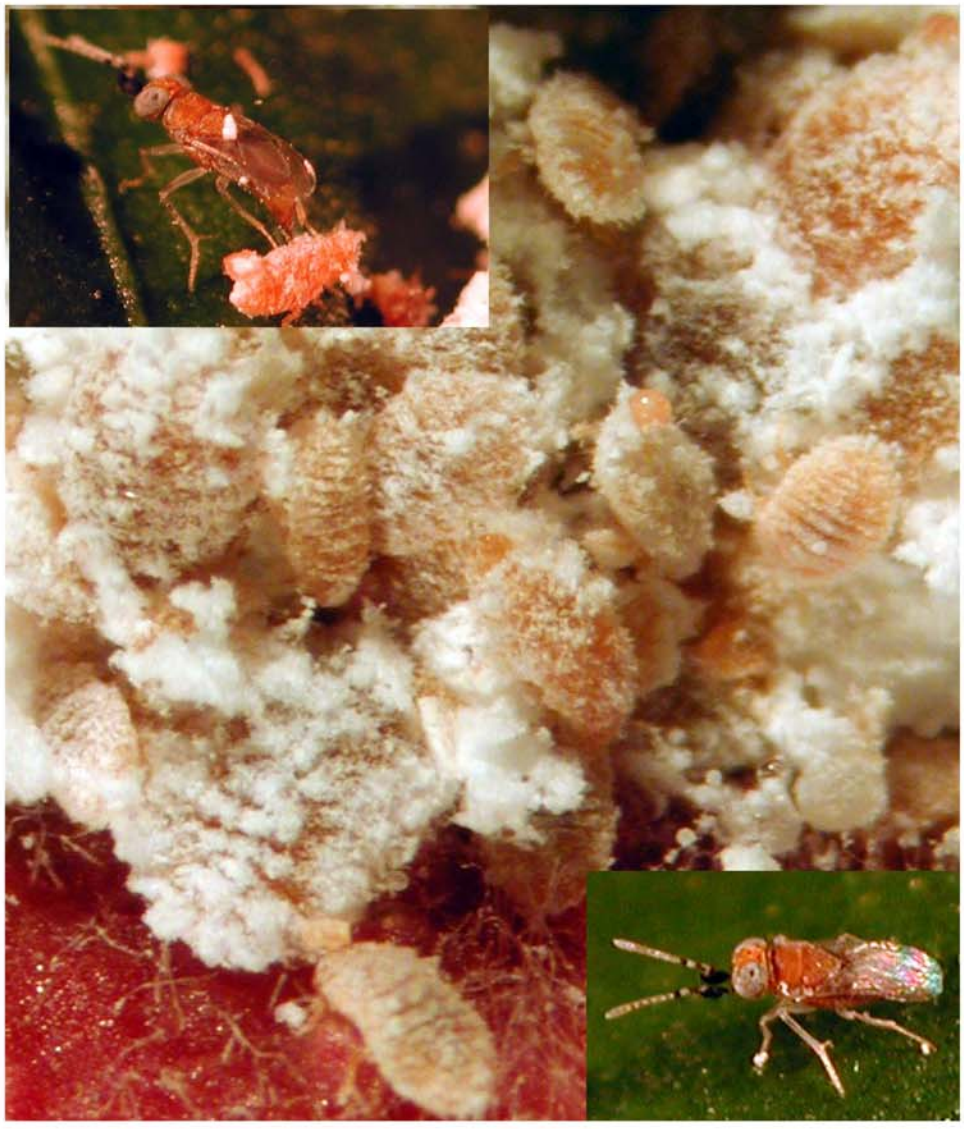
Biological Control Program
Annual Report
2005



California Department of Food & Agriculture

BIOLOGICAL CONTROL PROGRAM

2005 SUMMARY

DEVELOPED BY

Patrick Akers
Jim Brown
Kris Godfrey
Charles Pickett
Mike Pitcairn
William Roltsch
Baldo Villegas
Dale Woods

CALIFORNIA DEPARTMENT OF FOOD AND AGRICULTURE
PLANT HEALTH AND PEST PREVENTION SERVICES
INTEGRATED PEST CONTROL BRANCH

Cite as: Dale M. Woods, Editor, 2006. Biological Control Program Annual Summary, 2005. California Department of Food and Agriculture, Plant Health and Pest Prevention Services, Sacramento, California. 60 pp.

CDFA CONTRIBUTING PERSONNEL

Dr. Patrick Akers
Mr. Jim Brown
Dr. Kris Godfrey
Dr. Charles Pickett
Dr. Mike Pitcairn
Dr. William Roltsch
Mr. Baldo Villegas
Dr. Dale Woods

CDFA Technical Assistants

Mr. Ruben Aguilar
Ms. Christina Black
Mr. Dagne Demisse
Ms. Leann Brace/Horning
Ms. Claudia Erwine
Mr. Pushpinder Kumar
Ms. Michelle Lawson
Mr. Blake Lim
Ms. Viola Popescu
Ms. Laureen Wagoner
Mr. Lue Yang
Mr. Jose Zuniga

County Co-operator Acknowledgement

The CDFA Biological Control Program greatly appreciates the many biologists and agriculture commissioners throughout the state whose co-operation and collaboration made this work possible.

Cover developed by Baldo Villegas and William Roltsch. The pink hibiscus mealybug was first found on a variety of ornamentals in Imperial Valley, California in 1999. Pink hibiscus mealybug, *Maconellicoccus hirsutus* nymphs and adult females [center]; encyrtid wasp, *Anagyrus kamali* [lower right]; *Anagyrus kamali* parasitoid ovipositing into a mealybug nymph [upper left].

COOPERATING SCIENTISTS

Dr. Lars Anderson, USDA-ARS, Davis, California
Mr. Earl Andress, USDA-APHIS-PPQ, Brawley, California
Mr. John Andrews, University of California, Berkeley, California
Mr. David Asakawa, CDFA, Pest Detection/Emergency Projects, Goleta, California
Dr. Joe Balciunas, USDA-ARS, Albany, California
Dr. Jay Bancroft, USDA-ARS, SREC, Shafter, California
Dr. Bernd Blossey, Cornell University, New York
Mr. Gary W. Brown, USDA-APHIS-PPQ, Portland, Oregon
Dr. William L. Bruckart, USDA-ARS, Ft. Detrick, Maryland
Ms. Janet Bryer, University of California, Santa Cruz, California
Dr. Anne-Marie Callcott, USDA, APHIS, Gulfport, Mississippi
Mr. Gaetano Campobasso, USDA-ARS European Biological Control Laboratory, Rome, Italy
Dr. Nada Carruthers, USDA-APHIS, Albany, California
Dr. Ray Carruthers, USDA-ARS, Albany, California
Mr. Bob Case, Contra Costa County Department of Agriculture, Concord, California
Dr. Al Cofrancesco, United States Army Corps of Engineers, Michigan
Dr. Matthew Cock, CABI Bioscience, Delemont, Switzerland
Mr. Eric Coombs, Oregon Department of Agriculture, Salem, Oregon
Mr. Craig Conley, US Department of Interior, Bureau of Reclamation, Ephrata, Washington
Mr. Dominique Coutinot, USDA-ARS European Biological Control Laboratory, Montferrier, France
Mr. Massimo Cristofaro, Biotechnology and Biological Control Agency, Rome, Italy
Dr. Kent Daane, University of California Berkeley, Kearney Ag Center, Parlier, California
Ms. Jolene Dessert, Imperial County Department of Agriculture, El Centro, California
Dr. Joe M. DiTomaso, University of California, Davis, California
Mr. Krishna Dole, Texas A & M University, College Station, Texas
Dr. Stephen Enloe, University of Wyoming, Laramie, Wyoming
Mr. Larry R. Ertle, USDA-ARS, Newark, Delaware
Dr. Alison Fisher, USDA-ARS, Albany, California
Ms. Diana Fogle, CDFA, Plant Pest Diagnostics Center, Sacramento, California
Dr. John Gaskin, USDA-ARS, SREC, Shafter, California
Ms. Carolyn Gibbs, US Department of Interior, BLM, Susanville, California
Mr. Raymond Gill, CDFA, Plant Pest Diagnostics Laboratory, Sacramento, California
Dr. Andre Gassmann, CABI Bioscience, Delemont, Switzerland
Dr. John A. Goolsby, USDA-ARS, Queensland, Australia
Dr. Tom Gordon, University of California, Davis, California
Dr. Henri Goulet, Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada
Dr. Elizabeth Grafton-Cardwell, University of California, Kearney Ag Center, Parlier, California
Mr. Daniel Hamon, USDA-APHIS-PPQ, Western Region, Sacramento, California
Dr. Rich Hansen, USDA-APHIS, Bozeman, Montana
Ms. Kate Haas, Modoc County Department of Agriculture, Alturas, California
Dr. Mark Hoddel, University of California, Riverside, California
Dr. Kim Hoelmer, USDA-ARS, Beneficial Insect Introduction Research Unit, Newark, Delaware
Dr. Fred Hrusa, CDFA, Plant Pest Diagnostics Center, Sacramento, California
Dr. Eduardo Hummeres, University of California, Riverside, California
Dr. Marshall Johnson, University of California Riverside, Kearney Ag Center, Parlier, California
Dr. Walker Jones, USDA-ARS, European Biological Control Laboratory, Montferrier, France
Mr. Javid Kashefi, USDA-ARS European Biological Control Laboratory, Thessaloniki, Greece
Dr. William Kaufman, USDA-APHIS, Ft. Collins, Colorado
Mr. Dan Keaveny, CDFA, Integrated Pest Control Branch, Shafter, California

Dr. David Kellum, San Diego County Department of Agriculture, Escondido, California
Dr. Alan Kirk, USDA-ARS, European Biological Control Laboratory, Montferrier, France
Dr. Boris Korotyaev, Zoological Institute, St. Petersburg, Russia
Mr. David Kratville, CDFA, Integrated Pest Control Branch, Sacramento, California
Dr. William Longland, USDA-ARS, Reno, Nevada
Dr. Douglas G. Luster, USDA-ARS, NAA, Ft. Detrick, Maryland
Dr. Michael McGuire, USDA-ARS, SREC, Shafter, California
Dr. Russel Messing, University of Hawaii, Kapaa, Hawaii
Dr. Dale Meyerdirk, USDA-APHIS-PPQ, PDMP, Riverdale Maryland
Dr. David J. W. Morgan, CDFA, Pierce's Disease Control Program, Riverside, California
Dr. Joseph Morse, University of California, Riverside, California
Dr. Sergei Mosyakin, National Academy of Sciences of Ukraine, Kiev, Ukraine
Mr. Diego Nieto, University of California, Santa Cruz, California
Dr. Hannah Nadel, University of California Riverside, Kearney Ag Center, Parlier, California
Dr. David Oi, USDA-ARS, CMAVE, Gainesville, Florida
Mr. Bill Osterlein, Riverside County Department of Agriculture, Riverside, California
Ms. Carri Pirosko, CDFA, Integrated Pest Control Branch, Burney, California
Mr. Christopher Pirosko, Fall River Resource Conservation District, McArthur, California
Dr. Marcel Rejmanek, University of California, Davis, California
Dr. Frederick Ryan, USDA-ARS-SJUASC, Parlier, California
Mr. Steve Schoenig, CDFA, Integrated Pest Control, Sacramento, California
Dr. Rene Sforza, USDA-ARS, European Biological Control Laboratory, Montferrier, France
Dr. Andy Sheppard, CSIRO, Montferrier, France
Dr. Karen Sime, University of California, Berkeley, California
Dr. Greg Simmons, USDA-APHIS-CPHST, Phoenix, Arizona
Dr. Lincoln Smith, USDA-ARS, Albany, California
Dr. Norm Smith, Fresno County Department of Agriculture, Fresno, California
Dr. David Spencer, USDA-ARS, Davis, California
Dr. Jerry Stimac, University of Florida, Gainesville, Florida
Dr. Richard Stouthammer, University of California, Riverside, California
Dr. Sean L. Swezey, University of California, Santa Cruz, California
Ms. Lynne Turner, Lassen County Department of Agriculture, Susanville, California
Dr. Gillian Watson, CDFA, Plant Pest Diagnostics Laboratory, Sacramento, California
Dr. Robert Wharton, Texas A & M University, College Station, Texas
Dr. Tim Widmer, USDA-ARS, European Biological Control Laboratory, Montferrier, France
Dr. Rob Wilson, University of California Cooperative Extension, Susanville, California
Dr. James Young, USDA-ARS, Reno, Nevada
Dr. Frank Zalom, University of California, Davis, California

Preface

M. J. Pitcairn

Biological control is a global activity. The search for natural enemies for use as biological control agents occurs primarily overseas and is accomplished by several, highly-valued cooperators: the USDA Agricultural Research Service (both domestic and overseas scientists), the University of California, CABI-Bioscience, and the Biotechnology and Biological Control Agency (BBCA). All have contributed efforts in the foreign exploration and collection of biological control agents now established in California. Biological Control Program scientists have assisted with the foreign exploration work for some of the projects currently being pursued but it is simply not possible for the small number of Program scientists alone to perform the amount of work required by all of the projects now underway. Foreign exploration is important, but demanding, work and we are grateful for the valued efforts and cooperation in this activity by our several cooperators. For the olive fruit fly project, the fruits of their labor were realized with the release of *Psytallia loundsburyi*, a braconid larval parasitoid from southern Africa. Approval to release this species was granted by USDA-APHIS in 2005 and the first releases occurred in northern California in October 2005. One release occurred at a private orchard in Butte County located in the Sacramento Valley and a second release occurred at Ohlone College in Marin County located in the northwest region of the San Francisco Bay. Large-scale releases of *P. lounsburyi* are planned for 2006. This is the first of several natural enemies being examined for use as biological control agents against the olive fruit fly in California.

Once a biological control agent becomes established, implementation efforts are needed to assist with its distribution statewide. For this, the Biological Control Program relies on the cooperation of the California County Agricultural Commissioners and their staff to assist with the implementation effort. County biologists know their area and public better than anyone and provide an extremely valuable service through the education of the public and assistance with distribution efforts. The partnership between the Biological Control Program and the County Agricultural Commissioners has resulted in an efficient and effective vehicle for the transfer of biological control agents and information to public and private landowners statewide. Their assistance is greatly appreciated. The effectiveness of this partnership is demonstrated by the recent efforts to distribute the exotic rust, *Puccinea jaceae* var. *solstitialis*, on its host plant, yellow starthistle. To date, 38 counties have participated in the training workshops associated with this effort and releases have occurred in over 100 locations during the last three years (2003-2005). A truly monumental achievement is a very short period of time.

This year we've begun the first releases of several biological control organisms against the red imported fire ant (*Solenopsis invicta*) in southern California. Two pathogens, a microsporidium and a fungus, and a parasitic fly were released near fire ant mounds in Riverside County. Each of these organisms has shown promise in reducing the abundance of the red imported fire ant in the southeastern United States. We hope that they perform as well in California. Updates for several other projects are included in this report. I hope you enjoy this year's report.

TABLE OF CONTENTS

Insect Projects

Weed Projects

Pink Hibiscus Mealybug Parasitoid Insectary Production and Field Population Update

W. J. Roltsch, J. Zuniga, and R. A. Aguilar

The pink hibiscus mealybug (PHM), *Maconellicoccus hirsutus* (Green), is native to South Asia and was first detected in Imperial County, California in August of 1999. This was its first record in North America.

A cooperative biological control project against the PHM infestation in Imperial Valley was conducted from the fall of 1999 to 2004. Several populations of two encyrtid parasitoid species and one platygastrid species were mass reared and released as the centerpiece of the program. Population densities of the PHM and percent parasitism were monitored at a number of mulberry tree and carob tree sites. The population density of *M. hirsutus* within the first year of bioagent release was reduced by approximately 95%. Over the first four years from 2000 to 2003, the average regional population density of the mealybug exhibited a continued decline and continued at a very low density in 2004 as well (Figure 1). *Anagyrus kamali* Moursi was the predominant parasitoid, often parasitizing in excess of 50% of the mid to late stage *M. hirsutus* in the first two years following the parasitoid's release.

A detailed summary of this project was last provided in the 2004 CDFA, Biological Control Program Report. In 2005, limited sampling was conducted to determine if population densities were continuing at levels similar to those occurring in the past two years and to determine the status of the platygastrid parasitoid, *Allotropa* sp. nr. *mecrida*. The eight study sites, consisting of either mulberry trees or carob trees, were sampled once in August and again in September of 2005. Pink hibiscus mealybug densities were comparable to those recorded in recent years (Figure 1). The average densities in mulberry and carob were 3.6 and 4.5 second to adult PHM/terminal respectively in August and 1.6 and 0.3 respectively in September. *A.* sp. nr. *mecrida* was not found in the 2005 samples, indicating that it may have not established.

During 2005, although CDFA had no immediate need to produce parasitoids for release in the affected area of California, USDA contracted with CDFA to continue rearing of the parasitoids and provide them to the state of Florida. Three cultures were maintained for production; two cultures of *Anagyrus kamali*, one from Taiwan and one consisting of populations from China and Hawaii, and the third culture consisted of *Gyranusoidea indica* from Pakistan and Egypt. CDFA produced and mailed over 448,000 parasitoids to Florida (Table 1). In addition, 16,200 *Anagyrus kamali* from Taiwan were released in Imperial Valley and 10,550 were provided to Mexico to be released in Mexicali Valley. This particular strain had not been released in either the Imperial Valley or in Mexico during the primary phase of the program from 1999 to 2004.

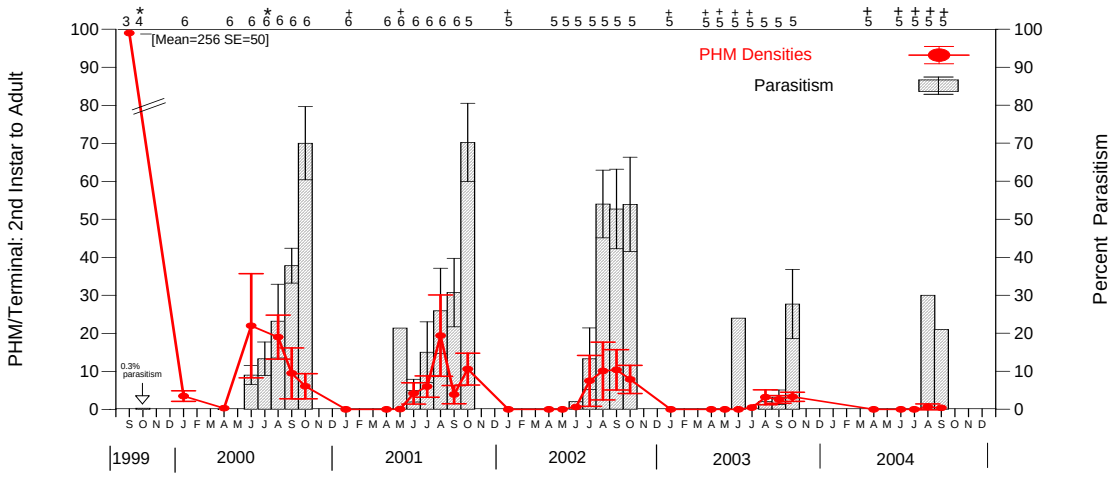


Fig. 1. PHM densities and parasitism at 5-6 mulberry tree sample sites.

Table 1. Number of parasitoids sent to Florida during 2005 from the El Centro insectary.

Month	*A. kamali* China/Hawaii	*A. kamali* Taiwan	*G. indica*	Monthly Totals
Jan.	2,800	8,200	15,900	26,900
Feb.	10,400	4,400	9,600	24,400
Mar.	2,000	3,200	9,200	14,400
Apr.	2,000	1,400	1,200	4,400
May	5,200		1,900	7,100
Jun.	9,600	9,400	10,800	29,800
Jul.	4,400	16,200	23,200	43,800
Aug.	15,800	13,000	38,800	67,600
Sep.	13,600	12,400	27,200	53,200
Oct.	14,800	6,400	32,000	24,400
Nov.	13,600	13,200	44,800	71,600
Dec.	10,400	9,600	32,000	52,000
Totals	104,600	97,400	246,600	448,600

Olive Fruit Fly Pest Management Project

K. M. Daane[1], M. W. Johnson[2], K. R. Sime[1], A. Kirk[3], R. Wharton[4], H. Nadel[2], R. Messing[5], C. H. Pickett, and F. Zalom[6]

Olive fruit fly, *Bactrocera oleae* (Gmelin) (Diptera: Tephritidae) (OLF) is the primary pest of olives in the Mediterranean basin, where the vast majority of the world's olives are produced. It is capable of infesting 100% of the fruit on a tree, rendering the harvest unmarketable. Following its introduction in 1998, this fruit fly pest is now firmly established in olive growing regions throughout California. We report on a multifaceted project aimed at providing pest management strategies for the control of olive fruit fly in California, with a focus on classical biological control. Additional research includes fly biology and impact of the fruit fly insecticide Spinosad on natural enemies that could be present in olive orchards.

