Biological Control Program 2004 Annual Report



California Department of Food and Agriculture



BIOLOGICAL CONTROL PROGRAM

2004 SUMMARY

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

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Cover developed by Baldo Villegas and Dale Woods. The rust fungus *Puccinia jaceae* var. *solstitialis*, was widely distributed as a weed biocontrol in 2004. Clockwise from upper left; mature pustules comprised of teliospores and urediniospores on a yellow starthistle leaf, teliospore (2 celled) and urediniosopres, removing a dew tent from a field inoculation, vacuum collecting spores in the greenhouse, and severely infeted yellow starthistle leaf (center).

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Preface

M. J. Pitcairn

Progress in several insect biological control projects has been significant in 2004. The team of University, USDA, and CDFA scientists working on the biological control of the olive fruit fly has completed host specificity testing of *Psyttalia lounsburyi*, the first of several parasites being examined for use in California. A petition for its release was submitted to USDA-APHIS but a decision on the permit is still pending. Foreign exploration for additional parasites of the olive fruit fly continued in 2004 and the efforts were highly productive with shipments of six species to the quarantine facility at the University of California, Berkeley.

Release of the third of three parasite species against the pink hibiscus mealybug was completed in 2004. The first two species readily established and built up high densities that resulted in a 99% decline in mealybug abundance. With the extreme decline in mealybugs, it is not certain if current populations can sustain a third parasite species. Monitoring will continue to document the resulting parasite complex that will emerge from this system.

Production of the yellow starthistle rust in our greenhouses was very successful. A total of 30 grams of rust spores was harvested during the summer and fall of 2003 and provided an abundant amount of release material for 2004. A total of 25 releases were performed in 20 counties statewide. Some infection by the rust was observed at all release sites except Santa Barbara where plants died shortly after inoculation. A second release was made shortly thereafter which did take.

It appears we've finally turned a corner in the establishment of the two *Galerucella* beetles on purple loosestrife in California. These two leaf beetles have been very successful at reducing the infestation of purple loosestrife in other states but previous attempts at their establishment in California have been largely unsuccessful. In 2004, high densities of these beetles were found at one location in eastern Shasta County. Damage to the loosestrife population was limited in space but striking where it occurred. More importantly, the damage showed a progression in degree through the stand of plants suggesting that the beetles were moving away from the immediate area and onto healthy plants nearby. It is hoped that both *Galerucella* species are firmly established at this location.

These are but a few of the highlights presented in this report. I hope you enjoy this year's report.

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Foreign Exploration for Parasitoids of the Olive Fruit Fly, Bactrocera oleae

K. A. Hoelmer^{1,2}, A. A. Kirk¹ and C. H. Pickett

Olive fruit fly, *Bactrocera oleae* (Gmelin) (Diptera: Tephritidae) 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 several years ago this fruit fly pest is now firmly established in olive growing regions throughout California.

Olive fruit fly larvae feed in the flesh of olive fruits, introducing bacteria and fungi which cause fruit deterioration. During the first generations of the season, larvae pupate inside fruit, while later generations drop from fruit into the soil for pupation and overwintering. There are usually several generations per year. Processors have a very low tolerance for infested fruit used for table olives, and only a 5-10% tolerance for fruit destined for olive oil production. Before the establishment of the olive fruit fly, the olive industry in California was nearly pest free, and this introduction challenges the survival of the olive industry in California.

As part of a statewide management program being developed for olive fruit fly, we have been searching for effective parasitoids that attack this pest in its native range, which we believe to be parts of eastern and southern Africa to south-central Asia wherever its wild host, *Olea europaea* subspecies *cuspidata*, occurs. A rich diversity of olive fruit fly parasitoids is known to occur in several parts of Africa, while southeast Asia remains largely unexplored. Although a considerable amount of biological information has been accumulated regarding native parasitoids and predators of the Mediterranean region, these species are not capable of keeping fly populations at low levels. The braconid wasp, *Psyttalia concolor*, has been extensively evaluated in augmentative biocontrol projects in Europe; however, these have not been implemented on a wider scale due to economic costs. The potential of African species has remained largely untapped for biological control.

Since the previous annual report, additional explorations for olive fruit fly and its natural enemies were made in South Africa & Namibia in May 2004, Réunion Island (June 2004), the Canary Isles (October 2004) and Morocco (November 2004) in habitats where *O. europaea cuspidata* and other subspecies of *O. europaea* occur. Collections of fly-infested olives were made from wild and cultivated olives, and parasitoids were reared from them in the quarantine laboratory at EBCL in Montferrier, France. The collections in southern Africa included the braconids *Psyttalia concolor* (or near *concolor*), *P. lounsburyi, Utetes africanus,* and several species of *Bracon*. Collections of olive fruit flies infesting wild olives in Reunion yielded *Diachasmimorpha fullawayi,* a rather polyphagous species known to attack other species of fruit fly. Small populations of *P. concolor* were obtained from Grand Canary Island and southwestern Morocco. Several shipments of *Psyttalia ponerophaga* collected in the Northwest Frontier Province of Pakistan were also received from CABI Bioscience cooperators in Pakistan.

During 2004, shipments of the following olive fruit fly parasitoids were sent from the EBCL quarantine to the UC Berkeley quarantine for further evaluation: 1) *Psyttalia lounsburyi* (originally collected at Burguret Forest and Mt. Elgon, Kenya 6 August 2002

and 2003); 2) *Psyttalia concolor/*nr.*concolor (B. oleae* strain) from Namibia, May 2004; 3) *Psyttalia concolor* (or near *concolor*) (*C. capitata* strain), Kenya; 4) *Psyttalia c.f. ponerophaga* from Cherat, Pakistan, October 2004, and 5) Utetes africanus and *Bracon celer* from Cape Province, Rep. South Africa, and Namibia, April-May 2004.

During 2005, field collections are planned to obtain more material from Namibia in southwestern Africa, Kenya in eastern Africa, the northwestern provinces of Pakistan, and southwestern China. In addition, new surveys will be made in northern India and southern China.

Foreign exploration and collection of natural enemies have been made possible in part by funds provided by USDA-APHIS and by a University of California Specialty Crop grant for foreign exploration and biological studies of new olive fruit fly natural enemies.

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Host Specificity Studies on Parasitoids of Olive Fruit Fly

H. Nadel¹, K. Daane², J. Andrews², and C. Pickett

The olive fruit fly is being targeted for classical biological control in California, especially in untreated urban olives and abandoned orchards found over much of the olive growing regions. An important aspect of the screening process for candidate biocontrol agents is their potential non-target impact on native and beneficial exotic fruit flies. As a first step to assess risk to non-target species, we examined the behavioral response and reproductive capability of several imported fruit fly parasitoids offered non-target fruit fly hosts and target olive fruit fly in choice and no-choice tests.

Several braconid parasitoids were imported from laboratory colonies in Hawaii, where they were reared on Medfly. They attack several fruit fly species, including olive fruit fly. They include two strains of *Psyttalia concolor*, one originally from Italy (*P. concolor*-T) and the other from Kenya (*P. concolor*-K). The others were *Diachasmimorpha kraussii*, originally from Australia, and *D. longicaudata*, originally from Asia. All are internal parasitoids, attacking mainly the third instar larva, and emerge from the host pupa.

Other braconid parasitoids were collected during foreign exploration on wild olive fruit fly in Africa and Pakistan and imported directly to the UC Berkeley quarantine, or after initial colonization at the European Biological Control Laboratory in France. *Psyttalia lounsburyi, Psyttalia nr humilis, Utetes africanus,* and *Bracon celer* originated in southern Africa. Two more strains of *P. concolor*, from Namibia (*P. concolor*-N) and from Kenya (*P. concolor*-K2, darker in color than *P. concolor*-K), were colonized, as well as *P. ponerophaga* from Pakistan. All attack mainly the third instar, emerging from the pupa, except *B. celer*, which feeds externally on the host larva and pupates near the remains. *P. lounsburyi* has so far been collected only on olive fruit fly. *Utetes africanus* failed to perform during the tests, so no results will be reported here. Work on *P. concolor*-N and *P. ponerophaga* is just starting.

The beneficial exotic fruit fly hosts we tested are the European *Chaetorellia succinea*, which is employed for biological control of yellow starthistle, and the South African *Parafreutreta regalis*, which will be released for control of Cape ivy. *Chaetorellia succinea* is a seed-head feeder, while *P. regalis* develops in stem galls. Both are gregarious and multivoltine. The native fruit fly we used is *Rhagoletis fausta* (black cherry fly), which is a solitary, univoltine, frugivore inhabiting bitter cherry in California.

Methods: Wood and mesh cages (ca. 30 cm³) with one glass side were used. Six to 15 female parasitoids were caged with multiple non-target hosts (offered in bouquets of host plant stems) for 48 hours in a no-choice test, after which olives infested with olive fruit fly were added to provide a choice for the next 48 hours. The hosts were offered as 2nd and 3rd instars, the majority as 3rd. Honey and water were provided. The number of searching and probing parasitoids on fruit, flower heads, or galls was recorded in three 10-minute periods during each of the no-choice and choice segments of the tests. The host plant material was isolated and held at least 6 weeks for parasitoid and fly emergence, then dissected. The number of emerged flies and parasitoids was recorded.

Unemerged puparia were also dissected and their contents identified as parasitized, unparasitized, or contents unknown.

