

Biological Control of Weeds
Joe Balciunas Biennial Research Report (1999-2000)

United States Department of Agriculture - Agricultural Research Service
Western Regional Research Center - Exotic & Invasive Weed Research Unit
800 Buchanan St., Albany, California 94710
(510) 559-5975 FAX: (510) 559-5982



Executive Summary

This report summarizes the research during the calendar years 1999 and 2000 that was directed by Research Entomologist, Joseph Balciunas, at the USDA-ARS Exotic & Invasive Weed Research Unit in Albany, California.

Our primary project during this period was yellow starthistle (YST), *Centaurea (Cnt.) solstitialis*. YST is the most widespread weed in California, and is among the most damaging in adjacent states. In 1996, we found an unintentionally introduced fly, *Chaetorellia (Ch.) succinea*, attacking YST in California and Oregon (see Sections II B-F in this report). Our surveys, in cooperation with CDFA, have shown this fly to have spread throughout California, to parts of Nevada, Oregon and Washington, sometimes infesting as much as 70% of a YST flower heads. Seed production in YST heads attacked by *Ch. succinea* can be reduced by as much as 80%. In order to determine the population dynamics of *Ch. succinea* and how the phenology of YST influences its impact, we established a long term study at a site, Wildcat Canyon Regional Park, 10 minutes from our Albany Lab. For the past two years, we have monitored the emergence of *Ch. succinea* at this site, and the flowering of YST. A large brood of *Ch. succinea* emerges in August, approximately a month after the peak flowering event in July, but relatively few *Ch. succinea* survive the winter.

Overseas scientists had previously rejected *Ch. succinea* as a potential biological control agent for YST because it might attack safflower. With the assistance of the CDFA, we conducted surveys to determine its distribution, and launched field and laboratory studies to determine its impact. We evaluated the host range of *Ch. succinea* on five safflower varieties and on native and exotic *Cirsium* thistles at our greenhouse facilities in Albany. *Ch. succinea* only developed on YST and all varieties of safflower tested. However, our field surveys during 1998 of safflower head samples collected from 45 fields in California, showed negligible impact on commercial safflower crops by this fly. However, the impact of this fly on native thistles was unknown. In July 1999, we conducted a survey of native and exotic *Cirsium* thistles at 15 sites in four California counties. We looked for *Ch. succinea*, and found no evidence that it had oviposited and developed on these thistles.

The weevil *Larinus curtus*, a biocontrol agent for yellow starthistle, was first released in 1992, but seems to have only marginally established. With assistance from state and university agencies, we documented this weevil's density and impact at all the original release sites in CA, ID, OR, and WA. Cooperating insect pathologists determined the infection rates of *Nosema* at each site. Although *Nosema* infection rates varied from zero to 50%, there seems to be little correlation with the lack of impact on YST by this weevil.

Six YST seed-destroying insects have been intentionally released and five have established. Despite these agents [and the unintentionally released *Ch. succinea*], YST continues to spread, and additional agents from overseas are needed. Joe Balciunas personally surveyed for additional agents in Eurasia during 1996 and 1997. He established both formal and informal agreements with overseas scientists to conduct research on a promising root-boring apionid weevil, and to search for additional potential agents. During 1999, the project leader reviewed the initial research by cooperators in Turkey, and planned for the expansion of next year's surveys of the natural enemies of YST. He also prepared protocols for our cooperators to initiate

comparative studies of YST ecology. This project should provide us with more information about the insects attacking YST in its native range, as well as other potential biological control agents for this weed, such as the promising apionid - *Ceratapion basicorne*.

Biological control on *Onopordum acanthium* (Scotch thistle), a very serious pest to grazing livestock in many states, began in 1996. We have ruled out one insect – *Lixus cardui* as a biocontrol agent due to environmental safety concerns. Currently we are finishing evaluating another *Lixus* new sp. from France, and a new species of *Trichosirocalus* weevils. The larvae of latter bore into the rosettes of Scotch thistle, and often kill the rosette. Unfortunately, we have found this weevil to oviposit on native thistles and artichoke - a commercially grown plant.

Cape ivy (*Delairea odorata*) is a South African vine that smothers native vegetation in riparian zones along the California coast, and may be toxic to aquatic organisms. Surveys began in South Africa in April of 1998 to identify the natural enemies. The third year of research in South Africa is wrapping up, and first potential agents will be imported in early 2001 to be tested in our quarantine, and, hopefully, will be released after we show that they pose no threat to native vegetation.

There has been increasing concerns about the safety of biological control, and standards for conducting research are needed. In July, during the Xth International Symposium for Biological Control of Weeds, the Code of Best Practices for Classical Biological Control of Weeds - authored by the project leader, was ratified. As this code is more widely adopted, the safety and acceptance of this sub-discipline of biological control will be greatly enhanced.

1999-2000 Biennial Research Report

prepared by Joe Balciunas, Chris Mehelis, and Maxwell Chau

Research presented in this report was performed under the guidance of Dr. Joe Balciunas.

List of Staff and Cooperators

Staff

Joe Balciunas, Project Leader (Jan. 1, 1996 to present)
Chris Mehelis, Biological Science Technician (April 28, 1998 to present)
Maxwell Chau, Biological Science Technician (March 1, 1999 to present)
Eve Lednický, Biological Science Technician (July 7, 1999 to present)

Cooperators:

Academy of Sciences - St. Petersburg, RUSSIA
CSIRO - Canberra, AUSTRALIA
ENEA - Rome, ITALY
Plant Protection Research Institute – Pretoria, SOUTH AFRICA
University of Çukurova - Adana, TURKEY
USDA-ARS, European Biological Control Laboratory (EBCL), FRANCE
USDA-ARS, Reno, Nevada
USDA-ARS, Fredrick, Maryland
USDA-ARS, Fresno, California
California Agricultural Commissioners & Sealers Association
California Department Food & Agriculture (CDFA)
California Exotic Pest Plant Council
California Native Plant Society
California Polytechnic State University, San Luis Obispo
California State Parks and Recreation
East Bay Municipal Utility District (EBMUD)
Golden Gate National Recreation Area
Nez Perce Indian Tribe - Bio-Control Center
Oregon Dept. Agriculture (ODA)
University of California - Berkeley
University of California - Davis
University of Idaho (UI)
University of Nebraska
Washington State University (WSU)

Publication of results prohibited: The results in this report are preliminary and tentative. In order to prevent the spread of out-of-date or inaccurate information, this report should not be quoted or cited without verifying accuracy with the Research Leader, Exotic & Invasive Weed Research Unit, USDA - ARS - Western Regional Research Center.

List of Acronyms and Abbreviations

List of Acronyms

ARS	Agricultural Research Service (an agency of USDA)
APHIS	Animal and Plant Health Inspection Service (an agency of USDA)
CA	California
CalEPPC	California Exotic Pest Plant Council
CDFA	California Department of Food and Agriculture
CINWCC	California Interagency Noxious Weed Coordinating Council
CNPS	California Native Plant Society
CSIRO	Commonwealth Scientific and Industrial Research Organization
EBCL	European Biological Control Laboratory, USDA-ARS
EIW	Exotic & Invasive Weed Research Unit, USDA-ARS, Albany, California
FY	Federal Fiscal year (Oct. 1 to Sept. 30)
ID	Idaho
IPM	Integrated Pest Management
NRA	National Recreation Area
NV	Nevada
OR	Oregon
ODA	Oregon Department of Agriculture
PPRI	Plant Protection Research Institute (an agency of the Agricultural Council of the Republic of South Africa)
ScT	Scotch thistle, <i>Onopordum acanthium</i>
TAG	Technical Advisory Group for Biological Control of Weeds
USDA	United States Department of Agriculture
WA	Washington
WRRC	Western Regional Research Center - USDA - Albany, California
YST	Yellow starthistle, <i>Centaurea solstitialis</i>

List of Generic Abbreviations

<i>Crd.</i>	<i>Carduus</i> thistles
<i>Crt.</i>	<i>Carthamus</i> thistles
<i>Cp.</i>	<i>Ceratapion</i> weevils
<i>Cnt.</i>	<i>Centaurea</i> knapweeds and starthistles
<i>Ch.</i>	<i>Chaetorellia</i> flies
<i>Cir.</i>	<i>Cirsium</i> thistles
<i>Eu.</i>	<i>Eustenopus</i> weevils
<i>Lr.</i>	<i>Larinus</i> weevils
<i>Lx.</i>	<i>Lixus</i> weevils
<i>Ono.</i>	<i>Onopordum</i> plants
<i>Sen.</i>	<i>Senecio</i> plants
<i>Tr.</i>	<i>Trichosirocalus</i> weevils

Table of Contents

Executive Summary	I
List of Staff and Cooperators	III
List of Acronyms and Abbreviations	IV
Table of Contents	V
List of Tables	VII
List of Figures	VIII
I. The Western Weeds Quarantine Facility	1
A. A Short History of the Western Weeds Quarantine Facility 1944-1998	1
B. Staff Changes during 1999-2000 at Biological Control of Weeds, Albany, CA	4
II. Yellow Starthistle (<i>Centaurea solstitialis</i> , Asteraceae)	5
A. Introduction	5
B. <i>Chaetorellia succinea</i>	7
1. Introduction	7
2. <i>Chaetorellia succinea</i> Host Range Tests	8
a. 1998-2000 <i>Chaetorellia</i> Choice and No choice Oviposition / Development Tests on Safflower	9
b. 1996-2000 <i>Chaetorellia</i> No choice Oviposition / Development Tests on Various Thistles of the tribe Cardueae	13
C. Surveys for Phytophagous Insects on Cardueae Thistles	15
D. Yellow Starthistle Research at Wildcat Canyon	17
a. 1999-2000 Wildcat Canyon Research	17
b. Wildcat Canyon seed bank and seedling recruitment studies	30
E. 1998-1999 <i>Chaetorellia</i> Distribution Surveys	34
F. <i>Larinus curtus</i>	40
1. Introduction	40
2. <i>Nosema</i> sp. Surveys	40
3. <i>Larinus curtus</i> Distribution and Impact Surveys	43
G. Yellow Starthistle Research in Eurasia	47
1. Introduction	47
2. Cooperative Research on Yellow Starthistle in Turkey	47
3. Joe's Asia Minor Trip	48
4. <i>Ceratapion basicorne</i>	49
III. Scotch thistle (<i>Onopordum acanthium</i> , Asteraceae)	53

A. Introduction	53
B. <i>Trichosirocalus</i> new species	54
IV. Cape ivy (<i>Delairea odorata</i> , Asteraceae)	59
A. Introduction	59
B. Cooperative Research in South Africa – the Native Home of Cape Ivy	60
C. 2000 Research in South Africa	63
D. Cape ivy Toxicity Tests	64
E. <i>Senecioneae</i> Germination and Test Plant Lists	66
V. Code of Best Practices	71
VI. Seed Inventory	73
VII. Selected Meetings and Travel by Dr. Joe Balciunas	75
VIII. Publications Issued or Submitted	79
IX. References Cited	81
X. Appendices	85
Appendix A- Protocols - 1998-1999 <i>Chaetorellia</i> Surveys	85
Appendix B- Protocols - 1999 <i>Larinus curtus</i> Surveys	86
Appendix C- Scotch thistle Research and Planning Meeting, Pendleton, OR, April 1 st , 1999	87
Appendix D- 1999-2000 South Africa Trip Logs	89
Appendix E- Quarantine Laboratory Shipment Records	92

List of Tables

Table 1. A list of agents imported and released in the U.S., for biocontrol of yellow starthistle.	6
Table 2. Oviposition / development by <i>Chaetorellia succinea</i> on five safflower varieties and on yellow starthistle controls in no choice tests.	10
Table 3. Oviposition / development by <i>Chaetorellia succinea</i> on five safflower varieties and yellow starthistle in choice tests.	12
Table 4. Oviposition / development by <i>Chaetorellia succinea</i> on various thistles of the tribe Cardueae in no choice tests.	14
Table 5: 1999-2000 tribe Cardueae thistle survey results.	16
Table 6. Data from 1999-2000 collections to Wildcat Canyon YST site.	20
Table 7: Summary of 1998-1999 <i>Chaetorellia succinea</i> distribution studies in California and Nevada using J. Balciunas' <i>Chaetorellia</i> sampling protocol.	36
Table 8: Summary of 1998-1999 <i>Chaetorellia succinea</i> distribution studies in Oregon using J. Balciunas' <i>Chaetorellia</i> sampling protocols.	37
Table 9: Summary of 1998-1999 <i>Chaetorellia</i> infestation rates on YST sampled in California using various sampling protocols.	38
Table 10. USDA <i>Larinus curtus</i> release and <i>Nosema</i> sp. test summary.	42
Table 11: Summary of 1999 <i>Larinus curtus</i> distribution and impact surveys.	44
Table 12: Summary of <i>Chaetorellia</i> sp. impact at <i>Larinus curtus</i> release sites.	45
Table 13: Summary of the impact of other YST agents at <i>Larinus curtus</i> release sites.	46
Table 14. Summary of <i>Ceratapion</i> sp. reared from various thistles of the tribe Cardueae.	50
Table 15. Results of <i>Ceratapion basicorne</i> day - night feeding studies.	51
Table 16. Scotch thistle biocontrol agents released in Australia.	53
Table 17: <i>Trichosirocalus</i> new sp. no choice oviposition tests, Fall-Winter 1999-2000.	56
Table 18: <i>Trichosirocalus</i> new sp. no choice development tests, Winter 2000-2001.	57
Table 19: <i>Trichosirocalus</i> new sp. choice oviposition tests, Fall-Winter 2000-2001.	58
Table 20: <i>Senecio</i> seeds tested for germination for future Cape ivy host range testing.	67
Table 21: A list of host plants we wish to test Cape ivy biological control agents on.	68