In anticipation of releasing new imported parasitoids into California, 12 release sites have been identified in California. These include three sites in Butte, two in Yolo, one in Amador, five in Santa Barbara, and one in Alameda County. Adult and larval populations of olive fruit fly are being monitored at these sites, as well as extant parasitoids. The most common parasitoid recovered from fly infested olives is a species of *Pteromalus*, followed by *Eurytoma* spp., and a eupelmid. The first releases of *Psyttalia lounsburyi*, recently permitted for field release, were made in October 2005 into one site in Butte County and one in Alameda. Less than 100 adults were released at each location. To date, no recoveries have been made.

Foreign Exploration. Collection trips were made, or material was shipped from collaborators, in South Africa, Namibia, Kenya, Pakistan, and China. During 2005, UC Berkeley Quarantine received seven shipments of olive fly parasitoids. Three of these shipments were from material collected in South Africa, Namibia, or Kenya during spring 2005, and then processed in the EBCL quarantine. Four other shipments came directly from South Africa to the UC Berkeley Quarantine. The parasitoid species and its origin are *Psyttalia lounsburyi* from West Cape, South Africa and Kenya; *Psyttalia concolor* from Canary Islands and Morocco; *Utetes africanus* from South Africa; *Psyttalia ponerophaga* from Pakistan; and *Fopius arisanus* and *Diachasmimorpha kraussii* from colonies maintained in Hawaii. The UC Berkeley Insectary & Quarantine currently has colonies of: *Psyttalia lounsburyi* (South Africa and Kenya), *Psytallia concolor* "Kenya," "Morocco," and "Canary Islands," *Psyttalia* sp. nr *humilis*, (from South African, referred to as "no marks"), *Diachasmimorpha kraussii*, and *Utetes africanus* (very weak colony). Maintenance of these cultures was made possible through improvements in rearing methodologies. The availability of green olives and a strong OLF colony helped tremendously. We are also working in collaboration with Dr. Carlos Crisosto (post-harvest pomologist, UC Davis) on long-term storage of olives under controlled temperature and atmospheric conditions which will make it easier to maintain colonies during winter and spring when olive quality and availability in the field declines.

Testing for Host Specificity. To select the appropriate natural enemy species for release against OLF, we are conducting non-target studies to determine the potential host range of each parasitoid species. Nine species of native and exotic tephritid species have been chosen for parasitoid specificity (non-target impact) studies. These flies represent a range of niche preferences including fruit feeders, seed-head feeders, and a gall maker. The tephritid species are: *Rhagoletis indifferens* (western cherry fruit fly), *Euphranta canadensis* (currant fly),

Chaetorellia succinea (yellow starthistle fly*), Neotephritis finalis* (sunflower fly), *Euaresta aequalis* (cocklebur fly), and *Parafreutreta regalis* (Cape ivy fly). Priority was given to testing *C. succinea* and *P. regalis*, which are beneficial exotic flies controlling weeds in California.

Based on initial laboratory results from "no-choice" host testing in spring 2005, most of the imported parasitoids, including material reared from OLF in South Africa, would be classified as generalist parasitoids (Table 1). Most importantly, we found that some species will "attack," probe the fly larvae with their ovipositor – but not develop in the beneficial tephritids used for biological control of yellow starthistle and Cape ivy fly. *Psyttalia lounsburyi* had the highest degree of host specificity of all those tested and did not attack or reproduce on any of the non-target flies and we have obtained a release permit for that species.

Table 1. Host testing results with olive fruit fly parasitoids

.	HOSTS				
	Target Pest	Native, Pest		Exotic, Beneficial	
PARASITOIDS	Olive Fly	Black Cherry Fly	Apple Maggot	Cape Ivy Fly	Yellow Star Thistle Fly
P. concolor –Tunisa	R	P	P	R	P
P. concolor Kenya	R	R	P	R	
P. concolor Namibia	R	--	--	R	
P. nr humilis	R	R		R	
P. ponerophaga	R	--	--	R	
P. lounsburyi	R			S	
D. longicaudata	R	R	P	R	
D. kraussii	R	R	P	R	R
B. celer	R	P		R	P
U. africanus	R*				

S = Host plant searched by parasitoid; P = host plant probed by parasitoid; R = parasitoid successfully reproduced in host; R* = showed no interest during study, but reproduced in colony; -- = not tested.

In fall 2005, we began a new series of non-target host specificity tests for *P. ponerophaga, P.* nr *humilis*, and *D. kraussii*, using large cages to better test parasitoid response to non-target hosts and host environment (plants). Parasitoids were tested against *Rhagoletis indifferens, N. finalis, C. succinea*, and *P. regalis*. Initial results, based on behavioral observations, suggest greater specificity of all three species towards olive fruit fly under these conditions than had been observed in smaller cages. We are currently waiting to finish rearing flies exposed during these experiments, which will provide more conclusive results. Most recently, in November and December 2005, we conducted "no-choice" trials for *Fopius arisanus*, testing its responses to olive fruit fly, *C. succinea*, and *P. regalis*. Observational data are inconclusive, as very little activity was seen. We are currently rearing the flies to confirm whether parasitism occurred.

Fly Biology. The field biology of olive fruit fly is being studied to develop management strategies for olive growers and to provide basic information on how these insects survive under California climatic and olive growing conditions. Studies observing olive fly adults in cloth cages hung within olive tree canopies indicate that some adults can live up to eight months under San Joaquin Valley conditions when ample water and honey-water (50%) are provided. About 50% of the test individuals lived six months. These data are similar to longevities observed in lab

colonies at room temperature. Several traps were compared for monitoring efficiency and data continues to be collected on the temperature and fly populations around the state.

Impact of Spinosad on natural enemies. The insecticide Spinosad (GF-12)® is increasingly being used in California for olive fruit fly control and needs to be evaluated for its effect on natural enemies. GF-120® caused low mortality (about 10%) of the generalist predator *Chrysoperla carnea*. Mean longevity of adult females that fed on GF-120® for 24 hrs (30.7 ± 18.5 days) was reduced by about 25% compared with females that fed on honey only (42.0 ± 17.4 days), and was slightly reduced compared with females that fed on blank bait (38.0 ± 16.0 days). Females that fed on GF-120® for 24 hrs laid fewer eggs (167 ± 127) than females fed on blank bait (234 ± 65), which may be partly explained by the shorter life span in females that ingested GF-120®. Interestingly enough, females that fed on blank bait laid 1.5-fold more eggs than females that ingested honey. Egg viability (about 24%) was similar for all treatments.

---

[1]Division of Insect Biology, University of California, Berkeley, California
[2]Department of Entomology, University of California, Kearney Ag. Center, Parlier, California
[3]USDA-ARS European Biological Control Laboratory, Montferrier, France
[4]Department of Entomology, Texas A&M University, College Station, Texas
[5]Department of Entomology, University of Hawaii, Kauai Research Station, Kapaa, Hawaii
[6]Department of Entomology, University of California, Davis, California

Developments in the Taxonomy of Natural Enemies of Olive Fruit Fly, *Bactrocera oleae* (Gmelin)

R. A. Wharton[1], K. Dole[1], R. Stouthammer[2], and C. H. Pickett

There is currently a great need for dissemination of information about the biology and taxonomy of natural enemies of the olive fruit fly. A primary vehicle for the communication aspects is our newly upgraded web site (hymenoptera.tamu.edu/paroffit) which was made public earlier this year includes direct links to pages for known olive fly parasitoids through a new olive fly entry point. Over a hundred images have been added, as have a number of web pages on non-opiine parasitoids of relevance to the olive fly program. Updates to the site can now be done directly, without the necessity of pulling down the web page and re-doing them as in our first version.

Our taxonomic work on *Psyttalia concolor* and related species has two purposes. One objective is to determine whether *Psyttalia concolor* is a single, widespread, more or less panmictic species. The alternative hypothesis is that there are either several cryptic species or genetically differentiated populations in each of the regions of interest (Mediterranean, Subsaharan Africa, and Middle East at least as far as east as Pakistan). A second objective is to find genetic markers for populations currently in culture and which have already been released in California. To facilitate an understanding of the nature of the problems we are attempting to address, a brief history of taxonomic problems in *Psyttalia* as well as a brief history of the some of the cultures is presented below.

***Psyttalia* taxonomy and related information.** There are about 50 described species in the genus *Psyttalia*. Many of these have host records, and all of the known hosts are in the family Tephritidae. All the specimens that we have seen from the olive fly program are members of what we are calling the *concolor* species group. This group includes, in the strictest sense, the following nominal species that should be considered in putting a name to your material:

Psyttalia concolor (Szépligeti, 1910) described from Tunisia
Psyttalia dacicida (Silvestri, 1912) described from Eritrea
Psyttalia fuscitarsis (Szépligeti, 1913) described from Tanzania
Psyttalia dexter (Silvestri, 1913) described from Senegal
Psyttalia humilis (Silvestri, 1913) described from South Africa
Psyttalia perproxima (Silvestri, 1913) described from Benin, also Ghana to Nigeria
Psyttalia ponerophaga (Silvestri, 1916) described from Pakistan
Psyttalia siculus (Monastero, 1931) described from Sicily

We know of no satisfactory way of distinguishing these morphologically, in part because morphological features noted by Silvestri in some of his original descriptions vary within a population based on such things as size of host. In the material from Pakistan collected by EBCL, for example, the distinctive wing feature noted by Silvestri in his original description of *P. ponerophaga,* is present in the left wing of one specimen, and absent in the right wing. Four of these nominal species, *concolor*, *dacicida*, *ponerophaga*, and *siculus*, were originally described from specimens reared from olives. Two similar species that could also be placed in this species group, *Psyttalia cosyrae* (Wilkinson) and *P. phaeostigma* (Wilkinson), have noticeably longer ovipositors, so we are excluding them from consideration for the moment, especially because

they have never been associated with olive fly. *Psyttalia lounsburyi*, also originally described from material reared from olives, is a distinctly darker species.

Psyttalia concolor was introduced to Italy in an effort to control olive fly shortly after its discovery in Tunisia. Its early use in Italy has been well documented, as has its subsequent use in augmentation programs following development of mass rearing techniques using Medfly as hosts. As a result of these efforts, there is now a considerable amount of information on the developmental biology of *Psyttalia concolor*, as well as other facets of its biology related to its utility for biological control of fruit pests. Although material from the Mediterranean region can be called *P. concolor* without much fear of contradiction, material from elsewhere is problematic. For example, during the exploration phase of the Oriental fruit fly program, several of the opiines from Kenya were variously identified as color varieties of *Psyttalia concolor* or as *Psyttalia perproxima* (Clausen et al. 1965). Material from the same localities had been identified as either *P. humilis* or *P. perproxima* during an earlier sampling program (Bianchi and Krauss, 1936). Difficulty in identification of these three species is still a problem, and uncertainty over whether or not they are distinct makes it difficult to correctly associate previously published host records. Similarly, two decades after the purposeful introduction of *P. concolor* to Italy to control olive fly, *Bactrocera oleae* (Gmelin), Monastero (1931) described *Psyttalia siculus* from Sicily as a parasitoid of *B. oleae*. Considerable debate ensued over whether *P. siculus* was actually distinct from *P. concolor* (Monastero, 1934; Delucchi, 1957). Fischer (1963, 1971, 1972) treated *P. siculus* as a subspecies of *P. concolor* with slightly longer ovipositor, and although he originally treated *P. humilis* and *P. perproxima* as synonyms of *P. concolor*, he later retained *P. humilis* as distinct from *P. concolor* "because of differences in morphology of developmental stages."

Brief history of the cultures. Several cultures were initially established in Kenya in 1998, then shipped to Guatemala and Hawaii for biological control programs against Mediterranean fruit fly. Material from both Guatemala and Hawaii was subsequently shipped to California for the olive fly program. The cultures originally established in Kenya came from two sources. One was initiated from wasps reared from Medfly in coffee that was field-collected from the central highlands in Kenya. The second culture was initiated from wasps obtained from puparia shipped to the ICIPE quarantine facility from Alfio Raspi's *Psyttalia concolor* colony in Pisa, Italy. To further complicate the issue, Alfio Raspi subsequently sent more material directly to Russell Messing from Italy for the olive fly program. Thus, the *Psyttalia* in various cultures now in California (Berkeley:UCB, Sacramento:CDFA, and Fresno:USDA) either originated from Raspi's culture from Italy (either from Italy to Hawaii to California or from Italy to Kenya to Guatemala to Hawaii to California) or they originated from Kenya (and came to California via Guatemala or Hawaii). Because these cultures have been variously labeled in each of the places where they have been kept, one of our objectives is to sort out the origins of these cultures (separate from the issue of what species names to put on them) and develop markers that will facilitate future identification.

We currently have 45 samples for analysis. These samples include representatives from all current cultures of olive fly-associated *Psyttalia*, all *Psyttalia* previously collected from olives, and several species of *Psyttalia* collected from other fruit-infesting tephritids for comparison. More specifically, we have samples of typical *P. concolor* originating from a mass-rearing program in Italy, and wild populations from olives in Morocco and the Canary Islands. From Subsaharan Africa, we have populations from Kenya, South Africa, and Namibia. We also

have samples from olives in Pakistan. The samples include field-collected material used to initiate cultures as well as material from some of these same cultures sampled over a period of several years. To facilitate the identification of the origin of the various cultures, a sample was field-collected from coffee in Kenya in June 2004 and fresh specimens were sent directly to UC Riverside from Raspi's culture in Italy.

The DNA work. DNA has been successfully extracted and amplified from 43 samples (two outgroups, recently obtained, have yet to be sequenced). The D2 expansion segment of the 28S rRNA gene has been sequenced for all of these samples. The D2 region contains roughly 600 base pairs, and though a bit too conservative for most of the questions we are addressing (only nine of the sites are variable), it is a gene that has been commonly used in systematics research. Thus, standard protocols are available, as are primers known to work for opiine Braconidae. The widespread use of the D2 region in systematics research enables us to treat it as an easy marker to ensure that the DNA in our samples is of sufficient quality to extract and use for comparative purposes. The conservative nature of this particular gene segment and the fact that few individuals have been sequenced per sample, means that much of the data provided in the following paragraphs should be treated as preliminary. Additional genes will eventually be sequenced to obtain more definitive results, as well as to obtain adequate data for publications. Primers have recently been obtained for an internal transcribed spacer region (ITS2), and sequencing for this gene region has now begun.

The most divergent D2 sequences obtained thus far are from the material collected from olives in Pakistan. Four individuals from the same locality, but collected at four different times over a period of two years, yielded identical sequences and these differed from all other populations sampled by at least two base pairs (with a four base pair difference between the Pakistan population and the typical *P. concolor* from the Mediterranean Region). This provides supporting data to suggest that the material from Pakistan is *Psyttalia ponerophaga* (Silvestri), and that *P. ponerophaga* is a distinct species relative to the similar-looking *P. concolor*. *Psyttalia ponerophaga* has not been collected or studied since its original description in 1916. Even though studies are on-going, we recommend that this population from Pakistan be called *Psyttalia ponerophaga*. As noted above, one of the primary morphological features used to distinguish this species in the original description (Silvestri, 1916), is variable in the material at hand.

Psyttalia lounsburyi, though easy to recognize because of its darker coloration and slightly shorter ovipositor, differs by only one base pair from *concolor*-like populations on olives in southern Africa, but there is a consistent, two base pair difference between *P. lounsburyi* and the typical *P. concolor* from the Mediterranean region. The *P. lounsburyi* from Kenya (3 different samples from Burguret Forest) does not differ in this regard from the one sample we tested from South Africa. The D2 sequence data, though preliminary, confirm our suspicions that the ICIPE, Kenya lab culture of *Psyttalia lounsburyi* that was initiated in 2002 and kept for over 20 generations became contaminated at some point with *P. concolor* (which was being reared in the same laboratory). It was from this culture contaminated with *P. concolor* that material was subsequently sent to EBCL under the name *P. lounsburyi*. The D2 sequence of the EBCL colony matches that of *P. concolor* from Kenya. The contaminated culture at ICIPE was destroyed in 2004, when we observed that there were no individuals present resembling *P. lounsburyi*. If the EBCL colony is still in existence, there seems little point in maintaining it. However, the suspicion of contamination was first noted shortly after the material was sent to EBCL from

ICIPE, and there has already been considerable discussion with EBCL staff about the merits of maintaining this culture.

The preliminary D2 data suggest that *Psyttalia perproxima* is distinct from *P. concolor*. The two species are very difficult to distinguish morphologically, but there are small, consistent differences in the D2 sequences that suggest the West African *P. perproxima* may also occur in the lowland tropical forests of coastal Kenya. This finding may not have relevance to the olive fly program, since *perproxima* is associated primarily with plants in the Rubiaceae, but it helps address the general issue of speciation in the *concolor* species group.

Hannah Nadel has made excellent observations on color differences in populations currently being cultured in California (the culture in Fresno differing from those in Berkeley and Sacramento). These correspond to slight differences we observed when cultures from Italy and Kenya were being maintained in Kenya a few years ago. However, the observed differences are subject to post-mortem changes, making assessment based on dead specimens challenging. More importantly, based on earlier studies that we did in Kenya (Kimani-Njogu et al. 2001), corroborated by studies this past year in California, the two forms interbreed.

Our results show that the material from the Mediterranean region (Italy, Morocco, and the Canary Islands) differs from the material field collected in both Kenya and southern Africa (South Africa, including "no marks," and Namibia) by only a single base pair in the D2 region. On this rather slim evidence, it is very likely that Dr. Yokoyama's culture in Fresno, received from Guatemala, is indeed the same as the culture initiated in Kenya from field-collected coffee and published under the name *P. concolor* and *P.* cf. *concolor* by Kimani-Njogu et al. (2001) and Wharton et al. (2000) respectively. The two cultures from Berkeley (one labeled Kenya and one labeled Tunisia) plus a sample from the culture in Sacramento labeled Tunisia are indistinguishable from Raspi's cultures from Italy. Berkeley's "Kenya" colony is therefore likely to be composed entirely or primarily of individuals originating from Italy. Our conclusions at this point are as follows: 1) cultures currently being maintained at Berkeley and Sacramento, that were sent from Hawaii, originated from Raspi's mass rearing program in Pisa, Italy; 2) the USDA culture being maintained in Fresno originated from wasps field-collected from coffee in Kenya; 3) material from Raspi's culture cannot at present be distinguished from material field-collected in Morocco and the Canary Islands; and 4) material originating from Kenya cannot at present be distinguished from material field-collected in South Africa and Namibia. Should the difference between Sub-Saharan populations and populations from the Mediterranean region hold up with additional testing, it should be possible to develop an easy assay for separating the two using RFLP because of the position of the base change in the D2 sequence.

Left unresolved at present (because they do hybridize) is whether the samples from Kenya, Namibia, and South Africa represent one or more species distinct from *P. concolor*. If they are distinct, the names *fuscitarsis*, *humilis*, and *dacicida* are available and it is uncertain which would be most appropriate. Specimens with larger numbers of flagellomeres have in the past been referred to as *dacicida* (as in Silvestri 1913 and Neuenschwander (1982), but as noted above, larger host produce larger wasps, which tend to have more flagellomeres.

---

[1]Department of Entomology, Texas A&M University, College Station, Texas
[2]Department of Entomology, University of California, Riverside, California

Biological Control of the Red Imported Fire Ant

K. Godfrey, B. Oesterlein[1], J. Stimac[2], D. Oi[3], and A. Callcott[4]

Established populations of red imported fire ant (*Solenopsis invicta*) were discovered in California in 1998. Since then, the red imported fire ant has been found in four southern California counties and at scattered locations in the San Joaquin and Central Valleys. Some of these infestations were eradicated, whereas for other infestations, insecticidal treatments are continuing. These treatments are expensive and in some areas, may raise environmental and health issues. Alternative control measures such as biological control need to be investigated because in the southeastern United States, eradication efforts based solely on insecticides have failed. Research on biological control agents of the red imported fire ant conducted in South America and the southeastern United States since the 1980's have identified three agents that have been used successfully to reduce and/or maintain ant densities at levels so low that spread is unlikely. The agents are a microsporidium, *Thelohania solenopsae*, a fungus, *Beauveria bassiana* (Strain 447), and a scuttle fly, *Pseudacteon tricuspis*. Each agent has established or demonstrated effectiveness in the southeastern United States. Their ability to be effective in southern California is unknown. Therefore, a study was conducted in Riverside County to investigate the effectiveness of each of these agents.