Results for the following parasitoids are reported: *P. concolor*-T, *P. concolor*-K, *P. lounsburyi, P. nr humilis, D. kraussii, D. longicaudata*, and *B. celer*. When offered a choice of non-target hosts and olive fruit fly, the parasitoids searched olives far more than non-target host material. The preference was significant (1-tailed paired t-test on the means) for all parasitoid species when the non-target host was *C. succinea* in yellow starthistle heads. When the non-target host was *P. regalis* in Cape ivy stem galls, the preference was significant for *P. concolor*-K, *P. nr humilis, D. kraussii*, and *B. celer*. The other species of parasitoids showed the same trend (mean searching females of *P. concolor*-T: olive 19.5, gall 3.5; *P. lounsburyi*: olive 1.8, gall 0.2, *D. longicaudata*: olive 15.0, gall 0.7) but more observations are needed to show significance. When the non-target host was *R. fausta*, all but *D. kraussii* searched significantly more on olives (mean searching on olives 11.5, cherries 0.0), but more observations are needed to confirm significance.

Most of the parasitoid species searched less in non-target hosts after infested olives were added than when no olives were present. However, a few searched non-target hosts more after addition of olives, while others did not search non-target hosts either before or after.

In all cases, the parasitoids probed more in olives than in non-target hosts. When *C. succinea* was the non-target host, the difference was significant for all parasitoid species except *P. lounsburyi* and *P. nr humilis*. When *P. regalis* was tested, probing was significantly greater in olive for *P. lounsburyi*, *P. nr humilis*, *D. kraussii*, and *B. celer*. Only *B. celer* of the parasitoids tested, showed significantly more probing in olive than cherry. The low response to cherry may have been due, in part, to lower numbers of cherry fruit fly larvae in the fruit compared to the infestation level in olives. Again, more observations would probably reveal significant differences in probing in olives rather than any of the non-target hosts.

Parasitoids were able to complete or nearly complete development on non-target hosts as follows: *D. kraussii* on *C. succinea*; all but *P. lounsburyi* on *P. regalis*; and *P. nr humilis, D. kraussii, D. longicaudata* on *R. fausta.* A preliminary test showed that *P. concolor*-N and *P. ponerophaga* also successfully reproduced on *P. regalis.* Data on host mortality due to parasitoid probes or oviposition activity are being analyzed.

Thus far, *P. lounsburyi* is the only species we tested that showed little or no interest in any of the non-target hosts, which indicates that it may be specific to olive fruit fly. However, its activity level was generally lower than all other parasitoids (except *U. africanus*, which showed no interest in any hosts during the study), so we cannot rule out that it would show some interest in non-target species under more favorable conditions. The generally weak response by all the tested parasitoids to the non-target species under severe confinement suggests that non-target risk for any of them is low. However, risk should be further assessed with tests under more natural conditions.

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Diaprepes Root Weevil: A New Threat to California Citrus and Nurseries

K. Godfrey and E. Grafton-Cardwell¹

Diaprepes root weevil (*Diaprepes abbreviatus*) is a serious threat to the citrus and ornamental nursery industries of California. This large, colorful weevil is native to the Caribbean region. It was accidentally introduced into central and south Florida in 1964 in a shipment of ornamental plants from Puerto Rico. Since then, it has spread throughout Florida where it sometimes causes serious damage to citrus trees by feeding on the roots. It also poses a threat to many ornamental plants and other crops such as papaya and sweet potato. In 2000, *Diaprepes* became established in a mature citrus grove in the Rio Grande Valley of Texas. It has been intercepted a number of times in California since 1974 in shipments of plants, in truck trailers, and in the cargo hold of aircraft. The weevils found in these interceptions were destroyed. However, the risk of introduction and establishment of *Diaprepes* into California is great because of the large volume of host plants brought into California. 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 weevil.

Educating the citrus, nursery, and regulatory personnel was done by producing hands-on educational materials and conducting training seminars. The hands-on educational materials included Riker mounts of the different morphs or color forms of the adult weevil, informational booklets, and a Powerpoint presentation. The Riker mounts, copies of both the booklets and Powerpoint presentations were given to each county agricultural commissioner's office, selected UC Cooperative Extension Office, and selected CDFA regulatory offices. The publication was published by the University of California Division of Agriculture and Natural Resources, and is available for free at http://anrcatalog.ucdavis.edu. The seminars were held in Orange, Tulare, and Santa Clara Counties.

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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. The parasitoid *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae) was collected in Australia and evaluated by Dr. D. Dahlsten (deceased) in 1999. The primary objective for 2004 was to characterize psyllid and parasitoid, population patterns in affected areas throughout the state.

Monitoring for post release parasitism was conducted at 61 and 59 locations statewide in 2003 and 2004 respectively. In most instances, these were locations where *P*. *bliteus* had been released from 2000-2002 by University of California and CDFA. The sample period ran from August through October. 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. Counts were made on 30 leaves and psyllid nymphs were inspected externally for signs of late stage parasitoid development (i.e., prepupal and pupal stages). In 2003, approximately 40 3rd-5th-instar nymphs (mummies not included) were randomly selected and placed in alcohol for dissection to assess parasitism in detail.



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



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

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 (Fig. 1). In 2004, parasitism was apparent at all survey sites. Estimates of the relative percent parasitism by site are illustrated (Figures 1 and 2). Values represent the proportion of all large lerps (i.e., 4th and 5th instar lerps) containing exit holes. On average, 22% and 34% of the lerps had parasitoid exit holes over all sites in 2003 and 2004 respectively. Most notably, Central Valley results indicate that the level of parasitoid activity had increased from that in past years.

Psyllid specimens that were placed in alcohol in 2003 were used to compare several approaches used to describe relative percent parasitism. After dissecting numerous specimens (26 sites, >20 specimens per site), correlation statistics were calculated comparing percent parasitism based on 1) dissection versus visual count (i.e., external examination), 2) visual count versus exit holes, and 3) dissection versus exit holes. Results are based on 4^{th} and 5^{th} instar data, despite it being known that parasitoids predominantly pupate and emerge from 5^{th} instar nymphs. The two instars were combined because large lerps were identified as representing those produced by 4^{th} and 5^{th} instar nymphs. This combination of 4^{th} and 5^{th} instar lerp sizes was found to be distinct from earlier sizes and easily counted by technicians.

Parasitism based on dissections of 4th and 5th instars was correlated to visual count estimates (r = 0.68) (Fig. 3a). Although it was expected that estimates based on dissections would be greater than for those based on a visual, external examination (because small parasitoid stages are detectable during dissection), this was not found to be the case. That is, the relative measure of parasitism based on dissection was estimated to be approximately three-fourths of that based on visual inspection. Because RGLP mummies were not included among specimens dissected, the dissection approach was restricted to detecting and recording parasitoid larval stages, thereby providing a more conservative measure of relative percent parasitism than if mummies were included. In comparison, the later approach of external examination was limited to detecting prepupal and pupal stages that are associated with RGLP mummies. In comparing visual counts (i.e.; external examination) to exit hole data, visual counts were determined to be weakly correlated (r = 0.51) with exit hole data (Fig. 3b). Lastly, dissection data were essentially uncorrelated (r = 0.11) with exit hole data (Fig. 3c). These findings were not unexpected, because lerps (including those with exit holes) produced weeks earlier are retained on leaf surfaces, thereby corresponding to a lengthy time period (i.e., weeks) of RGLP and parasitoid field activity, whereas visual counts and dissections pertain to a time frame much more immediate to the time of sampling. As a result, compared to dissection and visual count data, which are dependent on population activities close to the time samples are taken, exit hole data provide a generalized temporal view of parasitoid activity. The comparison of methods to estimate measures of relative percent parasitism suggests that generations of P. bliteus are somewhat discrete (i.e., only partially overlapping). On several occasions RGLP mummies containing parasitoid pupae were common in the visual examinations, whereas parasitoid larval stages were nearly undetectable during the dissection of psyllid nymphs from the same samples. This may account for the disparity between dissection and exit hole data at a number of sites leading to the overall low correlation between the two measures of parasitism (Figure 3c). A particular advantage of using lerp exit holes to characterize parasitism is that they are the most common sign of psyllid activity in the field. In contrast, it is difficult to collect enough psyllids for examination or dissection to derive a relative estimate of parasitism when psyllid densities

are low. As a result, we have used percent exit holes as the predominant means of presenting survey results.

In summary, *Psyllaephagus bliteus* has been released throughout the state and appears to be permanently established at most locations. Based on our relative assessment of parasitism, the parasitoid is very active (>10% of lerps with exit holes) at 70% of the sample sites in 2003 and 83% in 2004. Parasitoid activity was somewhat greater in the interior valley locations than observed in previous years. Photographs of representative trees affected by the RGLP have been taken at each site to compare with photographs in future years to document tree foliage status over time, in conjunction with population patterns.



Figures 3 a-c. Comparison of measures of relative percent parasitism. Estimates based on: adissection vs visual examination of nymphs; b- exit holes vs visual examination of nymphs; c- exit holes vs dissection of nymphs.

Distribution of Bemisia Parasitoids in Central California

C. H. Pickett, D. Keaveny¹, P. Kumar, and P. Akers

Several species of *Bemisia tabaci* parasitoids were released in the southern San Joaquin Valley from 1997 - 2000. They were released primarily into four study sites, one each in Fresno and Tulare counties, and two sites in Kern County. Typically, over 100,000 parasites were released weekly at each location with 4.05 million released in 1997, over 10 million in fall 1998, 3.2 million in 1999, and 124,000 in 2000. Much smaller numbers were released into several dozen sites over the same period of time. Since 2002, only one species of the five released in large numbers, remains, *Eretmocerus mundus* (Hymenoptera: Aphelinidae).

