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Annual Report
2003

California Department of Food & Agriculture

BIOLOGICAL CONTROL PROGRAM

2003 SUMMARY

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INTEGRATED PEST CONTROL BRANCH

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PREFACE

M. J. Pitcairn

It has been an exciting year for several of the projects pursued by the Biological Control Program. Most notably, the exotic rust, *Puccinea jaceae* var. *solstitialis* was approved for use as a biological control agent against yellow starthistle in North America. Approval of this rust came after over 25 years of quarantine evaluations by Bill Bruckart and his colleagues at the United States Department of Agriculture, Agricultural Research Service, and the Foreign Disease and Weed Science Research Unit at Fort Detrick, Maryland. Very few exotic plant diseases have been released in the United States for control of an exotic weed, and this is the first disease to go through the new permitting system established after the federal Plant Protection Act of 2000. This rust joins five insect biological control agents that were established between 1984 and 1992. All of the biological control insects attack the seed heads of yellow starthistle. The rust attacks the leaves and stem of the plant and it is hoped that damage caused by infections of this disease will complement and add to the damage caused by the insects. Together, the rust and insects may reduce the invasiveness of yellow starthistle. The first release of the rust occurred in July 2003 in Napa County. Since then, the rust was cultured in our greenhouses in Sacramento, and rust spores were collected weekly and stored for next year. It is anticipated that releases in 2004 will occur in at least 20 additional counties. Little is known about the ability of this rust to exist under environmental conditions in California where yellow starthistle is known to occur, and so much is needed to be learned before the full potential of the rust can be realized.

Among the insect projects, biological control of the pink hibiscus mealybug in the Imperial Valley appears to be another successful project. The pink hibiscus mealybug is a very serious pest of many agricultural and ornamental host plants, especially grapes, citrus, alfalfa, and cotton where mealybug populations can build up quickly and eventually kill the host plant. Once the pink hibiscus mealybug was discovered, the Biological Control Program initiated a comprehensive integrated control effort built around the mass rearing and release of three biological control agents that attack and kill the mealybug. If the mealybug had been allowed to proliferate and spread unchecked, control costs in grapes, citrus, alfalfa, and cotton were expected to have exceeded $700 million annually. Now, as a result of the release of three parasitic hymenoptera, the mealybug is under complete biological control and no additional control costs have resulted in any agricultural crops in the surrounding region. Release of the third parasite species is reported in this report.

This report updates several other projects involving biological control of the olive fruit fly, lygus bug, squarrose knapweed, and purple loosestrife. I hope you enjoy this year's report.

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Insect Projects

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Pink Hibiscus Mealybug Biological Control in Imperial Valley, California, 2003 Update

W. Roltsch, D. Meyerdirk[1], E. Andress[2], J. Brown, J. Zuniga, and R. Aguilar

The pink hibiscus mealybug (PHM), *Maconellicoccus hirsutus* (Green), was first detected in Imperial Valley, California in August 1999. Population densities of PHM on mulberry, carob, silk oak, hibiscus, and natal plum were determined to be high in several urban communities in southern Imperial Valley. Two parasitoid species, *Anagyrus kamali* Moursi and *Gyranusoidea indica* Shafee, Alam and Agarwal, were released at 10 sites in the fall of 1999. Subsequently, an insectary was established in El Centro for additional parasitoid production. The two species were then produced locally and released beginning in 2000. The culture of *A. kamali* that was propagated through 2001 originated from collections in China and Hawaii that were combined. *Gyranusoidea indica* was a combination of populations from Egypt, Pakistan, and Australia. In 2002, a population of *Anagyrus kamali* (collector: D. Gonzalez, University of California, (UC) Riverside) from southern Egypt was reared and released.

We received permits for rearing an additional parasitoid, *Allotropa* sp. nr. *mecrida* (Hymenoptera: Platygastridae), in November 2002. This population was collected in the very warm and dry climate of southern Egypt by Dr. Dan Gonzalez, UC Riverside in 2000. In 2003, we produced and released nearly 300,000 parasitoids (Table 1). They were either released in Imperial Valley or provided to Mexican authorities for release in the adjacent Mexicali Valley. Parasitoids were initially released at sites on the perimeter of the infested area. As the season progressed, releases were made at progressively interior locations. This approach avoided releases being made within several city blocks of long-term monitoring sites until the fall of 2003.

Table 1. Destinations of pink hibiscus mealybug parasitoid *Allotropa* sp. nr. *Mecrida* produced at the California Department of Food and Agriculture Insectary, El Centro, CA, in 2003.

Month	Imperial Valley	No. of Sites in Imperial Valley	Mexico	Monthly Parasitoid Release Totals
January	5,000	4		5,000
February	8,800	13		8,800
March	22,000	28		22,000
April	23,000	26		23,000
May	3,500	5		3,500
June	5,000	6	4,000	9,000
July	2,500	5	4,800	7,300
August	34,000	38	21,000	55,000
September	33,000	33	24,000	57,000
October	24,000	20	26,000	50,000
November	13,000	13	9,000	22,000
December	35,000	35		35,000
Total to Date	208,800	226	88,800	297,600

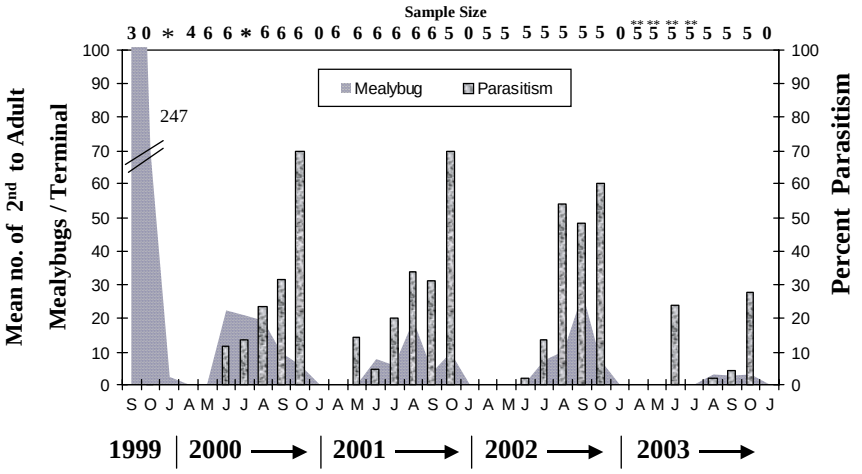

Figure 1. Pink hibiscus mealybug and parasitism on mulberry trees in Imperial Valley, California. Mulberry terminal samples in January are available with buds only. Sample size equals the number of sites sampled by date. [* = % parasitism only was calculated, ** = % parasitism was calcaulated for 1 or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

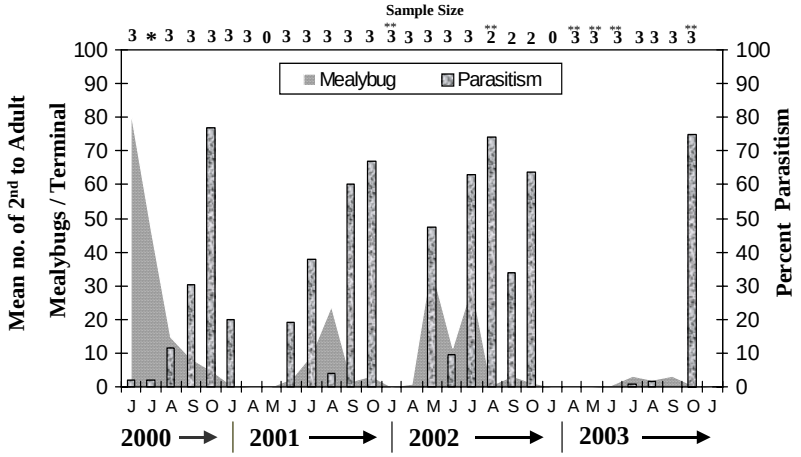

Figure 2. Pink hibiscus mealybug and parasitism on carob trees in Imperial Valley, California. Sample size equals the number of sites sampled by date. [* = percent parasitism only was calculated, ** = percent parasitism was calcaulated for 1 or 0 sites].

Monitoring of PHM population density and parasitism on mulberry and carob trees occurred primarily at the same sites (i.e., long-term sites) selected at the inception of the PHM project. Population densities on infested mulberry trees averaged over 200 mealybugs/terminal in September 1999 (Figure 1). Corresponding with the broad establishment of *Anagyrus kamali*, PHM densities have been consistently low for four consecutive years. *Gyranusoidea indica* is also established in Imperial Valley; however, its numbers are typically low during the year, particularly during the warmest months

from June through September. In 2002, less than 10% of all parasitoids collected during the year were *G. indica;* however, *G. indica* represented 21% of the primary parasitoids collected in October. Similar results have been recorded at three study sites consisting of carob trees (Figure 2). PHM densities were initially high on carob trees, but with the onset of parasitism, they have become considerably lower. In 2003, PHM densities were the lowest of all years to date (Figures 1 and 2) and *Anagyrus kamali* continues to be the dominant parasitoid. Due to very low PHM densities in 2003, it was not feasible to collect PHM specimens for assessing the percent parasitism at many sites. Overall, the percent parasitism in 2003 was considerably lower than in past years, presumably reflecting a density dependent relationship with PHM.

The impact of native (to Imperial Valley, CA) hyperparasitoid species on newly introduced primary parasitoid species is being monitored. A hyperparasitic species (*Marietta* sp.) was first collected in July 2000. At that time, its occurrence was quite rare. Dissected samples confirmed that the primary parasitoid, *A. kamali*, was under attack by *Marietta* sp. (Aphelinidae) and to a lesser extent by *Chartocerus* sp. (Signiphoridae). *Marietta* sp. was common through the remainder of 2000, as represented by the percent of PHM mummies from which hyperparasitoids emerged [(mean percentage, number of sample sites): late July 11%, five sites; late August 60%, six sites; September 16%, six sites; and October 51%, nine sites]. Hyperparasitoid attack of *A. kamali* declined after 2000 (Fig 3). In 2003, *Marietta* sp. was common during one sample date at two locations. Elsewhere, it was rarely found. From four samples taken during September 2003, hyperparasitism was estimated to be 32%. *Chartocerus* sp. was not collected in 2003.

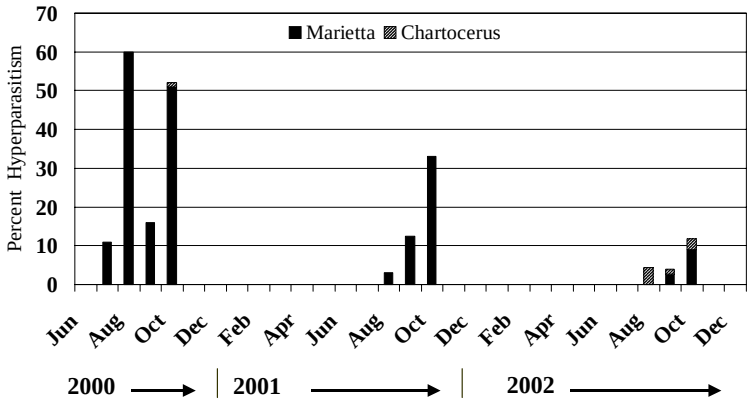

Figure 3. Hyperparasitism of pink hibiscus mealybug parasitoids in Imperial Valley, California.

A number of samples of two resident species of mealybug have been collected over several years to monitor for non-target impacts. Ten separate collections of the solenopsis mealybug, *Phenacoccus solenopsis* Tinsley and 13 collections of the striped

mealybug, *Ferrisia virgata* (Cockerell) have been made in Imperial Valley. The former species is native, whereas the latter is not a native species. To date, neither *A. kamali* nor *G. indica* have been recovered from either mealybug species, thereby demonstrating that they are either moderately or highly host specific. In summary, two biological control agents released against PHM have become widely established throughout infested areas of Imperial Valley, and one species has had considerable impact to date. The third newly released species has also shown strong signs of establishment to date. The average regional density of PHM has markedly decreased (>95% reduction) since 1999. Moreover, the distribution of PHM has remained unchanged continuing to be restricted to urban locations within the southern half of Imperial Valley.

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Releases of Parasitoids for Control of the Olive Fruit Fly

C. H. Pickett, R. Rodriguez, D. Asakawa[1], R. Messing[2], and H. Nadel[3]

Activities for the biological control of the olive fruit fly project in 2003 included sampling from former release sites in southern California, performing additional releases of *Psyttalia concolor* in Santa Barbara, and monitoring potential release sites in Yolo County. We also initiated a laboratory culturing program for *P. concolor*, which is reported by R. Rodriguez elsewhere in this volume.