List of Figures

Figure 1. Number of USDA-ARS [SY's] working on biological control of weeds at Albany and assigned overseas, from 1944-2000.	2
Figure 2. Current quarantine facilities at USDA's Western Regional Research Center.	3
Figure 3. A photo of the extra thoracic "spot" on <i>Ch. succinea</i> as compared to <i>Ch. australis</i>	8
Figure 4. The Wildcat Canyon site.	17
Figure 5. The Maddox head-classification scheme (Maddox 1981).	19
Figure 6. YST heads of each stage per square meter from 1999 and 2000 collections at Wildcat Canyon.	23
Figure 7. <i>Chaetorellia succinea</i> emergence per square meter from different YST head stages.	24
Figure 8. The mean female and male <i>Chaetorellia succinea</i> emergence per square meter from each collection for 1999 and 2000.	25
Figure 9. Charts of daily <i>Chaetorellia succinea</i> emergence per square meter for each collection during 1999 and 2000.	28
Figure 10. Summary of the YST seed bank at the Wildcat Canyon study site.	30
Figure 11. A summary of the year 2000's seedling recruitment study.	32
Figure 12. A map of the western US showing sites where YST was sampled for <i>Chaetorellia succinea</i> distribution, as well as impact of other YST biocontrol agents.	34
Figure 13. Slides of <i>Nosema</i> sp. from infected <i>Larinus curtus</i>	41
Figure 14. A map of <i>Larinus curtus</i> release sites and 1999 sampling sites.	43
Figure 15. <i>Ceratapion basicorne</i> illustration by an associate of Dr. Boris Korotyaev.	52
Figure 16. A <i>Trichosirocalus</i> new sp. adult, and its larval damage on ScT rosettes.	54
Figure 17. An example of no choice oviposition tests of <i>Trichosirocalus</i> new sp. on <i>Onopordum acanthium</i>	55
Figure 18. Cape ivy growing in some of its diverse habitats: A. On a stream bank in South Africa. B. In the Manuka Forest in Hawaii. C. On Mt. Sutro in San Francisco, CA.	59
Figure 19. <i>Diota</i> damage rating scale.	62
Figure 20: Average <i>Diota</i> damage rating per leaf for various host plants.	62

I. The Western Weeds Quarantine Facility

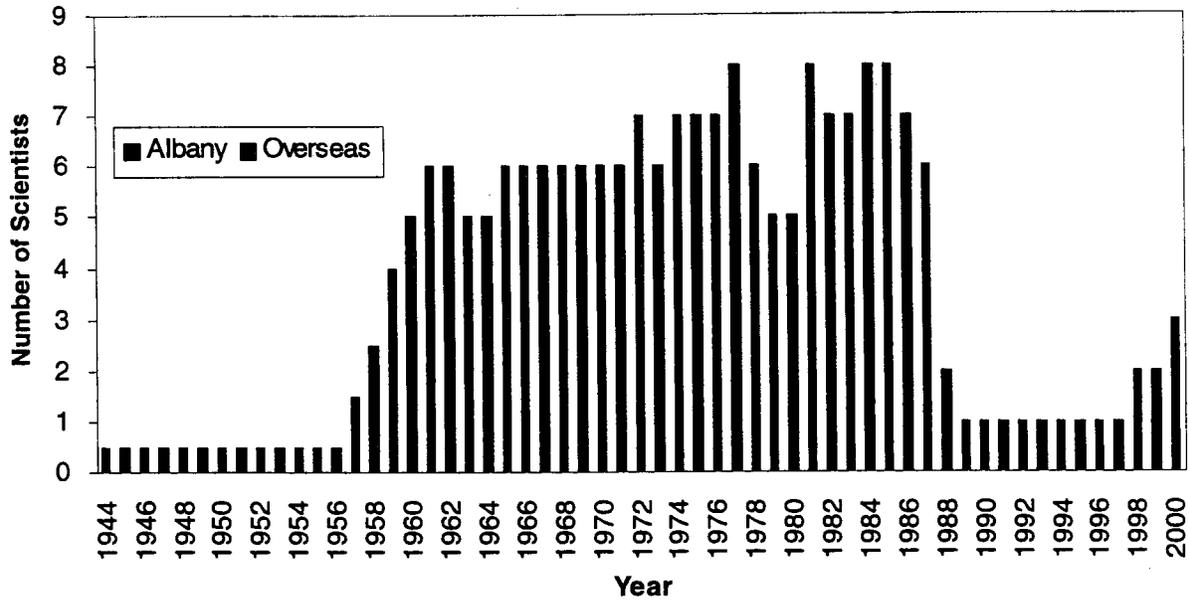
A. A Short History of the Western Weeds Quarantine Facility 1944-1998

Widespread exotic pests are obvious targets for classical (importative) biological control, which attempts to re-associate an exotic pest with carefully selected and screened natural enemies from its native range. Potential biocontrol agents for weeds undergo extensive host range testing to assure their safety to the environment. In the U.S., prior to release, an array of federal and state agencies review the host range tests and other information about the proposed agent. Ultimately formal approval for release is granted by the United States Department of Agriculture-Animal and Plant Health Inspection Service (USDA-APHIS), as well as by the state(s) where the release will take place. The feasibility of using classical biological control as a tool in managing exotic weeds was first demonstrated during the late 1920's and early 1930's, with the control of prickly pear cactus in Australia with *Cactorum*, a moth introduced from South America. Another Australian project, the control of *Hypericum perforatum*, caught the interest of Professor Harry Smith, Univ. of California - Berkeley, and Jim Holloway, USDA Bureau of Entomology and Plant Quarantine (now part of ARS). This bush - known in Oregon and Washington as "goat weed", and as "Klamath weed" in northern California - now has the "approved" common name of "St. John's wort." This European native is widely established at many temperate locations throughout the world, and had become a severe problem in the western United States, infesting over two million acres in California alone. They set up a cooperative project, and in 1944, imported three species of chrysomelid beetles, which the Australians had earlier imported from Europe for the control of this weed. One of the chrysomelid leaf beetles, *Chrysolina quadrigemina*, established readily and soon provided excellent control of Klamath weed in California, reducing infested acreage to one percent of the former level. Control of this weed in other parts of the Northwest, such as Washington and Oregon, has been less satisfactory, even after the release of several other agents.

The success of the *Hypericum* project led to the construction, in 1963, of USDA's first biological control of weeds laboratory, several miles NW of the Univ. of California's Berkeley Campus, on the University's Gill Tract in Albany. This particular site was chosen because of its proximity to the University's Division of Biological Control, easy access to the University libraries, proximity to an international airport, and its climate. The Mediterranean-type climate allowed the lab to work on weeds occurring throughout the entire U.S., even weeds in such diverse areas as Florida, the arid southwest, the Pacific Northwest, and the Great Plains. Dr. Holloway served as USDA's Director of this facility until his death in 1964. Dr. Lloyd Andres succeeded him and remained in that role until his retirement in 1988. The period from the mid-sixties through the early eighties was a golden era for weed biocontrol in the U.S. (Figure 1). The Albany scientists imported and tested over 70 insect species, of which 54 were released against 24 weed targets throughout the U.S. (Balciunas and Mehelis, 1999). Successful biological control projects during this period were tansy ragwort (*Senecio [Sen.] jacobaea*), puncturevine (*Tribulus terrestris*),

alligatorweed (*Alternanthera phylloxeroides*), musk thistle (*Carduus [Crd.] nutans*), and water hyacinth (*Eichhornia crassipes*). Also, during this period, the number of scientists in the United States, working on weed biocontrol increased to 15, of which the four scientists stationed at Albany served as a nucleus, directing many of the domestic projects throughout the U.S., as well as all of the foreign research programs.

Figure 1. Number of USDA-ARS scientists [SY's] working on biological control of weeds at Albany, and assigned overseas, from 1944 through 2000.



In 1986, USDA scientists moved nearby to a new quarantine facility at USDA's Western Regional Research Center (WRRC) (Figure 2). Ironically, in 1987, most of the staff were transferred to a new ARS weed lab in Bozeman, Montana, leaving the new Albany facility nearly vacant. Following Dr. Andres' retirement in 1988, Dr. Charles Turner was left as the sole scientist at the Albany quarantine. Despite several attempts at closing the quarantine facility, Dr. Turner was able to continue biocontrol research there through 1995.

Figure 2. Current quarantine facilities at USDA's Western Regional Research Center



At the end of 1995, after 11 years in Australia conducting research on biological control of *Hydrilla* and *Melaleuca*, Dr. Joe Balciunas transferred to the quarantine from USDA's Australian Biological Control Laboratory (ABCL). In a "job swap" with Joe, Dr. Charles Turner became the Director of ABCL in January 1996. Tragically, Charley was diagnosed with cancer at the end of his first year in Australia, and he succumbed to the disease after returning to his mother's home in Indianapolis, Indiana, on April 15, 1997. His interest and counsel on western weeds has been sorely missed.

Although Dr. Balciunas was able to obtain new, outside resources for research on new targets, "base funds" from USDA for the quarantine continued to be problematic. Insufficient base funding at the end of our federal [FY-97] fiscal year in Sept. 1997 led to the loss of our only permanent support staff-person, Kathy Chan. This resulted in termination/postponement of the host range testing in quarantine containment, and the focusing on field evaluations of *Chaetorellia* flies. Fortunately, Kathy transferred to another project at WRRC, so we are still easily able to access her knowledge about the quarantine facility and the research conducted here during her ten-year tenure.

Early in 1998, USDA permanently increased the base funds for the yellow starthistle (YST) project here by \$50,000 annually. Thus, we were able to advertise and hire a replacement permanent technical assistant. At the end of April 1998, Mr. Chris Mehelis, who completed his M.S. in Entomology at Oregon in 1995, joined the project. We quickly resumed quarantine evaluations of potential agents for YST [Section II] and Scotch thistle [Section III].

Additional positive changes continued during 1998. Our one-scientist project at Albany was administratively combined with the two scientists in Davis, California studying aquatic weeds

and the three scientists in Reno, Nevada who study range management, into the new Exotic & Invasive Weed (EIW) Research Unit. At the beginning of July 1998, Dr. Ray Carruthers, an entomologist who for past four years had served as the ARS National Program Leader for Biological Control, was added to our unit, and serves as Research Leader. Ray is based in Albany, and in addition to his administrative tasks, conducts research on biological control of saltcedar (*Tamarix* spp.) and giant reed grass (*Arundo donax*).

B. Staff changes during 1999-2000 at Biological Control of Weeds, Albany, CA

The past two years 1999-2000, have maintained the growth of the biological control of weeds group at Albany, now a part of the Exotic and Invasive Weed Research Unit (EIW), which had been formed in 1998. Joe Balciunas was able to add another full-time assistant, Maxwell Chau, in March of 1999, and a ½ time greenhouse assistant, Eve Lednicky, in July 1999. At the same time, with great relief, Joe was able to relinquish his Quarantine Officer duties to Nada Carruthers, a USDA-APHIS scientist detailed to Albany. At the end of 2000, the Research Leader's assistant, John Herr, took over the Quarantine Officer role.

In October 2000, a third full-time ARS scientist (SY) was added to the biocontrol of weeds group in Albany. Dr. Lincoln (Link) Smith, a Research Entomologist, formerly with the USDA-ARS biological control of weeds group in Sidney, Montana, accepted the position at Albany to work on biological control of yellow starthistle (YST) and Russian thistle (also known as tumbleweed). Joe has now focused his research on the expanding Cape ivy project, and is assisting Link in assuming the reins for the YST project.

II. Yellow Starthistle (*Centaurea solstitialis*, Asteraceae)

A. Introduction

Yellow starthistle (YST) has been the primary target for this project at USDA-EIW-Albany, and receives most of our research efforts. YST is an annual weed native to the eastern Mediterranean region of Eurasia. It was introduced into California more than 150 years ago (Maddox and Mayfield 1985). It is now the state's worst weed, and is also causing severe problems in parts of Oregon, Washington, and Idaho. Rangelands infested with YST are unproductive due to disruption of grazing by this weed's sharp spines and a neurologic disorder produced in horses if digested (Cordy 1978). In California, the area infested increased from an estimated 1.2 million acres in 1958 to 7.9 million acres in 1985 (Maddox and Mayfield 1985). YST's logarithmic range expansion continues. A 1997 survey by California Department of Food and Agriculture (CDFA) found this weed in 42 % (n = 1,935) of California's 4,638 townships - each six by six mi. square - and in 22 % (1,019 townships) the infestations are reported as "high" (Pitcairn *et al.* 1998). "High" abundance was defined as being, at a minimum, several miles of dense roadside infestation.

The control of YST will be costly and needs to be justified. Traditionally, most economic losses have been assigned to agriculture and included loss from deaths of poisoned horses, loss of production on infested pastureland, but also loss from control and management costs. However, losses also occur from roadside vegetation management, reduction of recreational value, and impact on biodiversity. An economic analysis will provide an estimate of the yearly savings once YST is controlled. Although California has the largest economic losses due to this weed, this information is needed for each state.

Overseas surveys to locate potential biocontrol agents for YST began in Europe 40 years ago, and seven insect species to date, all of which attack the flowers or seeds of YST, have been released in the United States for control of this invasive weed (Table 1).

Table 1. A list of agents imported and released in the U.S., for biocontrol of yellow starthistle.

Biocontrol agent	Date of release	Status
<i>Urophora jaculata</i> (Diptera: Tephritidae)	1969	Never established in North America
<i>Urophora sirunaseva</i> (Diptera: Tephritidae)	1984	Widely established, present at most YST infestations. Minimal impact on YST.
<i>Bangasternus orientalis</i> (Coleoptera: Curculionidae)	1985	Widely established, present at most YST infestations. Minimal impact on YST.
<i>Chaetorellia australis</i> (Diptera: Tephritidae)	1988	Established, but only at locations where <i>Cnt. cyanus</i> is also present. Minimal impact on YST.
<i>Eustenopus villosus</i> (Coleoptera: Curculionidae)	1990	Well established, being redistributed. Some localized reductions of YST populations.
<i>Chaetorellia succinea</i> (Diptera: Tephritidae)	1991	Accidentally introduced, well established in CA and parts of NV. May be reducing YST populations.
<i>Larinus curtus</i> (Coleoptera: Curculionidae)	1992	Established at a few release sites in CA, OR, WA, and ID. Limited impact.

