellow starthistle natural enemy, have been found on yellow starthistle and Sicilian starthistle in this area. Monitoring of Sicilian starthistle began on May 28, 1999. Forty flower heads were enclosed in small cotton bags each week for five weeks. Selected heads were just past full bloom with the florets beginning to oxidize. Seedheads were collected three weeks after the last bagging date of the season and taken to the laboratory and evaluated for evidence of attack by insects.

The false peacock fly, *Chaetorellia succinea*, seems fairly well adapted to Sicilian starthistle. The attack rate is fairly high and most larvae complete development to adult. The adult flies emerge very early in the spring and are ready to oviposit as soon as flowerheads are developed. During most of the sampling dates, *C. succinea* attacked about 20% of the sampled heads (Table 1.). The sharp dip on June 17 may represent a transition from the first to the second generation of this fly, or it may represent sampling error. In any case, the overall numbers of *C. succinea* successfully attacking Sicilian starthistle increased from 1998. The fly is also active throughout the entire growing season of Sicilian starthistle attacking 16% of the heads over the year. More importantly, *C. succinea* has a striking impact on seed production. Attacked heads produce only one third of the amount of seeds as non-attacked heads (Figure 1). *C. succinea* larvae seem to migrate significantly in the head, feeding on seed and supporting tissue. The host range of this accidentally introduced bioagent is currently being examined but clearly it can utilize resources other than yellow starthistle for survival.

Adults of *Lasioderma haemorrhoidale* 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 anobiid is apparently attracted to Sicilian starthistle as soon as it emerges, infesting 36% of the earliest heads. The infestation rate was drastically reduced in early June during 1999 as well as previous years (data not shown). Even when this insect was present, it did

not seem to affect seed numbers in 1999. This result is somewhat surprising as attacked heads are severely chewed. The damage, however, seems often limited to the receptacle and many of the larva die before they can impact the seed.

The overall attack percentage of Sicilian starthistle by *E. villosus* is nearly identical to that of *C. succinea*. In contrast, however, the attack is limited to the last half of the season (Table 1) and does not affect seed production (Figure 1.). It must be noted that the high attack rate at the end of the season is actually representative of feeding/oviposition, not larval development. Less than 4% of the heads had any evidence of larval chewing and in only one head did *E. villosus* develop to an adult (which died prior to emergence).

The introduction of classical biological control agents on 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 potential host range. For most biological control agents, this is limited to a few closely related species. Post-release monitoring of the sort described here can be used to confirm the anticipated host range and the continued safety to other plant species. Of the three insects described here, *E. villosus* is the only one to have undergone a pre-release evaluation for safety. The near failure of this insect on Sicilian starthistle supports the quarantine evaluations and speaks to the integrity of the review process. Both of the other insects were accidental introductions; neither received host testing evaluations and both can complete development on Sicilian starthistle.

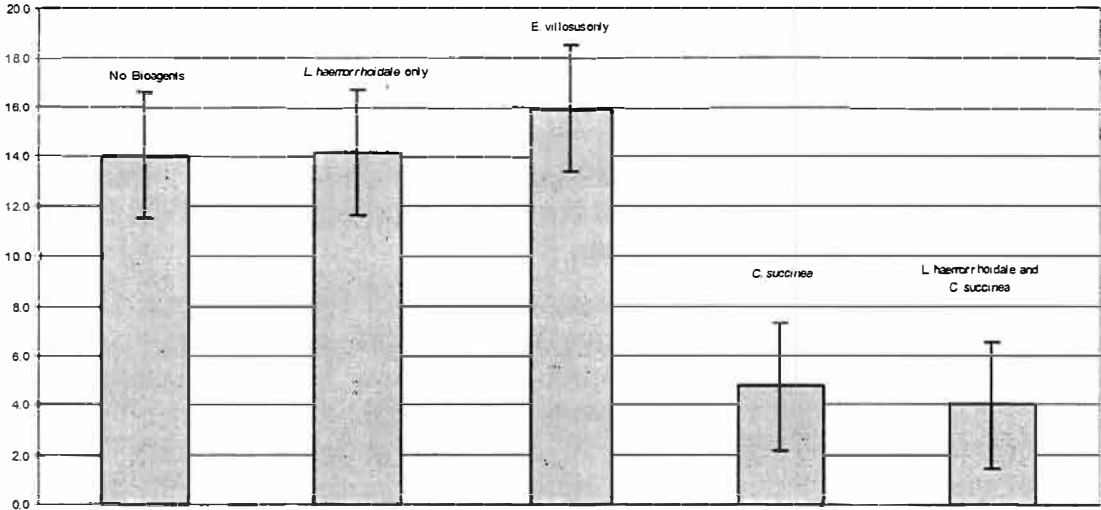
It would not have been surprising that an exotic weed such as Sicilian starthistle could be a host to yellow starthistle biological control agents as it is genetically similar, is similar in appearance and shares a portion of the geographic range. Consequently, several yellow starthistle biological control agents have the potential (none were tested) to attack this closely related species and we might even expect some level of control. Attacking these weeds, however, may actually be advantageous as they are not likely to become targets of direct biological control introductions.