Sampling of former release sites began on August 20, 2003 in Santa Barbara. At that time, olive fruit were so heavily attacked by the olive fruit fly (OLFF) that much of the fruit had fallen to the ground. A total of 53 olives were collected and returned to the laboratory in Sacramento in order to rear parasitoids. One adult *P. concolor* was recovered from olives collected on the ground. No parasitoids were recovered from samples collected October 7, 2003, the last date these former release sites were sampled.

Olives were also collected from two other areas in southern California where *P. concolor* was released between 1999 and 2001: the Jurupa Cultural Center in Riverside and private residential yards in Palos Verdes. No *P. concolor* were recovered from olives collected at either of these locations.

New releases of *P. concolor* were made in Santa Barbara on December 4, 2003 at a residential site that still had some infested olives remaining on the tree. A total of 600 *P. concolor* were released, 100 that we had reared in Sacramento and 500 that were shipped from Hawaii. Previous releases had not been made at this house, and no *P. concolor* was recovered from the 89 olives collected on the day of release.

Future release sites have been established in Davis, California where olive fly trap data has been collected over the last two years. The OLFF populations have been increasing rapidly at this site, with a five-fold increase in the number of flies caught per trap per day (Figure 1). An even greater increase was measured in the number of fly larvae per dry weight of olive over the same period of time. Additional releases of biological control agents are anticipated in 2004.

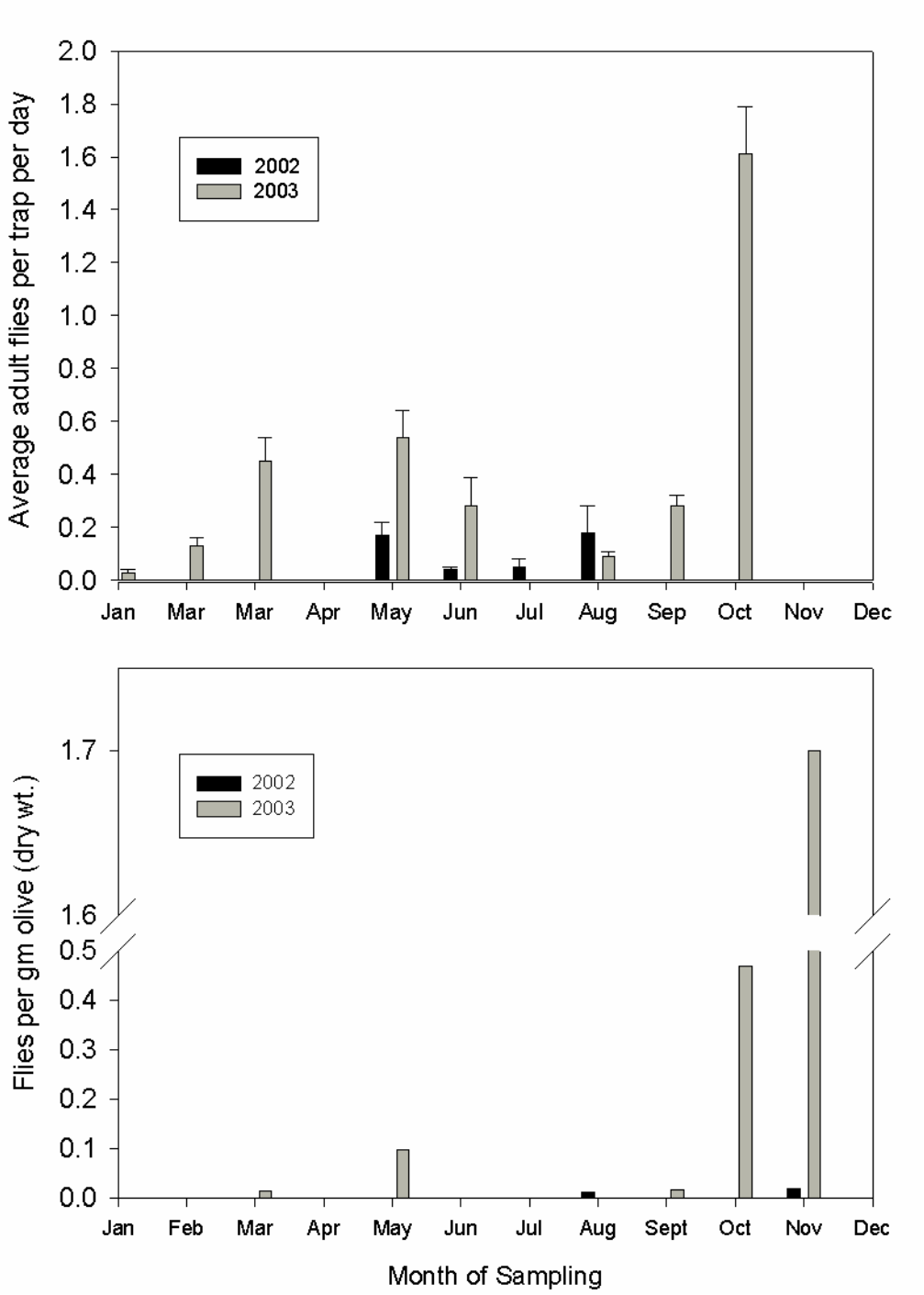

Figure 1. Olive fruit fly population at a future release site in Davis, CA.

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Indigenous *Pteromalus* on Olive Fruit Fly

C. H. Pickett and R. Rodriguez

Prior to releasing imported parasitoids for olive fly control, release sites are being surveyed for any extant parasitoids that could be attacking these flies. These surveys allow us to ensure that candidate parasitoids do not have existing populations in the release sites. In addition, we determine if any level of control is currently being exerted by indigenous natural enemies.

For the last four years, olives have been collected from current as well as future release sites. We released *Psyttalia concolor* at three locations in southern California from 1999 to 2003 (current sites). Additional locations in northern California were identified last year (future sites), and sampling commenced. At the Riverside and Santa Barbara sites, a species of *Pteromalus* was frequently recovered from olives that were heavily infested with olive fruit flies. The parasitoid has yet to be identified to species and may be undescribed. We have not seen this parasitoid at new sites in northern California where olive fly is still in low numbers. Due to the apparent association between *Pteromalus* and olive fly larvae in fruit, we designed a pilot study to determine if they could attack olive fly larvae.

In this study, *Pteromalus* sp. was exposed to pre-imaginal olive flies under three different regimes so that they could be exposed to the flies under a wide range of conditions: 1) olives with larvae and larvae created "windows" in them; 2) olives with late instar fly larvae, exit holes, tunnels, and pre-pupae and pupae; and 3) olives with parasitized (*P. concolor*) and non-parasitized larvae. All *Pteromalus* used in this study emerged from olives collected in Santa Barbara. Room temperature where the study was conducted was a constant 22°C. Results are shown in Table 1.

Condition 1. Five olives were exposed to olive flies for two days beginning October 15, 2003. Olives with window formation were selected and exposed to five *Pteromalus* on October 28. Seven weeks later, the waxed paper cans with insects were examined for parasitoids.

Condition 2. Five olives were exposed to olive fruit fly for three days beginning October 17. Olives were removed from cages with flies then held at room temperature on shelves in wax paper cans for eight days beginning October 20. They were exposed to five *Pteromalus* on October 28. Seven weeks later, the cans were observed.

Condition 3. Three olives were exposed to flies for three days beginning October 17. They were then held in waxed paper cans on shelves at room temperature for four days starting October 20. They were exposed to *P. concolor* for three days starting October 24. Olives were then removed and exposed to five *Pteromalus* spp. for 14 days. Seven weeks later, the cans were examined for insects.

Table 1. Development of olive fruit fly exposed to *Pteromalus* under three conditions.

Condition	No. Flies Produced		No. *Pteromalus*	
	Pupae	Adults	Start	Finish
1	12	5	5	5
2	7	5	5	5
3	7	5	5	5

Under all three conditions, no *Pteromalus* were generated, i.e. no reproduction was measured in presence of olive fly larvae or pupae. Under Condition 3, one *P. concolor* was produced, but again no *Pteromalus*. These preliminary results suggest under the conditions tested, *Pteromalus* did not attack olive fly. This study will be expanded this year.

Rearing the Olive Fly Parasitoid *Psyttalia concolor*

R. Rodriguez, C. H. Pickett, and M. Robertson[1]

Both natural and artificial rearing systems are being developed for the rearing of olive fruit fly (OLFF) parasitoids. Cultures of parasitoids are needed for host testing studies and for release into several sites. We began rearing OLFF in September 2002 using olives collected in the field. The flies reared easily, the only limitation being the seasonal availability of olives. Green, immature fruit are best, but they are not available from April to June. We have begun testing the use of fruit stored in a high nitrogen/low temperature environment with help from Hannah Burrack (Department of Entomology) and Bill Biasi (Department of Pomology) at UC Davis. Olives were field collected from roadside trees in Yolo County and divided into eight two-kilogram lots. The olives were then stored for about two months, from January 30 to April 1. Olives rapidly matured from green-purple to black after removal from storage. Olives proved unacceptable for fly production two weeks after removal from storage due to molding of the fruit. The rapid decay of stored fruit limited the production of *Psyttalia concolor* to those OLFF larvae that developed quickly and emerged from the olives. Green olives became available for rearing in mid June. The use of green olives increased parasite production due to an increase in the longevity of the fruit. We began rearing *P. concolor* on infested fruit beginning April 9, 2003. Rearing was conducted at around 22°C in our containment facility in south Sacramento. Olives were exposed to flies in a sleeve cage under artificial lighting (14:10, L:D).

The steps taken for producing parasitoids from these olives were:

1. Expose 80 to 100 green olives to OLFF for two to three days for oviposition.
2. Remove olives from OLFF and place on shelves in 0.5-gallon waxed buckets for three days. Buckets had a false bottom of hardware wire, the bottom covered with vermiculite.
3. Half of the olives from above were exposed to *P. concolor* for three days, the remaining half held for maintenance of the fly culture. Olives were held at room conditions on shelves, or in an environmental chamber set at 14:10, L:D, 25°C, and 75% humidity.
4. Parasitoids were collected as they emerged, approximately 22 days later.

A total of 1,190 parasitoids were successfully reared on olives from April 9 through December 29, 2003 with equal success for samples reared on laboratory shelves and in controlled environment chambers (Table 1). A rearing period of 18 weeks produced 633 parasites, with approximately 35 parasites produced per week (Table 2).

Table 1. Parasite production per cage on laboratory shelves versus controlled environment chambers from June 30 to October 31, 2003.

	No. Pupae Recovered	No. Male Parasites	No. Female Parasites	Total Parasites	Pupae/ Olive	Parasites/ Olive	% Parasitation
Shelves	23.27	3.52	3.20	6.729	2.095	0.606	28.91
STDEV	4.8	0.61	0.57	1.1	0.268	0.09	3.67
Chambers	23.04	3.73	3.15	6.88	1.93	0.57	29.89
STDEV	4.64	0.69	0.65	1.15	0.26	0.08	5.43

Table 2. Total parasite production for all cages and conditions June 30 to October 31, 2003.

Olives	Pupae Recovered	No. Adult Parasites	% Parasitism	Pupae/ Olive	Parasites/ Olive
1,068	2,154	633	29	2	0.59

As an alternative to the use of olive, we began rearing *P. concolor* with flies reared on an artificial medium with assistance from Dr. Mark Robertson. A laboratory strain of olive fly from Greece was used, since they rear much better than wild flies on this diet. Dr. Robertson has been sending us fly eggs, which we in turn rear on the artificial medium.

Protocol for rearing OLFF on an artificial diet:

1. Weigh out 250 grams of artificial diet and place in foam tray eight inches by four inches.
2. Add fly eggs to 20 milliliters of 0.2% propionic acid.
3. Sprinkle eggs with a 30-mill dropper over diet tray until all liquid has been dispensed.
4. Allow trays to incubate at 25°C and 50% humidity for 10 to 11 days.
5. Wash diet containing larvae through mesh screen with tap water at room temperature to free larvae from diet.
6. Add fresh diet to stinging dish (eight centimeter by one centimeter circular plastic dish with screened lid), add larvae to dish, add 20 ml of propionic acid, and then cover with screened lid.
7. Expose to *P. concolor* for 16 to 24 hours by placing the plastic dish inside a larger cage with at least 50 adult *P. concolor*; make sure that the diet does not dry out, add another 20 ml propionic acid if diet does dry out. The parasitoid cage is held at laboratory conditions 22°C and a 14:10 light:dark regime.
8. Remove larvae from the plastic dish and place into a foam tray as above, with fresh diet; and add 20 ml of propionic acid.
9. Place the foam tray into a large Tupperware® container that closes tightly, and has been lined with paper towels. Then place the container back into an environmental chamber set at 25°C and 50% relative humidity.
10. Larvae will begin to pupate in the next two to three days and will crawl out of the diet tray and under the paper towels.
11. Collect parasitoids as they emerge 12 to 14 days later.