The first insect *Urophora jaculata*, released in 1969, never established. Fifteen years later *Urophora sirunaseva* - a gall fly - was released, and *Bangasternus orientalis* - a seed head weevil - one year after that. Both are well established, but are not inflicting the kind of damage to YST that was hoped for. Two more seed head weevils - *Eustenopus (Eu.) villosus* and *Larinus (Lr.) curtus* were released in the early 1990s. The impact of *Lr. curtus* was studied in 1999 and is discussed in depth in section II, subsection E. *Eu. villosus* appears to be causing localized YST reductions at sites in Washington, Oregon, Idaho, and California. Cooperative studies with the California Department of Food and Agriculture (CDFA) Biological Control Program on the population dynamics of the biocontrol agents and their impact on YST seed destruction and are being conducted at three field release sites in northern California. *Eu. villosus* is now well established, and is currently the most important insect at the three sites, having increased to high levels and inflicting the most damage to YST. At two of the three sites, YST populations and/or seed production may be declining (Woods *et al.* 1998).

B. *Chaetorellia succinea*

1. Introduction

The fourth insect to be approved and released in the U.S. for control of YST was the seed head fly *Chaetorellia (Ch.) australis*. This fly's larvae feed inside the seed heads of YST, destroying most of the developing seeds. Mature larvae overwinter in old heads, and the adults emerge in the spring. Females oviposit on maturing buds, and more generations are completed before winter. Releases of *Ch. australis*, reared from YST heads shipped from Greece, began in 1988. By 1994, this fly had been released at 14 sites in California, Idaho, Oregon, and Washington. However, establishment was confirmed only at one site each in Oregon and Washington, and in 1995, at one of the Idaho sites (Turner *et al.* 1996). Establishment of this fly was not observed at any of the six California sites (Turner *et al.* 1996). However, in 1998 & 1999 we collected *Ch. australis* at one release site and off YST in adjacent areas (Section D). At the three sites (in Idaho, Oregon, and Washington) where it did establish, *Centaurea (Cnt.) cyanus*, (bachelor's button) was widespread. *Cnt. cyanus* is another exotic annual, closely related to YST, which is invasive in the Pacific Northwest. It was theorized that the early-blooming *Cnt. cyanus* flowers were acting as an alternate host until YST blossomed some weeks later (Turner *et al.* 1996).

Buoyed by these successful establishments, with the assistance of the CDFa, the colonization effort for *Ch. australis* in California was renewed, with releases at seven sites in seven counties during 1995, and a further 15 releases in 12 counties during 1996. Sites containing both *Cnt. cyanus* and YST were given the highest priority, and second priority was given to sites with early blooming YST. All flies released were those that emerged from YST heads (except one sample from *Cnt. cyanus*) collected at the Merlin, Oregon site. During CDFa's surveys at the end of 1995, populations of this fly were found at multiple locations in Humboldt and Trinity Counties in northern California. The populations in these counties were so large and wide-spread, that we surmised that they were the result of natural migration from the long-established populations at the Merlin, Oregon release site (107 mi. away), rather than from our releases earlier that year in Shasta and Siskiyou Counties. By late 1996, flies were recovered from all of the 1995-96 release sites, indicating at least temporary establishment.

The ease with which these flies from Oregon established at all sites, including those that lacked *Cnt. cyanus*, along with their rapid dispersal from the release sites, was unexpected - especially in light of the complete failure of the earlier releases in California. Specimens of the flies recovered from the field in California were submitted to two experts on fly taxonomy at the CDFa Plant Pest Diagnostics Center. Neither Dr. Louie Blanc nor Dr. Eric Fisher thought that these California flies fit the published description of *Ch. australis*, and Dr. Fisher identified them as either *Ch. succinea*, a similar species from Europe and Asia or *Ch. carthami*, an incidental pest of safflower in the Middle East.

After receiving these preliminary identifications, all further releases of *Chaetorellia* flies in California were immediately curtailed, due to the potential negative environmental effects of this

accidental introduction. We assembled *Chaetorellia* specimens recovered from field sites in California, Oregon, and Washington, and shipped these, along with voucher *Chaetorellia* specimens from those originally imported and tested at the ARS quarantine in USDA-Albany, California, to Dr. Ian White at the British Museum of Natural History, London for confirmation. Dr. White is an authority for the genus *Chaetorellia*, and had recently published a revision of this genus (White and Marquardt 1989). He confirmed that the majority of *Chaetorellia* specimens from California and Merlin, Oregon were, in fact, *Ch. succinea*. White and Marquardt (1989) place the nine known species of *Chaetorellia* into two groups, with *Ch. succinea* belonging to the *Ch. loricata* group, and *Ch. australis* to the *Ch. jaceae* group. *Ch. succinea* (and the other two species of the *Ch. loricata* group) each have an extra “spot” on each side of its thorax that is lacking in *Ch. australis* and the other five species in its group (Balciunas and Mehelis 1999). Since no other members of the *Ch. loricata* have been recorded in North America, we use this extra “spot” (shown in Figure 3) as an easy way to distinguish it from all other *Chaetorellia* flies found here. Additional details on the discovery and accidental release of this fly are presented in Balciunas and Villegas (1999).

Figure 3. A photo of the extra thoratic “spot” on *Chaetorellia succinea* (on left) as compared to *Chaetorellia australis* (on the right).



At the end of 1998, the results of the surveys in California showed that *Ch. australis* has established in a few scattered release sites, all of which have *Cnt. cyanus* in addition to YST. On the other hand, *Ch. succinea* is now well established and spreading rapidly. It is widespread from southwestern Oregon to as far south as Stockton, California. In late 1998, we also recovered this fly from several sites around Reno, Nevada. Since then *Ch. succinea* has continued to expand its geographic range.

2. *Chaetorellia succinea* Host Range Tests

The host range of *Ch. succinea* was evaluated on several commercially-grown safflower

(*Carthamus [Crt.] tinctorius*) varieties and on native and exotic *Cirsium (Cir.)* thistles at our greenhouse facilities in Albany. We conducted several no choice and choice oviposition / development tests on these targets using *Ch. succinea* that emerged from YST heads collected at Wildcat Canyon (see section II, subsection C), as well as *Ch. succinea* from other sites in California and Nevada. To date, *Ch. succinea* has not developed on anything except YST and the varieties of safflower we tested.

a. 1999 *Chaetorellia* choice and no choice oviposition / development tests on safflower

Scientists who had investigated the host range of *Ch. succinea* in Europe (Sobhian and Zwölfer 1985) expressed concern that this fly might attack safflower. Safflower is an important crop in California with almost 157,000 acres (valued at over \$65 million) grown in 19 counties in 1996. In 1997 we discovered *Ch. succinea* emerging from heads collected from a field of safflower variety Cargill 44 grown non-commercially in Tehama County. In 1998 and 1999 we revisited this site and from heads collected, estimated that the infestation rate was greater than 5% in 1998 (Balciunas and Mehelis 1999). In addition, during the same year, the CDFR conducted an extensive survey, sampling 42 commercial and 3 non-commercial fields in California, however, they found no *Chaetorellia* (Villegas *et al.* 1999).

We tested safflower varieties CalWest var. 4440, CalWest var. 1221, and CalWest var. 88-ol (oleic) for *Ch. succinea* oviposition and development because they are currently grown commercially (Woods, personal comm.). We also tested safflower variety Cargill 44 because it is the only variety we have found *Ch. succinea* on in the field. Safflower variety SeedTec 541 was tested in 1996, at that time it was favored by commercial growers.

We collected *Ch. succinea* within one to three days after they had emerged in our laboratory from YST heads collected from one of four field sites: Rancho Cordova - Sacramento Co., CA, Red Bluff - Tehama Co., CA, Willow Creek - Humboldt Co., CA, or Wildcat Canyon Park - Contra Costa Co., CA. We tested these newly emerged flies for oviposition and development on safflower by confining them in sleeve cages (72.9 x 41.9 x 48.9 cm) or screen cages (121.9 x 91.4 x 91.4 cm) to safflower plants consisting of one of five varieties for each test. Safflower plants tested each had at least one mature closed head appropriate for oviposition and development. For the duration of each test, confined flies were supplied a nectar source of Mountain Dew soda (Coca-Cola Company). Tests varied in length due to waiting time for YST heads to develop, but generally were run for 21 to 36 days to allow sufficient time for *Ch. succinea* oviposition, development and maturation on safflower heads.

After the tests were completed, flies were removed and the safflower were kept alive for at least three weeks to allow developing *Ch. succinea* to mature and were then monitored for adult emergence. The safflower heads were later decapitated and the heads were kept for 1-2 more weeks before dissecting to verify the presence or absence of *Ch. succinea*.

To confirm that flies used in these tests were ovipositional, we tested them for oviposition and development on greenhouse-grown YST plants (free of *Ch. succinea*), their host on their (presumed) native range. In these YST control post tests each YST plant had several mature closed heads appropriate for oviposition and development (Bu4 or older). Flies were confined to YST in sleeve cages for 21 days, then removed from the YST plants. YST plants were kept alive for one to two more weeks to monitor for *Ch. succinea* emergence. Once the plants dried out, the YST was decapitated. The heads allowed to dry out further for 1-2 weeks, and were dissected to confirm the presence or absence of *Ch. succinea*. Voucher specimens are kept at the USDA-WRRC.

Ch. succinea emerged from all safflower varieties tested in these no choice conditions (Table 2).

Table 2. Oviposition / development by *Chaetorellia succinea* on five safflower varieties and on yellow starthistle controls in no choice tests.

Test	Duration (days)		<i>Ch. succinea</i>		Safflower				Yellow starthistle control			
	Population†	n (♀)	Variety	Heads	% of heads developed	Heads developed / ♀	n (♀)	Heads	% of heads developed	Heads developed / ♀	P	
CH-1-96	63	RC	12	CalWest 4440	11	18	0.17	12	274	41	9.40	0.000*
CH-2-96	63	RC	12	SeedTec 541	15	0	0.00					
CH-1-98	14	RB	12	Cargill 44	13	17	0.17	4	43	26	2.80	0.335
CH-2-98	24	RB	12 ^a	SeedTec 541	15	13	0.17	4	29	17	4.25	0.028*
CH-12-99	36	W	7	Cargill 44	5	20	0.14	5	0 ^b			-
CH-21-99	31	WC	7	CalWest 1221	16	25	0.57	6	58	67	6.50	0.000*
CH-27-99	21	WC	10	CalWest 88-ol	14	21	0.30	2	29	45	6.50	0.000*
CH-28-99	21	WC	10	CalWest 1221	15	67	1.00	0 ^c				-

In the first two tests, one yellow starthistle control test was run simultaneously with the two *Ch. succinea* no choice oviposition / development tests on safflower, each using different flies. For remaining tests, the yellow starthistle control was exposed to flies that had survived the no choice oviposition / development test.

† *Ch. succinea* populations (all reared from yellow starthistle except RB - reared from safflower):

- RC - Rancho Cordova, Sacramento County, CA
- RB - Red Bluff, Tehama County, CA
- W - Willow Creek, Humboldt County, CA
- WC - Wildcat Canyon, Contra Costa County, CA

* significant ($P < 0.05$; Pearson's Chi-squared test).

^a Initially 12 males and 12 females, all died or escaped; later added 5 males and 12 females.

^b No flowering yellow starthistle available.

^c No female *Ch. succinea* adults survived on the safflower portion of the test.

For each experiment we compared the safflower and YST control tests using Pearson's Chi-squared test, to determine whether there were any significant differences in *Ch. succinea* oviposition / development. Because the number of females used in each test differed between the safflower and YST control tests, we divided the number of heads oviposited on by the number of females used in each test. In the safflower tests, this value was always less than one. To transform these values to a whole integer suitable for Chi-squared analysis, we multiplied the number of heads oviposited per female by 100, then rounded to the nearest whole number.

Interestingly in these no choice tests, *Ch. succinea* oviposited on all safflower varieties tested, in seven of the eight tests. However, of the six tests analyzed, four showed the number of YST heads oviposited to be significantly greater (P value < 0.05) than the number of safflower heads oviposited on.

We conducted choice experiments on the five varieties of safflower. Similar to no choice tests, male and female *Ch. succinea* were collected within one to three days after they had emerged from YST heads collected earlier from sites: Rancho Cordova - Sacramento Co., CA, Wildcat Canyon Park - Contra Costa Co., CA, Ione - Amador Co., CA YST or the Red Bluff site - Tehama Co., CA. These flies were confined in screen cages (121.9 x 91.4 x 91.4 cm) with 4 to 16 safflower plants comprising of one of five varieties of safflower for each test: SeedTec 54, CalWest var. 4440, CalWest var. 1221, CalWest var. 88-ol (oleic), and Cargill var. 44, as well as 4 to 10 greenhouse grown YST plants (free of *Ch. succinea*). These plants tested had mature closed heads appropriate for oviposition / development (Bu4 or older). In each test the confined flies were supplied a nectar source of Mountain Dew soda. Tests were run for 14 or 21 days to allow for *Ch. succinea* maturation and oviposition on to safflower or YST.

After the tests were completed, flies were collected. The safflower and YST were separated and kept alive for at least two weeks to allow any developing *Ch. succinea* to mature and were monitored for adult emergence. Later, heads of plants tested were dissected to confirm the presence or absence of *Ch. succinea*.

Ch. succinea either emerged from YST or developed to pupal stage in YST heads in all the experiments, but only developed in safflower variety CalWest 88-ol in these choice experiment conditions (Table 3).

Table 3. Oviposition / development by *Chaetorellia succinea* on five safflower varieties and yellow starthistle in choice tests.

Test	Duration (days)	<i>Ch. succinea</i>		Safflower				Yellow starthistle control			P *
		Population†	n (♀)	Variety	Heads	% of heads developed	Heads developed / ♀	Heads	% of heads developed	Heads developed / ♀	
CH-3-96	14	RC	8	CalWest 4440	53	0	0.00	384	39.6	19.00	0.000
CH-4-96	14	RC	8	SeedTec S541	48	0	0.00	328	42.4	17.40	0.000
CH-23-99	21	WC	20	CalWest 88-ol	19	0	0.00	74	91.9	3.70	0.000
CH-24-99	21	WC	20	CalWest 1221	27	0	0.00	66	69.9	2.30	0.000
CH-25-99	21	WC	20	CalWest 88-ol	24	4.2	0.05	87	64.4	2.80	0.000
CH-34-99	21	WC	13	CalWest 4440	15	0	0.00	33	9.1	0.23	0.002
CH-8-00	14	WC	10	Cargill 44	10	0	0.00	36	41.7	1.50	0.000
CH-9-00	14	I	10	Cargill 44	6	0	0.00	30	13.3	0.40	0.006
CH-11-00	14	SB	10	Cargill 44	6	17	0.10	7	85.7	0.60	0.000

†*Ch. succinea* populations (all reared from yellow starthistle):

- RC - Rancho Cordova, Sacramento County, CA
- WC - Wildcat Canyon Park, Contra Costa County, CA
- I - Ione, Amador County, CA
- SB - Sutter's Butte, Butte County, CA

* all tests were significant ($P < 0.05$; Pearson's Chi-squared test).