Additional monitoring of the parasitoid population was conducted in 2002, 2003 and 2004 to determine the presence and distribution of released parasitoids. The San Joaquin is a very large valley, over 300 miles long. Not until they have spread out across the Valley will the impact of released parasitoids be fully realized. Additionally, due to concerns regarding non-target impacts, we wanted to determine if species of whiteflies other than *B. tabaci* are attacked by the released parasitoids.

Two sampling protocols were conducted in late summer to early fall. The first used an existing sampling program managed by the Pink Bollworm Program, CDFA. Twice monthly from July through September, close to 5% of pink bollworm trap sites in the San Joaquin Valley were sampled. There were 89 sites in Kern County, 72 in Kings, 57 in Tulare, 125 in Fresno, 29 in Merced and 13 sites in Madera. Ten leaves, from ten separate plants, were selected from each sampled site. Leaves were taken from the fifth mainstem node below the terminal. Leaves from each site were placed in a separate container labeled with the county, site, number, and date of collection. Plants were within 10 meters of a pink bollworm trap. Leaves with high numbers of whiteflies (50 or more per leaf) were retained in one pint paper cans, then shipped to CDFA's Biological Control Program in Sacramento. Paper cans were held at room temperature for at least five weeks. The number of emerged parasitoids, native and exotic, and whiteflies were recorded. Parasitism was calculated by dividing the number of *Eretmocerus* (native and exotic) by the number of adult *B. tabaci* plus adult *Eretmocerus* (native and exotic).

The second sampling effort focused on identification of host whiteflies. Parasitoids were reared from isolated whitefly hosts to insure identification of host species. Two to three field samples were made from August to October. Leaves from weedy plants known to harbor *B. tabaci* were taken from locations near and distant from the four principal release sites, including the outlying area of Bakersfield where numerous much smaller releases were made. Leaves were shipped to the Sacramento Biological Control facility and processed for the presence of exotic parasitoids. Up to 40 late stage nymphs, from each sample location, were carefully removed from leaves. Nymphs were placed into plastic emergence trays (Pro-BindTM assay plate, 96, 0.3 ml wells, u-bottom, by Falcon[®]), one per well and incubated at room temperature. An absorbent paper cloth was placed between the top and bottom of the tray to prevent emerging insects from moving into adjacent wells. Each well was identified to collection site, date and host plant. Trays and a dish of salt slurry were placed in a plastic food container to maximize humidity. Recovered parasitoids and host exuviae were cleared then placed on a slide for identification to species or genus. Whitefly were identified using exuviae, according to Ray Gill (CDFA).

Collections of leaves came from Merced, Kings, Tulare, Fresno, and Kern counties. Most came from the latter three. Cotton leaf samples were retained by the Pink Bollworm project from 40 sample sites, placed into paper one pint containers and shipped to Sacramento. Additional samples from weedy plants for the parasitoid-host survey work came from nightshade (*Solanum* sp.), spurge (*Euphorbia* sp.), hollyhock (*Alcea rosea*), purple potato vine (*Solanum rautonnellii*), wild lettuce (*Lactuca* sp.), mulberry (*Morus* sp.), and sunflower (*Helianthus*). Two other species of whitefly were recovered from these plants, the banded wing whitefly, *Trialeurodes abutilonea* and the mulberry whitefly, *Tetraleurodes mori*. Exotic *Eretmocerus* were recovered only from *B. tabaci*. Native *Eretmocerus* spp. were associated with *T. abutilonea* infesting sunflower that had been collected from one site, and from mulberry whitefly infesting mulberry trees.

The only exotic parasitoid recovered with certainty was *Eretmocerus mundus*. Three other species were released from 1995 to 2000: *Eretmocerus emiratus*, *Er. hayati*, and *Encarsia sophia*. The proportion of *Eretmocerus* recovered from all samples (the Pink Bollworm survey and weeds samples combined) that were exotic has increased from the 2002 survey (Table 1). Increase has been greatest in the southern end of the San Joaquin where a higher number of parasitoids were released (Fig. 1, 2). This last summer, 92% of the *Eretmocerus* collected from weeds in the south region (Kern County) were exotic, up from 52% in 2002. The percent of exotics is higher in weeds than in cotton, regardless of region or year.

Year	Plant	Percent Exotic (males only)						
		North (n)	South (n)					
2002	Cotton	0.015(10)	0.32 (12)					
	Weeds		0.52 (2)					
2003	Cotton	0.047 (7)	0.50 (4)					
	Weeds		0.70 (7)					
2004	Cotton	0.07 (16)	0.58 (5)					
	Weeds	0.22 (4)	0.92 (9)					

Table 1. Parasitoids, species composition, by region. South = Kern County, north = other counties surveyed.

Patterns in parasitism, species composition, and whitefly densities on weeds and cotton suggest that introduced parasitoids may be beginning to have an impact on the regional densities of *B. tabaci*. For example, parasitism of *B. tabaci* on weeds has increased from 2002 to 2004, while whitefly densities have dropped (Table 2), and almost all parasitoids collected last year were exotic. On the other hand, parasitism in cotton dropped over this period of time, which is likely due to changes in pesticide usage patterns. Although the infestation level of *B. tabaci* does not appear to have changed over this period of time, leaf samples from this survey were not selected randomly. Only leaves with 'high' numbers of whiteflies on them were picked, in contrast to weed samples which were purely selected haphazardly. Furthermore, in Kern County, where exotics are most widely established, the number of infested cotton leaves in the 'high' category has dropped from 7% in 2002 to 1.7% in 2004 (from CDFA's final reports for the Pink Bollworm Surveys). One cotton field in Kern County (#213; W119.3043; N35.1203) had 82% parasitism with relatively low numbers of whiteflies (4.29 per gm).

Year	Plant	% Parasitism	Whiteflies per Gram Plant <u>+</u> 1SE	Number of Sites Sampled
2002	Cotton	18.0	33.74 <u>+</u> 7.8	29
	Weeds	8.0	21.16 <u>+</u> 12.6	6
2004	Cotton	8.0	36.6 <u>+</u> 6.81	40
	Weeds	30.0	12.7 <u>+</u> 5.6	21

Table 2. Parasitism by region (as above) and plant type.

Silverleaf Whitefly: Madera Eretmocerus Parasitoid Recoveries, 2002 Fresno Tulare San Benito Kings Monterey Release Sites major (4) Kern (6) minor Exotic vs Native Eretmocerus Males Plotted as Log(N+1) 2.5 = 316 1.25 0.25 Exotic Males Native Males Note: many points in clusters have been moved slightly from their original postions to permit better viewing of the data. 🖶 = No Eretmocerus Recovered = cotton acreage red by Patrick Akers, CDFA-Biological Control Program, April 2005 prepa

Fig. 1. Species composition and distribution of *Eretmocerus* in 2002.



Fig. 2. Species composition and distribution of *Eretmocerus* in 2004.

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Biological Control of the Pink Hibiscus Mealybug in Imperial Valley

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A cooperative biological control project against the pink hibiscus mealybug (PHM), Maconellicoccus hirsutus (Green), infestation in Imperial Valley, California was initiated in the fall of 1999. Subsequently, two encyrtid parasitoid species were mass reared and released. Population densities of mealybug 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 was reduced by approximately 95% (Figure 1). Over the first four years from 2000 to 2003, the average regional population density of the mealybug exhibited a continued decline. 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. Although Gyranusoidea indica Shafee, Alam & Agarwal was rarely found from spring through early fall, it was collected during the fall from branch terminal samples at levels representing as much as 20% of the parasitoid species composition. Hyperparasitism of A. kamali by resident species (Marietta sp. & Chartocerus sp.) was frequently over 35% during 2000. Hyperparasitism was considerably lower during each successive year, coincident with declining densities of both mealybug and the primary parasitoid host. Field collections of two non-target species of mealybugs common in Imperial Valley demonstrated that they are not being utilized as alternate non-target hosts by the newly introduced parasitoids.



Figure 1. Pink hibiscus mealybug and parasitism on mulberry trees in Imperial Valley, California. Mulberry terminal samples in January are available with buds only. Sample size equals the number of sites sampled by date. [*=% parasitism only was calculated, + = % parasitism was calculated for 1 or 0 sites.

In 2004, a third parasitoid species, *Allotropa* sp. nr. *mecrida*, was reared for a second year at the El Centro insectary and released locally. In addition, parasitoids were provided to Mexican authorities for release in neighboring Mexicali Valley, Mexico. As in past years, PHM densities

and parasitism were monitored at six mulberry sites and three carob trees sites. Lastly, corrugated cardboard tree band data collected from November to March of 2000/2001 were summarized to identify the primary life stages present during the winter, and to examine parasitoid abundance and species composition. This sampling method consisted of wrapping five, two inch wide corrugated cardboard bands around primary tree branches near the trunk of one tree per site in November and removing one band every three weeks for inspection and data collection. The last band was removed from each tree in March of 2001. Banding studies were conducted in the next two years; however, insufficient data were collected because PHM densities were too low.

A summary of the biological control agents released in Imperial Valley over the duration of the project is presented in Table 1. In 2004, over 100,000 *A*. sp. nr. *mecrida* were released locally and many were provided to Mexico. Average PHM densities were as low or lower than in 2003 when it was estimated that mealybug densities had declined >98% from fall 1999 densities. Results indicated that *A. kamali* continues to be the dominant biocontrol agent attacking the PHM. To date there is no evidence that *A. sp. nr. mecrida* has established in the area as no specimens were collected in 2004.