Three cohorts of OLFF larvae were reared on the artificial diet for 10 to 11 days prior to *P. concolor* exposure (Table 3). A total of 452 adult *P. concolor* were collected from the three cohorts four weeks sooner than parasites produced by larvae reared on olives (Table 4).

Table 3. Summary data for *P. concolor* produced from larvae reared on artificial diet.

Cohort	Date of Exposure	Number of Parasites Collected	Number of Puparia Collected	% Parasitism
1	December 17-23	95	336	28
2	January 21-22	278	704	39.4
3	March 1-2	79	186	42
Total	6 days of exposure	452	1,226	36.8

Table 4. Comparison of *P. concolor* rearing on olives versus artificial diet.

	Parasitoids Produced	No. Puparia Collected	% Parasitism	Weeks Reared	Parasites per Week
Olives	633	2,154	29	18	35
Artificial Diet	452	1,226	36.8	14	32

In summary, both rearing techniques produced about the same number of parasitoids per week. However, the artificial diet has more potential for producing a higher number of parasitoids, and most importantly, it does this without the need for fresh olives. Given more fly eggs, we could produce far more parasitoids. This system requires far less space as well. Furthermore, average parasitism was higher when using hosts reared on the artificial diet.

[1] University of California, Riverside, CA.

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

K. A. Hoelmer[1], A. Kirk[1], R. Wharton[2], and C. H. Pickett

The olive fruit fly (OLFF), *Bactrocera oleae* (Gmelin) (Diptera: Tephritidae), was first reported invading California in 1998 and is now firmly established in olive growing regions throughout California. This fruit fly is a very serious 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.

Olive fly larvae feed in the flesh of olive fruits, introducing bacteria and fungi, which cause fruit deterioration. During the first generations of the fruiting season larvae pupate inside fruit, while later generations drop from fruit into the soil for pupation and overwintering. There are usually several generations of OLFF per year. Processors have a very low tolerance for infested fruit used for table olives, and only a five to 10% tolerance for fruit destined for olive oil production. The olive industry in California has until now been almost pest free, and the introduction represents a severe challenge to the survival of the olive industry in California.

As part of a statewide management program being developed to control the olive fly, researchers propose to import effective parasitoids attacking this pest in its native home range, which we believe to be eastern and/or southern Africa or south-central Asia corresponding to the distribution of wild olive, *Olea europaea cuspidata*. A rich diversity of olive fly parasitoids is known to occur in several parts of Africa. Although a considerable amount of biological information has been accumulated regarding native parasitoids and predators in the Mediterranean region, these species are not capable of keeping fly populations at low levels. The braconid *Psytallia concolor* has been extensively evaluated in augmentative biocontrol projects but these have not been implemented on a wider scale due to economic costs. However, the potential of African species has remained largely untapped for biological control.

Collections of infested wild and cultivated olives were made during the fruiting season, which varies from the northern to the southern hemisphere. During 2002 and 2003, explorations for olive fly and its natural enemies were conducted by European Biological Control Laboratory staff or cooperators in the Republic of South Africa (two trips covering East and West Cape, Gauteng, Northwest, and Mpumalanga Provinces), Kenya (multiple collections in several highland areas), Pakistan (Northwestern Frontier Province), and southwestern China (Yunnan Province). Dr. Robert Wharton and Dr. Gerhard Prinsloo provided taxonomic assistance with identifications of parasitoids.

The survey in the Northwest Frontier Province of Pakistan was completed in late fall of 2003. Olive flies were reared from several sites. Infestation rates did not exceed 3% in the final samples collected in October. A braconid parasitoid very similar to *P. concolor* and identified by R. Wharton as *Psyttalia cf. ponerophaga* was reared from these flies. Pupal parasitism rates in October ranged from 33 to 60% at five different sites. We plan to obtain live specimens next season for rearing and evaluation.

Botanical records indicated that the eastern distributional limit of wild olive, *Olea europaea cuspidata*, is in the Yunnan and Sichuan provinces of China. There are also a number of scattered, small commercial olive orchards in these provinces. No published records of *B. oleae* exist for this region. Wild olive trees in fruit were found at several isolated locations during our survey, and several collections of wild and cultivated olive fruit were made, but no flies and therefore no natural enemies were reared from these. However, further surveys of wild olives in more isolated parts of western China are probably necessary before concluding that the fly is not present in the region.

We obtained quantities of the parasitoids (*Psyttalia lounsburyi*, *Psyttalia dacidida, Psyttalia spp. nr concolor, Utetes africanus, Bracon celer,* Bracon sp. A, Bracon sp. B, and Bracon sp.) during the surveys and collections in South Africa and Kenya. The most abundant of these were *Utetes africanus, Psyttalia lounsburyi,* and *Bracon celer* (from South Africa) and *Psyttalia lounsburyi* (from Kenya).

We continue to develop and refine our methods of rearing fruit fly hosts and parasitoids. During this process, we have been collaborating with Dr. Alfio Raspi and Dr. Augosto Loni (University Pisa, Italy). Olive flies adapted for rearing on artificial diet have been provided to EBCL by Dr. Basilios Mazomenos (Demokritos Institute, Athens) and Dr. Mark Robertson (UC Riverside). Attempts to switch *Psyttalia lounsburyi* from rearing on olive fly to Mediterranean fruit fly (which would facilitate rearing) have not been successful nor will females of this parasitoid accept olive fly larvae in artificial diet. Rearing of this parasitoid requires us to use fresh and cold-stored olives as the host medium for olive flies. For various reasons, our quarantine laboratory colony of *Psyttalia lounsburyi* has experienced several declines in numbers and sex-ratio reversals. At the beginning of 2004, the decline reversed, and the colony appears to be stable and building again. Several shipments of *Psyttalia lounsburyi* and *Utetes africanus* were made in 2002 and 2003 to our cooperators in California, Hawaii, and Italy for host-range evaluation and rearing development.

We will make additional collections during April in South Africa to obtain more *Psyttalia lounsburyi, Utetes africanus*, and *Bracon celer* as well as conduct the first surveys for olive fly natural enemies in Namibia in May and Reunion Island in June. Future trips are planned for the Canary Islands, southwestern Morocco, and southern Turkey. Depending on information about wild olive presence obtained from local sources, other surveys may include Madagascar; and further surveys may be made in southwestern China. Additional surveys are planned via co-operators in Pakistan, northeastern India and Nepal.

Acknowledgements:

Thanks are due to numerous local contacts during our travels, including Tammy Smith (Rhodes University, Grahamstown, South Africa), Johann Baard (Forestry, Knysna, South Africa), Dr. Stefan Neser and Dr. Gerhard Prinsloo (PPRI, Pretoria, South

Africa), Vaughn Walton (Infruitec, Stellenbosch, South Africa), and Zongqi Chen and Shen Ai Dong (CAAS, Kunming, China). Thanks also to Dr. Basilios Mazomenos (Demokritos Institute, Athens) and Dr. Mark Robertson (UC Riverside) for their continued support in providing an olive fly strain for culture in artificial diet. Funding for our work was provided in part by the California Department of Food and Agriculture (CDFA) and the UC Specialty Crops Program.

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Preliminary Host-Specificity Studies on Parasitoids of Olive Fruit Fly

H. Nadel[1], K. Daane[1], C. Funk[1], and C. H. Pickett

The olive fly is being targeted for classical biological control in California, especially in untreated urban olives and abandoned orchards found over much of the state's 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. While developing a protocol for host-specificity studies in quarantine, we studied the capacity of some imported fruit fly parasitoids to reproduce on two native and one exotic fruit fly species.

The parasitoids were imported from laboratory colonies in Hawaii. They attack several fruit fly species, including the OLFF. *Fopius arisanus* attacks eggs and emerges from the pupa, while *Psyttalia humilis*, *P. concolor*, and *Diachasmimorpha kraussii* attack mainly the third instar, emerging from the pupa.

Two univoltine fruit-feeding native host flies were exposed to the following parasitoids: *Rhagoletis completa* (walnut husk fly) and *R. indifferens* (western cherry fruit fly), and an exotic, mulivoltine, seed-head feeder, *Chaetorellia succinea*, which is employed for control of yellow starthistle.

Bitter cherry (*Prunus emarginata*) fruit were picked at various sites in the Sierra Nevada in August 2003 when much of the fruit was ripe. *Rhagoletis indifferens* prefers ripe fruit, but another cherry fly, *R. fausta*, attacks mainly unripe bitter cherry. The larvae of both fruit flies are solitary. The rate of infestation by the fruit flies varied considerably by site but was not determined before the study. A study replicate consisted of about 100 ripe or unripe fruit placed with four female parasitoids in plastic tubs with mesh lids for three days. Few *F. arisanus* females were available, so ripe and unripe fruit were exposed in open tubs inside the colony cage.

English walnuts infested with *R. completa* were collected in Alameda County and four to six walnuts per replicate were offered to about four female parasitoids in plastic tubs with mesh lids. Susceptible host stages were used. The larvae are gregarious, about eight to 15 per fruit. Unfortunately, despite intervention, many of the walnuts rotted, causing considerable mortality of larvae and pupae. Although no parasitoids emerged from surviving pupae, no firm conclusions can be drawn from this part of the study due to the rotting of the walnuts.

Chaetorellia succinea was reared from yellow starthistle inflorescences collected in Yolo County. After a mating and maturation period in cages, a female was isolated in a vial on an intact flower bud for 30 minutes to two days to oviposit, but was not observed. Oviposition by flies could not be confirmed. A female parasitoid was isolated for two to three days in the vial when the eggs or larvae were in susceptible stages. *Psyttalia concolor* was not used in this study.

The results are summarized in Tables 1 and 2. The only firm conclusions that can be made are that *P. humilis* and *D. kraussii* are capable of reproducing in larvae of *R. indifferens*. The results suggest that *F. arisanus* and *D. kraussii* cannot reproduce on *C. succinea*, but more data are needed.

Table 1. Results of parasitoid exposure to *Rhagoletis indifferens** in bitter cherry fruit.

	No. Replicates	Total No. Fruit	No. Fly Pupae	No. Parasitoids Emerged	
Ripe Fruit				Male	Female
Control	3	425	62	-	-
Fopius arisanus	1	120	31	0	0
Psyttalia humilis	4	420	18	4	7
Psyttalia concolor	-	-	-	-	-
Diachasmimorpha kraussii	1	125	2	2	0
Unripe Fruit					
Control	2	200	1	-	-
Fopius arisanus	1	75	12	0	0
Psyttalia humilis	3	314	1	0	0
Psyttalia concolor	-	-	-	-	-
Diachasmimorpha kraussii	-	-	-	-	-

* *Rhagoletis fausta* also infests bitter cherry, preferring unripe fruit. As we were uncertain of the identity of the fly larvae in the fruit, we used unripe fruit (green to orange) and ripe fruit (red and dark red) separately. Flies will be identified to species after they emerge in the summer of 2004.

Table 2. Results of parasitoid exposure to *Chaetorellia succinea* in yellow starthistle flowerheads.

	No. Replicates	No. Flowerheads Infested by Fly	No. Flies Emerged	No. Dead Fly Larvae	No. Parasitoids
Control	15	3	2	1	-
Fopius arisanus	20	7	10	1	0
Psyttalia humilis	10	0	0	0	-
Diachasmimorpha kraussii	10	4	2	2	0

[1] University of California, Berkeley, CA.

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

W. J. Roltsch, B. Villegas, D. L. Dahlsten[1], J. Brown, and L. Yang

Progress toward the biological control of the red gum lerp psyllid (RGLP), *Glycaspis brimblecombei* Moore, continued through 2003. In California, it is predominantly a pest of red gum eucalyptus, *Eucalyptus camaldulensis* Dehnh. The primary objective for 2003 was to survey for the establishment of the recently released encyrtid parasitoid, *Psyllaephagus bliteus*.