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, showed no evidence of attack on Sicilian starthistle. The hairy weevil, *E. villosus*, did find Sicilian starthistle to be somewhat attractive but was unable to complete development. Sicilian starthistle is not a preferred host for *C. succinea* as its attack rate and successful development is much lower than observed in nearby yellow starthistle. *L. haemorrhoidale* is less specific in its feeding habit and has been found on several Asteraceous weeds.

Table 1. Percentage of Sicilian starthistle seedheads that are attacked by various insects

	5/28	6/4	6/10	6/17	6/24	1999 Total
<i>C. succinea</i>	21%	22%	15%	3%	20%	16%
<i>L. haemorrhoidale</i>	36%	3%	3%	0%	5%	9%
<i>E. villosus</i>	0%	3%	5%	33%	38%	16%

Figure 1. Average number of seeds per head of Sicilian starthistle attacked by various insects.



Population Increases of *Bangasternus fausti* and *Larinus minutus* On Diffuse Knapweed in Trinity County

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

Diffuse knapweed, *Centaurea diffusa* Lamarck (Asteraceae), occurs in California as single plants or in small patches, and is under eradication in most areas of the state 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 currently established on diffuse knapweed in Trinity County, but the focus of this report is limited to results obtained with the two weevils *Bangasternus fausti* (Reitter) (Coleoptera: Curculionidae) and *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae).

In 1999, seed head samples were collected from sites where *B. fausti* and *L. minutus* had been released in previous years to compare population buildup of these weevils. These sites are distributed along Miller Road that runs north and south, extending from near the top of a ridge down toward the South Fork Trinity River. Four sites along this road were monitored and the results are shown in Table 1. The primary monitoring site is located between the lower and upper Miller Road sites. *B. fausti* was first released at the primary monitoring site in 1994, where it established and has maintained quite high populations. Additional releases were made two miles away at the lower Miller Road site in 1995. *Bangasternus fausti* has maintained significant populations at this lower site but competition with *L. minutus* may have limited the upper level of population densities. *B. fausti* continues to spread, albeit slowly, along Miller Road, and now seems established along the entire length of the road. *Larinus minutus* was released at three sites along Miller Road (bottom in 1995, lower and upper in 1996) and is still increasing, infesting nearly half of the heads at most of the sites.

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

Locations along Miller Road	<i>B. fausti</i>		<i>L. minutus</i>	
	1998	1999	1998	1999
Bottom	11	16	49	47
Lower	27	26	37	48
Mid (Monitoring site)	74	49	4	19
Upper	0	5	32	48

A more in-depth long term monitoring has been performed at the 'monitoring site'. Plant density and seed head attack measures have been collected from 1995 through 1999. The percentage infestation measure consisted of harvesting all or parts of 10 plants in mid autumn, removing and combining all seedheads, then dissecting and scoring a subsample of heads (minimum 100) for insects or empty larval/pupal cells under a microscope. A second set of 10 plants was collected and the seedheads from each plant were processed separately to compare plant-to-plant variation. Although there was similarity in damage caused by the two weevils, most damage could be ascribed to a single species, so percentage attack is reported for each weevil. Density estimates of reproducing knapweed plants at the monitoring site were also made at the same time. All reproductive plants were counted within a 0.25-m² frame placed at 15 contiguous locations along two permanent, parallel transects. The results are shown in Table 2.