			No. Rele		
Species	Year	Origin (strains)	Imp. Val.	Mexico	Source
 Anagyrus kamali	1999	China & Hawaii	4,500		ST & PR
	2000	China & Hawaii	167,550		CA
	2001	China&Hawaii	22,100	45,400	CA
	2002	Egypt	97,850 ^a	38,250	CA
Gyranusoidea indica	1999	Pakistan & Egypt	1,900		ST & PR
	2000	Pakistan & Egypt & Aust.	231,900		CA, ST & PR
	2001	Pakistan & Egypt & Aust.	39,800	70,075	CA
	2002	Pakistan & Egypt & Aust.	13,800		CA
Allotropa mecrida	2003	Egypt	208,800	88,800	CA
*	2004	Egypt	107,000	26,000	CA

Table 1. Annual releases of parasitoids in Imperial Valley, CA and adjacent Mexicali, Mexico.

From Feb. 2002 onward, *A. kamali* culture was from Egypt. Source-insectary: ST= St. Thomas, Virgin Islands, PR= Puerto Rico, CA= California

Winter banding data demonstrated that essentially all PHM life stages are present during early to mid-winter, whereas most specimens found in March are second and third instar nymphs (Figure 2a). This phenology pattern was corroborated by summer and fall samples from branch terminals and the few specimens found in January and early April on branch terminal samples. Ovisacs collected in March contained mostly dead eggs. It was also demonstrated that PHM mummies represent a large portion of specimens collected, and successful emergence of parasitoids is 80% or higher (Figure 2b).



Figure 2. Presence of *M. hirsutus* life stages and parasitoid pupal stage and exit holes on cardboard bands on mulberry trees during the winter and spring of 2000-2001. A - Mean life stage count per site at three-week intervals. B - Cumulative proportion of select life stages including parasitoid exit holes by sample date with associated standard error.

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Vine Mealybug Distribution and Biological Control

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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. To assist in the trapping effort, we conducted six trapper training sessions in the spring of 2004. Trapping was done in 44 counties and 21 of those counties were found to be positive for vine mealybug. In each of the positive counties, vine mealybug was found at a small number of locations. Most of these infestations are currently under an eradication program that appears to be reducing vine mealybug density. Certified blocks of grapevine nursery stock were trapped intensively to control spread. In a small number of instances, the blocks were positive for vine mealybug. The nursery stock from these blocks was hot water dipped when dormant to remove any remaining vine mealybugs.

For some vineyards in the state, eradication of the vine mealybug may not be possible. Therefore, a cooperative project was established to assist in the rearing of parasitoids of the vine mealybug. A colony of vine mealybug has been established at the CDFA – Biological Control Program in Sacramento. A colony of the parasitoid, *Anagyrus pseudococci*, will also be established. Large numbers of this parasitoid will be reared for use in augmentative releases in 2005.

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Gill's Mealybug, A Potential New Pest of Grapes

K. Godfrey

Gill's mealybug (formerly referred to as a striped mealybug) belongs to a species complex in the genus *Ferrisia* that includes the striped mealybug (*Ferrisia virgata*). In 2003, Gill's mealybug was recognized as being distinct from *F. virgata* and *F. malvestra* (two closely related mealybugs in the striped mealybug complex). Gill's mealybug is thought to be native to the southeastern United States and was possibly first introduced into California in the early 1960s. The first definite record of Gill's mealybug is from a sample from Shasta County in 1968. Currently, this mealybug can be found in Shasta, Sacramento, Tulare, El Dorado, Stanislaus, and Tehama Counties. Many of these infestations are present in ornamental plants (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 1990s. In El Dorado County, Gill's mealybug was found infesting about 10 acres of wine grapes in 2004.

The biology of Gill's mealybug is not well understood, and the limited observations that have been made were in pistachios. A limited investigation of the biology of Gill's mealybug in grapes began in August 2004 in a vinevard in El Dorado County with visual searches of the vines and deploying double-sided tape traps. Gill's mealybug was found inhabiting the aerial parts of the vine (leaves, canes, and clusters). Approximately 80% of the clusters were covered with honeydew and sooty mold and of these clusters, about 65% were infested with mealybugs. The clusters that were covered with honeydew and sooty mold, but no mealybugs, were not marketable at harvest. The vineyard was treated with an insecticide prior to harvest. The mealybugs continued to be found on the leaves and canes into early October. As the leaves began to senesce, the mealybugs moved downward onto the cordon and trunk. Limited root sampling was conducted, but did not reveal any mealybugs on the roots. During this sampling, mealybug mummies were found under the bark. Adult parasitoids emerged and were identified as Pseudaphycus meracus, a parasitoid known to attack mealybugs in the Ferrisia virgata complex in the southeastern United States. In addition, specimens were obtained of a parasitoid known to attack Gill's mealybug in Alabama. This parasitoid was identified as *Pseudaphycus meritorious*. More intensive studies of the biology of Gill's mealybug are planned for 2005.

Parasitoids of the Solanum Mealybug, a Cooperative Project with Israel

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The solanum mealybug, *Phenacoccus solani*, is an apparent native of North America and can be found throughout California. It is currently not a pest species in California, but causes extensive economic damage to peppers in Israel. During a 2004 field survey of a solanum mealybug population in southeastern Sacramento County, we found that the mealybugs were attacked by the parasitoid *Aenasius phenacocci*. This was the first record of this parasitoid attacking the solanum mealybug. A cooperative project was then established to facilitate biological control of solanum mealybug in Israel with parasitoids from California. Under the project, the CDFA, Biological Control Program will provide the parasitoids either by field collection or by rearing the parasitoid, and Dr. Mendel would make the necessary arrangements for importation of the parasitoid into quarantine in Israel. A laboratory colony of the mealybug was established in 2004, and plans have been made to collect the parasitoid to rear large enough numbers to ship it to Israel in late spring or early summer 2005.

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Colonization of Lygus Nymphal Parasitoids in California

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Lygus hesperus is native to western United States and is a pest to numerous field and seed crops. In California, it is a key pest of cotton and strawberries, both highly valued crops. Extensive surveys for natural enemies in the western United States have found one egg and two nymphal parasitoids attacking Lygus species, primarily L. hesperus. However, in central California, surveys in alfalfa by ourselves and others have failed to find any nymphal parasitoids. Recent limited collections along the central coast of California, a strawberry growing region, suggest Lygus spp. are either, attacked at very low levels by nymphal parasitoids associated with Lygus rugulipennis infesting alfalfa in central Europe. Van Steenwyk and Stern attempted but failed to establish Peristenus stygicus during the mid 1970s in the southern region of the San Joaquin Valley in central California. Importation of nymphal parasitoids into eastern United States during the 1980s, however, successfully reduced Lygus lineolaris infesting alfalfa, a close relative of L. hesperus.

Interest among Canadians in the importation of these same parasitoids in the late 1990s stimulated our interest in re-examining importation of *Peristenus* spp. into California. Several populations of *Peristenus stygicus* and *Peristenus digoneutis* were cleared through quarantine (USDA ARS, Delaware) and reared in Sacramento and released initially at a nearby study site of alfalfa. Populations of parasitoids were collected from southern France, central Italy and Spain by CABI Bioscience and the European Biological Control Laboratory, USDA-ARS. Beginning in 1999, parasitoids have been released at several sites in central California, both inland and on the coast.

Parasitism has increased each year at our original release site of alfalfa in Sacramento. Three years following our last releases there, we continue to find abundant numbers of both *P. stygicus* and *P. digoneutis*. Maximum summer parasitism has increased each year since releases were made, reaching 90% in summer 2004 (Fig. 1). Parasitized nymphs of *L. hesperus* and *Closterotomus norvegicus* have been collected from nearby vacant lots infested with black mustard and wild radish. Identification of adults is pending. These results indicate that these parasitoids are permanently established in the Sacramento region. Over the same period of time, maximum *Lygus* counts have varied from 3 to 14 per sweep, and appear to be declining.

In contrast to results at the first release site in Sacramento, parasitism at our other central California release sites, including one at UC Davis has yet to increase, despite additional releases in 2002 and 2003. However at one of our new central coast sites we recovered parasitoids, as larvae, at a control site 300 m from where they were first released six weeks earlier. Only the introduced parasitoids *Peristenus stygicus* and *P. digoneutis* were recovered, i.e. no native braconids (identification by H. Goulet, Agriculture and Agri-Food Canada). Native parasitoids, *Peristenus* nr. *howardi*, have been recovered from *C. norvegicus* at the same locations (identifications by H. Goulet).



Fig. 1. Density of Lygus and proportion parasitized. Monthly averages, April – October. North B St., Sacramento.

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Large Scale Production of the Rust Fungus, *Puccinia jaceae* var. *solstitialis*, for Biological Control of Yellow Starthistle, *Centaurea solstitialis*, in California

D. M. Woods and V. Popescu

The rust fungus, *Puccinia jaceae* var. *solstitialis*, was approved for release as a biological control of yellow starthistle, *Centaurea solstitialis*, in California in 2003. Decades of research and work went into the preparation of host specificity documentation. The majority of the research supporting the documentation was performed at the USDA-ARS Foreign Weed and Disease Research Unit quarantine facility by Dr. William Bruckart. Upon approval for field release in California, Dr. Bruckart provided CDFA a stock culture of rust spores. We used them to establish a larger supply of spores to begin field release in California. Methods and procedures were established for large-scale production of the rust for this field release as well as greenhouse research. Greenhouse research used a very small amount of spores while field releases in multiple counties in 2004 required a major increase in the scale of production.