Similar to the no choice experiments, we analyzed these choice experiments to determine whether there were any significant differences ($P < 0.05$) in *Ch. succinea* oviposition / development on safflower and YST using Pearson's Chi square test.

Of the nine choice experiments run, *Ch. succinea* oviposited on safflower in two; in all experiments YST were oviposited on. On a per female basis, the number of YST heads oviposited on was found to be significantly greater ($P < 0.05$) than the number of safflower heads oviposited on.

Although *Ch. succinea* oviposited on all varieties of safflower we tested under no choice conditions, under choice conditions, *Ch. succinea* rarely oviposit on safflower when given a choice of YST. Furthermore, despite an extensive search of 45 California safflower fields, we have not recovered *Ch. succinea* from safflower in the field, with the exception of one field of

non-commercially grown Cargill 44 variety safflower (Red Bluff site). For these reasons we believe that *Ch. succinea* poses a minimal risk to safflower. This is discussed further in Balciunas and Villegas (2001).

b. *Chaetorellia* no choice oviposition / development tests on various thistles of the tribe Cardueae.

Because of the concern that this accidentally introduced biocontrol agent might develop on native thistles, we evaluated its host range on several species of thistles from the tribe Cardueae in no choice tests (Table 4). These tests were run similar to the no choice oviposition / development tests on safflower varieties.

Table 4. Oviposition / development by *Chaetorellia succinea* on various thistles of the tribe Cardueae in no choice tests.

Test	Duration (days) of test	<i>Ch. succinea</i>		Test Plant	YST Control*						P	
		Population	n (♀)		Species	Heads	% of heads developed on	Heads developed on / ♀	n (♀)	Heads		% of heads developed on
CH-5-96	63	Sacramento	12	<i>Cirsium brevistylum</i>	38	0	0	0	274	41	9.40	<0.001
CH-1-99	35	Washoe, NV	9	<i>Cirsium brevistylum</i>	6	0	0	4	9	44	1.00	<0.001
CH-20-99	21	Safflower- Tehama	5	<i>Centaurea calcitrapa</i>	53	0	0	3	38	29	12.60	<0.001
CH-26-99	22	Contra Costa	5	<i>Carthamus baeticus</i>	8	0	0	4	15	66	2.50	<0.001
CH-30-99	21	Contra Costa	6	<i>Silybum marianum</i>	5	0	0	5	17	29	3.40	<0.001
CH-31-99	22	Contra Costa	10	<i>Carthamus baeticus</i>	22	0	0	6	13	54	2.30	<0.001
CH-32-99	21	Contra Costa	7	<i>Silybum marianum</i>	3	0	0	4	17	47	2.00	<0.001
CH-34-99	21	Contra Costa	4	<i>Carduus nutans</i>	2	0	0	3	14	57	2.70	<0.001
CH-1-00	21	Contra Costa	10	<i>Centaurea sulphurea</i>	12	33	0.4	9	10	50	0.55	0.015
CH-3-00	21	Ione	10	<i>Centaurea sulphurea</i>	8	25	0.2	6	29	48	2.33	<0.001
CH-5-00	21	Ione	3	<i>Cirsium ochrocentrum</i>	1	0	0	2	11	18	1.00	0.014
CH-6-00	21	Ione	6	<i>Centaurea sulphurea</i>	6	0?	?	6	12	67	1.33	<0.001
CH-7-00	21	Ione	5	<i>Cirsium occidentale</i>	5	0	0	2	13	31	0.50	<0.001
CH-10-00	14	Lafayette	5	<i>Centaurea maculosa</i>	10	0	0	3	6	67	1.33	<0.001
CH-11-00	14	Lafayette	8	<i>Cirsium brevistylem</i>	3?	0?	0?	7	17	53	1.29	<0.001
CH-12-00	14	various	8	<i>Centaurea cyanus</i>	30	0	0	4	12	58	1.75	<0.001
CH-14-00	14	Sutter's Butte	10	<i>Centaurea cyanus</i>	38	0	0	6	13	39	0.83	<0.001
CH-15-00	14	Sharpe Depot	6	<i>Cirsium occidentale</i>	7	0	0	0	-	-	-	-

* In the CH-5-96 test, the YST control test was run simultaneously with *Ch. succinea* no choice oviposition / development tests on *Cir. brevistylum* using different flies. Consequent tests used flies surviving no choice oviposition / development tests in post YST control tests.

We did not find any *Ch. succinea* oviposition or development on any of the thistle heads tested. We analyzed these tests to determine whether the differences were significant ($P < 0.05$) with Fisher's exact test. As expected, the number of thistle heads oviposited or developed on were significantly less than YST heads. These tests coincide with our 1998-2000 Cardueae thistle survey of which we did not find any plants oviposited on by *Chaetorellia* flies other than YST and safflower (Section C). We have not found *Ch. succinea* to oviposit or develop on anything except YST, safflower and *Cnt. melitensis* in the field.

C. Surveys for Phytophagous Insects on Cardueae Thistles

A survey of native and exotic Cardueae thistles (primarily thistles of the genus *Cirsium*) at 15 sites in one Oregon and four California counties was conducted in 1999 and 2000. The purpose of this survey was twofold. First, we checked if *Ch. succinea* - an accidentally introduced biological control agent for yellow starthistle - had oviposited and developed on these thistles; we found none. Secondly, we looked for other insects infesting these thistle heads.

We collected the above-ground vegetative portions of plants at field sites and brought these back to our laboratory. We segregated each plant's heads and stems in emergence boxes, which are sealed cardboard boxes (36.5 cm³) mounted with a protruding clear plastic shell vial. The vial provides a small source of light in the box which attracts adults emerging from the heads and stems. The vials were checked regularly to record the different insects emerging. Specimens are kept at the USDA-WRRC.

Table 5 shows the genus of insects that emerged from the thistles we collected. The most common insects to have emerged have been the introduced weevil *Rhinocyllus conicus*, two tephritid flies; *Orellia occidentalis* and *Paracantha gentilis*, and the sunflower moth *Homeosoma electellum*. Our 1998 survey with the assistance of CDFA, showed similar results.

CDFA continued surveys of *Cirsium* thistles in 1999. Their work is published in Villegas *et al.* 2000. To summarize: 17 species of thistles were surveyed in 1998 and 1999 and no *Chaetorellia* flies emerged from their collections.

Table 5: 1999-2000 tribe Cardueae thistle survey results.

County*	Location	Date	Species & Common name	YST present	Phytophagous insect emergence H-head, S-stem
Tehama	Driscoll Ranch, 6 miles NE of Red Bluff	7-19-99	<i>Carthamus tinctorius</i> Safflower	Yes	
Tehama	Driscoll Ranch, 6 miles NE of Red Bluff	7-19-99	<i>Centaurea solstitialis</i> Yellow starthistle	N/A	H-Anobiid, <i>Eustenopus</i>
Shasta	On Hwy 299, 2 miles E of Burney in road cut	7-19-99	<i>Cirsium occidentale</i> Snowy thistle	Yes	H- <i>Orellia</i> , <i>Paracantha</i> S-Pentatomidae
Shasta	On Hwy 299, 2 miles E of Burney in road cut	7-19-99	<i>Centaurea maculosa</i> Spotted knapweed	Yes	
Shasta	On Hwy 299, 2 miles E of Burney in road cut	7-19-99	<i>Centaurea solstitialis</i> Yellow starthistle	N/A	H- <i>Ch. succinea</i>
Shasta	Fall River Mills, in front of Ft. Crook museum	7-19-99	<i>Centaurea solstitialis</i> Yellow starthistle	N/A	H- <i>Ch. australis</i> , <i>Ch. succinea</i>
Shasta	Fall River Mills, in front of Ft. Crook museum	7-19-99	<i>Centaurea cyanus</i> Bachelor button	Yes	H- <i>Ch. australis</i>
Lassen	On Hwy 299, 1 mile W of Big Valley Summit	7-19-99	<i>Cirsium cymosum</i> Peregrine thistle	No	
Modoc	On Hwy 299, E of Adin near milepost 9	7-19-99	<i>Cirsium cymosum</i> Peregrine thistle	No	H- <i>Homeosoma</i> , <i>Rhinocyllus</i> S-Diptera, Hemiptera
Modoc	On Hwy 299, E of Canby prior to Co Rd 175	7-19-99	<i>Cirsium cymosum</i> Peregrine thistle	No	H-Diptera, <i>Homeosoma</i> , <i>Paracantha</i> , <i>Platyptilia</i> , <i>Rhinocyllus</i> S-Diptera, <i>Rhinocyllus</i>
Modoc	E of Alturas, Woodchuck Rd at Schuler ranch	7-20-99	<i>Cirsium ochrocentrum</i> Yellowspine thistle	partial	H-Coleoptera, <i>Homeosoma</i> , <i>Rhinocyllus</i>
Modoc	1.9 miles N of Co Rd 9 on Surprise Valley Rd.	7-20-99	<i>Cirsium douglasii</i> Swamp thistle	No	H- <i>Homeosoma</i> , <i>Rhinocyllus</i>
Modoc	12 miles S of Co Rd 9 on Surprise Valley Rd.	7-20-99	<i>Cirsium arvense</i> Canada thistle	No	H- <i>Homeosoma</i> , <i>Orellia</i>
Modoc	On Hwy 299, 2 miles W of Cedarville	7-20-99	<i>Cirsium occidentale</i> Snowy thistle	No	H-Coleoptera, <i>Homeosoma</i> , <i>Platyptilia</i> S- <i>Platyptilia</i>
Modoc	Alturas, corner of Co Rd 15 & refuge HQ Rd	7-20-99	<i>Cirsium vulgare</i> Bull thistle	partial	H- <i>Platyptilia</i> , <i>Rhinocyllus</i> S-Diptera
Curry - Oregon	Road 3680 Snow Camp Rd Jct.	8-31-00	<i>Cirsium remotifolium</i>	???	H- <i>Rhinocyllus</i> S- Hemiptera, Coleoptera

* All sites are located in California with the exception of the Curry, Oregon site.

D. Yellow Starthistle Research at Wildcat Canyon

In 1999, we initiated a study at Wildcat Canyon Park of the East Bay Regional Park District to obtain information on the phenology of YST, how it relates to the biology of *Ch. succinea*, and to monitor the impact of *Ch. succinea* on YST. Furthermore, we wanted to establish a reliable source of *Ch. succinea* for use in our laboratory host range tests.

We selected Wildcat Canyon as a site because of the abundance of YST and *Ch. succinea* there, as well as the site's close proximity to our laboratory. The site is about a 15 minute drive from our laboratory, and approximately one mile from the Alvarado staging area trail head. Vehicular access is restricted to a few authorized vehicles [including ours]. We selected a small patch (10-20m²) at the bottom of a hill, out of view from the trail above it.

Figure 4: The Wildcat Canyon site.



a. 1999-2000 *Chaetorellia succinea* research at Wildcat Canyon.

We confirmed the presence of *Ch. succinea* at the Wildcat Canyon site in April 1999. We believe that *Ch. succinea* arrived at the site late in 1998. We periodically checked the site until mid-July 1999, when the YST had begun to develop mature heads (Bu4). In 2000, we began collecting in June, as heads were developing sooner than the previous year. We then collected YST plants from the site about every two weeks. To collect YST plants we then clipped off at their base. We removed all YST plants within seven 0.05 m² plastic circles along a transect through the site. The circles were randomly selected along the transect; however, we ensured

that they were generally spread along the length of the transect. The height and number of heads in each size class was recorded, as was the number of plants in each circle.

We brought back all YST plants collected during these trips to our laboratory and used the Maddox head classification scheme to classify the age of the heads (Maddox 1981) (Figure 5).

We added another age grade - senesced heads - these are F2 heads excepts the florets has begun to brown. We removed the heads from each plant in our laboratory and segregated them in No. 2186 Dixie cups with clear plastic lids by collection date, then by circle number (within each collection date), and then head stage within each circle. With the exception of the weekends and holidays, we checked these heads daily for *Ch. succinea* emergence. The date and sex of all emerging flies was recorded. For weekends and holidays we divided the number of flies emerging by the number of days since the previous count, to arrive at a mean no. of flies per day.

Figure 5. The Maddox head classification scheme (Maddox 1981).

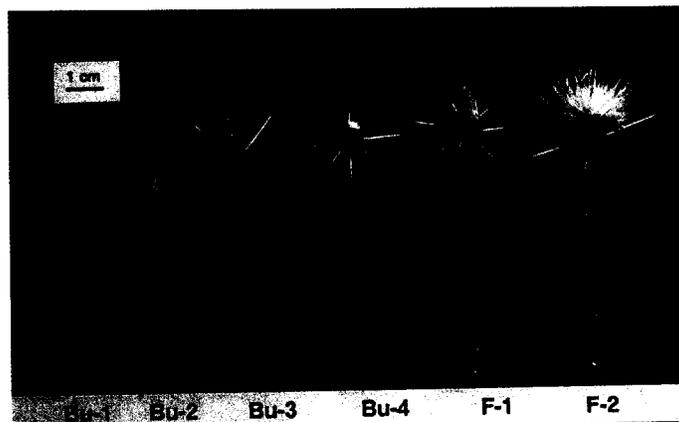


Table 6 presents our 1999 and 2000 collections and illustrates the mean height of YST, its density, and emergence of *Ch. succinea* per square meter from YST heads from each collection date.

Table 6. Data from 1999-2000 collections to Wildcat Canyon YST site.