The study was conducted at four sites in Lake Elsinore that were infested with red imported fire ant. In April, the fire ant nests at each site were mapped, and each nest sampled to determine the reproductive strategy of each nest (monogyne or polygyne nest) and to determine the presence of *T. solenopsae*. Determinations of the reproductive strategy and the presence of *T. solenopsae* were done with PCR methodology. All of the nests were found to be monogyne and no *T. solenopsae* was found. Plans for the release of *T. solenopsae* were temporarily suspended until polygyne colonies can be located because this microsporidian has a more successful association with polygyne colonies than with monogyne colonies.

To determine the effectiveness of the fungus, 20 nests at three sites were treated with a bait formulation. At 10 of the nests, the bait contained fungal spores (treatment), and for the other 10 nests, the bait did not contain fungal spores (control). The control was used to insure that the bait was attractive to the fire ants. All nests were sampled at two, seven, 14, and 30 days after treatment by gently disturbing the nests and collecting ants in a vial. The ants were frozen and later assessed for the presence of the fungus.

The two sites treated with the fungus had mixed results. At one site, all four ant nests were killed by the fungus treatment. At the second site, no nests were killed, but four nests decreased significantly in size and activity. At this site, the nests were in general, larger than those found at the other site. Control over these nests may have improved with a second application of the fungus about seven to fourteen days after the initial treatment. Overall in the fungus treatment, four nests were killed, four nests decreased in size and/or activity, and two increased in size.

At the control site, four nests stayed the same throughout the sampling interval, one nest increased in size, five nests decreased in size, two nests decreasing to zero. There was a tremendous amount of movement by the ants throughout the sampling period. It is possible that the two nests that decreased to zero had moved to another location (e.g., under the pavement,

farther into the bushes, etc.) and could not be found. Foraging ants could be found in the area of the two nests that decreased to zero, but the actual nest could not be located.

Releases of the scuttle fly, *P. tricuspis*, were made in June at two sites in Lake Elsinore. Approximately 10,500 fly pupae were received from Florida on June 9 and placed in emergence cages that were held at 27°C for fly emergence (Figure 1). The flies began emerging on the evening of June 12 and continued emerging through June 23. Each day, the adult flies were collected from the cage and placed in vials with approximately 20 – 50 adults per vial (Figure 2). The vials were placed in an incubator at 15.5°C until the following morning when they were taken to the field. The flies were released at a total of seven nests at the two sites between 8:00 am and 10:30 am. The releases were made early in the morning because the fire ants become inactive around 11:00 am because of heat. The details for the releases are in Table 1.

Figure 1. Emergence cage for scuttle flies that parasitize imported fire ants.

Figure 2. *P. tricuspis* in vials for field release.

Table 1. The total number of scuttle flies released by date and total at two sites in Lake Elsinore in June 2005. (The number of flies released at each nest was dependent upon the initial size of the nest.)

	Number of flies released by date (June)								
Nest	13	14	15	16	17	20	21	23	Total
PR-4	97	-	155	120	120	-	100	40	632
PR-5	-	-	-	80	100	-	-	-	180
PR-9	-	101	-	80	100	-	-	-	281
CAL-1	-	-	75	160	80	80	60	60	515
CAL-3	-	-	75	80	60	-	40	40	295
CAL-6	-	-	70	80	40	-	-	-	190
CAL-7	-	93	-	80	100	-	40	60	373

The success of the scuttle fly releases was monitored at both sites in July and August. To sample for the scuttle fly, an ant nest where the flies were released was disturbed and a few ants crushed to entice any flies in the area to come. The nest was observed for one minute. This procedure was repeated at least twice. Release nests were sampled on July 25 and again on August 23. In July, a single scuttle fly was observed at ant nest at each release site. Attempts to collect the flies were unsuccessful. In August, no flies were observed at either site. Monitoring will continue at the sites in June and August 2006.

[1]Riverside County Agricultural Commissioner's Office, Riverside, California
[2]Department of Entomology, University of Florida, Gainesville, Florida
[3]USDA-ARS, CMAVE, Gainesville, Florida
[4]USDA-APHIS, Gulfport, Mississippi

Vine Mealybug Distribution and Biological Control

K. Godfrey, G. Watson[1], and K. Daane[2]

The vine mealybug (*Planococcus ficus*) was first identified in vineyards in the Coachella Valley in 1994. From 1994 through 2002, the only method of detecting the vine mealybug was visually searching the vines. In 2003, a pheromone trap that attracts the males (the only winged stage of vine mealybug) was made commercially available, making trapping an option for growers, crop consultants, extension programs and county agriculture departments. Trapping occurred in many of the grape-growing counties during 2003-2004. In 2005, trapping was conducted in 31 counties, a reduction from that in 2003 and 2004 because no dedicated state funding has been obtained to maintain the program statewide. Funding was obtained, however, from USDA-APHIS to purchase pheromone traps and lures for use in the 2005 season. From the 2005 sampling, 20 counties were found to be positive for vine mealybug (either females or males found), along with three additional counties that may be close to eradicating vine mealybug from within their counties. The total number of infested sites statewide, however, is low and represents only a small percentage of the total grape acreage. A summary of the trapping and detection activities from 1994-2005 can be found in Figure 1.

For some areas of the state, eradication of the vine mealybug may not be possible. Therefore, a cooperative project was established in 2004 to assist in the rearing of parasitoids of the vine mealybug. A colony of vine mealybug and one of its parasitoids, *Anagyrus pseudococci*, has been established at the CDFA-Biological Control Program in Sacramento. These parasitoids will be field-released beginning in 2006.

[1]CDFA, Plant Pest Diagnostics Laboratory, Sacramento, California
[2]Division of Insect Biology, University of California, Berkeley, California

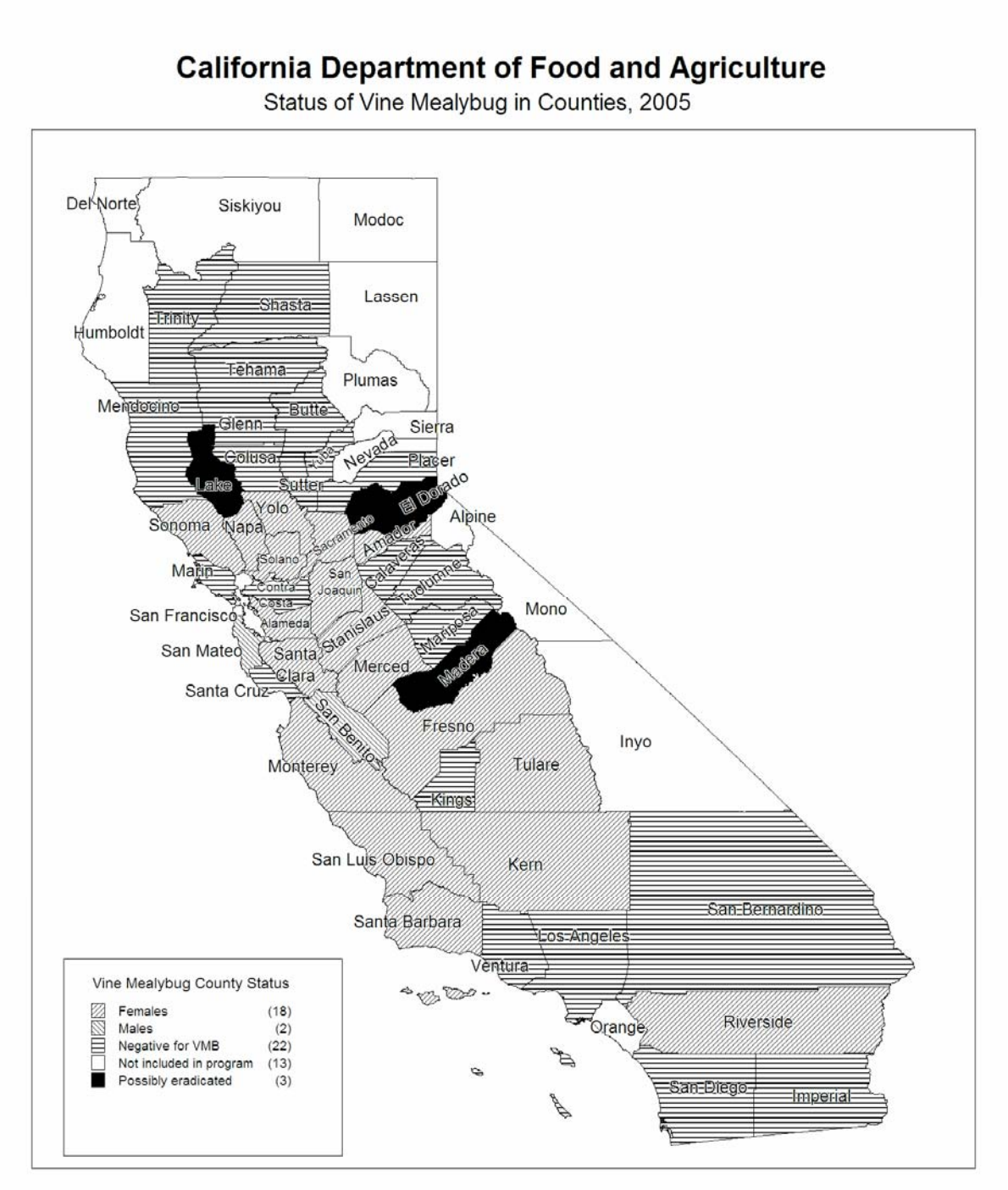


Figure 1. The distribution of vine mealybug in California from 1994 through 2005.

Gill's Mealybug: Status in California Grapes

K. Godfrey and L. Wagoner

Gill's mealybug (*Ferrisia gilli* Gullan) is thought to be native to the southeastern United States. It was probably first introduced into California in the early 1960's, and the first definite record of this mealybug is from Shasta County in 1968. Currently, this mealybug is found in Shasta, Sacramento, Tulare, El Dorado, and Tehama Counties with unconfirmed reports of the mealybug in Colusa and Madera Counties. Many of these infestations are present in ornamental plantings (mostly deciduous trees and shrubs) in urban areas. However, the most impacted area has been in Tulare County where Gill's mealybug has been infesting pistachios and almonds since the late 1990's. In El Dorado County, Gill's mealybug was found infesting about 10 acres of wine grapes in 2004, and an additional 25 acres of wine grapes were found to be infested in 2005. All of the wine grape acreage with Gill's mealybug was placed under the eradication program recommended by University of California Integrated Pest Management for vine mealybug. Because the biology of this mealybug was not well understood, a study was initiated to evaluate the eradication program and the threat this mealybug poses to grapes in California.

The study to evaluate the eradication program was conducted at three sites in El Dorado County using sticky tape traps and two-minute bark scraping samples. Sampling at Site 1 began on November 15, 2004, and continued until November 3, 2005. Site 1 was treated with foliar insecticides on November 15, 2004, and March 9, 2005, and with a systemic insecticide on July 5, 2005. Sampling at Sites 2 and 3 began on June 30 and continued until November 3. Sites 2 and 3 were treated with an insect growth regulator on July 26.

Results of the sampling suggest that the eradication program was successful at reducing densities of Gill's mealybug. At Site 1, first instar Gill's mealybug were found on trunk and cordon traps on March 17 (one nymph a trunk trap; one nymph on each of two cordon traps), March 30 (one nymph on a trunk trap), June 1 (one nymph on a trunk trap; one nymph on each of two cordon traps), and June 16 (one nymph on a trunk trap). In two-minute bark scraping samples, live Gill's mealybugs were found on June 1 (seven live nymphs, 20 dead nymphs, four dead adults, and nine emerged mummies (i.e., parasitized mealybugs)). From June 16 through November 3, no Gill's mealybugs were found on sticky tape traps and only dead Gill's mealybugs were found in the two-minute samples. At Sites 2 and 3, two first instar nymphs were found on a trunk trap on July 14 at Site 2, and one first instar nymph was found on a cordon trap on July 14 at Site 3. In two-minute bark scraping samples, one dead adult and four dead nymphs were found at Site 2 on August 9. No mealybugs were found in bark scraping samples at Site 3. From August 9 through November 3, no live Gill's mealybugs were found on sticky tape traps or in bark scraping samples at Sites 2 and 3. Monitoring will continue at these sites in 2006.

To begin to assess the threat Gill's mealybug poses to grapes, visual sampling of a variety of crops was conducted monthly at six sites in Tulare County. Sampling was conducted from April 20 through September 21. From this sampling, the mealybug has been found in persimmons and table grapes. Persimmon is a new host record for this mealybug. The density of Gill's mealybug in table grapes was extremely low, and was not found until the September sample. It appears from this sampling that grapes are not the most preferred host.

During this sampling, parasitized mealybugs were found. The parasites were identified as *Pseudaphycus* near *meracus* Gahan, and *Chrysoplatycerus* sp. (Hymenoptera: Encyrtidae). The

Pseudaphycus parasite was also recovered from El Dorado County and can be found in the eastern United States, the native region for this mealybug. The *Chrysoplatycerus* parasite could not be further identified because only one male was found and females are needed to separate the species in this genus. From a cooperator at the University of California-Davis, specimens of a parasite recorded to commonly attack Gill's mealybug in the eastern United States, *Pseudaphycus meritorious* Gahan, were obtained. All specimens are now maintained in the insect collection at the University of California Riverside.

Avocado Lace Bug Biological Control in Southern California

W. J. Roltsch, E. C. Hummeres[1] M. S. Hoddle[1], and J. G. Morse[1]

The avocado lace bug (AvLB), *Pseudacysta perseae* (Heidemann), was originally described from Florida in 1908. Its distribution in the United States includes Florida, Georgia, Louisiana, Texas and now California. Furthermore, this insect is native to southeastern Mexico, the origin of many commercial cultivars of avocado. It is also found in Bermuda, Dominican Republic, Puerto Rico and elsewhere in the Caribbean. It was first collected and recorded in California in the cities of San Diego and Chula Vista, in San Diego County during August of 2004. This piercing/sucking insect feeds on the leaves of avocado trees. Removal of fluids from the foliage causes a localized feeding spot in approximately the center of the leaves. Feeding wounds produced by the bug serve as entry points for anthracnose fungi, which create large, brown dead blotches on leaves. This symptom is sometimes confused with tip burn caused by high salinity, which occurs at the terminal end of the leaf, extending to varying degrees toward the center of the leaves. Avocado lace bug has the potential to become a pest of commercial avocados in California because leaf damage may be so severe that the production of fruit is impaired.

Beginning in mid-December 2004, the San Diego County Department of Agriculture and the Pest Detection/Emergency Projects Branch of CDFA conducted countywide surveys for this pest. These surveys included both urban settings and commercial avocado production areas. They determined that approximately 134 square miles of San Diego County were infested with AvLB. The infestation extended from the United States/Mexico border northward to Interstate 8 and from the San Diego Bay eastward to the Sweetwater Reservoir. One location north of Interstate 8, near Lake Murray, was detected during this survey. To date, no AvLB has been found in commercial orchards.

Because of the extensive establishment of this pest within the county of San Diego, the development of long-term management practices were selected over eradication. As a result, the use of classical biological control is being pursued as a means of contributing significantly, if not fully, to the long-term control of the AvLB. We initiated the monitoring of AvLB in the summer of 2005 to determine its seasonality and to determine if AvLB life stages (esp. eggs) are being parasitized by endemic parasitoids. Such work is important in prioritizing foreign exploration efforts (i.e. we would not want to go through time-consuming and costly importations into quarantine of a species already present in California). At the same time, a greenhouse and associated building in Chula Vista were refurbished to rear lace bugs for research purposes and to provide a field laboratory site for work within the infested geographic area. This included the construction of a screen breezeway between two small greenhouses, as well as frame, plumbing and electrical repairs. In addition, interior improvements to the former garage space were made to accommodate several work desks and provide space for holding specimens and several environmental growth chambers used to conduct research.

Plans are currently underway to obtain biological control agents from areas in Mexico and perhaps from Caribbean Island countries and Florida. The most likely candidates are egg parasitoids. When such parasitoids are collected and held in quarantine, AvLB eggs from the San Diego Insectary will be sent under permit to the University of California, Riverside quarantine facility to initiate and maintain parasitoid cultures.

As required by the USDA-APHIS for the issuing of permits for the release of promising parasitoid candidates, host range tests will be conducted utilizing other native or resident species of lace bugs in California. To this end, field population sources for these lace bugs are being identified. Initially, host range tests will include eggs of the Morrill lace bug, *Corythucha morrilli* Osborn & Drake; cotton (or sapote) lace bug, *C. gossypii* (Fabricius); Western sycamore lace bug, *C. confraternal* (Say); Ceanothus tinged, *C. oblique* Osborn & Drake; and the lantana lace bug, *Teleonemia scrupulosa* Stal.

Results of population monitoring in 2005 through early 2006 indicate that AvLB densities reach peak abundance in the fall. Plant damage paralleled these elevated densities (Figure 1). Variability was relatively high among sites during the height of seasonal activity. During monthly field sampling, we noted that all life stages of AvLB are present throughout the year. With limited monitoring to date, there is no evidence of parasitoids attacking ALB life stages in the San Diego region.

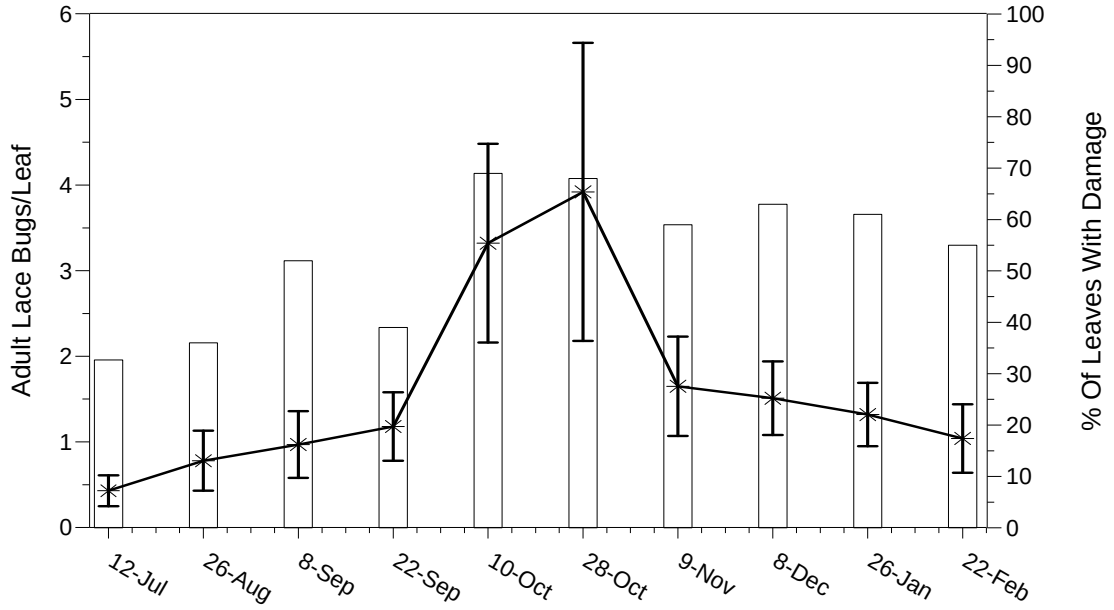


Figure 1. Avocado lace bug population densities (Mean ±SE) at six residential sample sites in southern San Diego County from summer 2005 to spring 2006. Leaf damage (open bars) represents the mean percentage of leaves per sample with evidence of lace bug feeding. Samples obtained from the lower canopy, consisted of 25 to 50 randomly selected leaves per site that were visually examined.