Both the percent of heads attacked by the weevils and the knapweed plant density increased significantly at this site through 1998 but declined in 1999 (Table 2). The decline in weevil numbers following the previous dramatic rise is disappointing, but may not signal the end of an overall increase in percentage attack. Perhaps the cooler than normal early- to mid-summer temperatures in 1999 delayed early flower head development, extending flowering beyond the ovipositional periods of one or both weevils. At the time of sampling (20 September), adult weevils were beginning to emerge from the older seedheads, while some of the plants were still producing flower heads. Another, more worrisome possibility was that the weevils may have interfered with each other instead of being complementary. There were numerous seedheads with *B. fausti* eggs that contained adults or other life stages of *L. minutus*, thus providing preliminary indication of interference. Further study will be done as the weevil populations expand and increase the mutual contact, to determine if interference is a potential problem. The reason(s) for the sudden drop in plant density following recent sustained increases are unknown, but again, weather patterns are suspected. There is, as yet, no distinct data to show that biocontrol agent attack was a significant factor in this decline.

Table 2. Combined percentages of attack of seedheads by combined weevils, 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
1999	69	54

Status of Introduced Biological Control Agents on Spotted Knapweed

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

The Biological Control Program of the California Department of Food and Agriculture began releasing biological control agents for spotted knapweed, *Centaurea maculosa* Lamarck (Asteraceae) in 1993. Since then, several natural enemies have been imported into California in an effort to establish enduring biological control. The current status of these agents at one monitoring site is shown in Table 1. Insect population estimates were based on samples collected at the end of the summer from a site along the Pit River near Big Bend California. Ten plants were collected along a transect through the release area. All seedheads were removed from the plants, combined, and a subsample (> 300 heads/year) was processed in the laboratory to determine the presence of the various seedhead-feeding insects. Additionally, 100 plants were uprooted at the site and their roots scraped and split to determine the attack by the root feeding insects.

The root-feeding insects have not fared well at this site. Despite repeated release efforts the yellow-winged knapweed root moth, *Agapeta zoegana* Linnaeus (Lepidoptera: Cochylidae), has not established a solid population. Adult moths are extremely rare in the field and larval numbers and level of associated root damage has plummeted following the initial population surge in 1997. The knapweed root weevil, *Cyphocleonus achates* (Fahraeus) (Coleoptera: Curculionidae) seemed to be very slow to establish but is now clearly established, although at a very low level infesting about 10% of the plants.

The knapweed seed head flies, *Urophora affinis* Frauenfeld (Diptera: Tephritidae), and *Urophora quadrifasciata* (Meigen) (Diptera: Tephritidae), are widely established in the United States and can achieve high population levels in some locations. *Urophora quadrifasciata* migrated to our site sometime before 1995. Adults of the first generation of the fly were highly visible in the field during 1999, but larvae are easily consumed by the seedhead weevils, which severely reduces the final infestation level. *Urophora affinis* appears to be still increasing, but even its hard galls are consumed by the weevils, limiting the overwintering population. The seedhead fly, *Terellia virens*, (Loew) (Diptera: Tephritidae), has remained at low level for several years and is hard to locate in the field.

The lesser knapweed flower weevil, *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae), established well at this site. Adults are visible during most of the growing season. Larvae appear to have a significant impact within infested seedheads, and most reach maturity, successfully producing visible exit holes. Two seasons of seed destruction data have been collected and are being analyzed. With over 55% of the seedheads being attacked by *L. minutus* for two years the total amount of seed production for the site should be reduced.

The yellow starthistle biological control insect, *Eustenopus villosus* (Boheman) (Coleoptera: Curculionidae), spread into the area and was noticed on yellow starthistle in 1997. By 1998, adults were noticed crawling on flowerheads of spotted knapweed. During the summer of 1999, yellow starthistle was so severely attacked by *E. villosus* that most plants died, and the others remained stunted most of the summer, recovering only when *E. villosus* had completed its adult feeding. Consequently, it appears that the excess *E. villosus* turned to spotted knapweed as a secondary host. Feeding and/or oviposition were detected on approximately one third of the heads in the area. Overall, nearly 22% of the spotted knapweed heads had both an oviposition

scar and evidence of larval feeding, or adult presence of *E. villosus*. A complete analysis of interaction of the two weevil species will require more involved analysis as the larval feeding and pupal chambers overlap in appearance.

Table 1. Infestation rates for biological control agents released on spotted knapweed at the monitoring plot along the Pit River.