Yellow starthistle seeds were sown on wet blotter paper for three to five days then transplanted to four inch plastic pots in a commercial soil mix at two plants per pot. Potted plants were grown on benches in a greenhouse for four to six weeks. Plants were then inoculated with a spore suspension of 50 mg rust spores in 100 mls. water and three drops Tween 20 (polyoxyethylenesorbitan monolaurate). The Tween 20 was used as a wetting agent. Inoculated plants were placed in the dark in a dew chamber for approximately 16 hrs of dew, and then moved to a greenhouse. Yellow starthistle plants were watered, and fertilized as needed while growing under 14:10 L:D lighting. Pest control with commercial insecticides was essential for whiteflies, mites, mealybugs and aphids. Fourteen days after inoculation we begin harvesting rust spores using a spore collector provided by Dr. Bruckart. The collector is a specially designed vacuum head implement that attaches to a large shopvac. Infected leaves are slid into the vacuum head and spores are sucked up and deposited in glass collection vials. Harvested spores were weighed and then stored at -70 C. Harvests took place three times per week with each plant repeatedly vacuumed as spores matured. Weekly production results are shown in figure 1.

We staggered new plant production and inoculations to maintain plants that were young, green and supple to withstand repeated harvest. Repeated use of the plants did, however, eventually lead to reduced leaf integrity and also a gradual decline in rust production per plant. Leaves would tear, become chlorotic and/or dry out such that pustules no longer produced spores. Repeated inoculations of older leaves did not prove to be worthwhile relative to establishing new plants. Weekly production varied greatly due to several factors including inconsistent numbers of inoculated plants, insect pest invasions, and age related decline of yellow starthistle foliage. Nearly 30 grams of spores were produced in 2004.

The following statistics summarize the life histories of individual inoculated plants.

- •Plant inoculation was at 4 weeks of age
- •Average age of rusted plants discarded for non-production = 90 days
- •Latent period (interval between inoculations and first spore production) was 10-14 days
- •Yield approximately 45 good days to harvest from each plant
- •Three harvests per week = 18-19 harvests per plant



Susceptibility of California Yellow Starthistle Collections to Puccinia jaceae var. solstitialis

D.M. Woods and V. Popescu

A high degree of host specificity has been considered one of the most desirable attributes of using rust fungi as biological control agents. Most rusts are limited to successful infection on only one or two species. In fact, some rusts are able to infect only specific cultivars or varieties of a host crop. Consequently, when utilizing rusts as weed biological controls, overly high specificity can be problematic. The exotic rust *Puccinia jaceae* var. *solstitialis* has been approved for release in California and is currently being distributed around the state. Prerelease quarantine testing of the rust confirmed that it was virulent on a small collection of yellow starthistle tested. We tested a large collection of yellow starthistle accessions from around California for resistance to the exotic rust, *P. jaceae* var. *solstitialis*. The biological control program has amassed a collection of over 200 samples of yellow starthistle collected from around the state. A total of 62 of these accessions were selected for testing with the rust. Samples were selected to represent the range and diversity of yellow starthistle infestations around the state. Figure 1 shows the locations of all of our yellow starthistle collections as well as the distribution of accessions used for this report.

Six plants of each accession (2 pots with 3 plants per pot) were inoculated with the rust and maintained overnight in a dew chamber. Plants were then transferred to a greenhouse and monitored for evidence of infection. Plants were evaluated at 8, 10 13 and 17 days post inoculation. At 17 days post-inoculation, all accessions showed pustules on at least two of the six plants, thus indicating that there was no immunity. All six of the plants were infected in 77% of the accessions. In two accessions, only three plants were infected, and in one accession, only two of the six were infected. Retesting of these three accessions resulted in infections on 6 of 6, 6 of 6, and 5 of 6 of the plants. The latent period, or interval between inoculation and symptom development, did not vary significantly from accession to accession.

There does not appear to be substantial resistance in yellow starthistle to the rust. Success or failure of the rust to establish in California will therefore be impacted more by environmental constraints than biological resistance.



Field Releases of the Rust Puccinia jaceae var. solstitialis in California

D. M. Woods and B. Villegas

A large-scale release program was initiated in California during 2004 to distribute the rust, *Puccinia jaceae* var. *solstitialis*. *P jaceae* is a new biological control of yellow starthistle, and the first plant pathogen released in the mainland United States under the modern review and permit system. The first field release occurred in 2003 at a single location in Napa County. Greenhouse research and rust production was also initiated in 2003 at our Meadowview facility and has continued since that time. Following the initial release, we began preparing for a larger release program for the state. Yellow starthistle is widely distributed in the state with the majority of counties having substantial infestations. In conjunction with the California Agriculture Commisioners Association, we selected a broad spectrum of counties to receive the first round of releases. Counties were selected to represent both the range and diversity of yellow starthistle habitat in the state. Additionally, selected counties provided a rough grid across the California yellow starthistle landscape.

Releases consisted of both informational workshops as well as a release event. A series of regional workshops were presented to train local biologists about the rust. Regulatory information, as well as biological information including inoculation techniques, was the focus issues. At the workshops, county agricultural biologists were provided with a 200 mg sample of rust spores and a 'dew tent' made of PVC pipes covered with black plastic sheeting. Biologists could then return to their site and perform the inoculation. Each inoculation and the dew tent itself was one square meter in size. Biologists removed the dew tents the following morning, then were expected to monitor the sites regularly for the first appearance of disease symptoms as well as natural spread from the site.

Workshops and releases began in April in the southern half of the state and progressed northward. A total of 25 releases were made in 20 counties during 2004 (Table 1). Inoculations were highly successful, as infection was noted in almost all sites. Many of the southern and central California sites experienced substantial drought during 2004 severely shortening the growing season for yellow starthistle. In fact, the plants at one site in Santa Barbara County died shortly after inoculation and the inoculation had to be repeated at a second site. The percentage of plants infected was very high in many of the Sacramento Valley and foothills sites. Several sites had substantial foliage injury from insects and spider mites complicating the monitoring effort. Although pustules can be found two weeks post inoculation in the greenhouse, it usually was about three weeks post inoculation before pustules were apparent for these field sites. Spread from the inoculated square meter could be confirmed in only two sites. Isolated pustules were found 2 and 10 meters away from the inoculation in Sonoma and Shasta Counties respectively. It is possible that spread occurred in additional locations but was not detected. Evidence of additional spread as well as overwintering and establishment will not be available until the 2005 season.

							Plants with
_		Release	2 week	3 week	1st	%	positive
County	Site	date	date	date	pustules	infection	infection
Napa	Atlas Peak - pike	7/9/2003			8/18/2003	5%	6 of 120
Napa	Atlas Peak - bowl	12/23/2003			3/17/2004	5%	4 of 80
Placer	Newcastle	29-Mar	12-Apr	19-Apr	12-Apr	100%	120 of 120
Shasta	Redding	30-Mar	13-Apr	20-Apr	13-Apr	95%	120 of 127
Napa	Yountville	5-Apr	19-Apr	26-Apr	29-Apr	34%	25 of 77
Sonoma	Sugarloaf Ridge	5-Apr	19-Apr	26-Apr	5-May	86%	300 of 350
Sonoma	Lakeville	6-Apr	20-Apr	27-Apr	5-May	87%	335 of 385
Merced	Snelling	8-Apr	22-Apr	29-Apr	29-Apr	25%	45 of 180
Contra Costa	Mt Diablo	9-Apr	23-Apr	30-Apr	29-Apr	15%	
Monterey	Priest Valley	13-Apr	27-Apr	4-May	11-May	10%	20 of 200
Monterey	FHL:Jolon Creek	13-Apr	27-Apr	4-May	21-May	58%	99 of 170
Monterey	FHL:San Miguelito	13-Apr	27-Apr	4-May	21-May	19%	29 of 152
Monterey	FHL:Del Venturi	13-Apr	27-Apr	4-May	21-May	65%	459 of 706
SLO	Santa Margarita Lake	13-Apr	27-Apr	4-May	12-May	32%	198 of 620
Santa Barbara	USFS:Upper Oso	14-Apr	28-Apr	5-May		0%	
Yolo	Madison	15-Apr	29-Apr	6-May	3-May	20%	110 of 550
Santa Clara	Morgan Hill	15-Apr	29-Apr	6-May	13-May	93%	623 of 672
Nevada	Penn Valley	16-Apr	30-Apr	7-May	18-May	29%	16 of 55
El Dorado	Lotus	16-Apr	30-Apr	7-May	7-May	98%	501 of 510
Tulare	Kaweah Oaks	20-Apr	4-May	11-May			
Tuolumne	Sonora	22-Apr	6-May	13-May	3-Jun	10%	30 of 300
Tehama	Red Bluff	27-Apr	11-May	18-May	11-May	33%	200 of 610
Mendocino	Ukiah	27-Apr	11-May	18-May	18-Jun	77%	10 of 13
Napa	Atlas Peak - photo	28-Apr	12-May	19-May	17-Jun	30%	50 of 150
Glenn	Artois	29-Apr	13-May	20-May	14-May	10%	24 of 240
Plumas	Quincy	3-May	17-May	25-May	26-May	25%	47 of 188
Solano	Putah Creek	3-May	17-May	24-May	24-May	97%	30 of 31
Nevada	Truckee	21-May	5-May	12-May	1-Jul	30%	26 of 86
Santa Barbara	USFS:Upper Oso 2	27-May	11-May	18-May	7-Jun	6%	6 of 100

Table 1. Release locations and infection successes of the rust fungus on yellow starthistle in California during 2004.