---

[1]Department of Entomology, University of California, Riverside, California

Colonization of *Lygus* Nymphal Parasitoids in the Monterey Bay Region

C. H. Pickett, M. L. Lawson, S. L.Swezey[1], D. Nieto[1] and J. Bryer[1]

Lygus hesperus Knight (Hemiptera: Miridae) is a serious pest to numerous field, fruit, and seed crops. It is a particularly serious problem to growers of strawberries in the Monterey Bay region of California. Currently Lygus is managed on most crops through applications of broad-spectrum insecticides. Cultural and biological alternatives are not considered useful. Importation of the nymphal parasitoid *Peristenus digoneutis* Loan (Hymenoptera: Braconidae) into eastern United States during the 1980's, however, successfully reduced *Lygus lineolaris* infesting alfalfa, a close relative of *L. hesperus*. *Peristenus digoneutis* has recently been reported attacking *L. lineolaris* infesting strawberries in New York. We have successfully imported and colonized both *P. digoneutis* and *Peristenus stygicus* Loan in Sacramento, California (see last years annual report). In 2002 and 2003, limited releases and recoveries were made of the same parasitoids in the Monterey Bay region. The purpose of this project was to release additional parasitoids at one new location and two former sites, and to continue monitoring another release site of wild vegetation.

Peristenus stygicus (ex. Granados, Spain) and *P. digoneutis* (ex. Catalognia, Spain) were reared and then released at three coastal locations: Harkins Slough (Watsonville), and two commercial, organic strawberry production sites near Castroville. *Peristenis stygicus* and *P. digoneutis* were reared on *Lygus hesperus* at CDFA's Biological Control Program's facilities in Sacramento. We also collected parasitized *Lygus* nymphs from a managed field insectary of alfalfa located at a state facility in Sacramento.

The two commercial organic strawberry sites use strips of alfalfa interspersed every 40 rows as a trap crop for *Lygus*. At the Eagle Tree site (Pacific Gold Farms, nr. Castroville), we released parasitoids into two 'release' strips, and monitored these and two additional non-release 'control' strips. Similarly, parasitoids were released into an edge, 'release' strip at the Elkhorn Slough site (Pacific Gold Farms, nr. Castroville), and monitored in this and a 40 meter distant strip of alfalfa. At Harkins Slough, parasitoids were released directly onto wild vegetation of black mustard, *Brassica nigra*, poison hemlock, *Conium maculatum*, and wild radish, *Raphanus sativus*, and the native *Atriplex triangularis*.

Each month, beginning in April and ending in December, release sites were monitored for the abundance of *Lygus* and released parasitoids. *Lygus* counts were made two ways depending on whether a sample came from strawberries or alfalfa. Alfalfa was sampled using a standard 37 cm diameter sweep net, taking 180 degree sweeps walking down a strip. Strawberries were sampled using a gas powered vacuum (a modified Stihl© leaf blower) and taking two to four sets of 300 suctions of strawberry plants randomly selected while walking across a field. After each group of 300 suctions, the contents of the organdy suction bag were emptied and *Lygus* nymphs placed into an alcohol filled vial.

Peristenus stygicus and *P. digoneutis* were released at three locations in 2005: two organic fields operated by Pacific Gold Farms (with site names of Eagle Tree and Elkhorn Slough) and into wild vegetation at Harkins Slough. A total of 4,878 *Peristenus* spp. were released at these sites (Table 1). Parasitoids were last released at a fourth site of wild vegetation bordering conventionally grown strawberries off Blackie Road. (Conlan Ranch Trust) in summer 2004 (655 *P. stygicus*, 25 *P. digoneutis*, 106 *Peristenus* spp.). Parasitoids have been recovered

within the same summer of release at all four locations. In 2005, *P. stygicus* were recovered in relatively high numbers in the control strips of alfalfa, at least 200 meters from where they were released, demonstrating their ability to disperse across strawberries (Figure 1). The same was also observed at the Elkhorn Slough site, where parasitoids were recovered from alfalfa strips 40 rows distant from the release strip of alfalfa. In addition, summer-long recoveries of *P. stygicus* at the Blackie Rd. site during 2005, one year after last released, shows that this parasitoid can survive on its own, without additional releases, for multiple generations when exposed to the Monterey region climatic conditions 12 months of the year (Figure 2). We also recorded at the same site their spread from wild vegetation into the adjacent strawberries treated with conventional pesticides, parasitizing up to 50% of sampled nymphs, at a time in the season when *Lygus* were especially low in numbers, i.e. less than four nymphs per 100 suctions. This demonstrates that *Peristenus* has a high degree of host specificity, i.e. they are able to search for and discover their hosts at very low numbers in strawberries.

The recovery of *P. stygicus* during summer 2005 from strawberries at the Eagle tree site demonstrates their ability to survive within a strawberry field interspersed with strips of alfalfa (Figure 3). Unlike the Blackie Road site, Eagle Tree lacks bordering vegetation capable of supporting *Lygus* or other closely related mirid bugs. The key to maintaining high, early season densities of *Peristenus* spp. in such a location may depend on preservation of these alfalfa strips from one year to the next, as was done at the Eagle Tree site.

Table 1. Number of *Peristenus* spp. released in 2005 in the Monterey Bay region.

Site	*P. stygicus*	*P. digoneutis*
Harkins	696	244
Eagle Tree	1716	980
Elkhorn	855	387
Totals	3267	1607

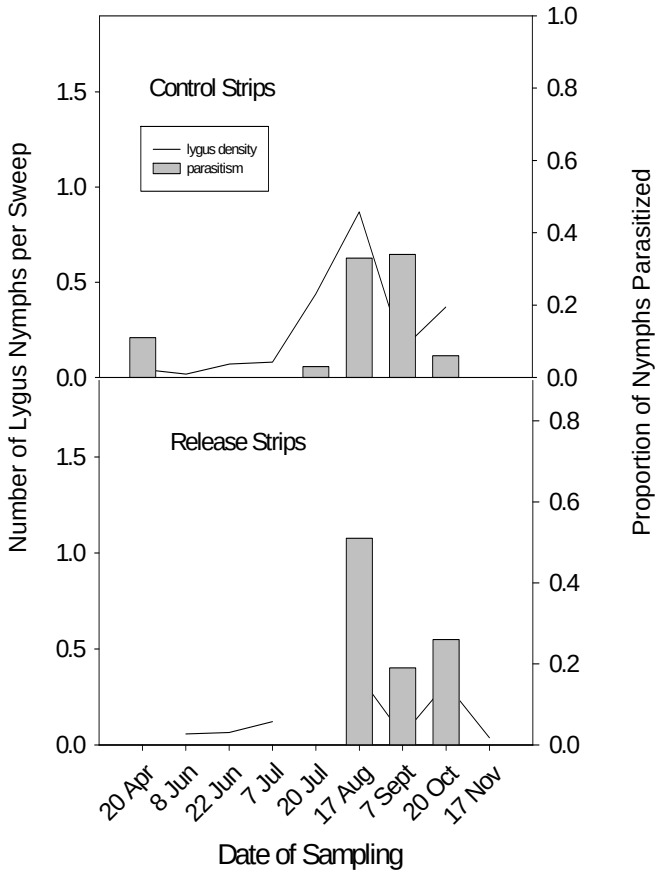


Figure 1. Eagle Tree 2005: Density of Lygus and Proportion Parasitized in Alfalfa Strips

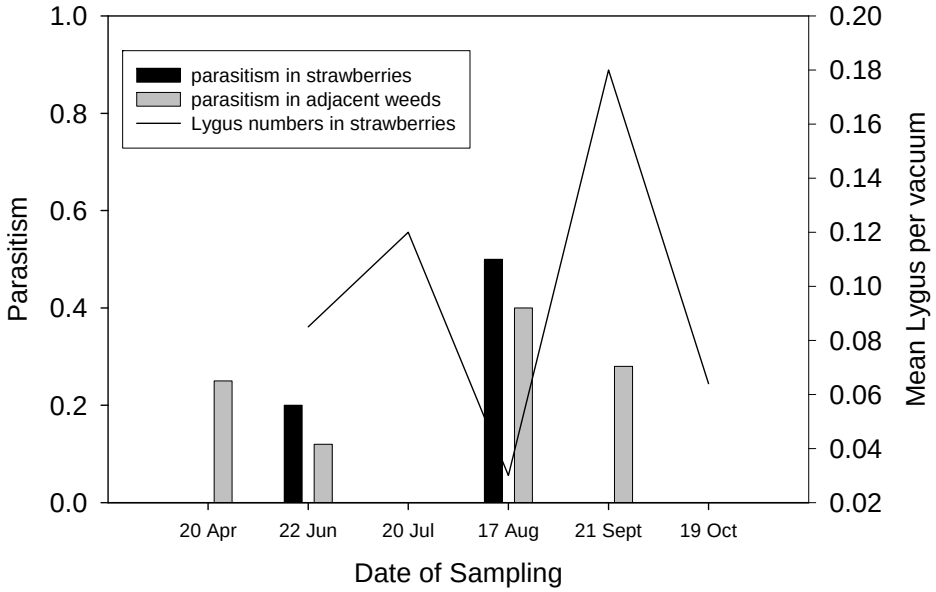


Figure 2. Blackie Road site 2005: Density of Lygus and Proportion Parasitized

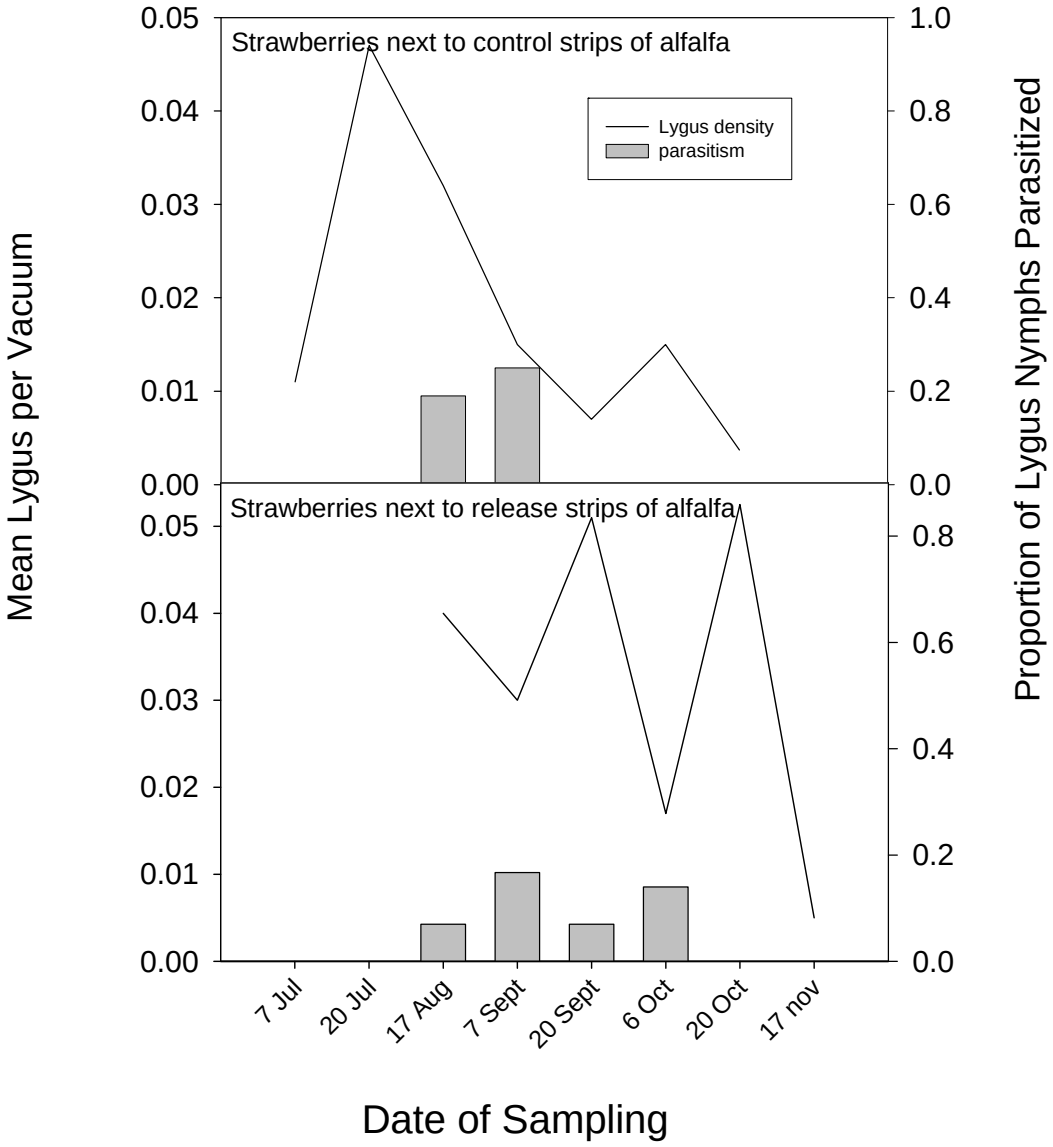


Figure 3. Eagle Tree, 2005: Density of Lygus and Proportion Parasitized in Starwberries

[1]Department of Environmental Studies, University of California, Santa Cruz, California

Asian Citrus Psyllid: A New Threat to California Citrus

K. Godfrey and B. Grafton-Cardwell[1]

The Asian citrus psyllid (*Diaphorina citri)* is a pest of citrus and close citrus relatives. It damages plants directly by injecting a salivary toxin into the plant that stops terminal elongation and causes the new leaves and shoots to be malformed. It also produces large amounts of honeydew that rain down on the plant, resulting in sooty mold infestations. The most severe impact of the Asian citrus psyllid is a result of its efficient vectoring of a bacterial disease called huanglongbing or citrus greening. The first American detection of Asian citrus psyllid was made in Palm Beach County in Florida in 1998. By 2001, the psyllid had spread to 31 counties in Florida with much of its spread due to the movement if infested nursery plants. In the spring of 2001, Asian citrus psyllid and one of its parasites was accidentally introduced into the Rio Grande Valley of Texas on potted nursery stock from Florida. In 2005, huanglongbing was found in citrus in southern Florida. With the disease and insect together in the United States, the threat from this psyllid has increased. The Asian citrus psyllid could invade California at any time, with the most likely sources of the infestation being Florida, Mexico, or Asia. Therefore, the University of California Exotic/Invasive Pest and Disease Program funded a grant to develop educational materials and to educate citrus, nursery, and regulatory personnel about this psyllid.

Educating the citrus, nursery, and regulatory personnel was done by producing a booklet on the Asian citrus psyllid and conducting training seminars. The booklet will be published by the University of California Division of Agriculture and Natural Resources in the spring of 2006 and be provided to each county agricultural commissioner's office, selected UC Cooperative Extension Office, and selected CDFA regulatory offices. The publication will also be available for free as an on-line publication. In 2005, educational seminars about Asian citrus psyllid were held in Riverside, Orange, and Tulare Counties. In 2006, seminars will be held in five counties throughout California.

[1]University of California Riverside, Kearney Agricultural Center, Parlier, California

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

W. J. Roltsch, B. Villegas, and L. Yang

In California, the red gum lerp psyllid (RGLP), *Glycaspis brimblecombei* Moore (Hemiptera: Psylloidea), is predominantly a pest of red gum eucalyptus, *Eucalyptus camaldulensis* Dehnh. To control this new pest, the parasitoid *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae) was collected in Australia and evaluated by Dr. D. Dahlsten (deceased) in 1999. The primary objective for 2005 was to characterize psyllid and parasitoid population patterns in affected areas throughout the state.

Monitoring for post-release parasitism was conducted at approximately 60 locations across the state each year from 2003 to 2005. In most instances, these were locations where University of California and CDFA had released *P. bliteus* from 2000-2002. The sample period ran from August through October of each year. With the exception of California's low desert, this is the seasonal time period when RGLP populations reach peak abundance, and red gum eucalyptus demonstrates considerable leaf loss and stress if under extensive attack. Samples consisted of 15 branch terminals, 30 to 45 cm in length, from three or more trees per site. Life stage counts were made on 30 leaves in the lab at which time psyllid lerps were examined for parasitoid exit holes and live nymphs were inspected externally for signs of late stage parasitoid development (i.e., prepupal and pupal stages). For presentation in this report, data pertaining to percent exit holes in the lerps of 4th and 5th instar psyllids are used to provide a relative estimate of parasitism at each site. This data presentation is based on work reported in 2004, which showed that exit hole counts are correlated with data resulting from the visual inspection of live specimens. Further, the use of exit hole data are preferable to visual counts or dissections for a limited survey of this type (i.e., one yearly sample per site) because it represents population events over a broader period of time (i.e., a cumulative record of perhaps two months) and is therefore not as sensitive to short term population fluctuations of both psyllids and parasitoids as are the methods of visual inspection and in particular, dissections.

By fall of 2003, *P. bliteus* had been recovered at all but two of the 71 locations where it had been released in past years (Figure 1). In 2004, parasitism was recorded at all survey sites, however in 2005 exit holes were not recorded in the leaf count samples at four sites (Figures 2 and 3). A few exit holes were noted at each of these sites when samples were being collected, therefore it does not appear that the parasitoid had gone extinct. However, it is rare. On average, 22, 34 and 27% of the lerps across all sites had parasitoid exit holes in 2003 through 2005, respectively. Parasitism is highest in coastal areas.

To further provide an additional geographical characterization of parasitism, 2004 parasitoid exit hole data were plotted against those of 2005 (Figure 4). Parasitism is greatest in areas near the coast, however, this was not true of all coastal locations. Although the highest levels of parasitism were found in sites along the coast, several intermediate valley sites supported high levels of parasitoid activity, while parasitism was low in others. Several foothill locations were found to support high levels of parasitism, however, parasitism fluctuated widely between years at several sites (esp. Calaveras and Mariposa counties). Parasitism was generally lowest in the central valley locations with less than 10% parasitism recorded both years in the counties of San Joaquin, Colusa, Yolo, Madera, Stanislaus, Tulare, Glenn and one of three

Sacramento County sites. Parasitism varied widely between years in the interior valley sites in San Joaquin County and one of the two Shasta County sites. In general, survey results illustrate a similarity in parasitoid activity between years by site. Of the 58 sites sampled, 76% of the sites varied by 20% or less between years. A correlation analysis of the data resulted in a correlation coefficient of r=0.61, further demonstrating that similar levels of parasitism occurred at most sites between the two years.

In summary, *Psyllaephagus bliteus* has been released throughout the state and appears to be permanently established at most locations. Based on our assessment of parasitism, the parasitoid is very active (i.e., >10% of lerps with exit holes) at 70% of the sample sites in 2003, 83% in 2004, and 66% in 2005. On average, the parasitoid performs less effectively in the interior regions of the state, presumably due to high summer heat and low humidity. Although parasitoid performance is very good at many coastal and near-coastal locations, it does perform poorly at some coastal sites. This is likely due to an entirely different, yet unknown, factor than that which is responsible for reduced performance in the interior sites.

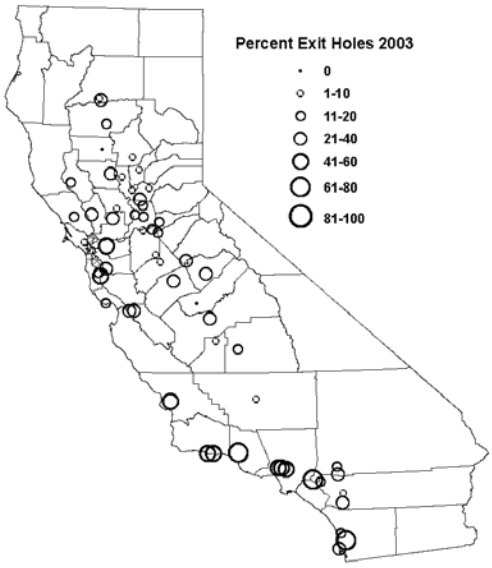


Figure 1. Fall survey 2003. Percent of lerps with parasitoid exit holes.

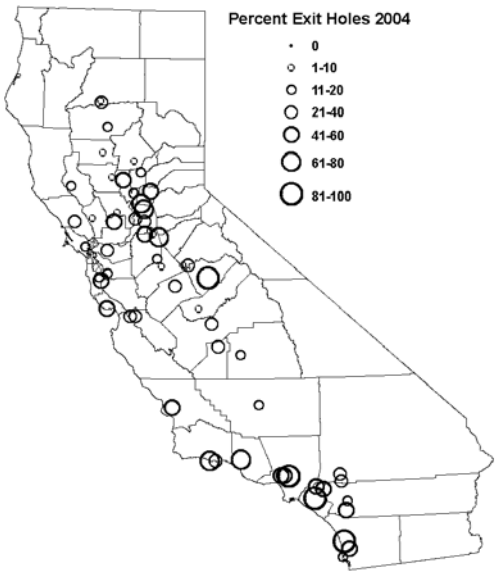


Figure 2. Fall survey 2004. Percent of lerps with parasitoid exit holes.