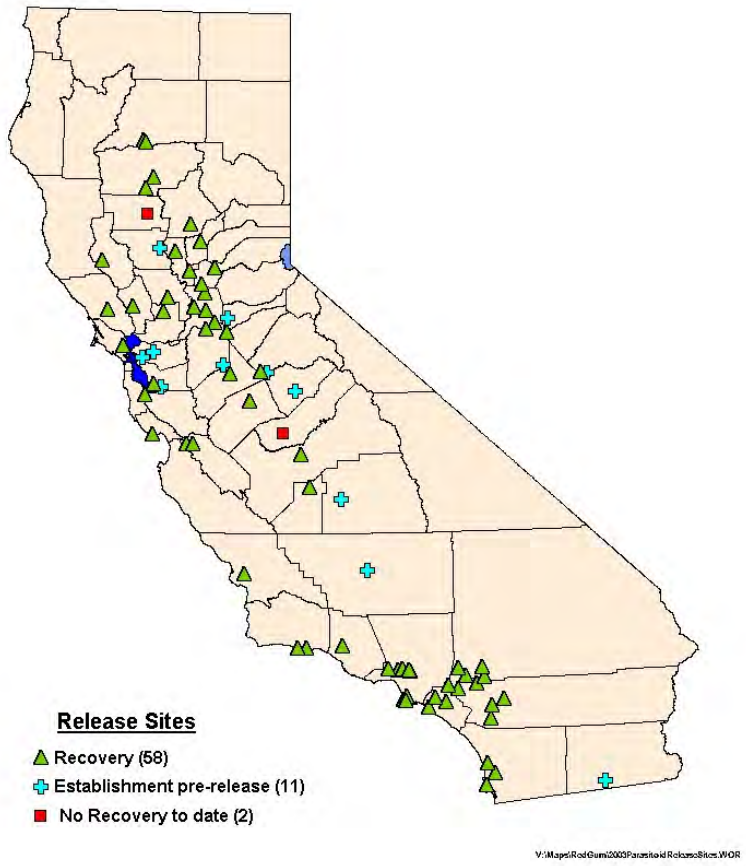

Figure 1. Recovery status of RGLP parasitoid at former release sites of the University of California and the CDFA. Updated fall 2003.

Monitoring for post-release parasitism was conducted at 61 locations statewide in 2003. In most instances, these sample sites represented the locations where *P. bliteus* had been released in previous years (2000 through 2002). The sampling period ran predominantly from August through October. With the exception of California's low desert, this is the season when RGLP populations reach peak abundance, and the red gum eucalyptus demonstrates considerable leaf loss and stress if under extensive attack. Exceptions included four southern California sites, that were sampled in November and December, and sites in the low desert region of Imperial Valley, which were sampled in late March. Samples consisted of 15 branch terminals, 30 to 45 centimeters in length that were collected from three or more trees per site. The intent was to obtain 100 leaves containing a minimum of 100 occupied lerps for population counts and for detecting signs of parasitism. Counts were made on 30 leaves and psyllids of all instars were inspected externally for signs of late stage parasitoid development.

Approximately 40 third to fifth instar nymphs were randomly selected and placed in alcohol for dissection to assess parasitism in detail. In addition, 50 leaves or more per site were held in large paper bags for 60 days for parasitoid emergence. This was an additional practice used for detecting the simple presence of parasitoids at each site.

By the fall of 2002, populations of *P. bliteus* had persisted at 54 of the 72 original release sites. Pre-release samples during 2002 releases found parasitoids present at only 12 locations prior to release.

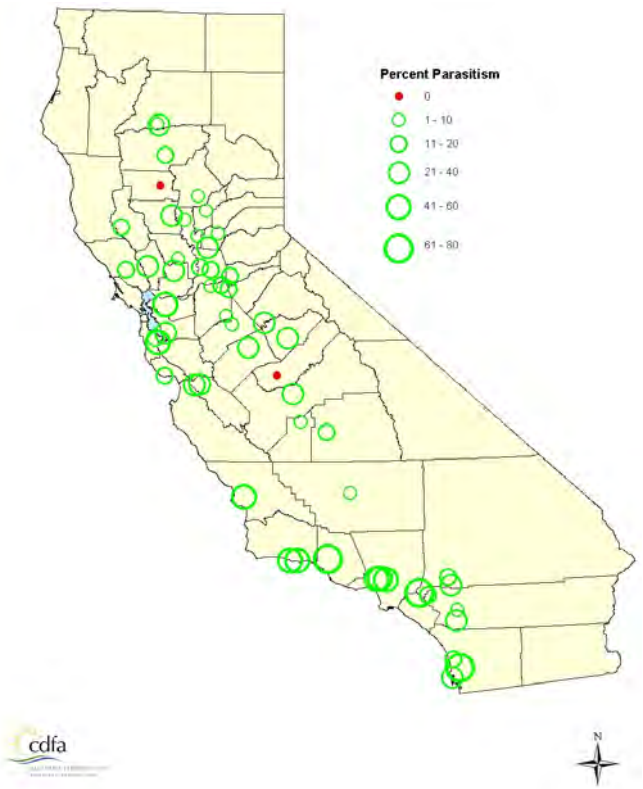

Figure 2. Percent of lerps with parasitoid exit holes. Fall 2003 survey by the CDFA Biological Control Program .

By the fall of 2003, *P. bliteus* had been recovered at all but two of the 71 locations where it had been released in previous years (Figure 1). At several of the 61 locations surveyed in 2003, the level of parasitoid activity was strikingly different from levels found in 2002. For example, at the Solano County site *P. bliteus* was nearly undetectable in 2002, whereas parasitism was common by 2003 during the October sample period. On average, 22% of the lerps over all sites had parasitoid exit holes. Estimates of this relative measure of parasitism are illustrated in Figure 2. Values represent the proportion of all large lerps (i.e., fourth and fifth instar lerps) containing exit holes. The relationship between this measure and that of the actual percent parasitism is under evaluation; nevertheless, it does provide a relative assessment of parasitism across the sites. Based on RGLP nymphs that were visibly parasitized (i.e., external appearance), 11% were fourth instars RGLP nymphs and 89% were fifth instars. The late larval and pupal stages of the parasitoid were not found in third instar psyllids. This supports observations that exit holes are limited to lerps produced by fourth and fifth instar red gum lerp psyllids.

In summary, *Psyllaephagus bliteus* has been released throughout the state and is well on its way toward permanent establishment at most locations. Based on our relative assessment of parasitism, the parasitoid is very active (>10% of lerps had exit holes) at 70% of the sample sites. Photographs of representative trees affected by the RGLP have been taken at each site so they can be compared with photographs in future years to document tree foliage status over time in conjunction with population patterns.

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Status of Introduced Silverleaf Whitefly Parasitoids in Imperial Valley, California

W. J. Roltsch, L. Yang, and L. M. Ragaini

An intensive effort was made to establish biological control agents for silverleaf whitefly, *Bemisia tabaci* B strain (= *B. argentifolii*) in the desert Southwest from 1994 to 1999. Greenhouse-reared aphelinid parasitoids in the genera *Eretmocerus* and *Encarsia* were released in large numbers (exceeding several million for many species) in commercial fields, refuge nursery plots, and urban yards by state, federal, and university scientists. This report examines exotic parasitoid establishment and relative abundance in two field plots and in the Imperial Valley communities of Brawley and El Centro. The two field plots were pesticide-free insectary garden plots (0.1-0.3 hectares each) located at the Imperial Valley Research Center.

In contrast to previous years, field plots did not contain a winter planting of either collard or sunflower. These winter-fallow plots were planted with two beds each of okra, basil, and cantaloupe, and four beds of cotton in March 2003. Samples (15 leaves per plant species per plot) were collected twice in the spring cantaloupe plantings (May and June) and four times from May to October for all other species. The parasitoid population from May through September consisted of over 90% *Eretmocerus*. The parasitoid population consisted of approximately 70% *Eretmocerus* species and 30% *Encarsia* by late October. *Encarsia sophia* represented 85% of the *Encarsia* collected, whereas 15% were *En. luteola* and *En. meritoria* combined. In total, 264 *Eretmocerus* parasitoids were isolated for identification from the field plots, along with *Encarsia* species. Of the entire collection of *Eretmocerus*, 99% of the male and 97% of the female *Eretmocerus* sampled across all plant species were identified as introduced, exotic species (Figure 1).

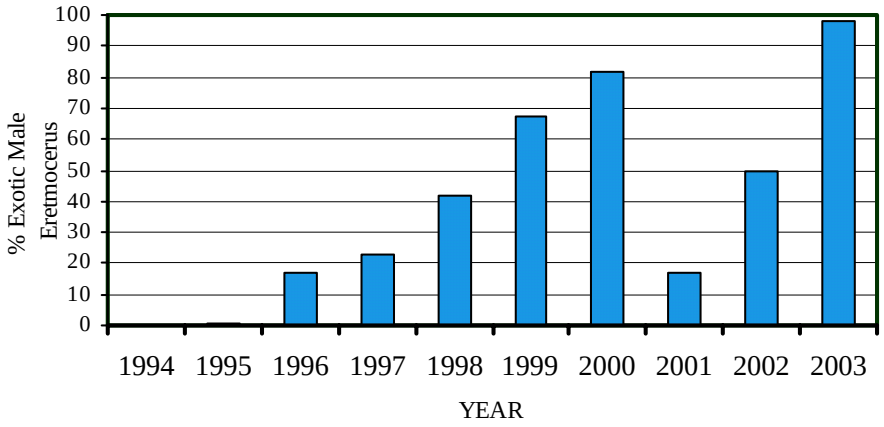

Figure 1. Establishment of silverleaf whitefly exotic parasitoids in the genus *Eretmocerus* in field plots in Imperial Valley, California. No releases were made in study plots following 1997.

The sex ratio of the exotic *Eretmocerus* was somewhat skewed with nearly 60% females. The dominance of exotic *Eretmocerus* is especially noteworthy, given that the native species, *Er. eremicus*, is a commonly occurring biological control agent in the region, especially from mid-summer through fall.

Urban samples came from hibiscus plants in each community sampled on four dates from late July through early October. Results from this urban survey of hibiscus paralleled those obtained from the field plots. Of a total of 195 *Eretmocerus* specimens, 97% of the males and 98% of the females were exotic species. The sex ratio was lower, with 48% of the population composed of females.

Species determination of exotic species is pending for the 2003 collection. Morphological identification (M. Rose) of *Eretmocerus* collected in 2002 determined that the predominant species was *Eretmocerus* sp. nr. *emiratus* (M96076 Ethiopia [48 specimens]). In addition, three specimens were *Er. emiratus* (M95104 United Arab Emirates) and three were *Er. mundus*. A number of specimens were not identifiable, but intermediate in form among these species. Polymerase chain reaction analysis of samples collected from 1997 to 2001 by the United States Department of Agriculture, Animal and Plant Health Inspection Service (USDA-APHIS) indicated that over 95% of the exotic *Eretmocerus* have been *Er.* sp. nr. *emiratus* and *E. emiratus*. The predominant *Encarsia* species collected, *En. sophia* (M95107 Multan, Pakistan), was very common by mid to late summer in the field nursery plots during several of these years. In contrast, *En. sophia* was not common until October during 2003.

In summary, three species of exotic *Eretmocerus* and one species of exotic *Encarsia* are established in Imperial Valley. Based on data collected in 2003, exotic *Eretmocerus* sp. nr. *emiratus* from Ethiopia is the predominant species among parasitoids that attack the silverleaf whitefly. Data from the two field plots combined with data from two communities suggest that this is a regional phenomenon.

Importation and Establishment of Lygus Parasitoids in the San Joaquin Valley and Central Coast of California

C. H. Pickett, K. Casanave, R. Rodriguez, D. Coutinot[1], L. Ertle[2], K. A. Hoelmer[2], M. McGuire[3], and J. Bancroft[3]

Lygus hesperus (Hemiptera: Miridae) is a pest to numerous seed crops including alfalfa (UC Cooperative Extension 2000). To control this pest in seed alfalfa, four to five applications of insecticides are required each season. It is also a serious pest of several field crops across the United States. In California, it causes about $30 million in damage to cotton each year. It is also a serious problem to strawberry growers along the central coast of California at an estimated cost of $40.3 million (www.ipm.ucdavis.edu, Zalom, pers. comm.). Currently Lygus is managed on most crops through applications of broad spectrum insecticides. Cultural and biological alternatives are not considered useful. Importation of nymphal parasitoids into the eastern United States during the 1980's, however, successfully reduced *Lygus lineolaris*, a close relative of *L. hesperus* that also infests alfalfa. We have successfully imported and established both *Peristenus stygicus* and *P. digoneutis* in one small plot of alfalfa in Sacramento. Although these parasitoids have been released at two other southern locations in central California, their populations have not increased at these locations as much as they have in Sacramento. Alfalfa cultural practices at our Sacramento release site differ from other locations and may be favoring establishment of these Lygus parasitoids. This last year, in addition to making releases of parasitoids, we modified sites to more closely resemble the agronomic practices of alfalfa grown at the Sacramento site.