	% Seedheads Infested			
	1996	1997	1998	1999
<i>Urophora quadrifasciata</i>	22%	15%	32.4%	5.6%
<i>Urophora affinis</i>	9%	14%	48.1%	36.9%
<i>Larinus minutus</i>	23%	11%	61.5%	55.8%
<i>Terellia virens</i>	-	5%	0.6%	0.4%
<i>Eustenopus villosus</i>	-	-	-	21.7%
	% Roots Infested			
<i>Agapeta zoegana</i>	-	26%	2%	0
<i>Cyphocleonus achates</i>	-	0%	12%	10%

Evaluation of Survivorship and Growth Habit of Scotch Thistle in Northeastern California

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

Field studies were initiated in 1996 at the Modoc National Wildlife Reserve and at Ash Creek Wildlife Area in northeastern California to develop baseline information on the biology of Scotch thistle in anticipation that biological control agents would be available for release in the next few years. However, the first candidate (*Lixus* species) was eliminated from further consideration because it readily attacked native *Cirsium* spp. during quarantine evaluations. Because it seems unlikely that biological control agents will be cleared through quarantine testing in the near future, we have scaled down the Scotch thistle field studies to concentrate on survivorship and growth habit in northeastern California. We also decided to prevent further seed production at both field sites by removing plants at the bolting stage to avoid jeopardizing attempts to eliminate Scotch thistle at these wildlife refuges.

The Modoc plot is comprised of four transects with 30 contiguous one m² quadrats each (total = 120 contiguous quadrats). The Ash Creek site is established on a small, low mound with a concentration of Scotch thistle plants surrounded by lower-lying vernal pools. Contiguous 1m² quadrats were arranged in six transects over a 12m by 15m area on top of the mound in a six by 15 grid pattern (total = 90 quadrats).

Modoc Site

1998 seedling cohorts. A total of 557 seedlings emerged and were flagged in 1998 (Table 1). Very few seedlings emerged during the early summer visits of 1998, although rainfall was plentiful until mid-June. Presumably, temperatures were too low in May and June for germination to occur. The greatest numbers of seedlings were flagged late in the season, on 19 August and 8 October 1998. A storm in late July provided approximately 30 mm of precipitation and subsequent storms provided additional rainfall in early and late September. Maximum daily temperatures during the late July and early September storms ranged from the mid-twenties to above 30^o C. By far, the greatest number of seedlings occurred in quadrats close to two large plants that dispersed their seeds in 1997. None of the 1998 seedling cohorts bolted in 1998, but 103 bolted in 1999. All of the 103 reproductive plants bolted 10 to 13 months from emergence. As of 20 October 1999, 175 plants remained alive. The other 279 died over the 18-month period with most dying during the summer of 1998.

Twenty-five percent of the August 1998 cohort of seedlings (n = 343) died in 1998 and 1999, and 47% (n = 162) remained alive on 20 October 1999. By contrast, 94% (n=188) of the 201 October 1998 cohort died in 1999, with half of those by 8 June 1999. Differences in root development of the two cohorts may have accounted for variation in mortality.

1999 seedling cohorts. Few seedlings were flagged during 1999, and most (43 of 46) died that same year. The weather pattern was generally dry after early March. There was a single-day storm (21mm) on 14 July and scattered precipitation in early and late August, but seedling emergence was poor. Either the moisture level and temperatures were inadequate to induce germination or, perhaps, there were few highly germinable seeds left in the seed bank due to previous germination and lack of new seeds after 1997.

Table 1 Modoc National Wildlife Reserve Site

Month of 1998 Cohort	Initial # of Plants	# Bolted In 1998	# Bolted In 1999	# Died		#Remaining Oct 1999
				1998	1999	
June ¹	2	0	2	0	0	0
July ²	11	0	6	3	2	0
August ³	343	0	95	21	65	162
October ⁴	201	0	0	0	188	13

¹June = 3 October 1997 – 23 June 1998

²July = 23 June – 14 July 1998

³August = 14 July – 19 August 1998

⁴October = 19 August – 8 October 1998

Ash Creek Site

1998 seedling cohorts. During initial setup of the field plot on 22 June 1998, all bolting and large rosette plants were removed leaving a total of 30 seedlings and 216 small to medium sized rosettes of variable ages that were flagged. A total of 82 seedlings were recorded in 1998 (Table 2). None of the 1998 seedlings bolted in 1998 or 1999. Only three of the seedlings survived to 20 October 1999. Of the 216 rosettes, 2 bolted and 51 died in 1998, and 4 bolted and all except one of the remaining plants died by 20 October 1999. The greatest mortality among the seedlings and rosettes occurred between 8 October 1998 and 7 June 1999 when 68%% of total flagged plants died. Reasons for the high mortality could not be determined, although we suspect that desiccation from cold temperatures and lack of soil moisture may have contributed significantly

Table 2 Ash Creek Wildlife Area Site

Month of 1998 Cohort	Initial # of Plants	# Bolted In 1998	# Bolted In 1999	# Died		#Remaining Oct 1999
				1998	1999	
June ¹	30	0	0	18	11	1
July ²	10	0	1	3	4	2
August ³	39	0	0	11	28	0
October ⁴	3	0	0	0	3	0

¹June = 8 October 1998 – 23 June 1998.