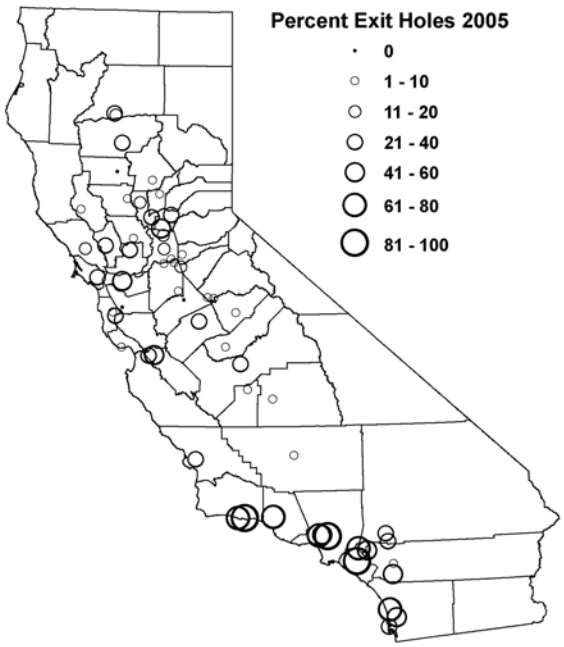


Figure 3. Fall survey 2005. Percent of lerps with parasitoid exit holes.

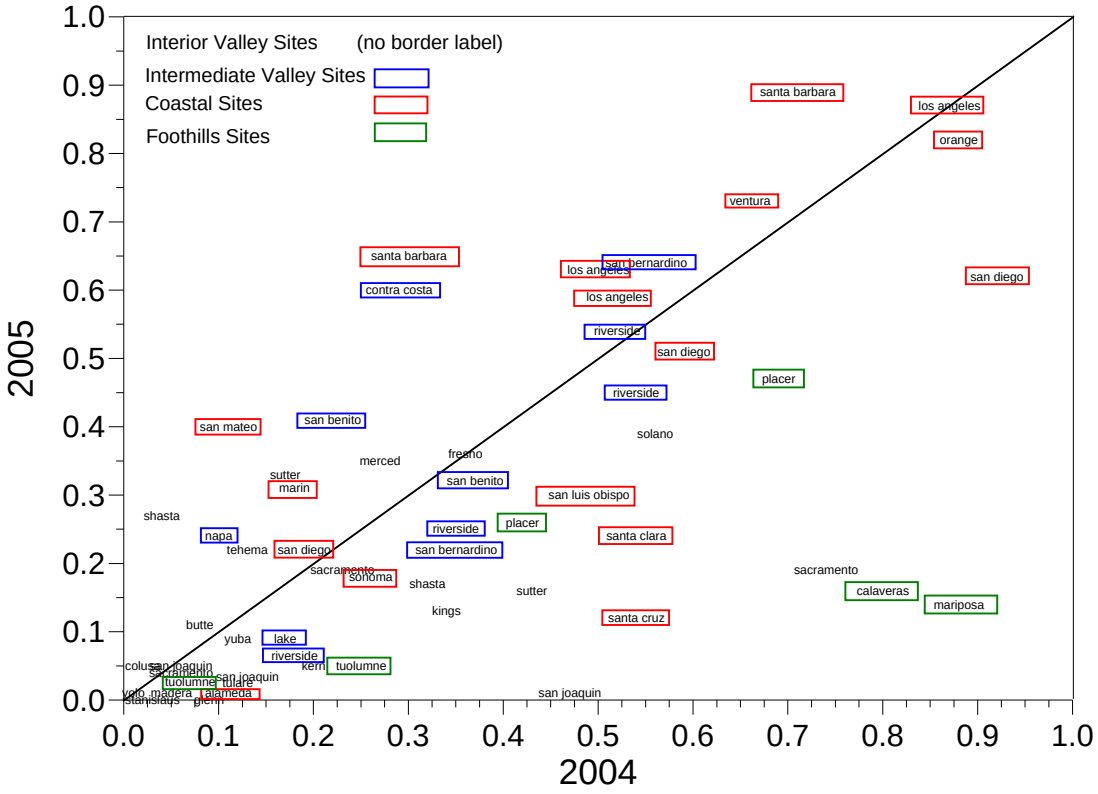


Figure 4. A comparison of the proportion of large lerps with parasitoid exit holes during 2004 and 2005. Transect line with slope of 1 for identifying increasing or decreasing activity between years by site.

Single Season Spread of the Yellow Starthistle Rust Fungus, *Puccinia jaceae* var. *solstitialis*

D. M. Woods, P. Akers, and V. Popescu

The rust fungus, *Puccinia jaceae* var. *solstitialis*, was approved for release as a biological control against yellow starthistle, *Centaurea solstitialis*, in California in 2003. A large-scale release and distribution program for the rust was initiated in 2003. The first phase of the project was the initiation of greenhouse production of the rust starting in 2003. A total of 25 releases were made in 20 counties in 2004 from the greenhouse-produced spores, with additional releases made in 2005. Most of the 2004 release sites did not experience any significant spread of the rust during the spring and summer of 2004. One site in Sonoma County exhibited greater spread than all the others and was selected for additional monitoring in 2005 to evaluate the potential for spread over a one-year time frame from the initial release.

The Sonoma site was located at a pheasant club in the southern portion of the county, south of Highway 37 and adjacent to the San Francisco Bay. Yellow starthistle was scattered among naturally occurring shrubs, particularly *Baccharis* plants, in an area bordered by cultivated grain fields and the San Francisco Bay. Selection of this specific site by the Sonoma Agricultural Commissioner's Office proved particularly valuable, because we learned that the combination of cooler temperatures with abundant moisture at this site kept yellow starthistle green and susceptible for an extended time during the spring and summer allowing the fungus to naturally spread. Extensive localized infection of up to six meters from the first release was noted in mid summer 2004 so follow-up was planned for 2005. The infestation was mapped August 19, 2005 by walking around the yellow starthistle infestation and examining plants for evidence of the rust. The area of plants with rust was related to GPS positions and mapped (Figure 1). Infection was highest in the area immediately around the 2004 release point and decreased in intensity with distance. There was some directionality to the degree of rust infection as the area east and somewhat north of the release site was most uniformly infected, following the prevailing wind pattern. Yellow starthistle was generally infected although to a lesser intensity throughout the primary weed infestation. The infected area comprised some 37.36 acres with the longest straight line spread of 1,890 feet. A few plants along the roadway into the club were not infected by the rust and may indicate the westward limits of spread. Spread was limited eastward and south by the lack of starthistle in the tidal flood zone. Spread west and north was limited by the lack of starthistle in the cultivated oats. The yellow starthistle rust seems quite able to spread unaided by human intervention under the conditions at this site. Additional studies of rust spread are needed under drier and hotter California conditions.

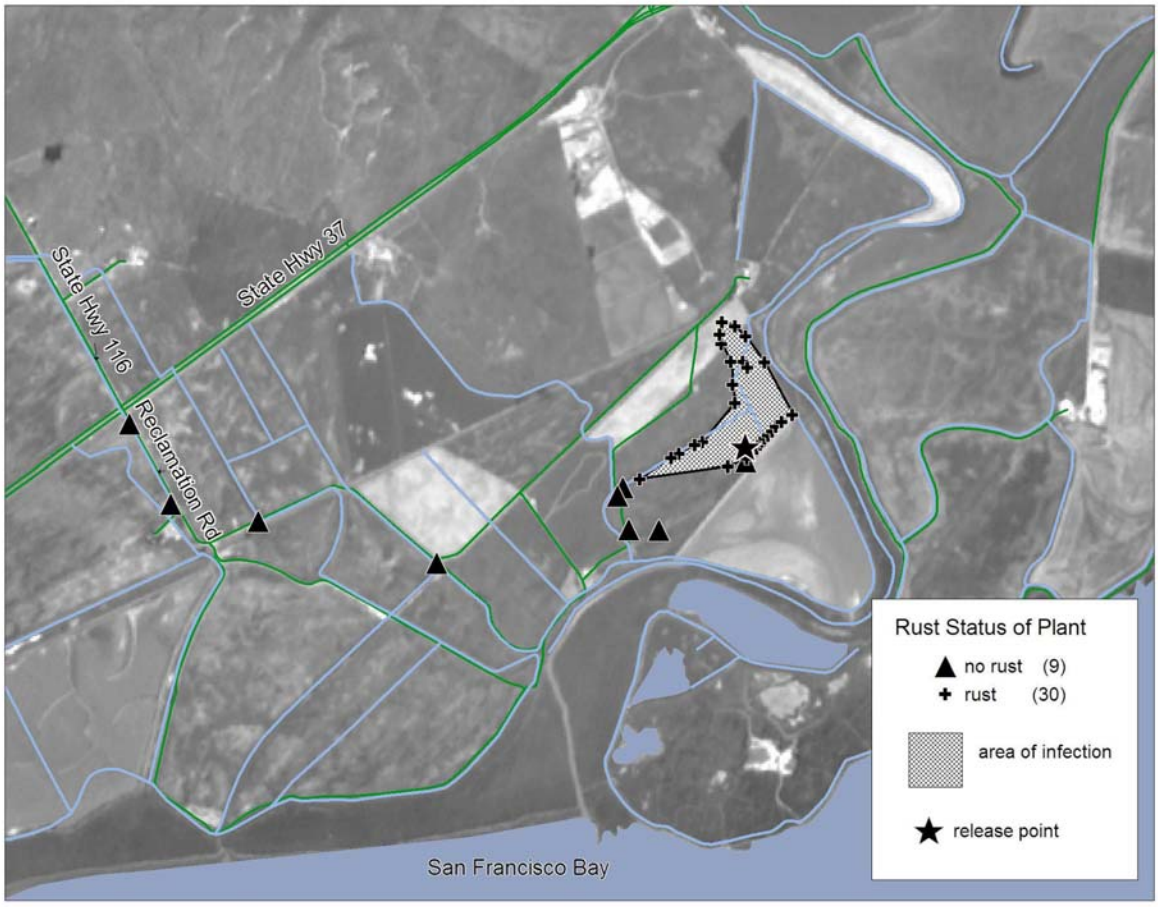


Figure 1. The extent of natural spread of the yellow starthistle rust, *Puccinia jaceae* var. *solstitalis*, one year after its initial release at a yellow starthistle site in southern Sonoma County.

Extended Distribution of the Rust *Puccinia jaceae* var. *solstitialis* in 2005

D. M. Woods and B. Villegas

The successful field releases of the rust *Puccinia jaceae* var. *solstitialis* to 20 counties in California during 2004 led to a larger scale release program for 2005. For the 2005 program, we offered at least one release of the rust to each county where yellow starthistle is a weed. All releases were made through the California Agriculture Commissioners Association. A total of 38 counties responded with interest to perform releases. A series of regional workshops were presented to train local biologists about the rust. Regulatory information, inoculation techniques, and biological information were presented at each session to enable local application and monitoring. As part of the overall training, a powerpoint presentation detailing the inoculation procedure was given to all workshop participants.

Field studies in 2004 had demonstrated that greater success might be achieved with a smaller amount of rust released at each site than was widely used in 2004. Consequently, we reduced the amount of rust applied at each location in 2005, which had the additional benefit of allowing more releases in 2005 with a similar amount of rust spores that were used in 2004. County agricultural biologists were provided with a 100 mg sample of rust spores (200 mg in 2004) and a 'dew tent' made of PVC pipe covered with black plastic sheeting. Biologists could then return to their sites and perform the inoculation, covering the plot overnight with the dew tent to maintain heat and moisture. Each inoculation was designed to be one square meter in size and remained covered overnight with the dew tent. Biologists removed the dew tents the morning following inoculation, then continued to monitor the sites regularly for the first appearance of disease symptoms (two to four weeks) as well as natural spread from the inoculated plants. Releases began in March (April in 2004) in the southern half of the state and progressed northward. A total of 76 releases were made in 38 counties during 2005 (Table 1). Although complete data has not yet been accumulated, preliminary data show that inoculations were highly successful, as infection was noted in almost all sites. Preliminary observations suggest that coastal environments may be the most successful releases as the rust has a longer season to develop and spread beyond the release site. The greatest spread from the 2005 releases appears to been a Contra Costa release and a Ventura release which moved some 20 meters outside the plot. The Ventura release plot continued to develop during the season, increasing from 32% of the plant infected from the original inoculation to 100% of the plants near the end of summer.

Table 1. Release locations, dates and successes of the rust on yellow starthistle in California during 2005. Data not yet available is left blank.

County	Site	Release date	% infection	County	Site	Release date	% infection
Alameda	Sunol	22-Mar		Placer	J. Wilson		
Alameda	Turner Dan	28-Mar	100%	Plumas	Indian Creek		90%
Amador	Fiddletown	21-Mar	1%	Plumas	Quincy Mound		90%
Amador	Jackson	24-Mar	25%	Riverside	Girl Scout Camp		85%
Amador	Fiddletown #2	9-May		Sacramento	Eagles Nest	21-Mar	70%
Butte	Butte Creek	14-Apr	70%	Sacramento	Sloughouse	17-Mar	30%
Butte	Dove Ridge	12-Apr	25%	San Benito	Hollister OVP		20%
Calaveras	1 - San Andreas		97%	San Benito	John Smith Rd		
Calaveras	2 - Jiny Lind		0%	San Benito			
Contra Costa	Briones	31-Mar	70%	San Bernardino	Chino Hills	7-Jun	
Contra Costa	Walnut Creek	26-Mar	30%	San Diego	Mt. Palomar #1	16-Mar	
El Dorado	Holm	19-Mar	80%	San Diego	Mt. Palomar #2	16-Mar	
El Dorado	Lime Mine	18-Mar	30%	Santa Barbara	E Camino Cielo	14-Mar	50%
Fresno	Fresno	14-Mar	97%	Santa Clara	Arastradero	11-Mar	100%
Glenn	G-T Canal	30-Mar	80%	Santa Clara	Foothills Park	10-Mar	85%
Glenn	Office	1-Apr	90%	Santa Clara	Quicksilver	11-Mar	0%
Humboldt	Bushnell	30-Mar	4%	Shasta	Churn Creek		
Humboldt	Ft. Seward	31-Mar	80%	Shasta	Turtle Bay		90%
Lake	Anderson Marsh	24-Mar	30%	Siskiyou	Iron gate Dam	4-Apr	1%
Lake	Kelseyville	29-Mar	80%	Siskiyou	Shasta Valley W.	5-Apr	
Lassen	Susanville	20-May	13%	SLO	See Canyon	9-Mar	95%
Los Angeles	Gorman		26%	SLO	Templeton	10-Mar	80%
Marin	Buck	28-Mar	100%	Sonoma	Sonoma	25-Apr	70%
Marin	Loma Verde	6-Apr	40%	Sonoma	Taylor Mtn	26-Apr	30%
Mariposa	Westfall Rd	24-Mar	10%	Sutter	Airport		20%
Mendocino	Lake Mendocino		75%	Sutter	Buttes		25%
Mendocino	Spy Rock		70%	Tehama	Paynes Creek	26-Apr	90%
Merced	O'Neil forebay	22-Mar		Tehama	School Farm	20-Apr	80%
Merced				Trinity	office	31-Mar	4%
Monterey	FHL:El Piojo	7-Mar	80%	Trinity	pond	31-Mar	6%
Monterey	FHL:Engineer Pond	7-Mar	100%	Tuolumne	Foothill Horizons	16-Mar	
Monterey	FHL:Grand Canyon	7-Mar	75%	Ventura	Lake Piru	23-Mar	32%
Monterey	FHL:Interlake Road	8-Mar	40%	Ventura	Reasoner Cnyn	2-May	14%
Monterey	Priest Valley #2	7-Mar	10%	Yolo	Capay	17-Mar	80%
Monterey	Priest Valley Rd	20-Apr	20%	Yolo	Madison	23-Mar	60%
Monterey	San Antonio Lake	7-Mar	57%	Yuba	1st		80%
Napa	La Posadas		10%	Yuba	2nd		50%
Napa	Chapelle		30%				
Napa	Napa Jct		20%				

Testing Release Strategies for the Rust Fungus *Puccina jaceae* var. *solstitialis*

A. J. Fisher[1], D. M. Woods, L. Smith[1], and W. Bruckart[2]

The rust fungus *Puccinia jaceae* var. *solstitialis* (*P. jaceae*) is one of few pathogens introduced to the United States for the classical biological control of an invasive weed. Since 2003, it has been distributed in 41 counties in California to control yellow starthistle, *Centaurea solstitialis*. Due to the limited number of fungal pathogens used in biological control programs, there is little information regarding optimal strategies for releases. For example, when is the best time to inoculate plants and what are the optimal conditions. After the permits for release were acquired, plants in Napa County were inoculated with the rust in July and December 2003. In 2004, the first statewide release program was initiated, and *P. jaceae* was introduced in 22 counties from late March to the end of May. Because many plant pathogenic fungi require moist, humid conditions for infection of host plants, release plots were covered with plastic tents overnight to ensure that the moisture requirement was met for at least 12 hours following inoculations in 2003 and 2004. In 2005, a field experiment was initiated to determine the optimal time of year for *P. jaceae* introductions and to determine if tents were necessary to achieve high levels of infection after plants were inoculated in the field.

In January 2005, permanent experimental plots were established in the coastal hills outside the cities of Napa, Napa County and in the central valley near Woodland, Yolo County. Six blocks, each comprised of seven permanent 1 by 1.5 meter plots, were installed at each site. Within each block, one plot was repeatedly inoculated every four to five weeks from January to June (January, February, April, May, June), five plots received single inoculations, (one plot for each inoculation date), and one plot was a non-inoculated control, for a total of seven plots. To determine if tenting is necessary to achieve high rates of infection after field inoculations, we divided the single inoculation plots into thirds to create modified split plots; a third of the plot was tented after inoculation, a third was not tented after inoculation, and the center third was a non-inoculated buffer space.

Plots were inoculated with a suspension of 100mg spores per m^2 in the late afternoon. Plots destined to be tented were covered with tents immediately after inoculation while the others were left non-tented. Tents were removed from tented plots an hour and a half after sunrise the next morning. Plots were evaluated for disease incidence one week after the first pustules emerged. Incidence was measured in tented and non-tented plots by counting the number of infected plants out of 50 evenly spaced plants per plot.

Disease incidence ranged from 10% to 80% in Woodland (Figure 1). Moderate to high rates of infection occurred at the Woodland field site after inoculation in January, February, May and June. In Woodland, there was a decline in infection after the April inoculation in both tented and non-tented plots. April was the only month in which it did not rain within 24 hours of inoculation. Plants in Napa also showed symptoms of infection after each inoculation, and the highest rates of infection, approximately 50% of plants infected, occurred in May (Figure 2). Tenting did not have an effect on disease incidence at the Woodland site. In Napa, a greater number of plants showed disease symptoms in the tented plots in both January and May (Figure 2). Similar to Woodland, in Napa there was also a decline in infection rates during the month of April.

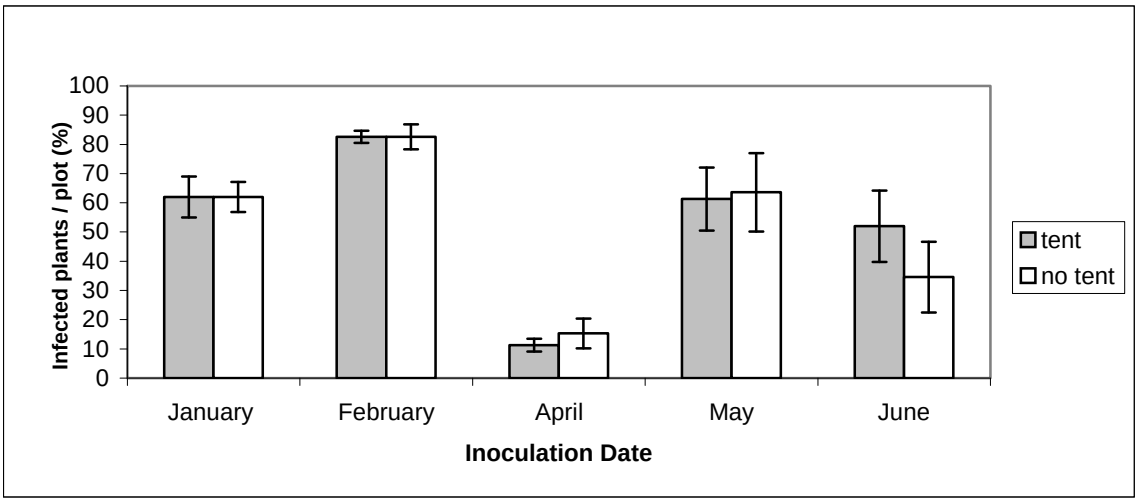


Figure 1. Percentage of plants infected with *P. jaceae* in Woodland (± stderr).