As in past years, parasitoids were reared at the CDFA's Biological Control Program's headquarters in Sacramento during the spring and summer of 2003. A total of 5,224 adult and 28,070 immature parasitoids were released at five locations over the same time period (Table 1). In addition to releasing parasitoids in central California, releases were made at two central coast locations, one for the first time. These were non-crop sites near strawberry production, a crop that also suffers from Lygus damage. Mark Bolda (UC Farm Advisor, Castroville) located the Castroville site, and Ramy Colfer (Mission Organics) helped locate a second site at Harkins Slough near Watsonville.

Parasitism of Lygus persisted and increased into 2003, two years after the last releases of *P. stygicus* and *P. digoneutis*. Parasitism reached 75% topping 2002's high of 60% (Figure 1). Maximum number of Lygus nymphs over the same years has varied from three to 14 per sweep, and as yet shows no sign of decline. On April 9, 2003, we sampled for Lygus in a vacant field 0.3 km from our alfalfa site. *Peristenus stygicus* was found attacking a mix of predominantly *Closterotomus norvegicus* (=*Calocoris*) (Hemiptera: Miridae), and *Lygus* infesting black mustard (*Brassica niger*). Over 60% of these nymphs (n=300) were parasitized. These two findings of increasing parasitism and expansion into outlying fields indicate that these parasitoids are most likely permanently established in the Sacramento region. In contrast, despite additional releases in 2002 and 2003, parasitism has yet to increase significantly at our other central California release sites, including one at the UC Davis (Table 2). On a more positive note, we were surprised by the discovery of parasitoid larvae at our new central coast site. This discovery was made 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).

Table 1. *Peristenus* field release data, 2001 to 2003.

Site	*P. stygicus* Released		*P. digoneutis* Released	Nymphs Transferred	Total Insects
	Spain	Italy			
Fresno County					
UC KAC[1]					
2001	0	2,054	1,736	600	4,390
2002	703	0	268	4,603	5,574
2003	1,065	1,201	0	3,316	5,582
Total	1,768	3,255	2,004	8,519	15,546
Kern County					
Poplar Ave					
2001	2,629	0	479	0	3,108
SREC[2]					
2001	0	1,732	1,889	0	3,621
2002	674	441	763	3,718	5,596
2003	1,305	967	0	4,982	7,254
Total	1,979	3,140	2,652	8,700	16,471
Madera County					
2001	1,050	0	123	0	1,173
2002	204	0	0	0	204
2003	0	0	0	0	0
Total	1,254	0	123	0	1,377
Merced County					
2001	2,324	0	0	550	2,874
2002	316	0	0	0	316
2003	0	0	0	0	0
Total	2,640	0	0	550	3,190
Monterey County					
Castroville					
2002	0	100	776	1,056	1,932
2003	602	630	295	2,906	4,433
Total	602	730	1,071	3,962	6,365
Sacramento County					
Sacramento					
2001	0	1,520	795	0	2,315
Santa Cruz County					
San Juan Bautista					
2001	1,100	156	53	300	1,609
Harkins Slough					
2003	1,200	1,602	0	4,732	7,535
Yolo County					
UC Davis					
2001	0	0	0	0	0
2002	57	596	1,101	4,450	6,204
2003	1,410	248	2,181	16,866	20,705
Total	1,467	844	3,282	21,316	26,909
Total Released					
2001-2003	14,639	11,247	10,459	48,079	84,424

[1] University of California, Kearney Agricultural Research Center, Parlier, CA.
[2] UC/USDA, Shafter Agricultural Research and Experiment Center, Shafter, CA.

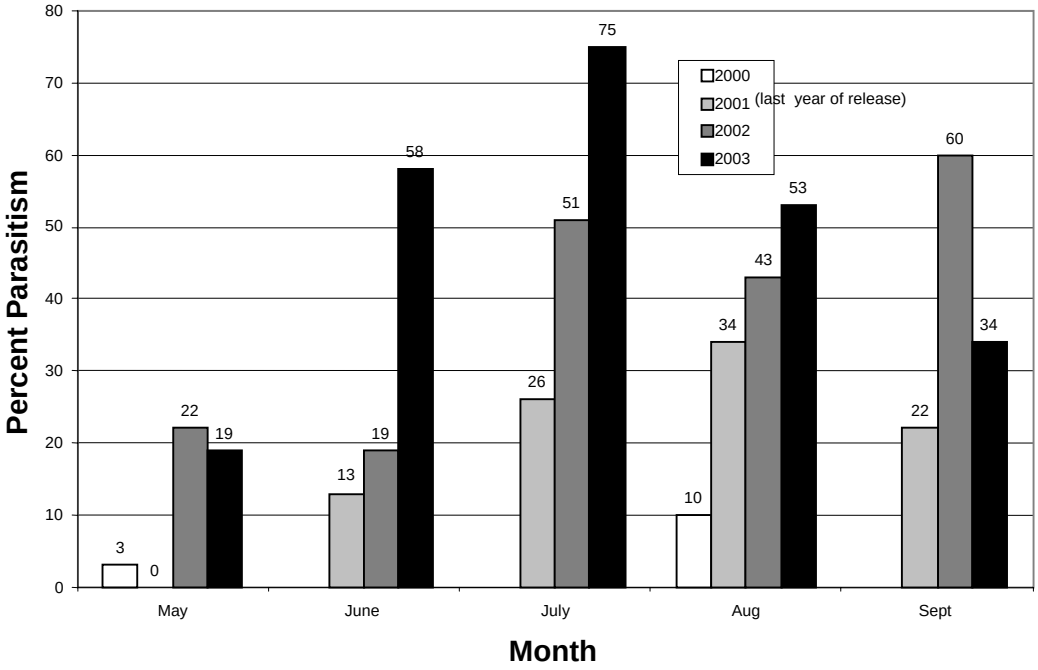

Figure 1. Parasitism of Lygus at the Sacramento site from 2000 to 2003.

Table 2. Recoveries of released parasitoids.

	Maximum Percent Parasitism and Sample Size (Number of Hosts Dissected)		
Location	2001	2002	2003
Sacramento	34.0(32)	60.0(30)	75.0(40)
UC Davis	4.0(50)	2.0(50)	3.5(28)
Merced	14.0(7)	0.0 (0)	
Madera	59.0(2)	0.0(14)	
UC KAC	12.0(25)	10.0(40)	3.3(30)
SREC	5.0(17)	0.0(40)	0.0(45)
Castroville[1]		24.0(25)	7.14(14)
Watsonville (Harkins Slough)[2]			2.5(40)

[1] Releases initiated summer 2002.
[2] Releases initiated summer 2003.

To date, *P. stygicus* has been the dominant species of parasitoid recovered at the Sacramento release site (Table 3). However, the relative proportion of *Peristenus digoneutis* among recovered parasitoids increased to 50% by October 2002. Due to problems with emerging adult wasps, we were unable to replicate the same degree of sampling in 2003.

Table 3. *Peristenus* species recovered at the Sacramento site.

	P. stygicus Recovered		*P. digoneutis* Recovered		Total Parasites Recovered
Date sampled	No.	Percent	No.	Percent	No.
July 2002	76	95.0	4	5.0	80
August 2002	86	82.7	18	16.3	104
October 2002	5	50.0	5	50.0	10
January 2003[A]	8	66.6	4	33.4	12
January 2003[A]	12	85.7	2	14.3	14
July 2003[B]	16	84.2	3	15.8	19

[A] All recoveries were made from soil samples **on two dates.**

[B] Two *P. stygicus* and three *P. digoneutis* recovered from soil samples.

Parasitoids are vulnerable to desiccation and predation as they pupate in the soil. The quality of the soil/thatch interface may also affect their survival. Since the beginning of this project in 1998, alfalfa at the Sacramento site was never baled, but was cut and allowed to decompose. In addition, the alfalfa watered with a sprinkler system rather than surface irrigated that could drown late instar parasitoids, and an insectary garden was planted adjacent to this plot. The organic matter resulting from the cut alfalfa has built up over the last five years. The hay was baled at other sites. Beginning in the spring of 2003, the cutting of alfalfa was changed at the UC Davis (20 km west of Sacramento), UC KAC (ca. 300 km south of Sacramento), and the Shafter UC/USDA Research Center (ca. 560 km south of Sacramento) to mimic that done in Sacramento. The UC Davis and Shafter UC/USDA also switched to sprinkler irrigation. Substantial build-up of thatch began by late summer at all locations. We also planted two rows of insectary plants at the UC Davis release site in the spring of 2003 to further mimic the Sacramento release site where *Peristenus* spp. have successfully colonized. These insectary plants consisted of St. Catherine's Lace (*Erigonum giganteum*), *Ammi visnaga*, and golden yarrow (*Achillea* sp.).

In January 2003, soil samples were taken at the Sacramento release site to verify the overwintering location of parasitoid pupae. We used the same technique to develop baseline information on the impact of the above cultural practices on survivorship of parasitoid pupae in the soil at other release sites in July and October 2003. Soil collections came from the upper two to five centimeters of thatch. Fifteen, one square foot samples were taken using a flat shovel across each release site. Each sample was placed into a paper bag and refrigerated at 50°F until further processing. Samples were then placed into paper cans fitted at one end with a glass vial for collection of emerging adult wasps over a two-month period.

Parasitoids have been recovered only from the Sacramento site (Table 4). Nearly one adult *Peristenus* per ft^2 thatch emerged from a collection made in January, then much fewer in July. No parasitoids were recovered from samples taken at the three other sites.

Table 4. Soil sample recoveries of *Peristenus* species from release sites in 2003.

	Number of Parasites Recovered			
Date Sampled	Sacramento	UC Davis	SREC[1]	UC KAC[2]
January	12	0	0	0
July	5	0	0	0
October	0	0	0	0

[1] UC/USDA Shafter Agricultural Research and Experiment Center.
[2] UC Kearney Agricultural Research Center.

In summary, the results from the last two years of work show that *Peristenus* spp. has most likely become permanently established in the Sacramento region. Recovering *P. stygicus* 300 meters from the original release site in an abandoned field near our release site shows that the population is expanding its range. However, although parasitism has continued to increase since releases ceased, there has been no indication of Lygus suppression at this location. Most likely until parasitoids have expanded several kilometers beyond the initial release site, migration of Lygus into the isolated alfalfa plot will affect our results. The impact on Lygus during a similar effort in eastern United States was not realized until seven years after initial releases. The poor recovery of *Peristenus* from other release sites in central California suggests climate, rather than cultural practices of alfalfa may be limiting its colonization. The rapid recovery of these parasitoids at our central coast sites also indicates a milder climate is important in initial establishment. Other, as yet unidentified native *Lygus* or *Closterotomus* parasitoids found at these sites, but not inland, further suggests that climate is hampering the establishment of the imported *Peristenus*. The continued poor recovery of *Peristenus* at our UC Davis site, only 24 kilometers west of the Sacramento site, on the other hand, suggests that perhaps an unknown factor, other than or in addition to, climate could also be affecting our ability to establish these parasitoids in central California. For example, continuous availability of Lygus to the parasitoids during summer months is likely critical to their initial colonization in alfalfa. Lygus numbers at the UC Davis have been less than half that at the Sacramento site. During mid-summer at the SREC site in 2003, the Lygus nymphal population for unknown reasons dropped to near zero. The release and monitoring of imported *Peristenus* spp. at UC Davis, UC KAC, and the central coast will continue in the summer of 2004.

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[1] USDA-ARS, European Biological Control Laboratory, Montpellier, France.
[2] USDA-ARS, Newark, DE.
[3] USDA-ARS, Shafter Research and Experiment Center, Shafter, CA.

Releases of *Hylobius* Root Weevils on Purple Loosestrife in California

B. Villegas and K. Martyn

The Biological Control Program received a total of 525 adult *Hylobius transversovittatus* weevils for release on purple loosestrife in California in 2003. The weevils were provided by Margorie Gilford, USDA-APHIS, Niles, Michigan. Shipment of these weevils was arranged by Richard Hansen, USDA-APHIS-CPHST, Fort Collins, Colorado. Releases of the weevils were made in Shasta County and Kern County.