Date	YST heads /m ²	Mean height (cm)	Density (YST plant/m ²)	Ch. emergence /m ²	Collection method used
4-28-99	170†			40.6‡	heads picked from 1998 YST plants to confirm <i>Ch.</i> infestation
5-5-99	50†			6.0‡	heads picked looking for <i>Ch.</i> infestation, collected 25 soil cores.
5-18-99	100†				heads picked looking for <i>Ch.</i> infestation
7-6-99	100†			1.0‡	heads picked looking for <i>Ch.</i> infestation
7-13-99	426†	90	39*	1.6‡	Picked YST following a transect
7-21-99	1200	37	366	8.5	Used 0.71 m ² quadrant - 260 YST plants
7-28-99	1460	35	1052	28.0	five 0.05 m ² circles
8-10-99	3254	51	523	117.2	seven 0.05 m ² circles
8-19-99	2849	46	617	54.4	seven 0.05 m ² circles
8-27-99	2926	49	577	111.5	seven 0.05 m ² circles
9-7-99	3924	55	483	241.4	seven 0.05 m ² circles
9-16-99	3577	46	706	262.9	seven 0.05 m ² circles
9-24-99	2457	51	680	114.3	seven 0.05 m ² circles
10-4-99	983	52	751	20.0	seven 0.05 m ² circles
10-14-99	3551	54	594	5.7	seven 0.05 m ² circles
10-22-99	4140	50	797	8.7	seven 0.05 m ² circles
11-1-99	2917	53	651	2.9	seven 0.05 m ² circles
11-12-99	3703	46	617	137.0	seven 0.05 m ² circles
11-22-99	3218	57	700	231.3	seven 0.05 m ² circles, 19 soil cores

* actual number of YST plants collected; could not be converted to /m² because area of collection is not known.

† actual number of YST heads; could not be converted to /m² because area of collection is not known.

‡ *Ch.* emergence per 100 heads; could not be converted to /m² because area of collection is not known.

Table 6. (continued)

Date	YST heads /m ²	Mean height (cm)	Density (YST/m ²)	Ch. emergence /m ²	Collection method used
2-17-00	-	-	-	-	YST seedling study, collected 14 soil cores
3-3-00	-	-	-	-	YST seedling study
3-20-00	-	-	-	-	swept flowering plants, collected 15 soil cores
3-21-00	-	-	-	-	YST seedling study
4-11-00	-	-	-	-	YST seedling study
4-26-00	-	-	-	-	collected YST from seven 0.05 m ² circles
6-16-00	194	45	520	0.0	seven 0.05 m ² circles
6-30-00	831	61	549	2.8	seven 0.05 m ² circles
7-10-00	1243	62	471	2.9	seven 0.05 m ² circles
7-19-00	2677	73	574	31.3	seven 0.05 m ² circles, collected 20 soil cores
7-28-00	1997	64	480	14.4	seven 0.05 m ² circles
8-7-00	2394	70	517	74.2	seven 0.05 m ² circles
8-17-00	2400	68	454	357.1	seven 0.05 m ² circles
8-25-00	2963	68	386	345.8	seven 0.05 m ² circles
9-1-00	3043	68	531	494.2	seven 0.05 m ² circles
9-11-00	3531	61	680	268.7	seven 0.05 m ² circles
9-20-00	3234	65	597	57.2	seven 0.05 m ² circles
9-29-00	3883	65	509	108.7	seven 0.05 m ² circles
10-13-00	3511	65	471	14.4	seven 0.05 m ² circles
10-31-00	3517	72	526	42.9	seven 0.05 m ² circles

During 1999, YST density ranged between 366 to 1052 YST plants per square meter, with a grand mean density of 651.1 per square meter (this includes all plants in each collection). During 2000, YST density was lower in range (386 to 680 m²) and in grand mean 519 m². This difference is significant (Student's T test: $t = 2.820$, $df = 26$, $P = 0.009$).

The *Ch.* emergence ranged between 2.9 and 262.9 per square meter in 1999, with a mean of 96.0 *Ch. succinea* emerging per square meter. The grand mean of *Ch. succinea* emerging per square

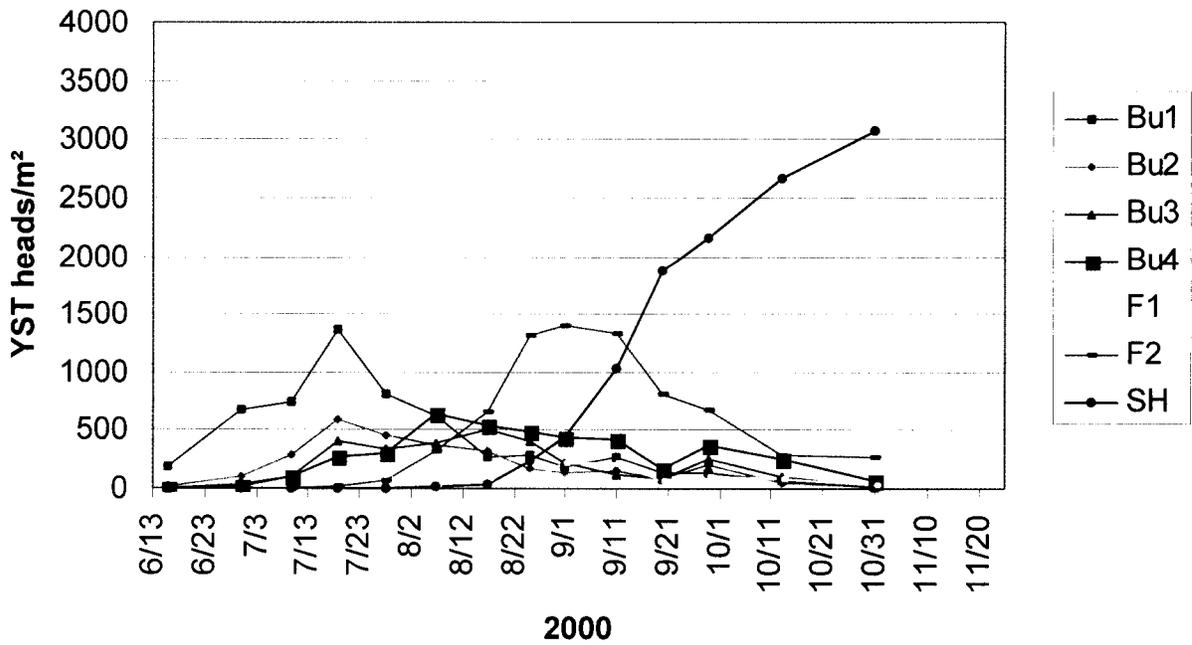
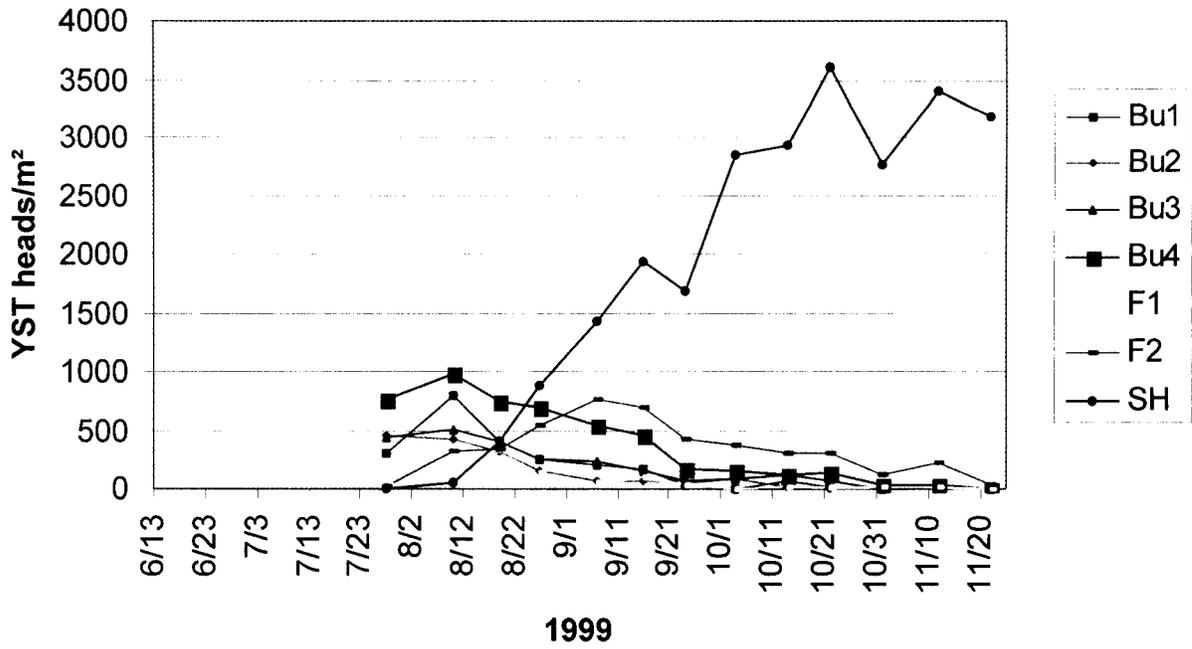
meter was greater in 2000 (129.6), but not significantly (T-test of *Ch. succinea* emerging per square meter in 1999 and 2000 transformed by square root, $t = -0.239$, $df = 26$, $P = 0.813$).

In 1999, the mean YST plant height per collection ranged between 35 and 57 cm, and increased as the growing season progressed. In 2000, the range of mean YST plant height per collection was 45 to 73 cm.

We observed an increase in *Ch. succinea* emergence (not significant), and a decrease in mean YST height (not significant) and mean YST density (significant) for the years 1999 and 2000. We would like to attribute the decreases in YST density to the increased fly population between the two years, however, most of the differences were not significant.

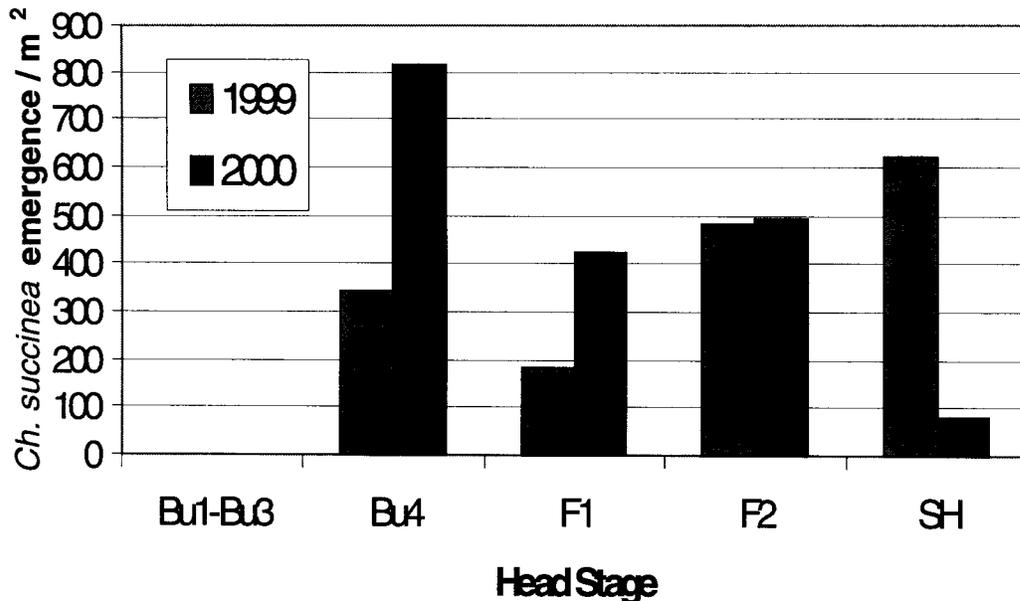
Figure 6 shows the heads per square meter for each head stage at each collection date for the years 1999 and 2000. As we expected, as the season progressed, so did the numbers of maturing flowers, until the last collection when virtually all the heads collected were senesced heads.

Figure 6. YST heads of each stage per square meter from 1999 and 2000 collections at Wildcat Canyon.



We wanted to see how the phenology of the YST related to *Ch. succinea* emergence. Figure 7 shows the number of *Ch. succinea* that emerged per square meter for each YST head stage for the two years at Wildcat canyon.