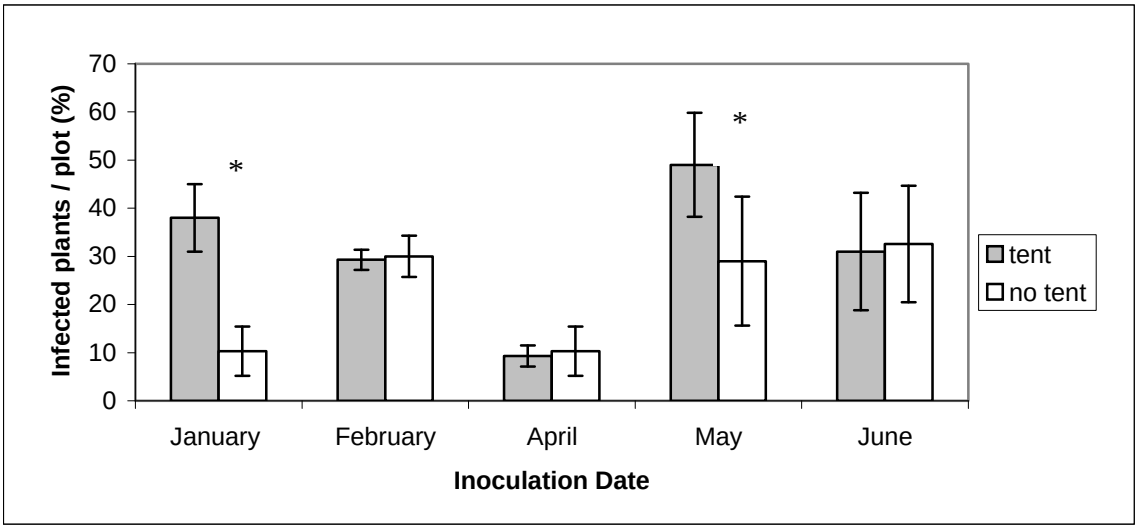


Figure 2. Percentage of plants infected with *P. jaceae* in Napa (± stderr). Differences between tenting treatments marked with an asterisk (*) are significant at the 0.05 level.

At the first inoculation date in January, yellow starthistle plants were small rosettes with approximately four to six leaves. At the last inoculation date in June, plants were flowering and beginning to senesce. Therefore, our results show that infection can be expected following inoculations with *P. jaceae* during most of yellow starthistles growing season. However, as temperatures rise, and the plant senesces, *P. jaceae* stops producing urediniospores, spores that re-infect new plants, and begins to produce teliospores, resting spores that germinate the next winter. By inoculating early in the growing season, there is the opportunity for multiple spore generations, which take approximately three to five weeks. Inoculating in the summer may result in infection, but the number of generations for secondary spread is likely reduced if the infection results in teliospore production.

Tenting increased the success of inoculations in Napa during the first release in January and then again in May. In January, the temperature outside tents dropped to 0^{o}C, while inside temperatures remained above freezing. Therefore, tents may protect release plots from the detrimental effects of cold. Tents had no effect during the other inoculation dates in Napa and had no effect at all in Woodland. One goal for future research will be to test our hypothesis that frost is detrimental to *P. jaceae* infection. If feasible, tenting can help increase infection after inoculation, and we have no data suggesting that tenting inhibits *P. jaceae* infection. However, our data shows that tenting is usually not necessary for successful inoculation and therefore, if a large-scale inoculation program were to be initiated, using aerial sprayers for example, it would not be hindered by the inability to cover large areas with tents. Our data agrees with the results presented in the 2004 CDFA annual report, that *P. jaceae* infects yellow starthistle following releases, and we would expect this agent to establish on plants within the State of California.

---

[1] USDA-ARS, Albany, California
[2] USDA-ARS, Ft. Detrick, Maryland

Releases of *Phrydiuchus tau* Warner for the Biological Control of Mediterranean Sage in Northern California

B. Villegas, C. Gibbs[1], K. Haas[2] and E. Coombs[3]

Mediterranean Sage, *Salvia aethiopis* L., (Lamiaceae), is widely distributed in the West with the main infestations being reported in Colorado, Nevada, California, Oregon, Idaho and Washington. In California, this weed is commonly called "medsage" and it occurs widely in Modoc and Lassen counties infesting open rangeland, roadsides, pastures, and meadows. Its native range includes the Mediterranean area of Europe and Northern Africa and into western Asia. Medsage is a strongly aromatic biennial plant that is distasteful to cattle and horses. It grows two to three feet tall and produces a stout taproot. Rosettes are produced during the first year averaging about a foot in diameter. During May and June of the second year, the plants bolt and produce a flowering stalk with numerous whitish flowers. After flowering, the plant dries up and the flowering stalk breaks off from the taproot and it tumbles across the open rangelands and roads spreading seed.

Two weevils were introduced into North America by the USDA-ARS for the biological control of medsage. The first weevil, *Phrydiuchus spilmani* Warner, was imported from Italy in fall of 1969 and 1970 and released in Lake County, Oregon, but did not establish. A second weevil, *Phrydiuchus tau* Warner, was imported from Yugoslavia and released in the same area of southern Oregon in the fall of 1971, 1972, 1973, and in the spring of 1972. *P. tau* became well established and movements of the weevils to other areas within Oregon and other states were started in 1976 (Andres, L. A., Coombs, E., and McCaffrey, J. P. 1995. In: Nechols, J. R. et al. (Eds) *Biological Control in the Western United States: Accomplishments and Benefits of Regional Research Project W-84, 1964-1989.* UC DANR, Oakland, CA. pp.303-305). In California, a total of 12 releases were made in the fall of 1976-1978 in infested areas of Modoc County (Table 1 & Figure 1). The release program was renewed again in October 2002 to include Lassen County and areas of Modoc County where *P. tau* was not found established. The collections for these releases were made in the fall of 2002 and 2003 from the Summer Lake area of southern Oregon while those in 2004 and 2005 were made from the Abert Lake area during May and June (Table 2 & Figure 1).

The weevil, *Phrydiuchus tau*, impacts medsage by adult-feeding on the foliage and larval feeding on the leaf midribs, petioles and crown of the plants. According to the literature, the adults estivate during the summer months and the summer diapause ends with the fall rains. In the fall, the adults enter the reproductive stage and oviposit on the undersides of the leaves and petioles of the medsage rosettes. The eggs hatch and the larvae mine their way from the midrib and leaf petioles down to the crown of the where the larvae finish their development in the spring. However, adults, eggs, and larvae may overwinter and continue their development through the spring months. In southern Oregon, the adult weevils were found emerging from earthen cocoons located near the medsage crowns just below the soil during May and June 2004 and June 2005. At this time, the adults were numerous and easier to collect than during the fall as was previously done. Collections were made in the spring of 2004 and 2005 in order to determine which collection dates lead to better establishment of the weevils in northern California (Table 2).

Table 1: Releases of *Phrydiuchus tau* root weevils on Mediterranean sage infestations in Modoc County during 1976-1978

CITY	LOCATION	WEEVILS	DATE	RELEASER
Davis Creek	3mi E Goose Lake on Hwy 9	250	11/5/1976	T Allen, R Dunkle, D Joley, K Wright
Davis Creek	2.5mi E Goose Lake on Hwy 9	250	11/5/1976	T Allen, R Dunkle, D Joley, K Wright
Davis Creek	1 mi SE Jct Hwy 47 & 395 (2.5mi E Goose Lake)	250	11/5/1976	T Allen, R Dunkle, D Joley, K Wright
Davis Creek	2.5mi N of Lake Mill Canyon Rd. (3mi S Goose Lake)	250	11/5/1976	T Allen, R Dunkle, D Joley, K Wright
Tulelake	Hwy 139, 3mi N of Clear Lake Reserve Rd.	250	11/5/1976	T Allen, R Dunkle, D Joley, K Wright
Tulelake	5.5mi N Jct Hwy 139 & Clear Lake Reserve Rd (S of Tulelake)	200	11/3/1977	T Allen, D Minnesang, W Sauer, M Pointere, Bud Greenbank
Alturas	1.5 mi N Jct Hwy 395 & 299 (6mi N Alturas)	200	11/3/1977	T Allen, D Minnesang, W Sauer, M Pointere
Davis Creek	Fandango Rd (3mi E Goose Lake)	200	11/3/1977	T Allen, D Minnesang, W Sauer, M Pointere
Alturas	0.7mi W on Sage Dr. from Jct of Thomas Creek Dr	150	11/3/1978	T Allen, K Wright
Alturas	1.0mi W on Sage Dr. from Jct of Thomas Creek Dr	150	11/3/1978	T Allen, K Wright
Davis Creek	2.5mi E Goose Lake on Hwy 9	300	11/3/1978	T Allen, K Wright
Tulelake	5.5mi N Jct Hwy 139 & Clear Lake Reserve Rd (S of Tulelake)	150	11/3/1978	T Allen, K Wright
	TOTAL RELEASES:	2600		

Table 2: Releases of *Phrydiuchus tau* root weevils on Mediterranean sage infestations in Modoc and Lessen counties during 2002-2005

COUNTY/CITY	LOCATION	WEEVILS	DATE	RELEASER
Lassen County				
Susanville	Belfast Tablelands dirt Rd off Center Rd	200	10/25/2002	C Gibbs
Susanville	Belfast Tablelands dirt Rd off Center Rd	200	11/3/2003	C Gibbs
Susanville	Belfast Tablelands dirt Rd off Center Rd	300	5/21/2004	B Villegas & C Gibbs
Susanville	Belfast Tablelands dirt Rd off Center Rd	250	6/9/2004	C Gibbs
Susanville	Belfast Tablelands dirt Rd off Center Rd	250	6/9/2004	C Gibbs
Susanville	Belfast Tablelands dirt Rd off Center Rd, (Pete's Valley)	444	6/8/2005	C Gibbs
Susanville	Belfast Tablelands dirt Rd off Center Rd, (Pete's Valley)	438	6/8/2005	C Gibbs
Ravendale	BLM, Ravendale	600	6/8/2005	C Gibbs
Susanville	Old Susanville dump, Hwy 139, N of Lassen College	349	6/8/2005	C Gibbs
Susanville	Cramer Rd, just north of Rd 27A (Center Rd)	750	6/9/2005	B Villegas
Modoc County				
Alturas	11mi S Alturas (BLM) E Hwy 395	750	6/9/2005	B Villegas
Alturas	4mi N Alturas (XL Ranch W of Hwy 395 over RR)	750	6/9/2005	B Villegas, J Moreo, L Smith
	Grand Total:	5281		

[1]United States Department of the Interior, Bureau of Land Management, Susanville, California
[2]Modoc County Department of Agriculture, Alturas, California
[3]Oregon Department of Agriculture, Salem, Oregon

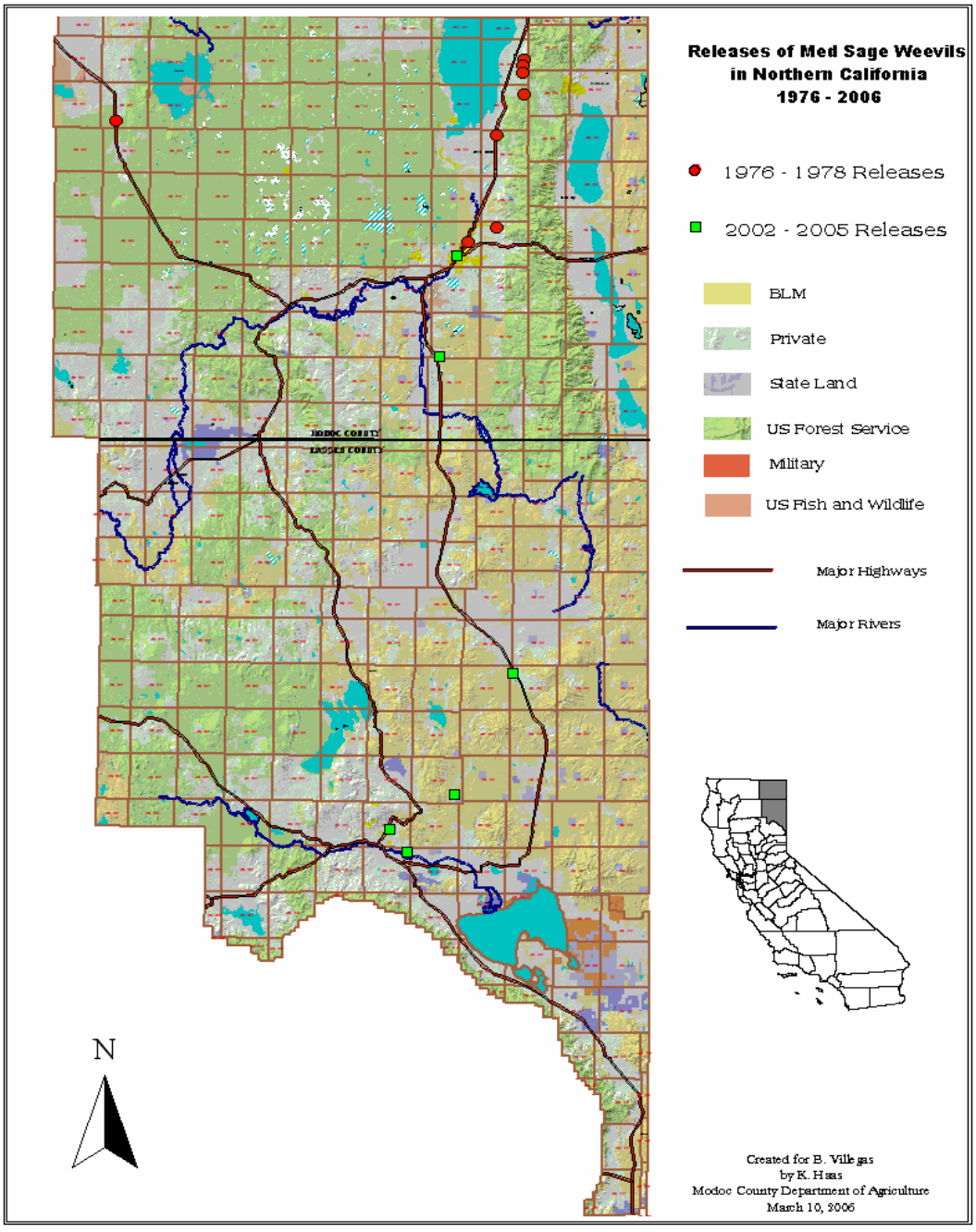


Fig. 1: Releases of *Phrydiuchus tau* Warner for the Biological Control of Mediterranean Sage in Northern California, 1976-2006.

Releases of Puncturevine Weevils for the Biological Control of Puncturevine in Lassen County, Northern California

B. Villegas, R. Wilson[1], L. Turner[2], and C. Pirosko[3]

Puncturevine, *Tribulus terrestris* L. (Zygophyllaceae), is Mediterranean in origin and occurs in many parts of the world. It was first reported in California in 1903, and by 1912, it was considered a serious weed pest. In 1965, Maddox and Andres (1979) estimated that some 360,000 hectares (approximately 900,000 acres) of land in California were infested with puncturevine and even today it is still a common weed in many counties in California. Puncturevine infestations can be an extreme nuisance around homes, orchards, roadways, bicycle lanes, and recreational areas because of its spiny seedpods commonly called "Goatheads". Puncturevine has also been suspected of poisoning livestock.

In 1956, the Biological Control of Weeds Laboratory of the United States Department of Agriculture Agricultural Research Service [USDA-ARS] and the University of California, initiated a cooperative biological control program on puncturevine. Through this program the weevils, *Microlarinus lareynii* (Jacquelin du Val) [the seed-infesting weevil, Figure 1], and *Microlarinus lypriformis* (Wollaston) [the stem-infesting weevil, Figure 2] (Coleoptera: Curculionidae), were introduced from Italy into California in 1961 for the biological control of puncturevine. Numerous releases of these two weevils were made throughout California by USDA-ARS and University researchers and State and county agencies, which contributed to the successful dispersal of these biological control agents.

At high elevation areas in California, the two weevils have not been able to keep puncturevine under control. Several attempts have been made in the past to establish the two weevils at high elevation infestations in Inyo, Mono, Siskiyou, Shasta, Modoc and Lassen counties from weevils collected in areas of the San Joaquin Valley as well as from southeastern Colorado. Attempts to establish the weevils in high elevation areas of California also were made in 1993 with weevils collected from areas near the 1000 ft. in elevation area in the Abruzzi Mountains in Italy.

In 2005, at the request of the Lassen County Agricultural Commissioner and the Lassen County Agriculture Board, another attempt to establish the two weevils was made in Lassen County. Approximately 2700 weevils, were mass collected in July 2005 in Tulare, California and released at five sites in Lassen County. The sites were selected in order to assure that the weevils could survive the cold winter temperatures common in the area. Fall surveys of the release sites revealed live weevils as well as exit holes in the seedheads and the stems at all the release sites. The sites will be surveyed again in 2006 to determine winter survival of the weevils.

Figure 1. *Microlarinus lareynii* and seedhead damage

Figure 2. *Microlarinus lypriformis* and stem damage

[1] University of California Cooperative Extension, Susanville, California
[2]Lassen County Department of Agriculture, Susanville, California
[3]CDFA, Integrated Pest Control, Burney, California

Biological Control of Water Hyacinth I: Population Dynamics of the Weevil *Neochetina bruchi* in the Sacramento-San Joaquin Delta, 2003-2004

R. P. Akers, C. Black, and M. J. Pitcairn

The Sacramento-San Joaquin Delta can support heavy infestations of the non-native water hyacinth (*Eichhornia crassipes*), and control costs approximately $2.9 million per year. In the early 1980's, two species of *Neochetina* weevils were released in the Delta for control of the weed. The weevils had little obvious effect, such that weed managers thought they had gone extinct, until a focused survey in 2002 demonstrated that *N. bruchi* had survived and was indeed fairly common in the Delta. In many parts of the world, the weevils have provided significant control, which leads to the question, why aren't they doing better here? Preliminary monitoring in 2003 indicated that there were few adult weevils on plants in late spring but numbers began to increase in the latter half of June. This observation led to the hypothesis that the winter is a time of heavy mortality and thus a barrier to sustaining high populations. We undertook a study of the weevil's population dynamics to address this question.

Methods. Preliminary surveys showed that there was a significant and apparently long-standing population of weevils in Whiskey Slough (San Joaquin County), in a large (approx. 100 by 400 m) patch of hyacinth that had not been sprayed for several years. Such a situation is relatively uncommon in the Delta, where most hyacinth infestations are subject to active control. The infestation at Whiskey Slough is in a sheltered, very slow-moving channel and receives few disturbances. The infestation completely fills the channel almost the entire year, and the plants have the tall (>60 cm), slender petioles that are typical of established, crowded infestations. A contrasting population was monitored in Rock Slough (Contra Costa County). The infestation there is in a slightly sheltered, shallow bay along an open, flowing channel. The infestation receives more disturbances from wind and currents than at Whisky Slough, and, as a result, its size is small (about 20 by 100 meters at the largest) and variable. Often, many of the plants have the short (< 20 cm) stature and bulbous petioles that are typical of new, uncrowded infestations.

Ten adult and 10 daughter plants were sampled every two weeks from each location. [A definition of "daughter" plants: water hyacinth usually reproduces by sprouting new (i.e., "daughter") plants on short stolons. The stolons are brittle, and the daughters often separate from the parent because of wind, currents, or because a parent dies. We were interested in whether the weevils used the parents differently from the daughters.] All plants were taken to the laboratory and examined for adults, eggs, larvae, and pupae. The plants were also evaluated for their size, number of leaves, number of daughter plants, and dry weight. Sampling began at Whiskey Slough in September 2003, and in October at Rock Slough. Sampling continued into late November of 2004.