A total of 325 weevils were sent to the Shasta County Agricultural Commissioner's Office. Kevin Martyn made the field releases on August 15, 2003. A levee break and subsequent flooding meant that one of the sites had to be a new site as the expected site was underwater. Figure one shows the location of these as well as past releases in the Fall River Valley.

The Kern County release was made with Robert Wegis of the Kern County Agricultural Commissioner's Office. A total of 200 *Hylobius* weevils were released at the Smith Ranch near Onyx in Kern County on August 14, 2003.

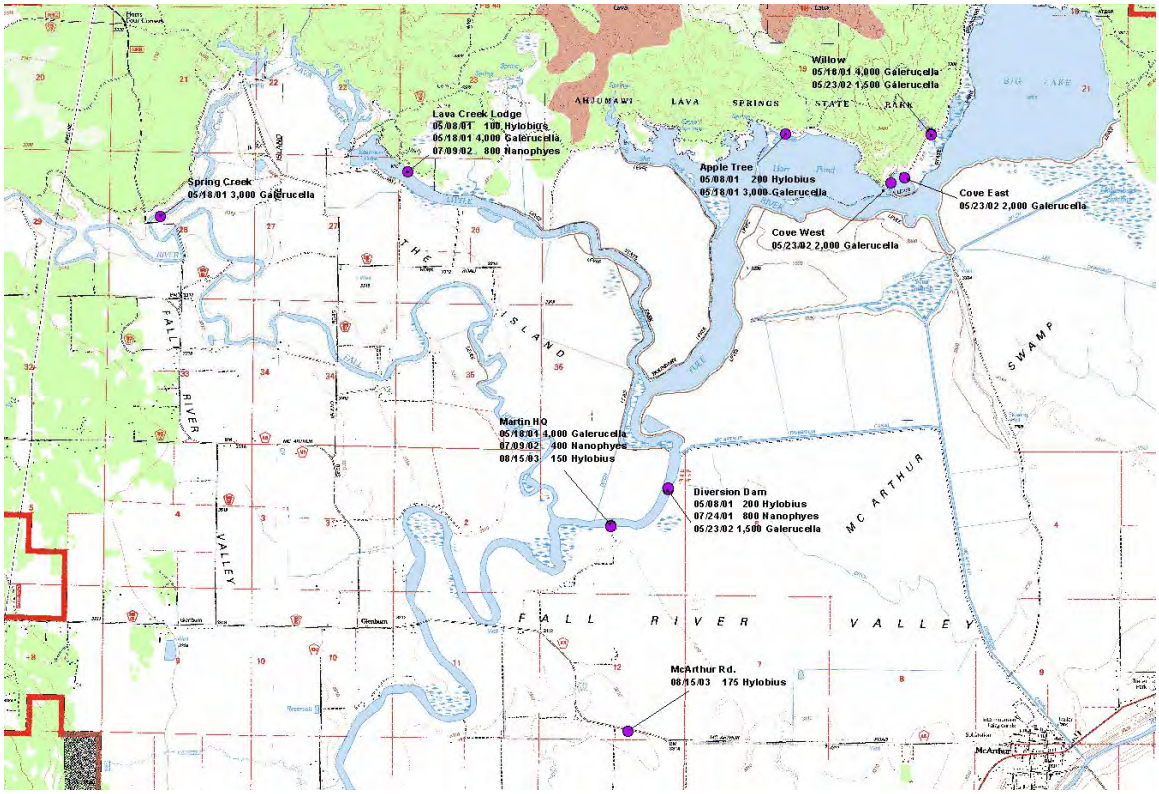

Figure 1. Release locations and release totals of purple loosestrife biological control insects in the Fall River Valley.

[1] Shasta County Department of Agriculture, Redding, CA.

Continued Success with Biological Control Agents of Squarrose Knapweed

D. M. Woods and B. Villegas

Squarrose knapweed, *Centaurea squarrosa*, the most widely distributed knapweed in California, was not targeted for biological control until 1995. The far greater prevalence and distribution of spotted and diffuse knapweeds in North America focused selection of potential natural enemies to these weeds. Host specificity testing prior to introduction suggested that several of these insects could attack more than one species of knapweed so we began releasing biological control agents on squarrose knapweed that had been selected for, established on, and collected from spotted and diffuse knapweed. Following a brief project in Siskiyou County in 1996, we began a larger, monitored project in the Pittville area of Lassen County in 1998. Two species of seedhead weevils, *Bangasternus fausti* (Reitter) (Coleoptera: Curculionidae), and *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae), established rapidly increasing to extremely high levels so that well over 95% of the squarrose seed was destroyed on squarrose plants around the Pittville release site. This early success encouraged us to expand bioagent distribution and monitoring to additional sites to confirm establishment success and impact.

Bioagent population development and impact on squarrose knapweed seed production are shown in Figures 1a and 1b. Site A is the original Pittville release site for *Larinus minutus*. The weevil *B. faustii* was released about 50 meters south of this site. The total infestation rate (either weevil), increased very quickly and has remained over 95% for five years (1999 through 2003). While non-attacked seedheads usually contain one to two seeds per head (mean = 1.44 seed/head), the high infestation rate has reduced mean seed production to less than 0.05 seeds per head, a decrease of 99% (Figure 1b years 2000 through 2003). Site B is several hundred meters east of site A and no insects were intentionally released at this site. The two year delay in bioagent establishment and ultimate seed reduction at site B reflects the time needed for natural dispersal of the biological control insects from site A. Following the delay, however, the 95% attack rate and 99% seed reduction applies. Site C is located several miles distant from sites A and B and did not have insects prior to a large release in 2001. The weevils established readily at site C, however, essentially eliminating seed production at this site in two years. Site D is located about 200 meters north of site C and seems to be confirming the Site B results. Weevil migration from site C to site D has already occurred and the site is perhaps one year behind results from site C. The dramatic results at these sites are being followed by additional monitoring at these sites as well as at new sites infested with squarrose knapweed.

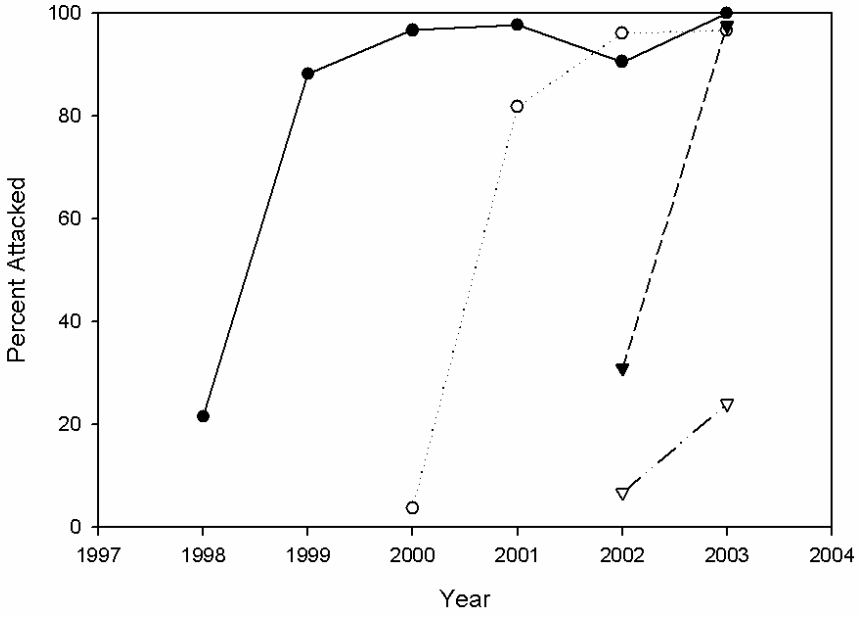

Figure 1a. Seedhead attack of squarrose knapweed

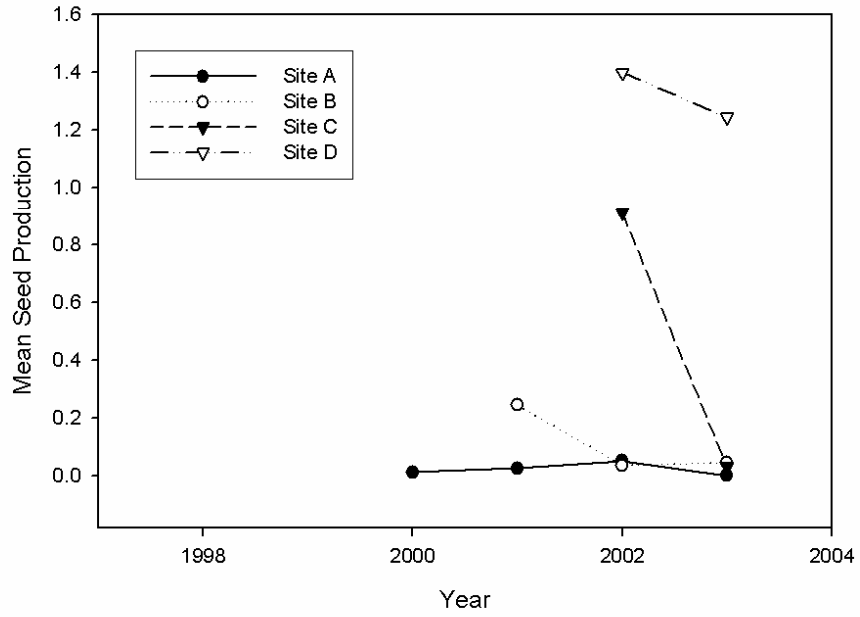

Figure 1b. Seed production of squarrose knapweed

Releases of Three Insects for the Biological Control of Squarrose Knapweed in Northern California in 2003

B. Villegas and C. Pirosko

Three biological control agents (the rootboring beetle *Sphenoptera jugoslavica* Obenberger, and two seedhead weevils, *Bangasternus fausti* (Reitter) and *Larinus minutus* Gyllenhal) were released onto squarrose knapweed in Lassen, Modoc, and Shasta counties during June and July 2003. The releases of these three agents were focused in two large infested areas found during a delimination survey of squarrose knapweed in the fall of 2002. Since these areas were relatively free of these biological control agents at the time of the survey, they were given high priority for releases during the 2003 field season.

Approximately 10,000 *Bangasternus fausti* seedhead weevils were collected from a diffuse knapweed infestation near Medford, Oregon, and released at two sites off of Highway 89 north of Hat Creek in Shasta County on June 11, 2003. On June 30 and July 1, 2003, additional collections of the seedhead weevils, *Larinus minutus* and *Bangasternus fausti*, took place at a well-established knapweed site near Pittville in Lassen County, California. The weevils were released at targeted infestations located along Highway 89 north of Hat Creek in Shasta County and along Muck Valley Road located south of Highway 299 in Lassen County (Table 1).

On June 27, 2003, a total of 200 *Sphenoptera jugoslavica* rootboring beetles were obtained from Steve Miller and Larry Skillestad, USDA-APHIS-Plant Protection and Quarantine, in Spokane, Washington. These rootboring beetles were released at four sites: two along Highway 89 in Shasta County, one along Muck Valley Road in Lassen County, and one site northwest of Lookout in Modoc County (Table 2).

Table 1. Releases of the seedhead weevils, *Bangasternus fausti* and *Larinus minutus*, for the biological control of squarrose knapweed in Northern California in 2003.

BC Agent	Counties	Location	Sites	No. Released	Date
Bangasternus fausti	Shasta	Hwy 89 at Kane Ranch	1	6,000	June 11
Bangasternus fausti	Shasta	Hwy 89 at Charlie Bone Rd	1	5,000	June 11
Larinus/Bangasternus mix	Shasta	Hwy 89 at Kane Ranch	1	4,800	June 30
Larinus/Bangasternus mix	Shasta	Hwy 89 at Charlie Bone Rd	1	3,600	June 30
Larinus/Bangasternus mix	Lassen	Hwy 299 and Muck Valley Rd #1	1	2,400	July 1
Larinus/Bangasternus mix	Lassen	Hwy 299 and Muck Valley Rd #2	1	2,400	July 1
Larinus/Bangasternus mix	Lassen	Hwy 299 and Muck Valley Rd #3	1	1,200	July 1
Larinus/Bangasternus mix	Lassen	Hwy 299 and Muck Valley Rd #3	1	1,200	July 1
Larinus/Bangasternus mix	Lassen	Hwy 299 and Muck Valley Rd #4	1	2,400	July 1
Larinus/Bangasternus mix	Lassen	Hwy 299 and Muck Valley Rd #5	1	1,200	July 1
Larinus/Bangasternus mix	Lassen	Hwy 299 and Muck Valley Rd #6	1	600	July 1
Larinus/Bangasternus mix	Lassen	Hwy 299 near MP21.50 (Ash Creek)	1	600	July 1
Larinus/Bangasternus mix	Lassen	BigValley Lumber Co	1	1,200	July 1
Total			13	32,600	

Table 2. Releases of the rootboring beetle, *Sphenoptera jugoslavica* Obenberger, for the biological control of squarrose knapweed in Northern California in 2003.