²July = 23 June – 14 July 1998

³August = 14 July – 19 August 1998

⁴October = 19 August – 8 October 1998

1999 seedling cohorts. A total of 196 new seedlings were flagged during 1999, all on 7 or 30 June 1999. None of these plants bolted, and all except 27 were dead by 20 October. Dry weather during spring and summer is thought to be responsible for the high rate of mortality. Seedling emergence for the year was less than we expected considering how many plants were present in 1998. However, without fresh seed input since 1997, the seed bank may have had a limited number of germinable seeds, and rainfall and suitable temperatures for germination may not have overlapped during the summer.

Size-dependent Reproduction by Scotch Thistle in Northeastern California

M. J. Pitcairn, D. B. Joley, L. Braddock, E. Stallions, and V. Popescu

Scotch thistle, *Onopordum acanthium* L. (Asteraceae), is an exotic noxious weed under eradication in California. This weed invades pastures and rangelands and quickly dominates the area due to its prolific seed production and long-lived seed bank. Despite efforts, eradication has proved difficult. Recently, the USDA-ARS Exotic and Invasive Weed Management Research Unit in Albany, CA, has begun screening insects as potential biological control agents against this weed. If insects are approved for introduction, it is critical to determine their impact against Scotch thistle in California. For this, pre-release field studies on scotch thistle were initiated to provide information on growth habit, reproductive phenology, survivorship, and fecundity. These studies will provide baseline information for before and after comparisons to estimate the impact of any introduced biological control agents. Here we report on the study documenting the relationship between plant size and seed production.

Two field sites were selected in northeast California: one in Modoc County near Canby and the other in Lassen County at the Ash Creek Wildlife Refuge (CA Dept. of Fish and Game) near Bieber. Scotch thistle populations studied were not subject to recent herbicide treatments or other control measures. Scotch thistle at the Canby site consisted of several hundred plants over ten acres of pastureland. A study area of approximately 30 x 40 meters was located near the eastern edge of the property. The Ash Creek site consisted of approximately 200 plants located on a raised mound approximately 40 x 50 meters in area. At both sites, bolting rosettes of different sizes were intentionally selected to provide samples throughout the local size range. Rosette size was estimate by measuring the width of the area occupied by the swirl of rosette leaves. Two width measurements were taken: at the widest width and at 90° to this measurement. On June 1, 1999, 63 bolting plants were selected at Ash Creek and 75 bolting plants selected at Canby. These were allowed to grow, produce flowers and set seed. To estimate seed production, samples of senesced flower heads were enclosed with cloth bags to collect seeds (n=217 and 205 at Canby and Ash Creek, respectively). Flower heads were selected based on size to ensure that heads throughout the size range were selected. The field sites were visited and heads bagged on three dates: June 30, July 21, and August 11, 1999. Heights of all plants were measured on September 1, 1999, then plants were harvested, enclosed in paper bags, and transported to the laboratory for processing. In the laboratory, all unbagged flower heads were identified and counted as flowering or not flowering. The outside diameter of mature heads (those that flowered) was estimated by measuring their largest width to the nearest millimeter and again at 90° to this measurement. For the bagged heads, all filled seeds were counted (viability was not tested) and outside diameter of the head estimated as above. Although, the plants and their flower heads have been processed, drying and weight determination is still underway. Here we report preliminary results on the number of filled seed and head size for the two Scotch thistle populations examined.

At Ash Creek, 56 of the initial 63 bolting rosettes produced flower buds; of these, 47 plants produced heads that completed flowering (nine produced only immature heads). Plant height ranged from 13 to 156 cm and mature head production ranged from 1 to 82 heads per plant. At Canby, 17 plants were damaged apparently by farm equipment or automobile and were not harvested. Of the remaining 58 initial rosettes, 51 plants produced at least one head that

flowered. Plant height ranged from 19 to 166 cm and mature flower production ranged from 1 to 206 heads per plant.