Results and Discussion – Whiskey Slough: Weevil densities were very high at Whiskey Slough in the fall of 2003. They fell gradually through winter and spring to very low levels by late spring and early summer. Although they increased several-fold from that point until the fall of 2004, they never regained the levels of fall 2003 (Figure 1).

Population details varied slightly for different life stages. In late summer 2003, the abundance of larvae declined after an apparent peak in August. Nonetheless, larval numbers remained very high into early September, exceeding 10 larvae per plant. Larval numbers steadily declined over the next six weeks but stabilized at approximately four to five larvae per plant

through December. Adult numbers steadily increased from August into November and remained high from mid-October into January, varying between five to seven adults per plant.

In January 2004, numbers of all stages began to fall steadily until they reached very low levels in April. Adults and larvae remained very low, approaching 0.1 per plant, almost through June, only beginning to increase again in July. The population appeared to go through two generations in 2004, with the first bout of egg-laying occurring in March and April, and the second bout in late July through early September. The other life stages do not show as clear-cut a pattern, but generally follow the same trends.

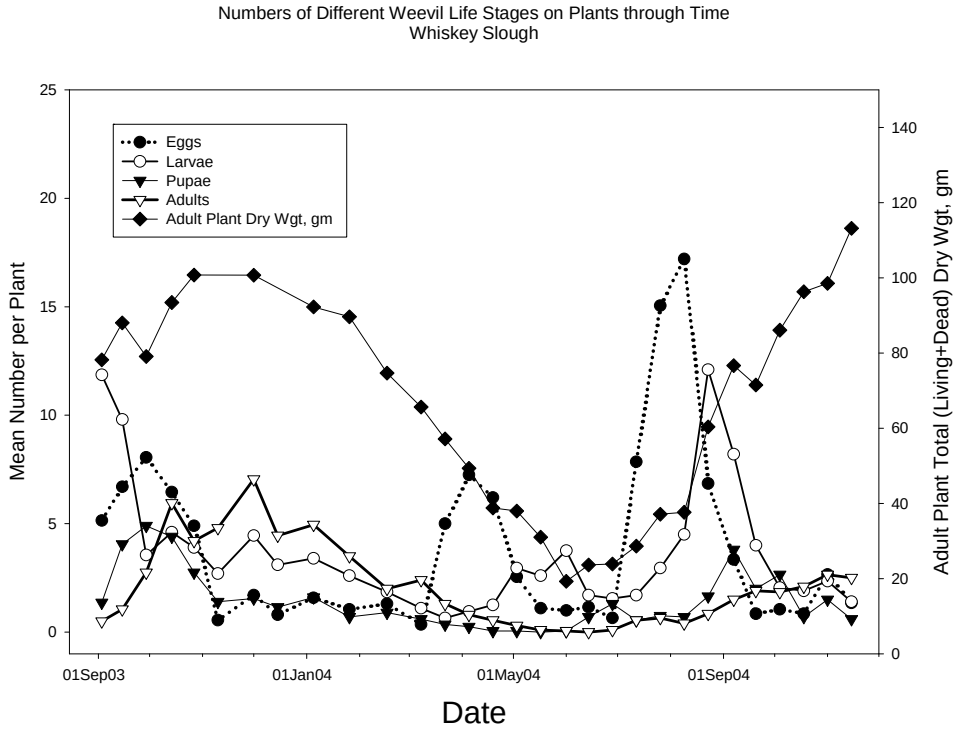


Figure 1. Densities of different life stages (numbers per plant) of the water hyacinth weevil, *Neochetina bruchi* and dry weights of adult plants at Whiskey Slough, San Joaquin County, California. Weevil results include counts from both adult and daughter plants.

The decrease in the weevil population in the winter and spring of 2004 seemed to parallel the decrease in the average dry weight of the plants, although the decline in the weevil populations appeared to lead the decline in plant weights by about a month (Figure 1).

The literature suggests that plant mortality should appear when larval densities reach about three to five per plant. Even though larval densities at Whiskey Slough have reached three times that level, significant mortality of plants is not apparent. Water primrose appears to be replacing hyacinth in the slough, which may be a sign of decreased competitiveness on the part of the hyacinth. Still, no areas of open water occur.

Rock Slough. The weevil densities at Rock Slough were substantially lower than those at Whiskey Slough (Figure 2.). Larval densities ranged between one and five larvae per plant, except for a decline to very low levels in April of 2004, similar to that at Whiskey Slough. After the spring of 2004, populations began to increase again. The peaks in the number of eggs per

plant again suggested that two generations occurred during the growing season. The changes in numbers of larvae, pupae, and adults were much more gradual. Larval densities peaked broadly in August through September 2004, at about four larvae per plant. Pupae may have shown a small peak in mid to late September, which would be consistent with the slowly rising adult populations after mid October. There was a small peak of adults in May and early June, possibly indicative of a small migration of adults into the area.

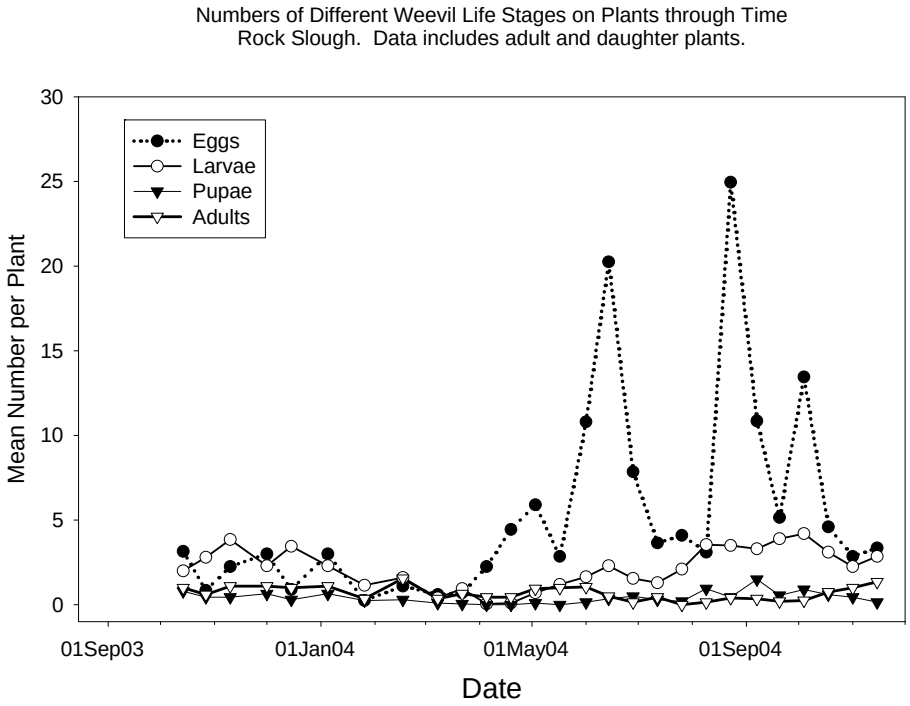


Figure 2. Larval and adult densities (numbers per plant) for the water hyacinth weevil, *Neochetina bruchi* at Rock Slough, Contra Costa County, California. Results include counts from both adult and daughter plants.

Interestingly, egg deposition tended to be higher at Rock Slough than at Whiskey despite a higher number of weevils present at Whiskey Slough. The plants at Rock Slough tend to have the bulbous morphology typical of open-grown hyacinth, while the plants at Whiskey Slough have the narrow, elongate leaf stalks typical of crowded populations. The literature shows that *Neochetina bruchi* prefers to lay its eggs in bulbous leaf stems.

Plants Escaping Attack: Hyacinth is able to grow well in spring despite the weevils because the plant population enters the winter with a significant portion that escaped attack (Figure 3). In addition, during winter attacked plants apparently die preferentially, and any new daughters are not attacked, so the portion of unattacked plants increases over the winter. Accordingly, when the next growing season arrives, the plants have a core of healthy, unattacked plants that are able to grow and reproduce rapidly, beyond the ability of a depleted weevil population to keep up.

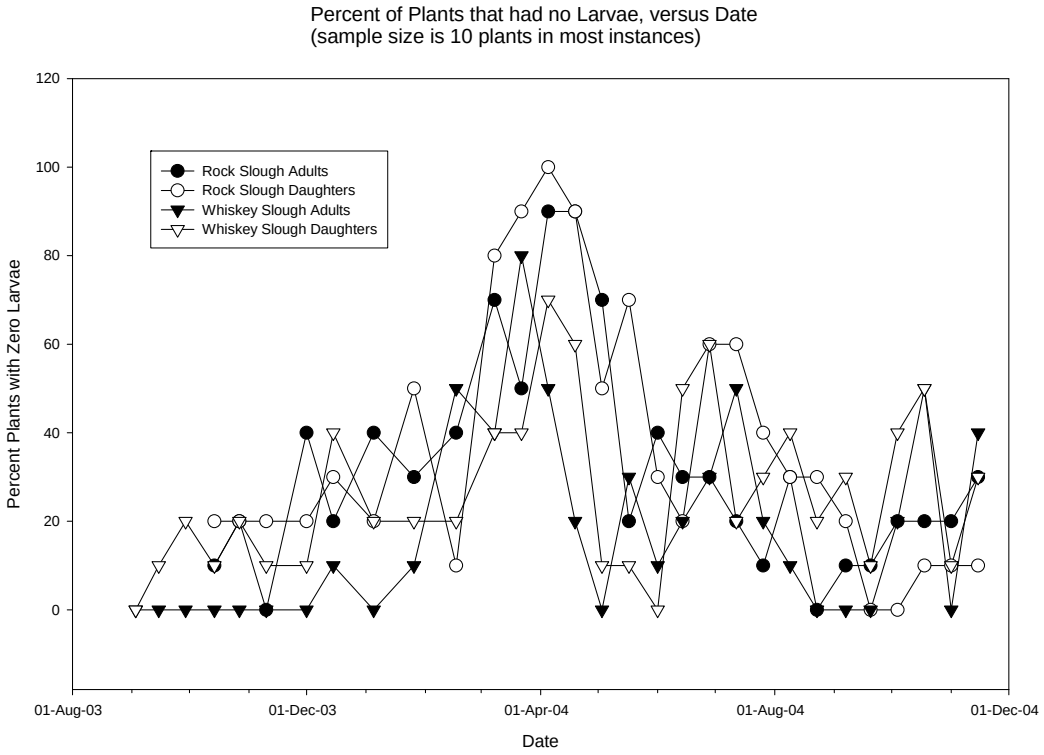


Figure 3: The percent of plants that had no larvae, by location and plant growth stage.

The observations of plant and weevil numbers in Whiskey and Rock Sloughs lead to the impression that the growing season in California is not quite long enough for the weevils to bring their population growth to bear on destroying the plants. *N. bruchi* has a relatively long interval between periods of reproduction, due to its life cycle of about 55 days, which is long compared to many crop insects. During bouts of reproduction, the populations can increase significantly, because each female lays about 150 eggs. In California, the populations reach damaging numbers in late October and November, just as the cold weather sets in and initiates the long, slow decline until next summer. Badly damaged plants sink, taking juvenile weevils with them and leaving undamaged plants to rapidly re-establish the infestation at the next growing season. This may not be the entire explanation for the lack of observed plant mortality, as larval numbers still reached over four or five larvae per plant for at least several weeks at both Rock and Whiskey Sloughs. Such numbers have been associated with plant mortality in other parts of the world, so it is suspicious that we are seeing as little mortality as we do in California. Some other factor may be at play, such as low humidity that limits the activity of saprophytes that could take advantage of the infection courts provided by the weevil damage.

Biological Control of Water Hyacinth II: Adult Longevity of *Neochetina bruchi* vs. Winter Food Quality

R. P. Akers, C. Black, and M. J. Pitcairn

Neochetina bruchi was released in the Sacramento-San Joaquin Delta in the 1980s for the control of hyacinth. Recent monitoring has showed that the weevil can build high populations there. Unfortunately, those populations develop just before winter, and then decline steadily until the beginning of the next summer, falling to very low densities. As a result, undamaged plants quickly recover the following season.

Mortality during the winter of eggs, larvae, and pupae is easy to rationalize, as these stages are essentially trapped on a single plant and die with it. Adult weevils can easily walk to another plant, however, and reasons for their losses are not so obvious. A winter visit to a hyacinth site provides a possible clue: the mass of dead and damaged leaves. Below the dead leaves, some green usually remains at the heart of the plant, but one wonders if the little remaining food is of good quality. We set out to test this possibility, extending an earlier experiment where we determined the longevity of weevils on leaves either from the field or from a greenhouse, from spring into the following winter. The present study ran through the winter to late spring. We also ran parallel observations of plant conditions and feeding in the field.

Methods: Feeding Test. Adult weevils were held on leaves with their stems submersed in water; in quart Mason jars in the greenhouse. Weevils were collected from Whiskey Slough and presumably emerged in summer and fall. The leaves used in the jars were either from plants grown in the greenhouse with abundant fertilizer, or were collected from plants in and near Whiskey Slough. Leaves were further classified into two types: either fully expanded leaves, or the youngest, still-expanding leaf on the plant, which is found wrapped around the petiole of the next youngest leaf. We called these “furled” leaves. This leaf is among the most protected on the plant and usually remained green even in winter. The leaves and water were refreshed two or three times a week. Any dead weevils were recorded and removed from the jars.

Field Observations: Every two weeks, a 20x50 cm quadrat was placed at six random locations within the hyacinth mat at Whiskey Slough. All plants with >50% of their crowns inside the quadrat were counted. Daughter plants were counted if the stolon was more than approximately 5 cm long and had more than 5 cm of roots. After counting, a total of forty plants were collected from and around the quadrats. In the laboratory, every plant was searched for weevils and the presence or absence of a furled leaf was noted. For 15 plants selected randomly from the 40, we measured the length of the longest leaf, the length of the longest leaf where at least 75% of the leaf was still green, the width and length of the leaf blade of the latter leaf, and the number of green petioles. We also rated every leaf on the percentage of green, with 1=>95+% green; 2=95-75%; 3=75-50%; 4=50-25%; 5=25-5%; 6=all dead (<5% green). Once a month beginning in February, we saved the mature leaf that had the most green (usually the same as the longest leaf that was at least 75% green) from 20-40 of the plants. We then used a flatbed scanner and a plant pathology program (called Assess), which is used to quantify leaf lesions, to quantify adult feeding scars on the leaves, as well as leaf area.

Results. The food from the field compared favorably with food from the greenhouse (Figure 1). Overall, the leaf sources that led to the longest life spans were from the field, in particular the field expanded leaves. When compared within either the greenhouse- or field-

collected leave types, furled leaves tended to result in a higher rate of mortality than expanded leaves. Overall, it is clear that the food available from the field was not materially worse than that from the greenhouse, even in late winter/early spring, at least in terms of its effect on longevity. These results are very similar to earlier experiments, reported in the 2004 annual summary.

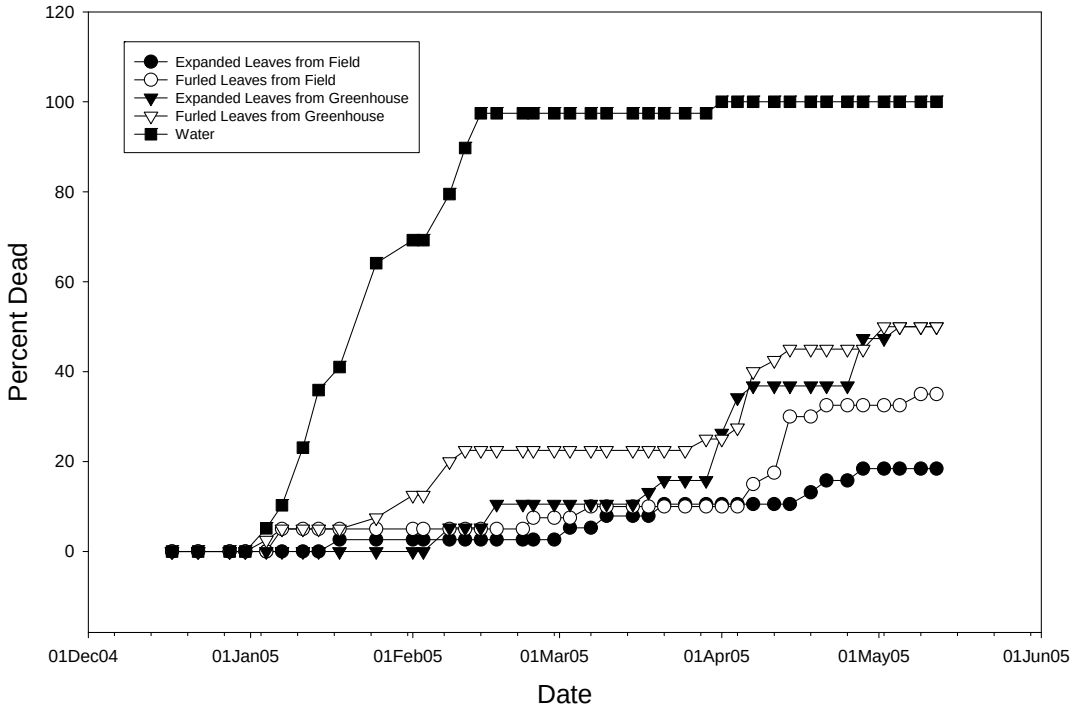


Figure 1. Mortality curves for adult weevils fed on water hyacinth leaves from differing sources. Water = no food. "Field" leaves collected from Whiskey Slough.

In addition to food quality in the field being adequate, the weevils in no way came close to eating all the food that was available in the field (Figure 2). Accordingly, the weevils apparently did not suffer for lack of food.

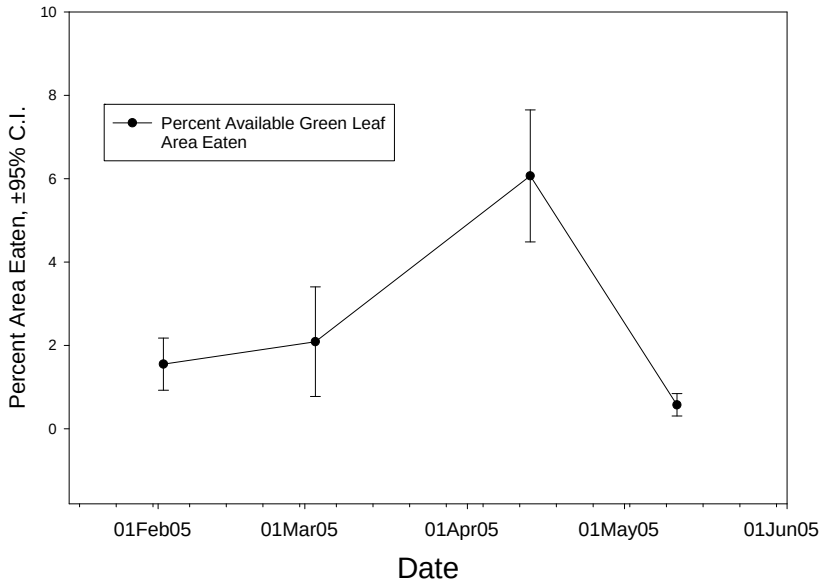


Figure 2: The amount of the available green leaf area eaten by adult weevils at various visits during the winter, Whiskey Slough, 2004-2005.

Biological Control of Water Hyacinth III: Above-Freezing Temperatures as a Limiting Factor in the Effectiveness of the Weevil *Neochetina bruchi*

R. P. Akers, C. Black, and M. J. Pitcairn

Neochetina bruchi was released in the Sacramento-San Joaquin Delta in the 1980s for the control of water hyacinth. Recent monitoring has shown that the weevil can build high populations in late summer/fall. Unfortunately, those populations decline steadily over winter, falling to very low densities by the summer. As a result, the weevils do not exert consistent enough pressure to bring the weed under control. The decline in numbers occurs for the adults as well as the juvenile stages. Food quality or quantity do not appear to be limiting during the winter (see elsewhere in this report.) Another possibility is that low temperatures are detrimental to the adults. The literature indicates that the developmental threshold for the weevil is about 10º C., and the weevils are thought to not have a true form of hibernation or diapause. They must simply endure the prolonged low level of metabolism that the Delta winters impose. Even though temperatures in the Delta generally do not reach freezing long enough to form ice on pond surfaces, average temperatures remain around 7º C. for at least two months. We set out to test the possibility that such relatively mild temperatures are still detrimental.