Update on the Long-term Monitoring of the Combined Impact of Biological Control Insects on Yellow Starthistle

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

Five exotic insect species have been established in California for biological control of yellow starthistle. Three species, *Bangasternus orientalis* (Capiomont) (Coleoptera: Curculionidae), *Urophora sirunaseva* (Hering) (Diptera: Tephritidae), and *Eustenopus villosus* (Boheman) (Coleoptera: Curculionidae), are widespread. The two other species, *Chaetorellia australis* Hering (Diptera: Tephritidae) and *Larinus curtus* Hochhut (Coleoptera: Curculionidae) are abundant in the Pacific Northwest but occur in low numbers in California. A sixth species, the seedhead fly, *Chaetorellia succinea* (Costa) (Diptera: Tephritidae), was accidentally introduced into western North America in 1991 and is now widespread throughout California and the Pacific Northwest. All of these insects attack the flower heads of yellow starthistle and destroy developing seeds.

Evaluations of the impact of individual insect species on yellow starthistle seed production in California suggest that no single agent acting alone would effectively reduce yellow starthistle abundance. Rather, a combination of the current, and possibly, future natural enemies may be necessary to control this noxious weed. A study was initiated in 1993 to evaluate the population buildup, combined impact, and interaction of all available biological control insects on yellow starthistle. Field sites were established in Yolo, Placer, and Sonoma Counties to represent three different climatic regions where yellow starthistle occurs in abundance. Four insects (B. orientalis, U. sirunaseva, E. villosus, and L. curtus) were released at each site in 1993 and 1994 and long-term monitoring of the weed and insect populations was initiated. A fifth insect, C. succinea, invaded these sites on its own between 1996-1998. The Yolo County site is open Sacramento Valley rangeland located west of Woodland; the Placer County site is at 1300 ft elevation in the Sierra Nevada foothills east of Auburn; the Sonoma County site is at 1200 ft elevation in the Coast Range foothills southeast of Santa Rosa. Various aspects of the plant-insect interaction are being monitored annually, including canopy cover estimates of yellow starthistle and competing flora, yellow starthistle seedling recruitment, adult plant density, seedhead numbers, seed production, and insect infestation rates. Preliminary results from 1995-2004 are presented in Table 1. Monitoring at the Placer County site was discontinued after 2001 because the property was sold and the new owner did not want to be a part of the study.

Ten years after the initial releases, we have evidence that attack by these biological control agents has reduced seed production by yellow starthistle at all three sites. The weevil, *E. villosus*, has become the most abundant insect at all three sites. In addition to seed destruction by larvae, adult *E. villosus* feed on and kill young developing buds. The loss of early buds produces a change in plant architecture with the damaged plant dominated by stem material. Instead of flowers born on the tips of longs stems, new flowers are produced on short stems (<1 cm) arising from the leaf axils along the main stems. The attack rates of *E. villosus* showed a similar pattern at all three sites: an initial steady increase then a leveling off after four to five years. Attack rates by *E. villosus* over the last three to four years ranged from 45-65% at Placer County, 51-74% at Yolo County, and 55-82% at the Solano County site.

The occurrence of *B. orientalis* was initially high in 1995-97 but has declined to less than 1% at all three sites. In like manner, the gall fly, *U. sirunaseva*, increased initially then declined to attack rates less than 1%. Interestingly, it has rebounded in 2003 and 2004 to 6-8% in Yolo County and 9-11% in Sonoma County. The false peacock fly, *C. succinea*, was first recovered in 1996 at the Yolo County site and in 1998 at the Placer and Sonoma County sites. While population densities initially increased, attack rates declined in 2001 but have rebounded since then. It is not clear if *C. succinea* populations have leveled off or if they will continue to increase. Attack rates over the last three years ranged from 11-28% at the Yolo County site and from 8-25% at the Sonoma County site. Interestingly, it appears that 2001 was a poor year for both *U. sirunaseva* and *C. succinea* as populations of both species declined severely at all three sites. Since then, both species increased in abundance at the Yolo and Sonoma County sites. The incidence of *L. curtus* has been low (<1%) at all three sites and may now be absent from the Yolo County site.

Yellow starthistle seed production and plant abundance have declined steadily at the Sonoma County site. The rapid increase of *E. villosus* appears to have resulted in a steady decline in the number of flower heads and the number of seeds per head. The percentage of mature heads infested by at least one biological control insect increased from 23% in 1995 to 89% in 1998 and has remained high since then (range 74-88%). In addition, there has been a concurrent decrease in seed production (14,167 to 270 seed per sq. m) and seedling density (897 to 84 seedlings per sq. m). While there was an increase in total seed production (seeds/m²) in 1999 and 2000, it has been substantially reduced the last four years. The decrease in seed production in adult plants. Attack by *C. succinea* has increased slowly to 25% in 2004. Attack by this fly combined with the attack by *E. villosus* has resulted in a high attack rate to the annual crop of seed heads each year and it is likely the combined attack of these insects that has produced the decline in seed production at this site.

As observed in Sonoma County, yellow starthistle densities at the Yolo County site also show a steady decline in seed production and a concomitant decline in seedling and adult plant abundance. There was no yellow starthistle seed produced within the study plot in 2004. Estimates of attack rates and seeds per head were obtained from plants growing outside and away from the study plot. Some recruitment from the seed bank was observed in Fall 2004.

It is unfortunate that access to the Placer County site was lost as this site had shown little change in seed production and plant abundance. It was hoped that *C. succinea* would have eventually increased in abundance to the level necessary to substantially decrease seed production. Attack by *C. succinea* at this site had declined in 2001 but, because the fly rebounded at the other two sites, we would have expected the same at this site.

These observations provide evidence that these natural enemies have reduced yellow starthistle seed production in at least two of three sites. While *E. villosus* is clearly the most important insect, the complementary attack by *C. succinea* appears to be a critical addition to the overall attack rate on yellow starthistle seed production.

Table 1 Status of yellow starthistle and its natural enemies at three multi-agent research sites.

Placer County												
<u>Plant</u>		95	96	97	98	99	00	01	02	03	04	05
Seedlings/squar	re meter	-	651	669	883	666	842	762	-	-	-	-
Adult plants/squ	uare m	332	83	108	151	54	109	138	-	-	-	-
Heads/ square n	neter	679	280	438	378	256	355	388	-	-	-	-
Seed/head		8.4	18.4	15.1	7.8	17.0	11.2	14.1	-	-	-	-
Seeds/square m	eter	5,704	5,152	6,614	2,948	4,372	3,976	5471	-	-	-	-
Insect & releas	se year											
B. orientalis	93	7%	1%	2%	2%	1%	1%	1%	-	-	-	-
U. sirunaseva	93	5%	4%	10%	13%	3%	8%	0%	-	-	-	-
E. villosus	93	54%	56%	57%	65%	45%	46%	60%	-	-	-	-
L. curtus	94	0%	0%	1%	1%	1%	1%	0%	-	-	-	-
C. succinea	-	0%	0%	0%	0%	6%	18%	7%	-	-	-	-
Heads w/1 or n	nore sp	62%	60%	67%	74%	52%	63%	66%	-	-	-	-
Yolo County												
<u>Plant</u>		95	96	97	98	99	00	01	02	03	04	05
Seedlings/squar	re meter	-	1095	1928	1076	642	992	840	187	488	2	1
Adult plants/squ	uare m	975	323	205	437	94	333	68	49	103	0	-
Heads/ square r	neter	1193	575	346	838	252	443	65	213	12	0	-
Seed/head		24.4	28.0	14.5	16.4	17.9	9.8	11.2	15.1	13.8	21.4*	-
Seeds/square m	eter	29,109	16,100	5,017	13,743	4,511	4,341	728	3,216	166	0	-
Insect & releas	se year											
B. orientalis	91	2%	1%	4%	1%	1%	1%	0%	1%	0%	0%	-
U. sirunaseva	93	13%	19%	12%	18%	7%	13%	1%	2%	8%	6%	-
E. villosus	93	5%	20%	26%	53%	20%	46%	51%	55%	74%	51%	-
L. curtus	94	0%	1%	1%	0%	0%	0%	0%	0%	0%	0%	-
C. succinea	96	0%	2%	8%	11%	28%	21%	2%	28%	11%	13%	-
Heads w/1 or m	nore sp.	19%	37%	42%	66%	49%	62%	52%	76%	82%	63%	-

Sonoma Count	у											
<u>Plant</u>	-	95	96	97	98	99	00	01	02	03	04	05
Seedlings/square	e meter	-	897	823	624	235	1020	310	234	450	56	84
Adult plants/squ	are m	241	233	223	231	65	435	31	145	130	17	-
Heads/ square m	neter	547	442	508	486	414	625	116	321	171	29	-
Seed/head		25.9	15.6	8.6	7.7	13.8	8.1	12.2	9.1	5.3	9.3	-
Seeds/square me	eter	14,167	6,895	4,369	3,742	5,713	5,062	1,415	2,921	906	270	-
Insect & release	e year											
B. orientalis	94	6%	10%	5%	1%	1%	1%	1%	1%	1%	0%	-
U. sirunaseva	94	5%	17%	21%	23%	21%	21%	1%	19%	9%	11%	-
E. villosus	94	13%	37%	79%	80%	59%	70%	66%	63%	82%	55%	-
L. curtus	94	0%	1%	1%	1%	1%	1%	0%	1%	1%	1%	-
C. succinea	-	0%	0%	0%	2%	9%	8%	13%	24%	8%	25%	-
Heads w/1 or me	ore sp.	23%	58%	86%	89%	75%	81%	74%	85%	88%	80%	-

*Estimated from plants growing outside the study plot

Size Variation in the Weevil, *Larinus minutus* Emerging from Spotted, Diffuse and Squarrose Knapweeds

D. M. Woods

The lesser knapweed flower weevil, *Larinus minutus* (Coleoptera: Curculionidae) feeds preferentially on diffuse knapweed in Europe. However, field observations in Europe and host testing prior to field release in the United States confirmed that the weevil also attacks other knapweeds, particularly spotted and squarrose knapweed. In California, the weevil has successfully established on all three knapweeds. While the adult weevil has been reported to feed extensively on knapweed foliage in other states, in California impact is primarily expressed as seed destruction by the larvae. Our preliminary evaluations of seed destruction by *L. minutus* suggest that the weevil has varying degrees of success in the three knapweed species; spotted, diffuse and squarrose. Since these knapweeds produce different size seedheads and also seed numbers, the relative size of *L. minutus* to these seedheads may explain the variation in success. We have been investigating size of adult weevils emerging from the three hosts as a part of this analysis.