There was a clear correlation between head diameter and number of filled seeds in both populations (Figure 1a,b). The regression lines for this relationship are: Canby, $y=13.997(x)-199.856$ ($r^2=0.82$, $F=996.81$, $P<.01$); Ash Creek, $y=11.968(x)-189.057$ ($r^2=0.82$, $F=943.37$, $P<.01$). The regression lines intersect the x-axis at 14.28 mm (Canby) and 15.80 mm (Ash Creek) indicating that the minimum size for a head to produce filled seed is approximately 14-15 mm. The slope of this relationship was higher at Canby indicating that more seed per head were produced at this site than similar sized heads at Ash Creek.

Individual seed weight was determined by weighing all seed in a head and dividing the result by the number of seed present. The results show that individual seed weight was not equal among heads; rather seeds from heads with many seed were, on average, heavier than seeds from heads with fewer seed (Figure 1c,d). There was an increase in individual seed weight as the number of seeds per head increased until approximately 200-250 seeds per head. In heads with >250 seeds, individual seed weight appeared to decline (Figure 1c). In both populations, there was a strong correlation between individual seed weight and head size, indicating that larger heads produced heavier seed (Figure 1e,f).

Using the above relationships, total seed production per plant was estimated by using head diameter to estimate number of filled seeds for individual heads and summing all heads on a plant. At Ash Creek, seed production ranged from 0 to 6,256 seeds per plant; at Canby, seed production ranged from 0 to 35,086 seeds per plant. The number of filled seed increased linearly with the number of heads per plant (Figure 2).