County	Site	Sites	No. Released	Release Date
Shasta	Hwy 89 at Kane Ranch	1	90	June 30
Shasta	Hwy 89 at Charlie Bone Rd	1	25	June 30
Lassen	Hwy 299 at Muck Valley Rd	1	25	July 1
Modoc	Lookout	1	25	July 1
Total		4	165	

[1] CDFA, Integrated Pest Control, Burney, CA.

First Field Release of *Puccinia jaceae* var. *solstitialis*, a Natural Enemy of Yellow Starthistle

D. M. Woods, W. L. Bruckart[1], V. Popescu, and M. J. Pitcairn

The first efforts to develop a plant pathogen as a biological control of yellow starthistle, *Centaurea solstitialis* L., occurred in 1978. A rust, *Puccinia jaceae* var. *solstitialis*, was collected in Turkey, brought to the United States, and maintained at the USDA-Agricultural Research Service Foreign Disease-Weed Science Research Unit quarantine greenhouse in Frederick, Maryland. Extensive laboratory and greenhouse experiments provided the host specificity data that was used in a permit request submitted in 2000. The request to release the rust as a biological control in California received final approval in mid June 2003 for a limited number of field releases.

The first release took place on July 9, 2003, at an isolated ranch east of Napa, California. The conditions were poor for release of a pathogen of this nature (too hot and dry with mature, dry plants). However, efforts were made to increase the likelihood of success. The square meter of yellow starthistle to be inoculated was watered with 25 gallons of water to hydrate the soil and increase humidity. A suspension of 40 milligrams of urediniospores in 250 milliliters water and eight drops of a wetting agent was sprayed on the plants at 7:00 p.m. when the plants were no longer in direct sunlight. A plastic tent on a PVC frame was placed over the inoculated plants overnight to increase humidity and simulate dew. The tent was removed in the morning and then replaced again the following night for a second treatment of dew.

A second release was made on July 30, a few meters away from the first release site. Again, the inoculated plants were tented so they could receive two nights of dew. During an August 1 inspection of sample leaves from the first release, we detected the presence of a very few uridiniospores of the rust. A few more pustules appeared during the next week, but most pustules quickly transitioned to producing teliospores. This suggests that the rust perceived a need to transition into the overwintering mode to survive the heat of summer.

We are currently propagating the rust in our greenhouse in Sacramento and storing spores to prepare for eventual statewide release of the biological control agent in 2004.

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[1] USDA-Agricultural Research Service, Foreign Disease-Weed Science Research Unit, Fort Detrick, MD.

Impact of Biological Control Insects on Yellow Starthistle at One Site in Yolo County

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

Biological control of weeds is an inherently long-term process requiring a sustained commitment to post-release monitoring to demonstrate successes and impacts. In 1993, we set up three sites where we could conduct long-term evaluations of the success of biological control on yellow starthistle. This report presents a preliminary look at data collected from one of those three sites. The site is former pastureland west of Woodland California, in Yolo County. Five seedhead attacking insect species were released from 1993 to 1995, and all five species established to varying degrees. They included three weevils, *Bangasternus orientalis* (Capiomont) (Coleoptera: Curculionidae), *Eustenopus villosus* (Boheman) (Coleoptera: Curculionidae), and *Larinus curtus* Hochhut (Coleoptera: Curculionidae), the gall fly, *Urophora sirunaseva* (Hering) (Diptera: Tephritidae), and the false peacock fly, *Chaetorellia succinea* (Costa) (Diptera: Tephritidae). Details of the releases and research methods were presented in earlier reports and are not repeated here.

Attack rates on seedheads have increased progressively over several years, reaching over 70% in the most recent two years (Figure 1). The weevil, *E. villosus,* accounts for the largest proportion of this attack. Seed destruction is the primary direct effect of the seedhead-feeding agents, although the hairy weevil also causes dramatic mortality to flower buds prior to seed production. Seed destruction measures began in 1995, one to two years after the agents had been released but while attack rates were still at low levels. Beginning in 1997, the first declines in seed production were evident (Figure 2). Seed production per seedhead has remained low since that time. Additionally, with a progressive decline in yellow starthistle plant numbers (Figure 3), the total seed production for the site has decreased even more rapidly.

Plant height and density are the most visible factors affecting the public's view of a successful control program. Although plant height was not measured in this study, the progressive decline in plant numbers associated with increases in both the population of biological control insects and the amount of seed destruction suggests that successful long-term control is probably occurring at this site.

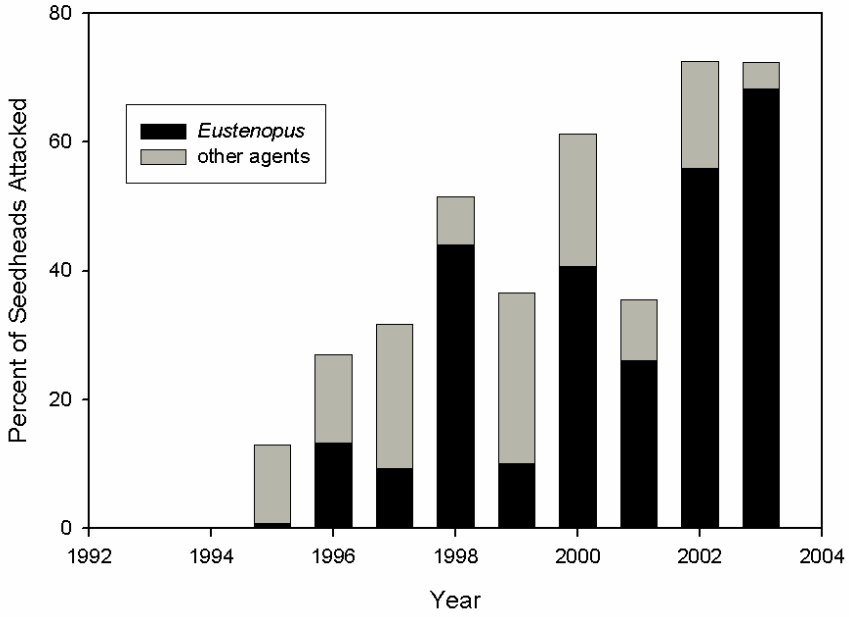

Figure 1. Attack of yellow starthistle seedhead by biological control insects

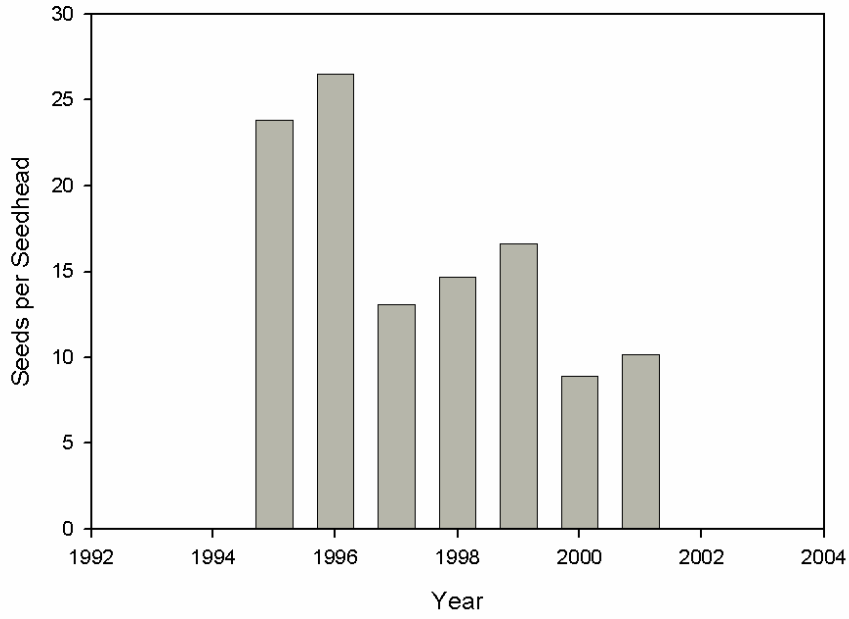

Figure 2. Mean seed production of yellow starthistle seedheads

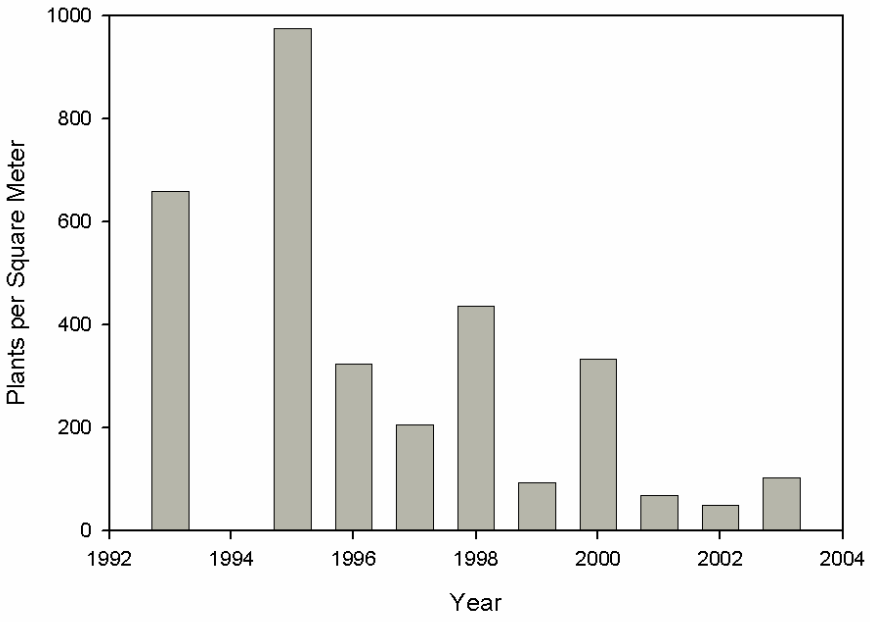

Figure 3. Density of yellow starthistle at Yolo County biological control site

Biological Control of Water Hyacinth in the Sacramento/San Joaquin Delta

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

Water hyacinth (*Eichhornia crassipes*) is a native of the Amazon River basin in tropical South America, but it has spread throughout much of the world and has come to be regarded as one of the worst aquatic weeds. It was introduced into the United States in 1884 as an ornamental plant, and by 1897, it was interfering with shipping in the waterways of the southeastern United States. The plant appeared in California in 1904. Infestations now occur in many natural and man-made waterways below 600 feet elevation in the Central Valley, San Francisco Bay, and the South Coast. The Sacramento/San Joaquin Delta supports heavy infestations of the weed.

In 2002, the CDFA entered into a contractual agreement with the Department of Boating and Waterways to determine the status of biological controls established in the Sacramento/San Joaquin Delta for control of water hyacinth. As part of this effort, a field survey was performed to determine the status and abundance of three biological control insects released in the 1980s. From the survey it appears that only the weevil *Neochetina bruchi* occurs in California, and that both the weevil, *Neochetina eichhorniae*, and the moth, *Sameodes albiguttalis*, failed to establish or are no longer present. In addition, *N. bruchi* was fairly common and abundant at certain times of the year. In 2003, a field study to determine the seasonal occurrence of the eggs, larvae, and adult stages of *N. bruchi* was initiated at two locations in the Sacramento/San Joaquin Delta: Whiskey Slough (San Joaquin County) and Rock Slough (Contra Costa County). A total of 10 adult plants and 10 daughter plants were sampled every two weeks from these locations. All plants were taken to the laboratory, examined for weevil adults and pupae, and then dissected for eggs and larvae. Sampling at Whiskey Slough began in September and at Rock Slough in October.