Adult *L. minutus* weevils were originally collected from diffuse knapweed in Montana and Washington in 1995 and were released at single sites of spotted and diffuse knapweed. Additional weevils were collected from diffuse knapweed in Oregon and released on squarrose knapweed starting in 1998. All three hosts currently support strong populations of the weevils. As part of a larger study, we yearly collect mature knapweed plants from the field and transport them to the laboratory for analysis. Adult weevils emerging from those plants are identified and stored dry in glassine envelopes in the laboratory. Weevils were individually measured in length and width at the longest point with electronic calipers. Weevils were then individually weighed. Weevils were collected during 1999-2002 from spotted and squarrose knapweeds and during 1998-2002 from diffuse knapweed.

Weevils were considerably shorter, narrower and particularly lighter when they emerged from squarrose than diffuse, and diffuse than spotted (Figure 1). Since the original source for all these weevils was diffuse knapweed, the current weevil size is likely reflective of the weevils adjusting to the innate size and thus food quality/quantity of the individual host plant. Spotted knapweed is considerably larger and squarrose is considerably smaller than diffuse. In spite of the reduced weevil size in the smaller headed knapweeds, our preliminary results (manuscript in preparation) show that the weevils are dramatically more effective in seed destruction on the smaller headed knapweeds.



Figure 1. Effect of the host plant, (spotted, diffuse or squarrose knapweed), on the length, width, and weight of emerging *Larinus minutus* weevils.

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

R. P. Akers, M. J. Pitcairn, L. Ragaini, and R. Weaver

Water hyacinth (*Eichhornia crassipes*) is a native of the Amazon River basin, but it has spread through much of world to become one of the world's worst aquatic weeds. The Sacramento-San Joaquin River Delta can support heavy infestations, leading to a costly control effort. In the early 1980s, two species of *Neochetina* weevils were released in the Delta for biological control of the weed. They provided little obvious control, such that weed managers thought the weevils had gone extinct, until a focused survey in 2002 demonstrated that *N. bruchi* had survived and was indeed fairly common in the Delta. *N. eichhorniae* apparently did go extinct. In many parts of the world, the weevils have provided significant to excellent 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 bottleneck to the weevil's sustained production in the Delta. We established a study of the weevil's population dynamics to address this question.

Methods. Two locations in the Delta were selected for our studies: Whiskey Slough (San Joaquin County) and Rock Slough (Contra Costa County). The Rock Slough infestation is in a poorly sheltered, shallow bay along an open, flowing channel, and it receives more disturbances from wind and currents than at Whisky Slough. As a result, the infestation is small (about 20 by 100 meters) and variable. Many of the plants tend to be of the short (< 20 cm) stature and have the bulbous petioles that are typical of new, uncrowded infestations. The infestation at Whiskey Slough is a large (approx. 100 by 400 m) patch of hyacinth that had not been sprayed for several years. It 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.

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

Results and Discussion – **Whiskey Slough.** Larval and adult weevil densities were very high at Whiskey Slough in the fall of 2003. They fell off gradually through the winter to very low levels by late spring and early summer, 2004. Although they increased several-fold from then until the fall of 2004, they never reached the levels of fall 2003 (Figure 1).

The details of the dynamics differ slightly for different life stages. Larval numbers fell after a peak in July and August 2003, yet remained very high into early September, exceeding 16 larvae per plant. Larvae steadily declined over the next six weeks but stabilized at approximately five larvae per plant through December. As expected, changes in adult numbers occurred later

than those for larvae, increasing from August into November and remaining high into January, varying between 7-12 adults per plant.

The curve for egg numbers in the fall of 2003 is perplexing. It began to rise as the larval numbers were still high, almost before the rise in the number of adults. It seems as though the newly emerged adults laid very heavily then shut down nearly all egg laying in late October, even though adult numbers continued to increase into December. Egg deposition continued despite the cold temperatures ($<50^{\circ}$ F) experienced in the winter of 2003, albeit at low levels considering the density of adults.

From January through April 2004, numbers of all stages fell steadily to very low levels, generally less than one per plant. They remained very low and only began to increase 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 in late July through early September. The other life-stages do not show as clear-cut a pattern, but generally follow the same trends. The perplexing behavior of egg-laying did not repeat in 2004, although once again egg numbers increased rapidly in late July and early August before there was any real sign of an increase in the numbers of adults. This may be partially explained by literature that shows that, although the weevil adults can be very long-lived (up to nine months), they tend to concentrate their egg production in the first several weeks of their adulthood. Also, the second bout of egg-laying in 2004 peaked in the last week of July, while the peak observed in 2003 was about the first week in October. 2003 appeared to be warmer than 2004 and the October 2003 peak in eggs may have actually been a third peak for that year. There was a possible, almost negligible, third peak in egg production in 2004 (about four eggs per plant in 2004 versus 13 in 2003) about the first week of November, about a month later than in 2003.

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

The weevil population at Whiskey Slough was quite high, as high as reports of populations in other parts of the world where they have devastated the hyacinth. The literature suggests that plant mortality should begin to occur when larval densities reach above five per plant. Even though densities at Whiskey Slough reached three times that level, mass mortality of plants is not apparent. In one confined area of the Slough, water primrose replaced perhaps 40% of the water hyacinth in 2004, which may be a sign of decreased competitiveness on the part of the hyacinth. Still, no areas of open water occurred. The lack of mortality will be explored further in 2005.



Figure 1. Larval and adult densities (numbers per plant) for the water hyacinth weevil, *Neochetina bruchi* at Whiskey Slough, San Joaquin County, California. In the legend, Plt Wt = average dry weight of plants

Rock Slough. The weevil densities at Rock Slough were substantially lower than those at Whiskey Slough. Larval densities ranged between one and five larvae per plant, except for a decline in April 2004, similar to that at Whiskey Slough. Populations began to increase again after April. The peaks in the number of eggs per plant again suggested that two or three generations occurred during the season. Compared to eggs, the changes in numbers of larvae, pupae, and adults were much more gradual, without sharp peaks. Larval densities increased gradually and peaked broadly in August through September, 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 indicating a small migration of adults into the area.

Egg deposition tended to be higher at Rock Slough than at Whiskey Slough despite the higher number of adults at Whiskey Slough. Rock Slough receives more disturbance than Whiskey Slough and the plants tend to have the short, bulbous morphology typical of opengrown hyacinth. Plants at Whiskey Slough have the narrow, elongate leaf stalks typical of crowded populations. The literature shows that *N. bruchi* prefers to lay its eggs in bulbous leaf stems.



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

The observations of plant and weevil numbers in Whiskey and Rock Sloughs give the impression that the growing season in California is not quite long or warm enough for the weevils to bring their population growth to bear on damaging the plants. *N. bruchi* has a relatively long interval between bouts of reproduction, due to its generation time of about 55 days, which is long compared to many crop insects. During bouts of reproduction, the population can increase dramatically because each female lays about150 eggs. However, in California, the populations reach high densities 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 less damaged plants to rapidly re-establish the infestation the next spring. 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 see 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: Adult Longevity of *Neochetina bruchi* vs. Winter Food Quality

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

Neochetina bruchi was released in the Sacramento-San Joaquin Delta in the 1980s for the biological control of water hyacinth. Recent monitoring has showed that the weevil can build high populations in the Delta. Unfortunately, those high populations develop just before winter. Populations then decline steadily until near the beginning of the next summer, with adult numbers falling to well below one weevil per plant. As a result, the weevils cannot exert a level of damage concerted enough to bring the weed under control.

The losses of eggs, larvae, and pupae during the winter are easy to rationalize, as these stages are immobile and essentially trapped. If the weevils badly damage a plant during the summer, it will likely die or sink during the winter. Any juvenile stages of the weevils on the plant will die with it, leaving relatively undamaged plants to quickly recover when temperatures increase the following season. Adult weevils are not so constrained, however, and walk quite readily. The reasons for their losses are not so obvious.

A winter visit to a hyacinth site suggests one possible reason. The many dead and damaged leaves, and the generally sorry appearance of the plants, lead to the idea that the food supply may be very poor during this season. Below the cover of dead leaves, often a good deal of green remains at the heart of the plant, but one still wonders if the remaining food is of good quality. We set out to test this possibility.

Methods. Beginning in early April 2004, we kept adult weevils in canning jars with leaves from different sources, or with access to water alone. The weevils were collected from Whiskey Slough and presumably were adults that emerged the previous fall and winter. The leaves used in the jars were either from plants grown in the greenhouse with abundant fertilizer, or were collected from plants in the field (Whiskey Slough area). The leaf types were further separated into 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. This leaf is among the most protected on the plant and usually remains green. We called these leaves "furled" leaves. The leaves and water were refreshed two or three times a week. Any dead weevils were recorded and removed from the jars. The experiment was run in the greenhouse.