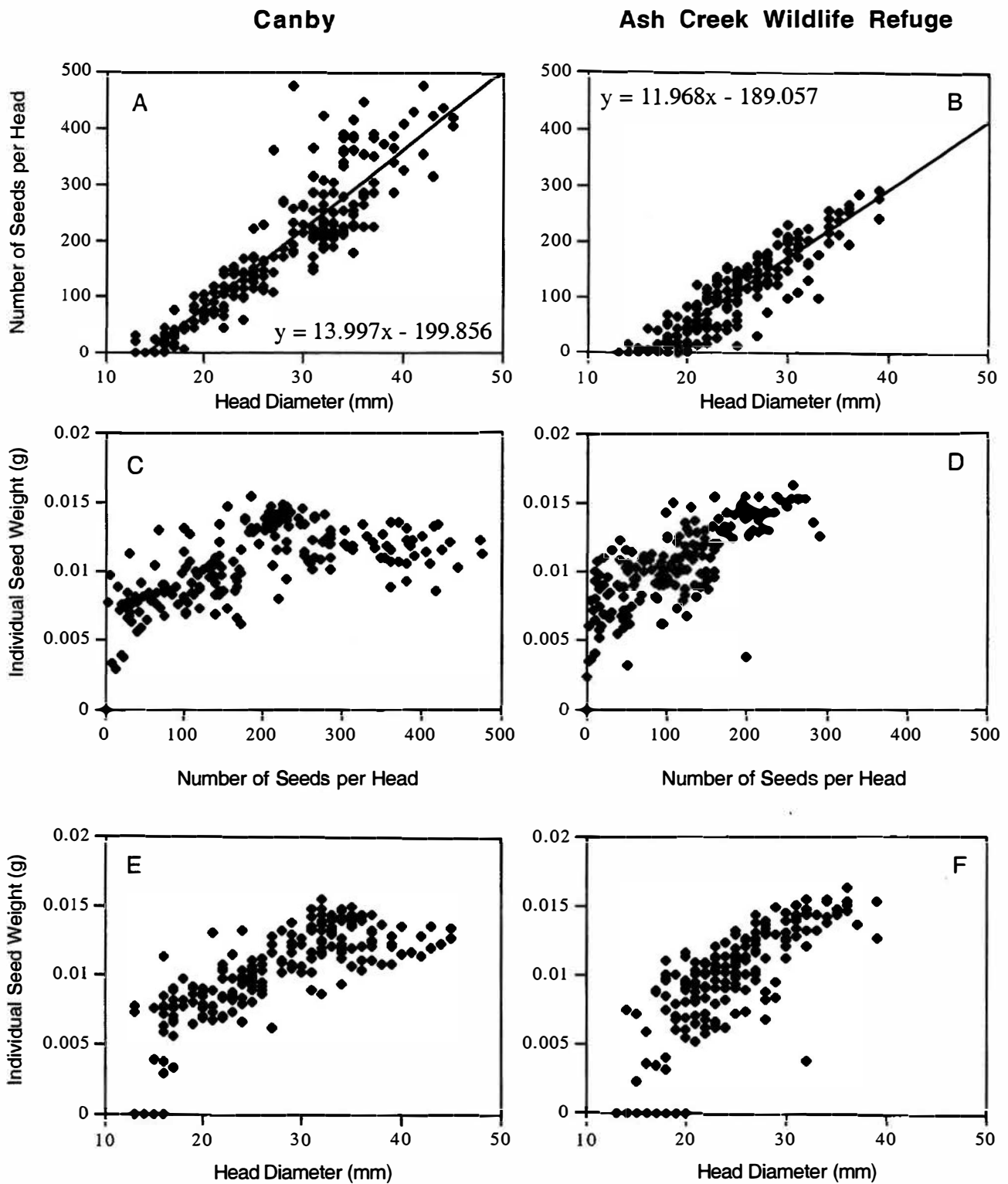


Figure 1. Production and weight of seeds from two Scotch thistle populations in northern California. A,B: seeds per head as a function of head size; C,D,: mean seed weight as a function of seeds per head; E,F: mean seed weight as a function of head size.

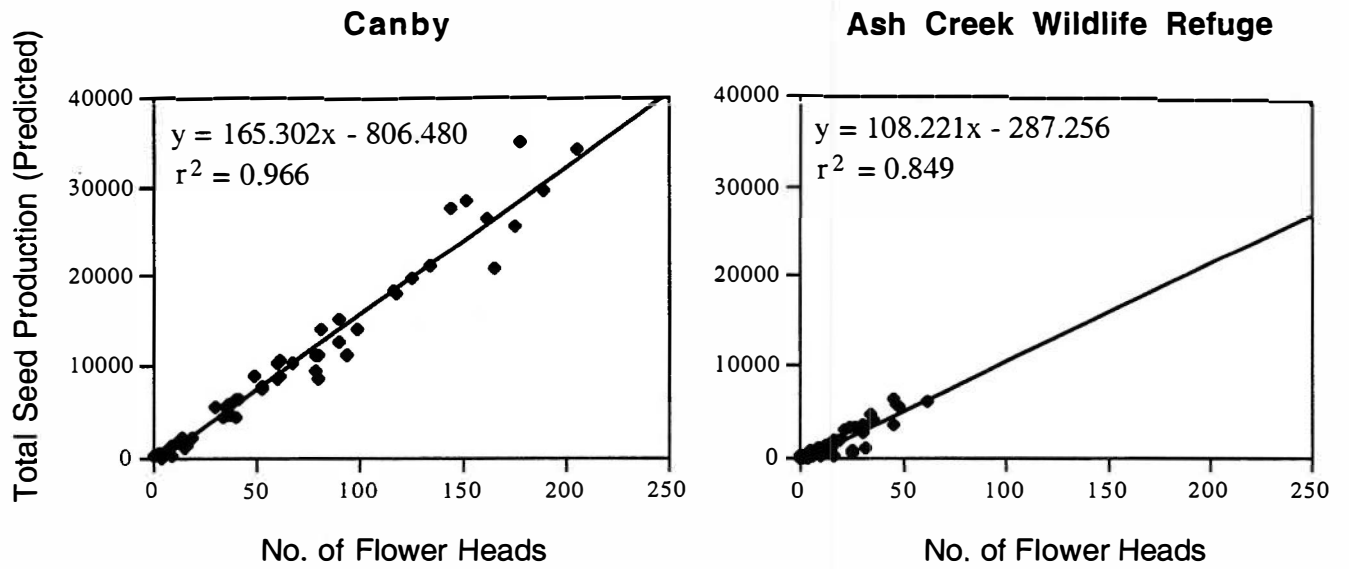


Figure 2. Estimated seed production by Scotch thistle plants at two sites in northern California. Seed production was estimated by measuring individual mature heads, estimating seed production using the regression equations in Figure 1 A & B, then summing estimates for all heads on a plant.