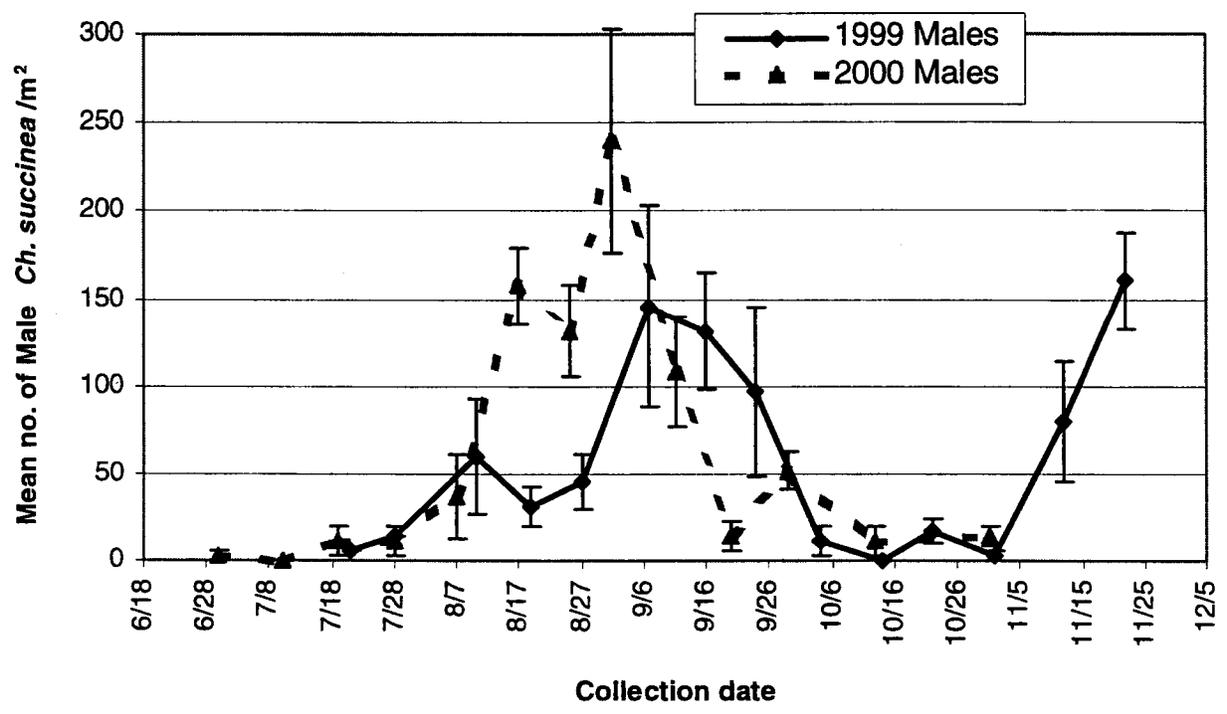
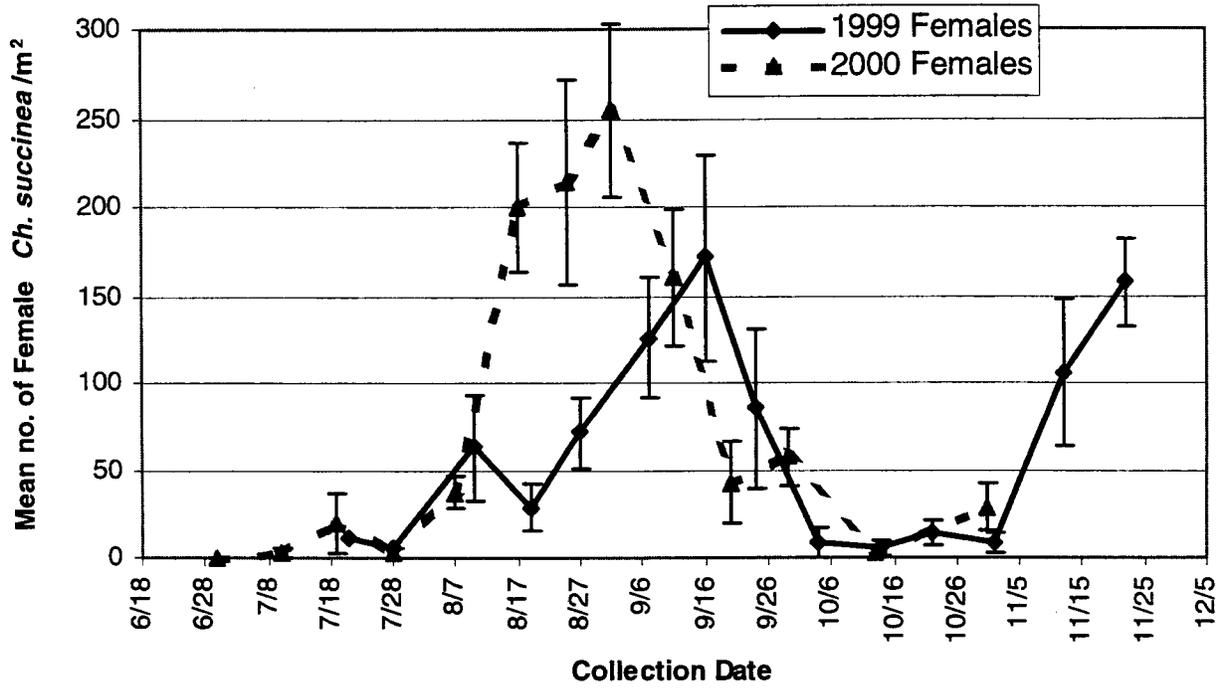
Figure 7. *Chaetorellia succinea* emergence per square meter from different YST head stages.



No flies emerged from YST heads stage Bu1-Bu3. Interestingly, we had opposite linear trends for *Ch. succinea* emergence for the two years. In 1999 the no. of flies emerging per m² was greatest in the senesced head stage and lowest in the Bu4 stage, in 2000 the values were opposite. We suspect that this is because we started sampling in 2000 earlier and ended sooner, than we did in 1999. Had we have collected later into Nov. of 2000 as we did in 1999, it is likely that we would have had a greater number of flies emerge from senesced heads, as we did in 1999 (Figure 8).

For each collection, we counted the number of female and male flies that emerged in each of the seven circles, then determined the mean no of flies emerging per circle for both sexes, this number was converted to m². We plotted these means with their standard errors for each of the collection dates for years 1999-2000 (Figure 8).

Figure 8. The mean female and male *Chaetorellia succinea* emergence per square meter for each collection during 1999 and 2000. Error bars indicate the standard error.



More flies emerged in 2000 than 1999. Additionally, three possible emergence peaks can be seen for 1999 and again, in 2000. From this, we hypothesize that there are four generations of *Ch. succinea*; three that emerge later in the summer and into the fall, and a fourth that overwinters and emerges early in the spring.

The first emergence peak (shown in figure 8) is miniscule in comparison to the second peak, it shows up in mid-August of 1999, but earlier (late July) for 2000. The second emergence peak is obvious in both years. Again it is earlier in 2000, compared to 1999. The third peak is reduced. It shows up earlier for 2000 (late Sept.) and in mid-Oct. for 1999. The overwintering generation is shown for 1999.

Figure 9 shows the daily emergence of adult flies per m² for each collection date over time for both years. Interestingly, the late increase in 1999 *Ch. succinea* emergence shown in Figure (above) is overwintering flies emerging in the spring of the following year. This is not seen in the *Ch. succinea* emerging in 2000. We believe we ended our collections prematurely, had we have collected through the month of November, as we did in 1999, we may have had overwintering flies emerge in the spring of 2001.

We plan to continue this study for a third year in 2001. We will continue our collection visits to further elucidate the number of generations of *Ch. succinea* that emerge throughout the year and measure the effect of this population of biological control agents on their target.

Figure 9. Charts of *Ch. succinea* daily emergence per m² per collection from years 1999 and 2000.

Daily *Chaetorellia succinea* emergence per m² (1999)

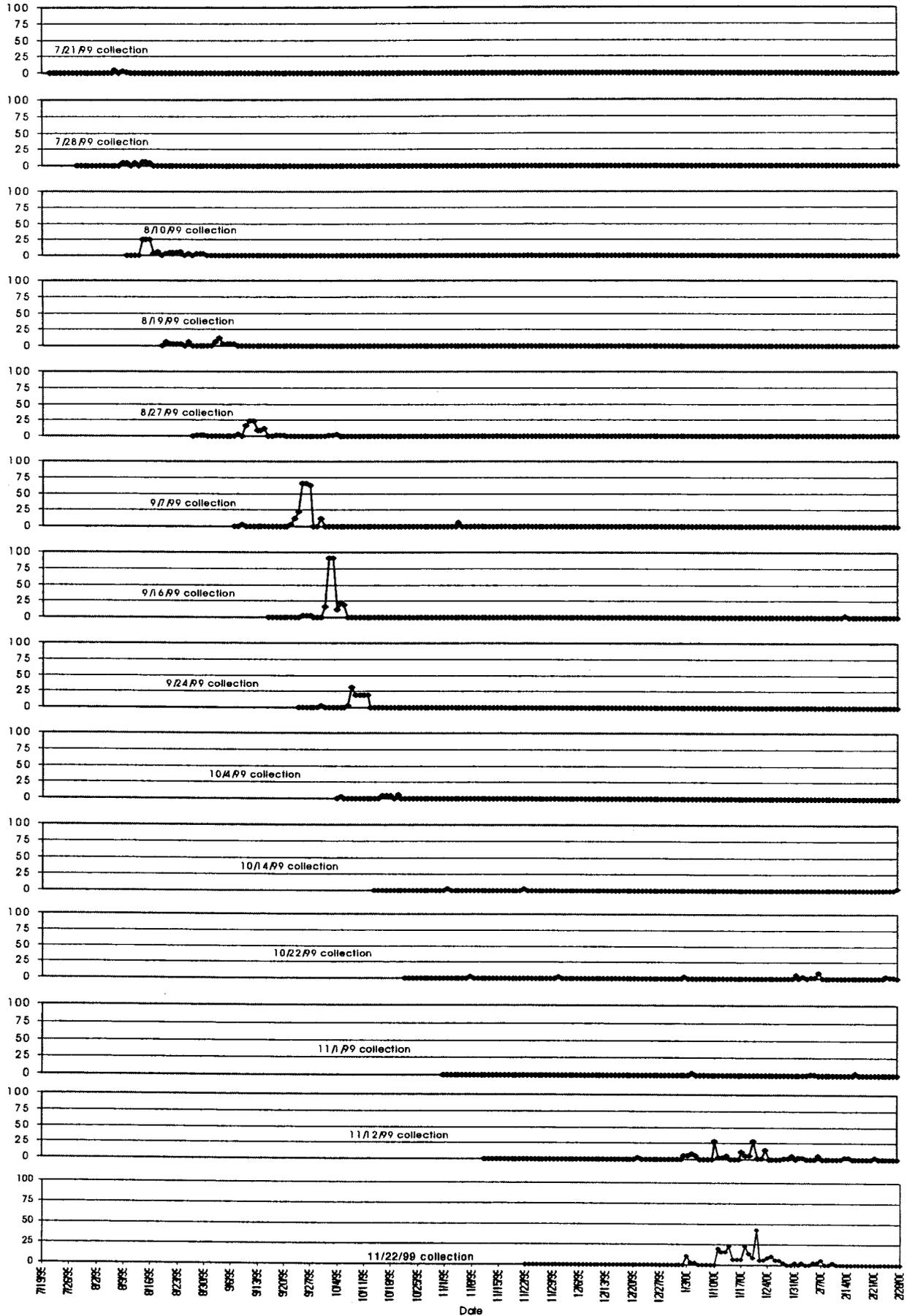
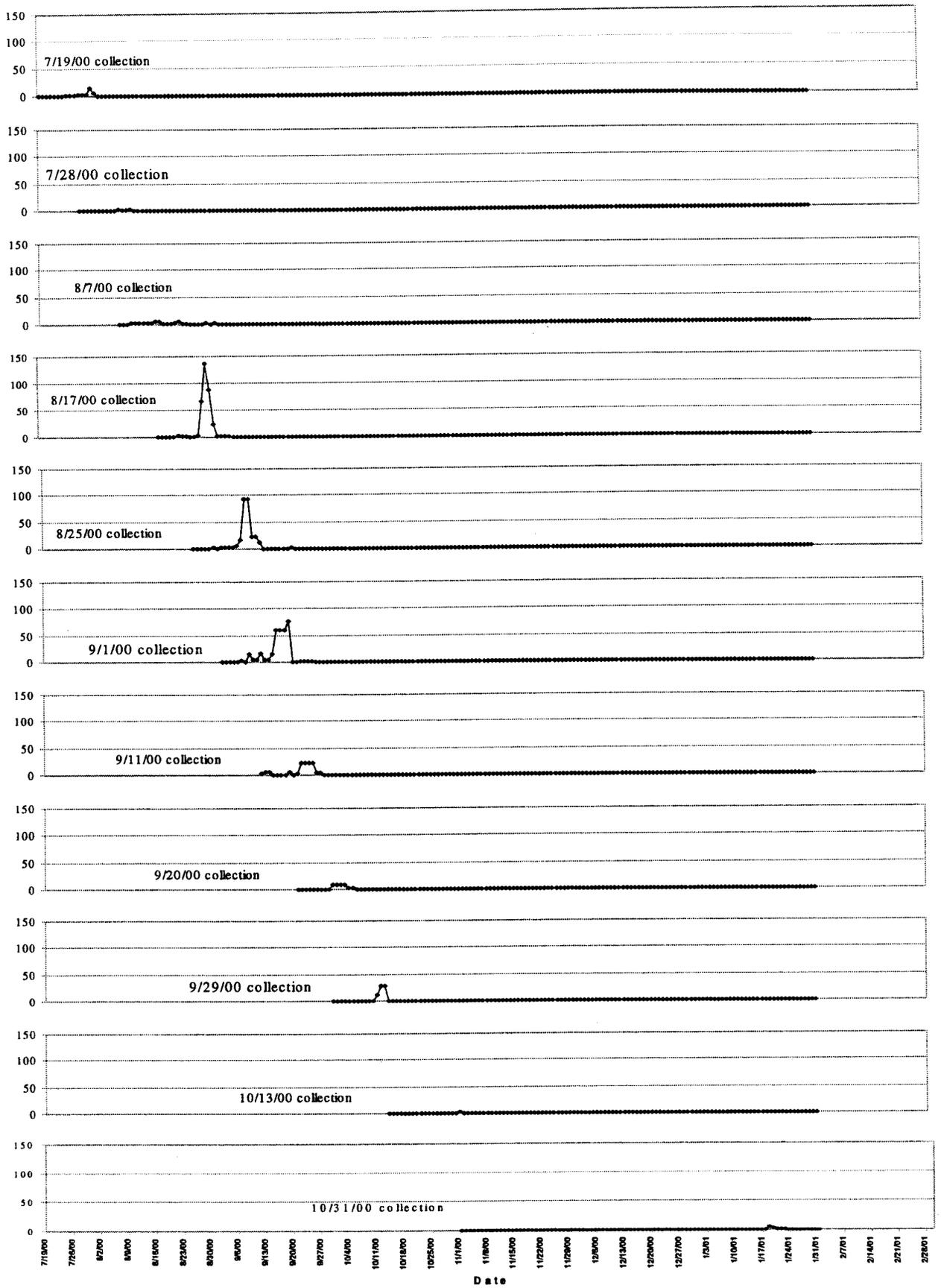


Figure 9. (continued)

Daily *Chaetorellia succinea* emergence per m² (2000)

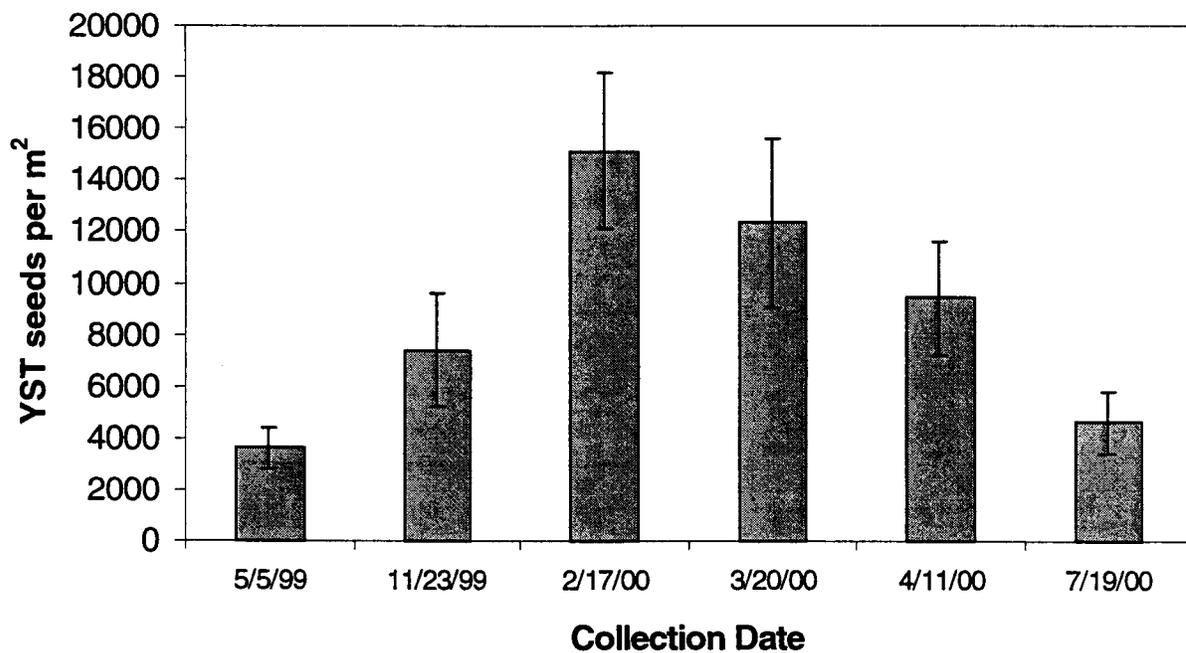


b. Wildcat Canyon seed bank and seedling recruitment studies.