Methods: Adult weevils were held at constant temperatures of 7, 15, 24, or 32º C. in environmental chambers, in 13:11 light:dark cycles. Weevil adults were recovered from a mat of hyacinth in the greenhouse, which was searched two or three times a week. From informal tests re-searching freshly searched plants, we believe we found probably better than 90% of the weevils that were on the plants at any one time. Therefore, we knew the date of emergence of the adults to within two or three searches, or about a week. Weevils that emerged on a particular date were marked with a distinctive pattern of colored dots using Testor acrylic paints. Earlier tests indicated that the paints were not toxic. The weevils from one emergence date were spread as evenly as possible among the treatments. They were held in 1-quart Mason jars on leaves with the stems submersed in water. The leaves and water were usually changed three times a week, or twice a week in a few instances. Dead weevils were removed from the jars, sexed, and the date of their deaths recorded. Sex ratios were not adjusted at the start of the experiment. About 80 weevils were started for each treatment; about eight weevils were placed in each a jar.

Results: Temperatures that are above freezing, but still cold, are hard on the weevils (Figure 1). Their lifespan at 7º C is a maximum of about 60 days, and even 15º C reduces the lifespan. By the time of preparing the data for this report, eight weevils (about 10%) were still alive in the 24º C treatment and six were alive in 32º C, with lifespans already beyond 160 days. Thus, temperatures that are only slightly below the developmental threshold not only arrested the weevil's metabolism but reduced survival.

The average daily temperature in Stockton, at the eastern edge of the Delta, is 7º C for all of December and January. In our experiment, two months exposure to 7º C resulted in complete mortality, so the weevils actually survive better in the field than they did in the experiment. Several factors might be at work in the field. First, the weevils can move down into the plant and close to the water, which will provide cover and buffer temperatures. Second, the temperatures often increase above 7º C during the day in the winter, perhaps allowing the weevils some period of increased metabolism. Third, constant temperatures, as used in this experiment, often result in shorter lifespans for insects than do more naturally varying temperature regimes with similar

amounts of heat accumulation. Despite these mitigating possibilities, simple exposure to prolonged cold obviously decreases the weevils' longevity.

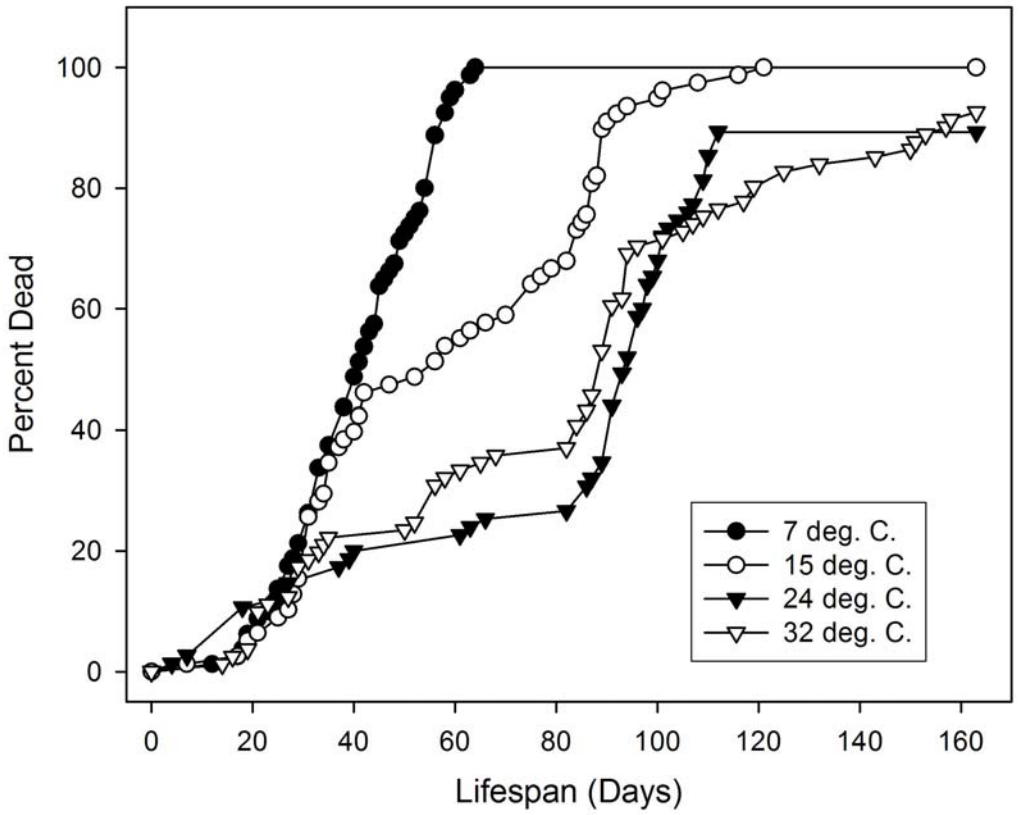


Figure 1. Longevity of adult weevils at various constant rearing temperatures

Comparisons of Impact Measures for Biological Control on Spotted and Squarrose Knapweeds

D. M. Woods

Defining success in weed biological control depends largely on the specific goal for the biological control effort but is also affected by the choice of measure. Squarrose and spotted knapweeds are principally eradication targets in California, so we employ biological control as a practical, cost effective method of reducing spread outward from large infestations until chemical and/or physical controlsare utilized. Consequentially, seed destruction measurements are appropriate techniques for these species as indicators of success in limiting spread of propagules and have been reported in previous reports. Regardless of our stated goals, most people want to visually see a change in plant population in order to accept a program as a success. This report discusses methods other than seed destruction measures as alternative impact measures of the biological control program on spotted and squarrose knapweeds.

Permanent transects were established in existing stands of spotted and squarrose knapweed where large numbers of biological control agents had previously been released. Repeated measurements were taken at each site to investigate trends in plant population as the biological control agents increased in number. Plot setup varied somewhat for the different sites but generally involved a series of one-quarter square meter or one square meter plots. Plant cover estimates were made in early summer when green plant diversity was at peak. An additional end-of-summer cover estimate was made at some locations for mature weed cover. Counts of plant (weed) number, seedhead number, stem density and mature plant height were also made at the end of summer. Repeated photopoint records were made as often as possible.

Cover estimates were made at three squarrose knapweed sites in Lassen (one site) and Shasta counties (two sites). Each site has two or three plots and multiple quadrants measured within each plot. Results are shown in Table 1. Spring cover estimates represent the plant canopy cover provided by seedlings, rosettes and the prebolting portions of squarrose knapweed within the quadrant. Fall cover is primarily bolted, mature, seedhead bearing plants of squarrose but additional estimates were made of ‘all-other-plants’. Over the three years of data collection, none of the sites shows any dramatic change in plant cover either in spring or fall. The three sites varied from each other with the Peterson site having the most complete cover of squarrose and Pittville the least. There was some variation year to year at each site, but no distinct trend was apparent.

Stem density also does not appear to be a simple, useful measure to evaluate squarrose knapweed changes over a three-year period. Total squarrose density measures taken in the fall include bolted plants as well as non-bolting seedlings and rosettes. At the two newest sites, Kane and Peterson, total plant numbers increased dramatically in 2005. This increase is however, primarily the result of moderate summer weather conditions allowing an unprecedented number of seedlings to survive the traditional summer drought. The Pittville site is an older site with a long history of data collection. Plant density visually declined dramatically three to four years after the first biocontrol releases in 1998, but prior to full scale monitoring. The lack of long-term data of more than three years limits an interpretation of plant population related measures so far at these sites. Our previously reported results show a rapid increase in biocontrol insect number over a three to five year period. Seed destruction has also dramatically increased. The

limited long-term data suggests that knapweed plant population are likely to decline four to five years after releases of biological control agents. In one location, (Peterson) spring cover declined over the three years and fall cover by other species increased. Hopefully, this trend represents a real phenomenon and will continue at this site and begin to appear in other sites. Three years does not appear to be sufficient to demonstrate impact utilizing these measures at these sites.

Table 1. Impact measures at three squarrose knapweed biological control sites in Lassen (Pittville) and Shasta (Peterson and Kane) counties.

Site	First release of Insects	2001	2002	2003	2004	2005
		Spring – percent cover by squarrose knapweed				
Pittville	1998			15	15	21
Peterson	2002			42	34	30
Kane	2003			33	32	35
		Fall – percent cover by squarrose knapweed				
Pittville	1998			No data	32	27
Peterson	2002			33	42	33
Kane	2003			No data	30	29
		Fall – percent cover by non-knapweed				
Pittville	1998			No data	36	42
Peterson	2002			13	20	39
Kane	2003			No data	16	19
		Squarrose density –plants per sq. m				
Pittville	1998	80	27	12	10	7
Peterson	2002			13	11	25
Kane	2003			28	36	87
		Squarrose knapweed stem density–stems per sq. m				
Pittville	1998			42	57	44
Peterson	2002			71	123	71
Kane	2003			136	153	96

Spotted knapweed has been more successfully controlled statewide through eradication efforts in California so the biological control effort is limited to one site in a remote portion of Shasta County. Insects were released in 1993-1995 and plant population measurements were initiated in 1995. Data was collected for all years but only alternate years are shown in Table 2.

Table 2. Cover and density measures taken over an eight-year period at the biological control of spotted knapweed research site in northern California.

	1997	1999	2001	2003	2005
Percent cover by spotted knapweed - spring		30.8	30	30	27.5
Spotted knapweed density (per sq. m.)	20	18	10	5	10
Spotted knapweed stem density (per sq. m.)	41	46	44	14	9

The longer series of data collection for spotted knapweed rather than squarrose has yielded some positive results. Spring estimates of spotted knapweed cover have not changed markedly over the last six years (the only period we collected them). In contrast, total spotted knapweed density and stem density have both declined over the eight-year period. These numbers support a general perception of the site that the plants are less vigorous and are declining in health. The seedhead attack rate by the biological control insects has been high at this site for several years averaging over 60% for eight years. Visual based evaluations as well as plant population studies are an inherently long-term process to demonstrate results, especially when biological control is the primary control method.

Purple Loosestrife: An Emerging Success for Biological Control in California

D. M. Woods and V. Popescu

There have been several reports of partial to near complete control of purple loosestrife by biological control organisms in many parts of the country. Until recently, the loosestrife infestations in California have eluded this goal. Four species of loosestrife biological control organisms were introduced into California between 1996 and 1998. Initial indicators of establishment were poor so a second thrust of introductions began and involved releases of larger numbers of insects at each site as well as introductions to wider areas. During 2004, the first evidence of significant impact was noted at some stands of purple loosestrife around Big Lake in Shasta County. On closer inspection we have observed dramatic damage at both the individual plant level as well as at the plant population level. The damage is largely caused by the leaf feeding beetles, *Galerucella calmariensis* (L.) and *G. pusilla* (Duftschmidt). Damage first shows up as chewing damage to the leaves progressing to a scorching and apparent death of entire plants. Large patches of plants seem completely dead, looking somewhat like herbicide applications. However, a progression of symptoms is detectable through the stand with early symptoms on the leading edges and dead or partially recovering plants on the interior of the stand.

We established permanent monitoring sites at three locations in the Big Lake area beginning in 1998 where we hoped to document long-term impacts on established purple loosestrife stands. The results we have obtained recently indicate the dramatic potential of the loosestrife biological control agents. The 'Lava Creek – terrestrial' site was selected as a low density site that does not remain flooded about three meters from a lake in Shasta County. Although there was a slight decline in the mean number of influoresences per meter and total plant height over the 1998-2002 period, this did not seem connected to biological control agents as their populations and damage remained very low over this period. By 2004, the insect population had dramatically increased eliminating all flowering and thus impacting plant height at this site.

The 'Lava Creek – Aquatic' site was established in 2001 less than 10 meters from the other site. However, this transect was established in a dense monotypic stand of loosestrife that remains in about a foot of water year-round. The leaf feeding beetles increased rapidly on these plants, again eliminating production of reproductive flower stalks by 2005. Additionally, severe feeding stunted all vertical growth. A sampling error resulted in the loss of influoresence data in 2003.

Biological control insects have not prospered at the Diversion Dam site, roughly 10 miles from the Lava Creek sites, yet on the same water system. Loosestrife has flourished at this site in the absence of control measures. A flood in 2003 prevented access to the site. The contrast between the Diversion Dam and Lava Creek sites provides evidence of the dramatic impact associated with the biological control agents. The biological control of purple loosestrife in the Fall River Valley of Shasta County appears to be an emerging success. Additional monitoring sites were established in Butte County during 2003 to extend the data gathering and evaluations.

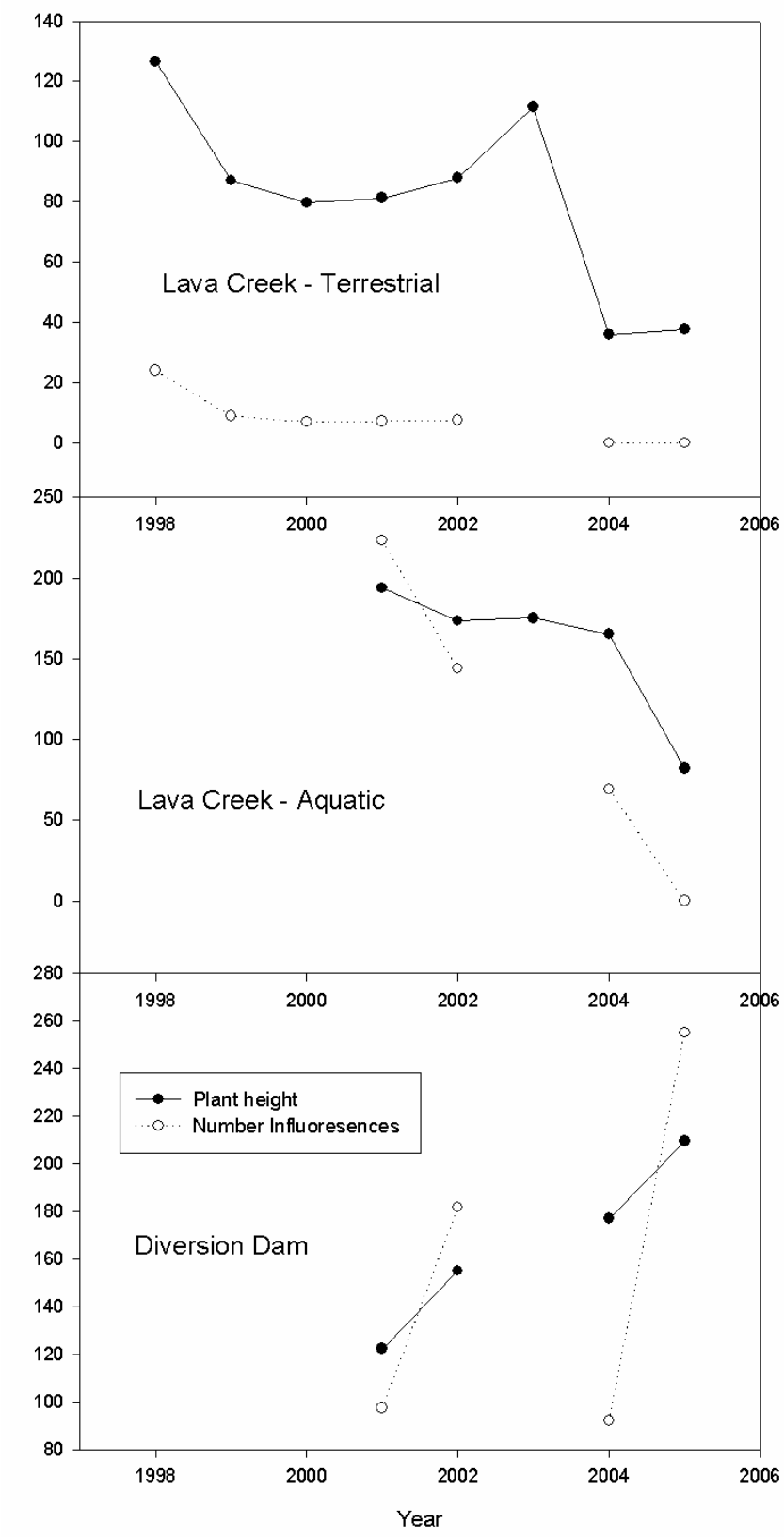


Figure 1. Plant height and number of influoresences at three purple loosestrife sites in northern California.

Redistributions Purple Loosestrife Biological Control Agents from Shasta County Nursery Sites to Other Purple Loosestrife Infestations in California

B. Villegas, D. Kratville[1], N. Smith[2], and C. Pirosko[3]

In 2004 three purple loosestrife (PLS) biological control agents were found well established in the McArthur area of eastern Shasta County, and in large enough numbers for redistribution releases to other parts of the state. With the announcement of successful establishment as well as indications of control over PLS, requests for redistribution releases came from County Agriculture Departments and weed management area groups in California suffering from PLS infestations (Figure 1).

In order to insure success and initial establishment insect biological control agents, six site selection criteria were established. If the infested area did not meet most or all of the criteria, no releases were recommended and other control measures were recommended. Candidate sites were evaluated for these criteria:

1) At least one acre in size with numerous plants of all ages within the site.
2) The water level should be stable and not constantly infuenced by changing water levels.
3) Some of the plants in the site should be out of the water, as some of the insects pupate at the base of the plants.
4) Site should be away from any pesticide use or drift
5) The site should not be heavily grazed by cattle
6) Site should not be disturbed by mowing, cultivation or other actions.

Sonoma County

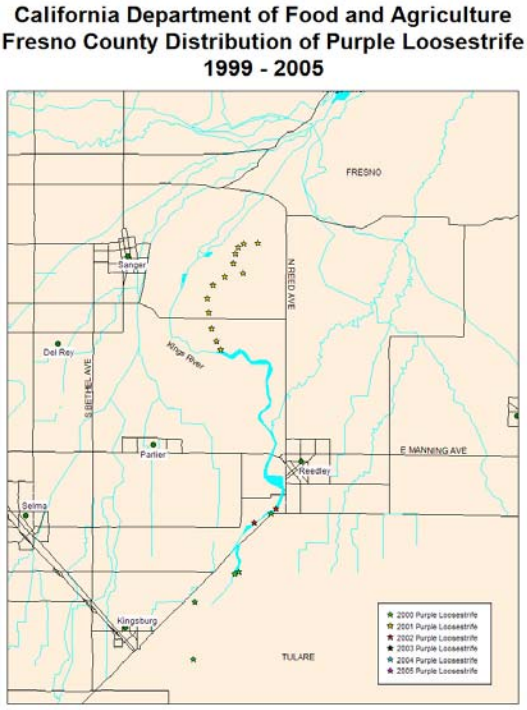
The Sonoma County PLS infestation is centered in the Sebastopol area near the Laguna. PLS plants were found along some of the small creeks that fed the Laguna; however, the distribution of PLS was found to be too spotty with not enough plants in any one area to support the biological control agents. A continued eradication/control program was recommended.

Humboldt County

The Humboldt County infestation is along areas of the Eel River near Weott. Site visits were made in September 2004 for potential 2005 releases. One site was identified that would make a suitable biological control site if PLS plants could be found away from the river currents. A large concentration of young plants about an acre in size were found along the banks of the river not too far from a paved road that follows the river in the area. However, on subsequent visits, it was noted that river current had been so strong during the 2005 winter-spring season that the young plants seen the previous season had been uprooted and washed away and no suitable release sites were found the rest of the season. Consequently, all plans for any biological control releases were discontinued and other control methods were recommended for the area.

Fresno County

The Fresno County PLS infestation is along small creeks that feed into the Kings River near Sanger, CA. Suitable sites were found along private lands bordering one such creek and releases were scheduled for May 2005. The main concern in the Fresno County release sites was possible problems with asynchrony between the biological control agents collected in Shasta County with the advanced plant development in Fresno County.



Approximately 3200 *Galerucella* leaf beetles (consisting of *G. pusilla* and *G. calmariensis*) were released in two locations in a large infestation of purple loosestrife in Sanger, CA. Approximately 100 adults of the flower weevil, *Nanophyes marmoratus,* were also released at the same time. Additional releases are being planned for the 2006 season at the same site as well as other suitable release sites in the area.

---

[1]CDFA, Integrated Pest Control, Sacramento, California
[2]Fresno County Department of Agriculture, Fresno, California
[3]Watershed Coordinator, Fall River Resource Conservation District, McArthur, California

California Department of Food and Agriculture
Statewide Distribution of Purple Loosestrife
1999 - 2005