Endemic Natural Enemy Fauna of Scotch Broom in the Central Sierra Nevada Foothills of California

M. J. Pitcairn and R. K. Wall

Scotch broom, *Cytisus scoparius* (L.) is a noxious exotic leguminous shrub native to central and southern Europe that is impacting millions of acres in the Pacific Northwest and California. Infested forestlands require higher regeneration costs and face increased risk of wildfire. Infested rangelands offer reduced forage for livestock and wildlife. Once present, Scotch broom forms dense stands that prevent establishment of native or other desirable plant species. The soil seed bank commonly ranges from 30,000 to 100,000 seeds per square meter and seeds remain viable for many years. Scotch broom continues to extend its range and increase in density in established areas.

Scotch broom is perhaps a greater problem in Australia and New Zealand where it also increases in abundance without check. To assist in its control, a cooperative research effort was established among Australia's Commonwealth Scientific and Industrial Research Organization (CSIRO), New Zealand's Land Care Institute, the Oregon Department of Agriculture, and CAB International to develop a biological control program against this noxious weed. The initial results look promising and may result in several new natural enemies for use as biological control agents in the western United States. Recently, several public land management agencies and private landowners have expressed interest in joining the Scotch broom research effort and are currently engaged toward that end.

In anticipation of the arrival of new biological control agents against Scotch broom, a survey was initiated to assess the occurrence of endemic arthropod fauna in California. Data from this survey will allow comparison with the natural enemy complex 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 alstromeriana* on poison hemlock), thus, this survey will show if identified potential biological control agents are already here prior to their release.

The fauna of Scotch broom was surveyed at nine sites in the central Sierra Nevada foothills in Amador, El Dorado, and Nevada Counties. The sites form a north-south transect from Nevada County in the north to Amador County in the south. All nine sites were heavily infested with Scotch broom infestations ranging from 0.5 to over 40 acres in size. All sites were visited for one to two hours every three weeks from late flowering in June 1999, through onset of dormancy in November. 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 removed by beating branches into a beating sheet. Stems were removed and examined externally under a microscope for mites, thrips, and aphids. Immature arthropods, egg masses, galls, and infested stems were returned to the laboratory and placed into rearing containers to obtain adult specimens for identification. Internal feeders were surveyed by splitting and examining stems for feeding damage under a microscope. Survey of the flowering period (April-May) will occur in 2000.

Distribution of Purple Starthistle in the San Francisco Bay Region

D. M. Woods, D. A. Mayhew and J. M. Gendron¹

The California Department of Food and Agriculture maintains several eradication and control programs for noxious and exotic weeds. Purple starthistle, *Centaurea calctrapa* L., (Asteraceae), does not usually fall into the category of plants warranting eradication activities. Since the Biological Control Program has an active program on the related weed, yellow starthistle, we are often asked to pursue a biological control program on purple starthistle. In order to quantify the extent of the purple starthistle problem, a region-wide survey was initiated in the San Francisco Bay counties. These counties have traditionally been the most severely infested counties.

Commercial county maps that had a grid of legal sections were used in the survey. A section is a one square mile block. Each section of the maps were marked for the abundance of purple starthistle with a score of 0 for no plants, 1 for low abundance, 2 for high abundance, or left blank if unknown. Any rating of purple starthistle abundance, is necessarily subjective because of the large sample area, however, the following guidelines were provided:

Low abundance:

- a. Only a single plant was found in the section.
- b. The only plants found were scattered plants confined to the roadsides.
- c. Plants were scattered throughout the section, but didn't occur in high densities.
- d. No dense patches or few, small dense patches (<10 acres) were observed.

High abundance:

- a. Plants were quite dense along roadsides.
- b. Plants not confined to roadsides, but observed throughout neighboring fields.
- c. Dense patches of plants >10 acres.
- d. Everywhere you looked you saw purple starthistle plants.

Despite the subjective nature of the rating system, this information provides a qualitative estimate of how widespread purple starthistle has become and indicates geographic areas of its highest abundance.

Commercial county maps with the section grid were first distributed in 1997, to Weed and Vertebrate Control Program district staff stationed within the Bay Area. The biologists themselves completed some of the maps, whereas other maps were distributed to the county biologists in their district. All maps were returned to the Biological Control Program, the information transferred into a GIS database and a preliminary map was produced. The preliminary map is shown on the following page. The survey shows that purple starthistle is found throughout the Bay Area with the largest infestations in Napa, Solano and Sonoma Counties. Additional infestations exist both within and outside the surveyed area and will be added to a future updated map.

¹CDFA, Integrated Pest Control Branch

**California Department of Food & Agriculture
Integrated Pest Control Branch**