Results. As can be seen from Fig. 1, the food from the field compared favorably with the food from the greenhouse. Overall, the food sources that led to the longest life spans were from the field, in particular the field expanded leaves. Preliminary statistics indicate that the differences between the different food treatments (i.e., excluding the water treatment from the analysis) are very highly significant. In the early portion of the study, before mid-July, there were clear signs that the furled leaves (from either the greenhouse or the field) resulted in a higher rate of mortality than expanded leaves (from either greenhouse or field). The growing site, however, had a mixed effect, with the greenhouse furled leaves resulting in higher mortality than the field furled leaves. The pattern changed in mid-July with a rapid acceleration of the mortality in the treatment with greenhouse-expanded leaves. The cause of this shift is not clear; there were no obvious changes in the growing of the greenhouse plants to account for it. Overall,

however, 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.



Fig. 1. Mortality curves for adult weevils fed on water hyacinth leaves from differing sources. Water = no food; GH expanded = fully expanded leaves from plants grown in the greenhouse; GH furled = emerging new leaves still wrapped around the next-youngest leaf's petiole, from plants grown in the greenhouse; Fld expanded = fully expanded leaves from plants taken from Whiskey Slough; Fld furled = emerging new leaves, from plants taken from Whiskey Slough

One point Fig. 1 makes clear is the long potential life span of the weevil. The age of the weevils was unknown at the start of the experiment, but they were field collected at Whiskey Slough a few days before. Most of them probably emerged as adults sometime during October to December 2003. The last weevil died on 25 January 2005, so it is possible that some of the weevils in the experiment lived for over a year. Another point the figure makes is that the weevils have substantial energy reserves. Even in the water treatment, the life span was 24.6 ± 3.0 days (mean $\pm 95\%$ CI), and most of these weevils had probably already survived a few months through the winter.

Observations of Biological Control Impact on Purple Loosestrife in California

D. M. Woods and B. Villegas

Biological control of purple loosestrife has been a successful venture in many parts of the country with notable successes in nearby Oregon and Washington. Indications of this potential success were occasionally evident soon after the project commenced. Therefore, we established monitoring plots in California associated with two of the earliest releases in the state to extend the monitoring to a new climate for purple loosestrife. Releases of several biological control agents were also made in other locations not as conducive to detailed monitoring. Seven years after the release of the biological control insects, our monitoring efforts have not confirmed significant damage to, or population reductions of, purple loosestrife at either of the two original monitoring sites in Shasta County.

Extensive field surveys were made to all former release sites in the state to evaluate the establishment success. On one of the monitoring visits during 2004, we noticed striking damage to mature loosestrife stands around Big Lake in Shasta County. On closer inspection and follow-up visits 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 beetle, *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.

The biological control of purple loosestrife around Big Lake in Shasta County appears to be an emerging success. Unfortunately, prerelease or early release plant measures were not collected at this site to augment this success story.

Releases of Four Insects for the Biological Control of Purple Loosestrife During 2004 in California

B. Villegas, C. Conley¹, G. W. Brown², and K. Martyn³

Two weevils, *Hylobius transversovittatus* Goeze (Coleoptera: Curculionidae), a root boring weevil, and *Nanophyes marmoratus* (Goeze) (Coleoptera: Curculionidae), a flower-bud weevil, along with two leaf-feeding beetles, *Galerucella calmariensis* L., and *G. pusilla* (Dufft.) (Coleoptera: Chrysomelidae), were released at three purple loosestrife populations during 2004. The primary focus of these releases was to establish strong populations in the afterbay area of Oroville Dam, Butte County, in order to evaluate the effectiveness of these insects in the Central Valley of California. The releases in Butte County were coordinated with the Butte Weed Management Area Group under the leadership of the Butte County Department of Agriculture. Releases were also made in Kern and Shasta Counties.

Approximately 21,000 *Galerucella* leaf beetles (Table 1) were released on May 19-20, 2004. The beetles were collected in the Moses Lake area of central Washington about 10 miles west of the 2001-2002 collection sites. The predominant species collected was *G. calmariensis*. Approximately 7,400 adults of the flower weevil, *Nanophyes marmoratus*, were released in the three infestations: 3,000 in Butte County, 2,200 in Kern County, and 2,200 in Shasta County. The flower weevils were collected from established populations near Ontario, Oregon by Marjolein Schat and Kerby Winter (USDA-APHIS PPQ, Portland, OR). A total of 600 *Hylobius transversovittatus* root weevils were received by Nada Carruthers (USDA APHIS-PPQ, Albany, CA) from Margorie Gilford at the USDA-ARS Purple Loosestrife Laboratory in Niles, Michigan. About 200 weevils were released at each of the three infestations in Butte, Kern, and Shasta counties.

The two *Galerucella* leaf beetles are well established at several sites in Shasta County and visible damage has started to take place. It is hoped that the leaf beetles will continue to spread to other sites within the Fall River Mills and Glenburn areas of Shasta County. In the Onyx area of Kern County, the leaf beetles are also well established, but cattle that wander through the infested area are impacting populations.

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County	City	Location	Release date	Biocontrol agent	No. released
Butte	Oroville	Oroville Afterbay	5/19/2004	Galerucella beetles	3,000
Butte	Oroville	Oroville Afterbay	5/19/2004	Galerucella beetles	3,000
Butte	Oroville	Oroville Afterbay	5/19/2004	Galerucella beetles	3,000
Butte	Oroville	Oroville Afterbay	5/19/2004	Galerucella beetles	4,000
Butte	Oroville	Oroville Afterbay	5/20/2004	Galerucella beetles	2,000
Butte	Oroville	Oroville Afterbay	5/20/2004	Galerucella beetles	2,000
Butte	Oroville	Oroville Afterbay	5/20/2004	Galerucella beetles	2,000
Butte	Oroville	Oroville Afterbay	5/20/2004	Galerucella beetles	2,000
Total					21,000
Butte	Oroville	Oroville Afterbay	6/25/2004	Hylobius weevil	200
Kern	Onyx	Onyx: Smith Ranch	6/24/2004	Hylobius weevil	200
		ALSSP: Big Lake			
Shasta	Glenburn	Cove, west	6/24/2004	Hylobius weevil	200
Total					600
Butte	Oroville	Oroville Afterbay	5/22/2004	Nanophyes weevil	500
Butte	Oroville	Oroville Afterbay	5/22/2004	Nanophyes weevil	300
Butte	Oroville	Oroville Afterbay	6/24/2004	Nanophyes weevil	200
Butte	Oroville	Oroville Afterbay	8/24/2004	Nanophyes weevil	2,000
Kern	Onyx	Onyx: Smith Ranch	6/23/2004	Nanophyes weevil	200
Kern	Onyx	Onyx: Smith Ranch	8/24/2004	Nanophyes weevil	2,000
		ALSSP: Big Lake			
Shasta	Glenburn	Cove, west	6/24/2004	Nanophyes weevil	200
		ALSSP: Big Lake			
Shasta	Glenburn	Cove, west	8/24/2004	Nanophyes weevil	2,000
Total					7,400

Table 1. Insects Released in California in 2004 for the Biological Control of Purple Loosestrife

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

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Mediterranean sage, *Salvia aethiopis* L., (Lamiaceae), is widely distributed in the Western United States. In California, it occurs widely in Modoc and Lassen Counties infesting open rangeland areas, roadsides, pastures, and meadows. Its native range includes the Mediterranean area of Europe and Northern Africa and into western Asia. Mediterranean sage is a strongly aromatic biennial plant that is distasteful to cattle and horses. It grows 2-3 feet tall and produces a stout taproot. Rosettes produced during the first year average about a foot in diameter but in well watered soils; the rosettes may exceed two feet in diameter. During the second year the plants bolt producing a flowering stalk with numerous whitish flowers which produce many seeds. After flowering the plant dries up, breaks off from the taproot and 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 Mediterranean sage. The first weevil, *Phrydiuchus spilmani* Warner was imported from Italy in 1969 and released in the Summer Lake area of southern Oregon, but did not establish. A second weevil, *Phrydiuchus tau* Warner, was imported from Yugoslavia and released in southern Oregon in 1971 where it became well established. Starting in 1976-1980, there were several collections of *Phrydiuchus tau* from southern Oregon and subsequent releases by personnel from the Biological Control Program in cooperation with the Agricultural Commissioners' offices from Modoc and Lassen Counties. Establishment was observed at several sites in Modoc County. However, recent surveys of these release sites in Modoc and Lassen did not recover the weevil.

Efforts to collect *Phrydiuchus tau* in southern Oregon and release them in Northern California were started in November 2002 and 2003. Two small collections of the weevils were made at a large pasture off Hwy 31 in the Summer Lake area northwest of Lakeview, Oregon. Approximately 200 weevils were collected during each of the visits and released in the Belfast Tablelands area east of Susanville, Lassen County, California. Subsequent surveys of the release sites did not reveal any signs of establishment by the weevils from these two releases.

In 2004 a different release strategy was tried. Rather than collecting the weevils in the fall of the year, the weevils were collected in the spring shortly after pupal emergence. These were released in the Belfast Tablelands area of Lassen County. The first collection took place on May 20, 2004 in the Lake Abert along US Highway 395 in southern Oregon. On that date, many teneral weevils were observed emerging from earthen pupal cases located just below ground level. Close examination of the damaged plants revealed extensive root and crown damage by the weevil larvae. A second collection took place on June 9, 2004 and at that time the weevils had finished emerging from the soil and were visible feeding on the rosette leaves or sheltered under the foliage near the crown of the plants. Approximately 300 weevils were collected on May 20, 2004 and 500 weevils were collected on June 9, 2004. Monitoring for colonization and establishment will begin in spring 2005.

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