Throughout 1999 and 2000 we periodically collected soil cores from our study site to determine the number of YST seeds in the “seed bank” at various times throughout the year. The soil cores were collected along a transect with an Oakfield soil corer (Oakfield Apparatus, Inc., Oakfield, WI) by taking a step, then taking a soil core, take a step, take another soil core. The area of the soil corer is $2.27 \times 10^{-4} \text{ m}^2$. The number of soil cores per visit varied but ranged between 14 and 33. Soil cores were brought back to our laboratory and soaked with water to break up the soil. The resulting solution was examined for YST seeds. YST seeds were recovered and dried. We planted these YST seeds to obtain germination rates and confirm that the seeds recovered were YST seeds.

Figure 10 shows the collection dates for soil core extractions and the number of YST seeds found per m^2 . The maximum number of seeds was 15,000 per m^2 .

Figure 10. Summary of the YST seed bank at the Wildcat Canyon study site. Error bars indicate the standard error.

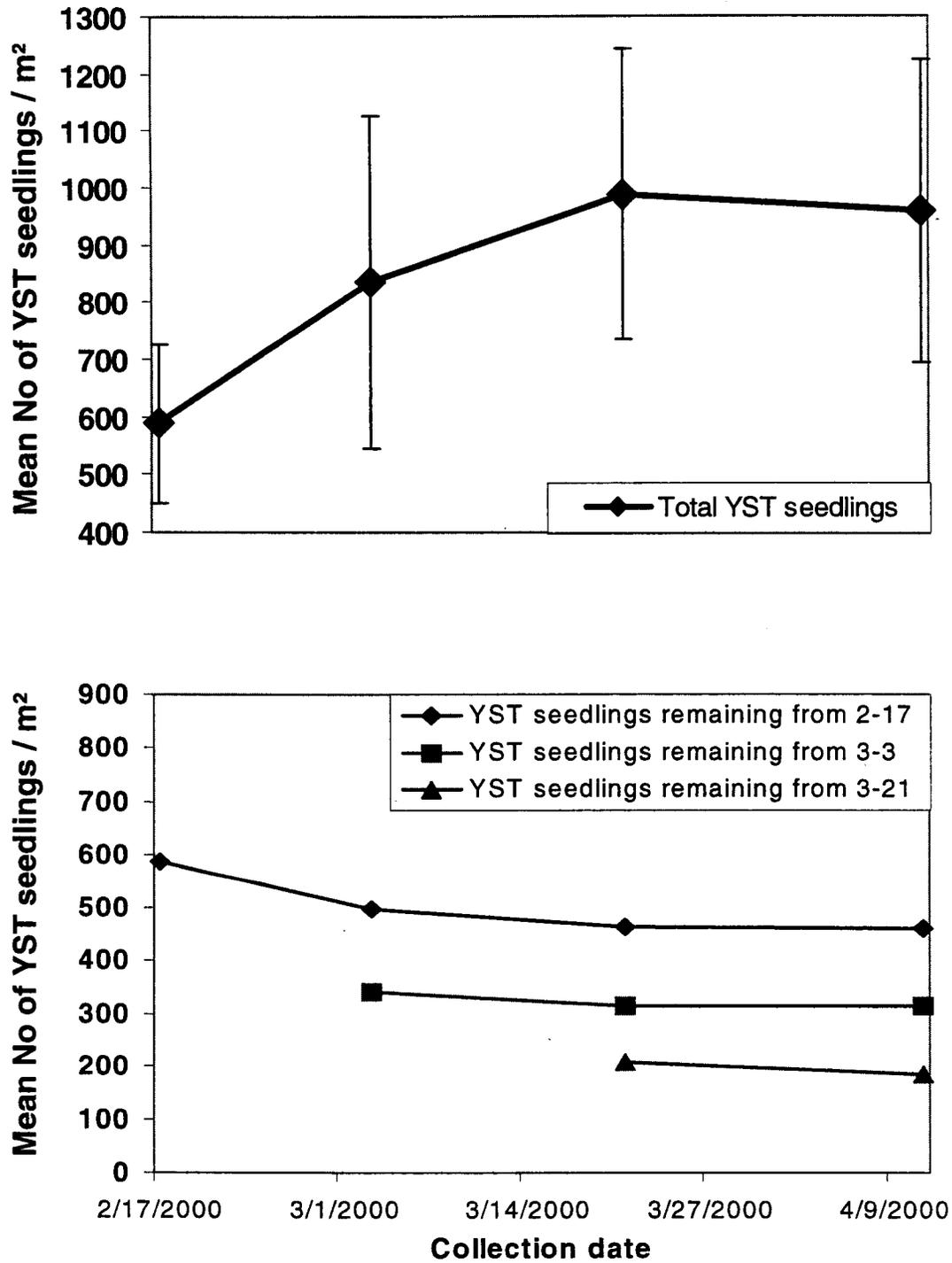


Generally numbers of YST seeds were lowest during the summer months and highest in the early months of the new year when YST skeletons began dropping heads.

In early 2000 we initiated a seedling recruitment study to initially quantify the number of YST seedlings present and then follow these seedlings, as well as newly emerging seedlings, in a predetermined area throughout the spring. During the first trip we randomly selected five 1/20 m² circles in our Wildcat Canyon YST study site using the same semi-rigid plastic hoops we used in previous Wildcat canyon studies. The area of each circle was staked, to be located on future trips. All YST seedlings were marked with same colored toothpicks for tracking at later visits. YST skeletons, emerging thistle seedlings, and other debris were removed to assist in the identification of each seedling more efficiently.

For each subsequent visit previously marked YST seedlings were counted and noted if missing. Newly emerging seedlings were marked with different colored toothpicks. We visited the site four times over the late winter - early spring. We ended the study when no more seedlings were emerging. Figure 11 shows the date of each of the visits and the number of YST seedlings present at each collection date.

Figure 11. A summary of the year 2000's seedling recruitment study. The top graph shows the recruitment of YST seedlings, the bottom graph shows the number of surviving YST seedlings. Error bars indicate the standard error.



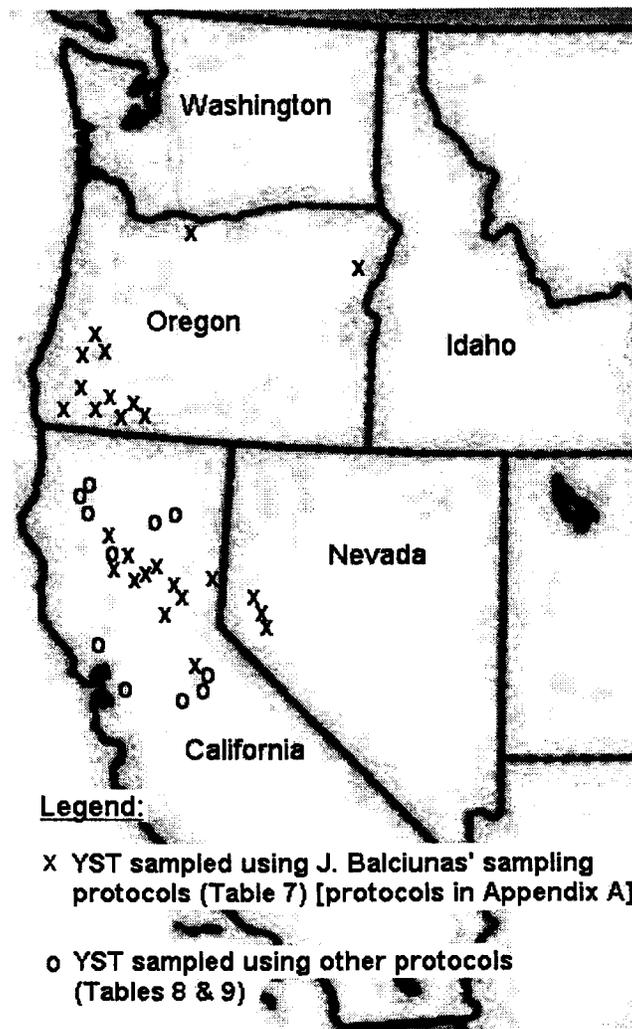
As the study progressed the number of YST seedlings increased and then leveled off. The rate of mortality was generally low. The number of seedlings dropped after the first visit and then held steady as the growing season progressed.

Dr. Lincoln Smith plans to continue this research in 2001 with a more extensive look at emerging seedlings, using more samples to better understand seedling survival.

E. 1998-1999 *Chaetorellia succinea* Distribution Surveys

With the assistance of colleagues in California, Nevada, and Oregon we conducted surveys in these states to estimate the distribution, rate of infestation, and impact of *Ch. succinea* on YST in 1998 and 1999. We used sampling protocols developed by the project leader - J. Balciunas. These sampling protocols are outlined in detail in Appendix A and are briefly discussed below. A map of the sites sampled is shown below (Figure 12).

Figure 12. A map of the western US showing sites where YST was sampled for *Chaetorellia succinea* presence or distribution and impact, as well as impact of other YST biocontrol agents.



Using J. Balciunas' sampling protocols, we randomly picked YST plants along a transect by taking a step, picking the nearest YST plant and removing its heads, and repeating this, until 300

heads were collected from each site. The height of each plant was measured and we estimated the density of YST at each site using a frame of known size. All of the YST plants were counted and then extrapolated to YST plants per square meter.

We sampled 16 sites in California and Nevada and had initially planned to hold each collection of YST heads and monitor for *Ch. succinea* emergence at each site, as the protocols developed had called for. However, because of delayed emergence by overwintering pupae, we randomly selected 50 of the 300 heads and dissected them to estimate the *Ch. succinea* infestation rate. We identified several *Chaetorellia* adults to species when they emerged, all were *Ch. succinea*. We assumed the other flies to be *Ch. succinea* since *Ch. australis* have only been found at a handful of sites in California, not of these sites were ones we sampled from.

We also counted mature viable YST seeds from each head split and compared the numbers of seeds in YST heads infested with *Ch. succinea* to those of heads not infested, to determine the percent of seed reduction attributed to *Ch. succinea*. (Table 7)

Table 7: Summary of 1998-1999 *Chaetorellia succinea* distribution studies in California and Nevada using J. Balciunas' *Ch. succinea* sampling protocol.

Date	County	Site	YST Density (Plants / m ²)	YST Mean height (cm)	Heads split (n)	% infested w/ <i>Ch</i>	Mean no. of YST seeds/ undamaged head	Mean no. of YST seeds/ <i>Ch</i> damaged head	% seed reduction from <i>Ch</i>	Notes
<i>California</i>										
11-?-99	??	Honey Lake	350	23.5	50	12	14.9	7.5	50	??
11-9-99	??	Hwy 395 N. Davis Creek	?	?	50	16	13.4	4.4	67	<i>Eustenopus</i> release site
9-20-99*	Amador	Castle Oaks	100	NA	1623	39	15.0	5.0	67	<i>Larinus</i> release site
9-28-99*	Butte	Sutter Butte ridge	189	NA	649	51	17.3	4.2	76	<i>Larinus</i> release site
10-28-98	Butte	Openshaw Ranch off Hwy 149	249.6	23.6	?	?	?	?	?	<i>Eustenopus</i> release site
11-2-98	Butte	Sutter Butte flats	150.4	21.0	100	20	12.9	7.4	43	<i>Eustenopus</i> release site
11-2-98	Butte	Sutter Butte ridge	163.2	28.2	100	46	16.0	3.3	79	<i>Larinus</i> release site
10-5-98	El Dorado	Folsom Lake	NA	32.8	50	40	13.7	4.6	66	<i>Eustenopus</i> release site
10-27-98	Glenn	Across from GCD Ag office	579.2	38.3	?	?	?	?	?	<i>Eustenopus</i> release site
11-98	Lassen	Bull Flat Rd.	3.7 (?)	27.4	50	28	20.8	14	33	near NV state line - very dry area
11-2-99	Plumas	Quincy Airport	?	45.5	50	34	1.2	2.0	0	multi species release site
10-28-98	Shasta	Turtle Bay	422.4	29.2	?	?	?	?	?	off Hwy 99E
10-28-98	Tehama	Edwards Ranch	246.4	35.1	?	?	?	?	?	off Hwy 99E
<i>Nevada</i>										
11-10-98	Washoe	Rancho San Rafael	4.1 (?)	26.4	50	60	10.6	3.5	67	sagebrush community
11-16-98	Washoe	Valley Rd.	14.7 (?)	46.0	50	24	10.6	1.6	85	industrial area in Reno
11-16-98	Washoe	Windy Hill	16.3 (?)	36.6	50	50	9.0	3.5	61	roadside suburban area of Reno

* Sampled YST using *Larinus curtus* survey protocols (Appendix B)

Ch. succinea was found in abundance at all 16 California and Nevada sites sampled. 33 percent of all YST heads split (from these sites) were infested with *Ch. succinea*. 15 of the sites (94%)

showed seed reduction of which we attributed to *Ch. succinea*. The percentage of seed reduction attributed to *Ch. succinea* ranged from 50 to 85 percent, with a mean of 64 percent.