Whiskey Slough: Larval and adult weevil densities were very high at Whiskey Slough (Figure 1). Sampling in late summer showed that larval numbers were still very high, exceeding 16 larvae per plant in early September. Larval numbers steadily declined over the next six weeks but remained steady at approximately five larvae per plant through December. In contrast, adult numbers steadily increased from early September through mid October then appeared to level off during November and December, fluctuating between seven to 12 adults per plant. The number of weevil larvae and adults recorded in October through December at Whiskey Slough is much higher than published reports of weevil populations in other locations. Interestingly, plant mortality has been reported to occur at densities above five larvae per plant, yet no mortality appears to have occurred in plants at Whiskey Slough with larval densities three times higher. This lack of mortality associated with larval feeding will be further explored in 2004. Another interesting observation was the continued deposition of eggs by weevils despite the cold winter temperatures. It was commonly thought that because this insect is a tropical species with no diapause it would cease egg deposition once the temperatures dropped below 55^{o} F. At Whiskey Slough, however, egg deposition of *N. bruchi* has continued despite daytime temperatures below 55^{o}F in December and January.

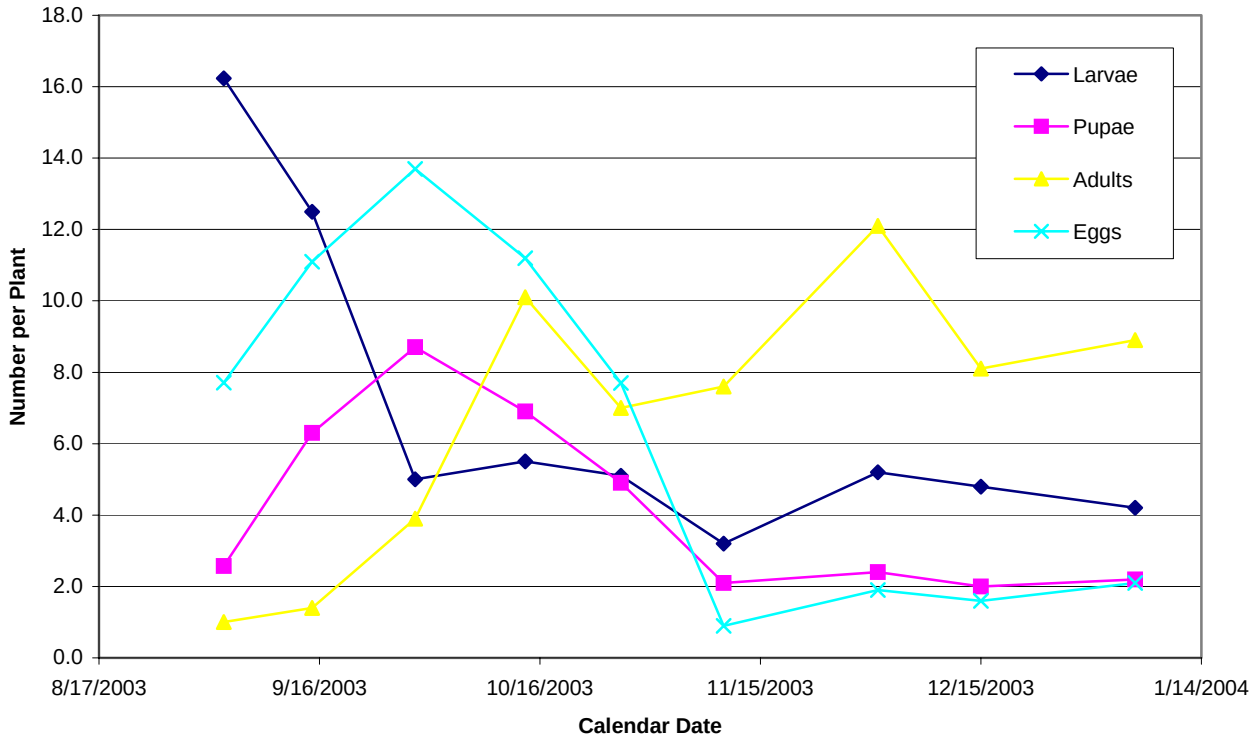

Figure 1. Larval and adult densities (numbers per plant) for the water hyacinth weevil *Neochetina bruchi* at Whiskey Slough in San Joaquin County, California, from September 3, 2003 to January 5, 2004.

Rock Slough: The weevil densities at Rock Slough were substantially lower than those observed at Whiskey Slough (Figure 2). Larval densities ranged between one and five larvae per plant and showed no clear increasing or decreasing trend. Pupal and adult densities at Rock Slough, however, were similar to those observed at Whiskey Slough with approximately one pupa and one adult per plant. The lack of change in adult numbers was similar to observations at Whiskey Slough for the same time period. Interestingly, egg deposition in November and December was higher at Rock Slough (one to four eggs per plant) than observed at Whiskey Slough (one to two eggs per plant) despite a higher number of weevils present at Whiskey Slough. Field monitoring at both the Whiskey Slough and Rock Slough sites will continue in 2004.

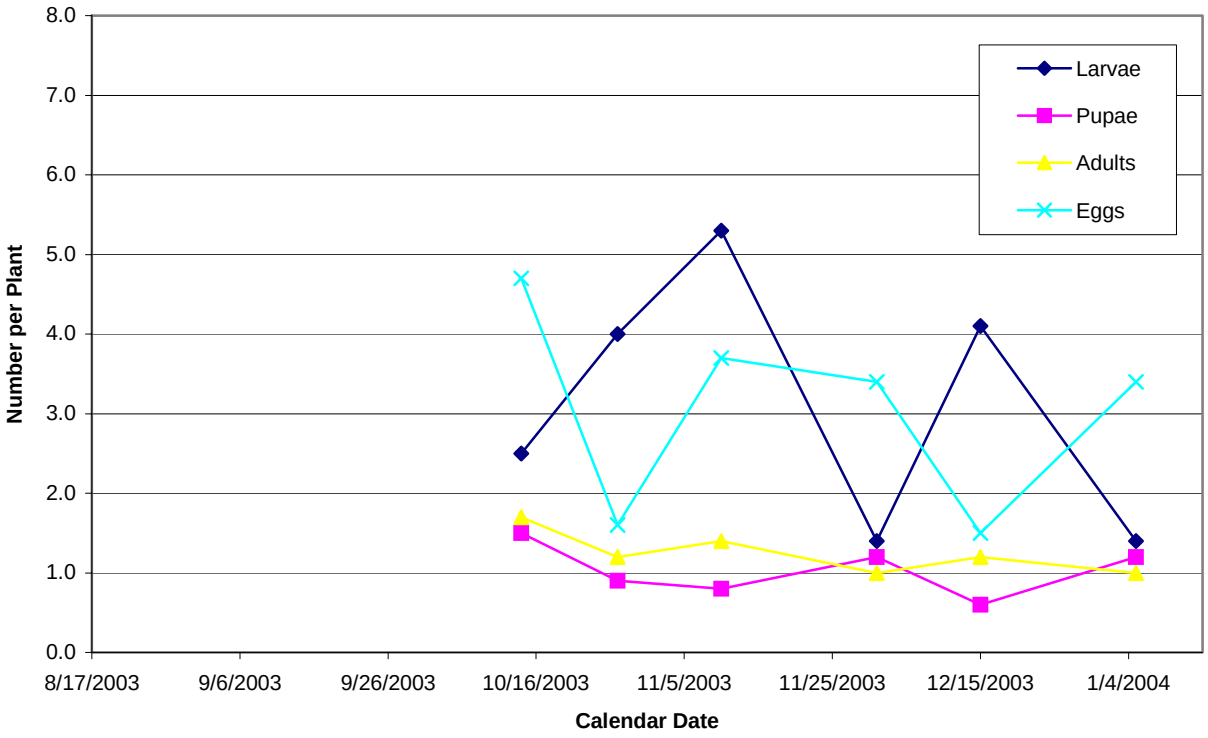

Figure 2. Larval and adult densities (numbers per plant) for the water hyacinth weevil *Neochetina bruchi* at Rock Slough in Contra Costa County, California, from October 14, 2003 to January 5, 2004.

Spotted Knapweed Seed Destruction by Seedhead Attacking Insects

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

Five seedhead-feeding biological control agents are currently established on spotted knapweed, *Centaurea maculosa* Lamarck (Asteraceae), in California. Three of these, *Urophora affinis* Frauenfield (Diptera: Tephritidae), *Terellia virens* (Loew) (Diptera: Tephritidae), and *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae), were intentionally released whereas *Urophora quadrifasciata* (Meigen) (Diptera: Tephritidae) migrated from knapweeds in Oregon and *Eustenopus villosus* (Boheman) (Coleoptera: Curculionidae) spread from yellow starthistle. Since one of the impacts of each of these insects is believed to be the direct destruction or displacement of seeds within the seedhead, we conducted a seed destruction study from 1998 to 2002 at study areas along the Pit River in Shasta County. Individual seedheads with oxidized flowers were enclosed in cotton bags every other week during the flowering season over the five-year period (approximately 50 bagged heads per bagging date per year). At the end of the season, the bagged, mature seedheads were dissected and inspected for viable seed as well as evidence of attack by the seedhead insects. Three sites were evaluated along a two-mile stretch of the river. The hill and plot sites were chosen because insects were intentionally released there while the Log Jam site was chosen as the result of natural movement of the insects, principally the two fly species leaving the slower moving weevils behind.

Seed production and impact of individual insect species are shown in Table 1. Seed production for the clean seedheads (no insects) was fairly consistent from year to year at each individual site. Variation from site to site is likely due to plant age structure and soil type. The Log Jam site consists of relatively young, three to four year old vigorous plants, compared to the older plants populating the other sites. Both the plot and the Log Jam sites are rock and sand soils with no shade, while the hill site is partially shady with sandy soil. Neither of the two *Urophora* gall flies produced dramatic impacts by themselves, in spite of occasionally producing as many as seven to nine galls in a seedhead. Results with *Terellia virens*, which were detailed in last year's annual report, were also equally disappointing because the species caused very little seed destruction. The yellow starthistle hairy weevil, *E. villosus*, had an enormous effect in the few instances when it was present. Heads that *E. villosus* oviposited in produced virtually no seed at all. The hairy weevil, however, greatly prefers yellow starthistle to spotted knapweed. In fact, the only substantial attack on spotted knapweed occurred when early season drought compelled a large population of hairy weevils to leave the shriveled prebloom yellow starthistle and moved to a second quality host. The weevil, *Larinus minutus*, has consistently been the major impact on spotted knapweed at our sites, destroying 60 to 90% of the seeds compared to numbers in clean seedheads.

Many of the seedheads were attacked by more than one agent and these seedheads are included only in the site total for each year, not under individual agents. The most common combinations were one or more of the gall flies along with *Larinus minutus*. Galls were usually partially to completely destroyed in these co-infestation cases. It is interesting, however, that both species of gall fly could coexist within a single seedhead.

Table 1. Seed production of spotted knapweed at three sites along the Pit River in Shasta County. Values are mean seed per seedhead. Superscripts represent the number of samples when the sample size is very small (n=one to three). The dashed values indicate that no collected sample met the criteria.

Hill	1998	1999	2000	2001	2002
No insects	15.7	14.6	16.0	15.5	10.7
Larinus minutus only	0.4	2.8	3.1	5.5	0.9
Eustenopus villosus only	-	0.5	1.7	7^{2}	-
Urophora affinis only	17.2	9.5	13.4	11.1	2.0^{3}
Urophora quadrifasciata only	12.3	11.7	10.6	11.3	-
Terellia virens only	-	-	-	18.5^{2}	-
Site total	11.3	5.9	5.8	8.7	1.4

Plot	1998	1999	2000	2001	2002
No insects	9.5	14.5	12.0	12.4	-
Larinus minutus only	3.2	1.6	1.7	4.2	1.7
Eustenopus villosus only	-	2.4	1.4	3.0^{2}	0^{1}
Urophora affinis only	8.6	2.4	6.1	5.2	4.0
Urophora quadrifasciata only	4.0	6.3	11.4	7.5	0^{2}
Terellia virens only	-	-	8.0^{1}	8.0^{1}	-
Site total	3.4	2.0	2.9	5.4	1.3

Log Jam	1998	1999	2000	2001	2002
No insects	-	-	-	17.6	17.8
Larinus minutus only	-	-	-	5.7^{3}	0^{1}
Eustenopus villosus only	-	-	-	-	-
Urophora affinis only	-	-	-	12.2	11.1
Urophora quadrifasciata only	-	-	-	11.5	9.6
Terellia virens only	-	-	-	11.9	9.0
Site total	-	-	-	13.8	8.0