We regressed YST height to the percentage of YST heads infested for each site to see whether or not sites with a greater number of heads infested had smaller plants, perhaps as a result of *Chaetorellia* damage to the plant. We found no correlation ($r^2 = 0.1$) between the size of infestation and the height of YST plants. We also thought that sites with a greater density of YST would have a larger infestation or more heads infested with *Chaetorellia* than sites with less YST. We regressed YST density (YST / m²) to the percentage of YST heads infested with *Ch. succinea* at each site and again found no correlation ($r^2 = 0.01$).

Twelve sites were sampled in Oregon, 10 of these were located in the southwest region of the state and the other two were located in the northeastern part of Oregon. For these collected samples, the *Chaetorellia* larvae and pupae that were collected from dissected YST heads were allowed to emerge. We did not count seeds in the heads we dissected and thus were unable to estimate the percent of seed reduction attributed to *Chaetorellia*. We were able to determine the species of each *Chaetorellia* fly that emerged. The results of the Oregon surveys are summarized in Table 8.

Table 8: Summary of 1998-1999 *Chaetorellia succinea* distribution studies in Oregon using J. Balciunas' *Chaetorellia* sampling protocols.

Date	County	Site	YST Density (Plants/m ²)	Heads dissected	% infested w/ <i>Ch. australis</i>	% infested w/ <i>Ch. succinea</i>	Notes
11-18-98	Baker	Copper Butte	>100	301	1.7	0.3	
11-10-98	Douglas	Myrtle Creek	4.9	300	0.0	12.7	<i>Eu. villosus</i> release site
1-12-99	Douglas	Myrtle Creek	6.0	300	0.3	25.7	2 nd sample from Myrtle Creek
11-10-98	Douglas	Roseburg	72.2	341	0.3	4.1	
11-18-98	Jackson	Obenchain	32.7	319	1.6	10.0	
11-17-98	Jackson	Blackwell Rd.	61.6	307	0.0	22.1	
11-18-98	Jackson	Dead Indian Rd.	74.6	299	2.7	14.4	
11-17-98	Josephine	Merlin	53.0	305	8.2	13.8	
11-18-98	Klamath	Klamath Falls	91.1	309	7.4	13.3	
11-18-98	Klamath	Buck Butte	57.9	301	1.3	6.0	
11-19-98	Klamath	Klamath River Canyon	83.1	313	1.0	12.8	
11-12-98	Wasco	Kelly cut off	102.1	317	3.5	0.0	

Chaetorellia flies were found at all sites sampled in Oregon with an average of 13.5% of heads infested at all sites. Interestingly, this is a lower rate of infestation than the average of YST heads infested with *Chaetorellia* sampled in California, but this is probably because not all flies

emerged as adults???? Of the 12 Oregon sites, the percent of YST heads infested with *Ch. succinea* was greater than the percent of heads infested with *Ch. australis* at all sites except for the two northeastern Oregon sites (Wasco and Baker County).

We sampled YST at a number of sites in 1998 and 1999 for *Ch. succinea* to determine whether or not the YST was infested, to determine infestation rates, or to collect *Ch. succinea* to use in host range tests. In these “initial” surveys, we also estimated the infestation rate of *Ch.* species at these sites. These *Chaetorellia* infestation rates are shown in Table 9.

Table 9: Summary of 1998-1999 *Chaetorellia* infestation rates on YST sampled in California using various sampling protocols.

Date	County	Site	Heads split	% infested w/ <i>Ch</i>	Method of collection	Notes
7-19-99	Tehama	Driscoll Ranch	262	44	YST plants recently ploughed under, collected all available	11 plants collected
9-20-99 *	Amador	Dry Creek-Hwy 124	100	21	randomly selected 30 <i>Cnt. melitensis</i>	<i>Cnt. melitensis</i> sample
10-6-99	Contra Costa	Deerhill Rd., Lafayette	101	61	YST plants selected on transect	collected 10 YST plants, heads split Bu4 or older
9-20-99	San Joaquin	Sharpe Depot	200	70	YST plants randomly selected	collected 10 YST plants, heads split Bu4 or older
7-19-99	Shasta	2 mi E of Burney on road cut	50	16	YST plants randomly selected	
7-19-99	Shasta	Fall River Mills-Ft. Crook museum	50	28	YST plants randomly selected	former <i>Ch. australis</i> release site
9-17-98	Napa	Yountville Yard	50	52	YST heads collected randomly for <i>Chaetorellia</i>	1997 YST study site
8-5-98	Siskiyou	Slough Rd, near Weed	50	12	Collected 300+ YST heads along transect	From CDFA (1998 <i>Cirsium</i> survey)
8-5-98	Humboldt	Berry Summit-Titlow Rd. Site 2	50	48	Collected 300+ YST heads along transect	From CDFA (1998 <i>Cirsium</i> survey)
8-7-98	Humboldt	Berry Summit-Titlow Rd. Site 3	50	10	Collected 300+ YST heads along transect	From CDFA (1998 <i>Cirsium</i> survey)

* This collection, although not of YST, is included for comparison.

Similar to the aforementioned *Ch. succinea* distribution studies, we found either *Ch. succinea* or *Ch. australis* at all sites we sampled (Table 8). Interestingly, we also found *Ch. succinea* infesting *Cnt. melitensis* - another exotic weed - at one site.

Surveys using J. Balciunas' sampling protocols have shown *Ch. succinea* to have spread throughout areas sampled in northern California and northwestern Nevada, since its accidental release in 1991. Our surveys, in cooperation with CDFA, have shown this fly to have spread throughout California, to parts of Nevada, Oregon and Washington, sometimes infesting as much as 70% of a YST flower heads. Seed production in YST heads attacked by *Ch. succinea* can be reduced by as much as 80%.

F. *Larinus curtus* Research

1. Introduction

Larinus curtus is another a biological control agent for yellow starthistle, which was introduced from Greece and first released in 1992. This flowerhead weevil destroys seeds during its larval stage. It was released at sites in California, Idaho, Oregon, and Washington from 1992-1995, but only became established at a few sites (shipment records are summarized in Appendix E). A *Nosema* sp. protozoan infecting this weevil was detected in 1995, and further releases were curtailed. In 1997, we surveyed the original release site at Sutter Butte Heights, Butte Co., CA, and it seemed that this weevil was dispersing from the release point. In that year, several populations of *Lr. curtus* were found well established in Oregon, and Idaho. Later in 1997, CDFA renewed distributions of this weevil in California (Villegas *et al.* 1998).

In November of 1998, we rechecked the original release site. At this time the *Lr. curtus* adults were overwintering in the soil, but YST heads damaged by these weevils remained. We found nine percent of YST heads damaged by *Lr. curtus* at its original site and two percent of heads damaged by *Lr. curtus* at a *Eustenopus* release site approximately one mile away (Balciunas and Mehelis 1998). However, *Eu. villosus* was more prevalent at its site of release, with 27 percent of the heads damaged by this weevil.

In 1999, we sought to determine why this biological control agent has only established marginally in some areas, whereas in other areas it seemed relatively abundant.

2. *Nosema* sp. surveys

CDFA suggested that one of the reasons for poor establishment could be due to *Nosema* sp. infections (Villegas *et al.* 1998). Initially, all shipments coming into the USDA-Albany quarantine were checked for *Nosema* sp. by insect pathologist Dr. G. Thomas of the Consulting Diagnostic Service, Berkeley, CA. All shipments released were determined to be free of *Nosema* sp. prior to release. In conjunction with our survey of *Larinus* release sites for distribution and impact in 1999 we checked all release sites not previously checked for *Nosema* sp. (Table 10). Cooperating insect pathologists Dr. G. Thomas and Dr. J. Siegel (USDA-ARS-Fresno, CA) determined the infection rates of *Nosema* sp. at each site.

Figure 13: Slides of *Nosema* sp. from infected *Larinus curtus*.

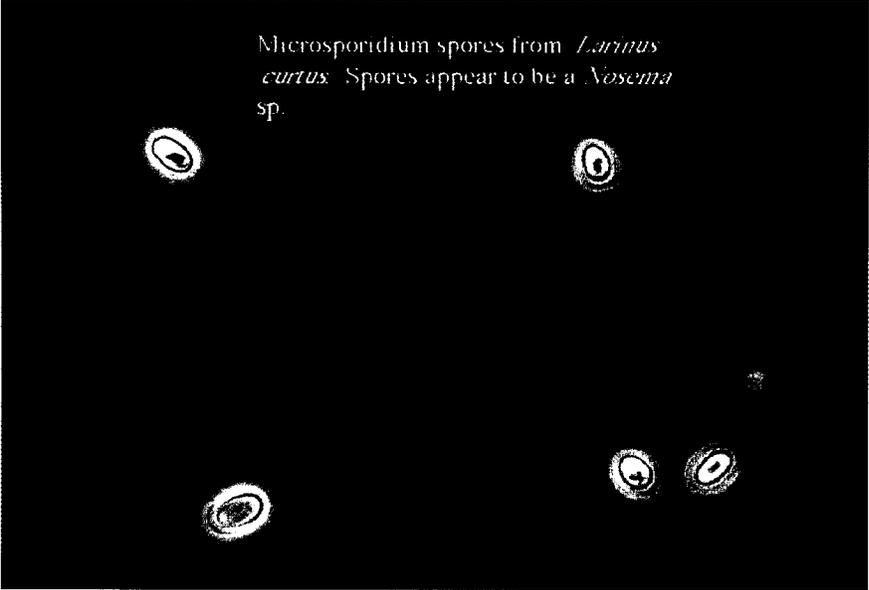


Table 10. USDA *Larinus curtus* release and *Nosema* sp. test summary.

County & State released in	Release site	Date <i>Larinus</i> checked for <i>Nosema</i> spp.	No. of <i>Larinus</i> checked for <i>Nosema</i> spp.	% of <i>Larinus</i> with <i>Nosema</i> spp.	Determined by
Butte, CA	Sutter Butte ridge	7-13-95	10	40	G. Thomas
	Sutter Butte ridge	8-18-99	11	39	G. Thomas
	Sutter Butte ridge	9-1-99	15	33	J. Siegel
Amador, CA	Castle Oaks	7-26-98	60	0	G. Thomas
	Castle Oaks	7-26-98	20	0	G. Thomas
	Castle Oaks	7-29-99	45	0	G. Thomas
Amador, CA	5 mile Drive	7-29-99	27	0	G. Thomas
Amador, CA	2.5 miles N of Ione	7-26-99	20	0	G. Thomas
Amador, CA	Sutter-Ione Rd & Hwy 124	7-26-99	20	0	G. Thomas
Placer, CA	Dry Creek	8-13-99	20	20	G. Thomas
Sonoma, CA	Sugar Loaf Ridge	8-4-99	15	7	G. Thomas
Yolo, CA	Rominger Ranch	8-16-99	8	0	G. Thomas
Nez Pierce, ID	7 miles S. of Peck	8-3-95	6	17	G. Thomas
	7 miles S. of Peck	9-3-99	22	50	J. Siegel
	7 miles S. of Peck Larvae	9-?-99	9	11	J. Siegel
	7 miles S. of Peck Teneral Adults	9-?-99	45	9	J. Siegel
Clearwater, ID	SW of Dworshak Dam	8-2-95	9	0	G. Thomas
Baker, OR	Copper Butte	8-2-95	24	17	G. Thomas
Wasco, OR	Kelly Ridge – The Dalles	7-26-98	36	0	G. Thomas
Whitman, WA	Colfax	9-3-99	24	21	J. Siegel
Whitman, WA	Union Flat	8-2-95	10	0	G. Thomas

Fourteen sites were checked between July of 1995 and Sept of 1999. Of these 14 sites, six were found to have *Lr. curtus* weevils with *Nosema* sp. infection. The number of weevils with

Nosema sp. ranged from 7 to 50 percent at the sites checked. We believe that *Nosema* sp. may be a contributing factor to poor *Lr. curtus* establishment, but there are probably other more important factors since the other 8 sites were *Nosema* sp. free, and at these sites *Lr. curtus* had not established well.

3. *Larinus curtus* distribution and impact surveys

With assistance from state and university agencies, we conducted surveys documenting this weevil's density and impact at original USDA release sites in four states.

Figure 14. A map of *Larinus curtus* release sites and 1999 sampling sites.



We sampled each site using protocols developed by Dr. Joe Balciunas (Appendix B). These protocols estimated YST density, determined the avg. height of YST plants sampled, and number and size of heads at each site. Furthermore, we split all of the heads we collected and determined

the amount of damage caused by each biocontrol agent found. The results are summarized in Tables 11,12, and 13.

Table 11: Summary of 1999 *Larinus curtus* distribution and impact surveys.

Statistics	CA		OR				WA		ID		
	Castle Oaks	Sutter Butte	Copper Butte	Kelly ridge	Myrtle Creek	Pendleton Airport-Dump	Colfax	Union Flat	Dworshak	Harrison	Schembeckler
USDA <i>Larinus curtus</i> release sites (1992-1995)	X	X	X	X	X		X	X	X	X	
Presence of <i>Nosema</i> spp. detected	N	Y	Y	N	N		Y	N	N	Y	
YST plants / m ²	100	189	46	206	30	73	29	53			
YST heads / m ²	3658	1890	92	357	141	173	1069	1900			
No. of YST heads split	1623	649	81	72	78	259	439	327	363	811	214
Avg. YST head width (mm)	7.75	7.53	6.36	6.40	5.58	6.46	6.32	9.04	7.10	8.90	7.70
Avg. no. of seeds in undamaged heads	15.0	17.3	11.3	5.5	9.2	17.5	5.9	11.4	9.1	15.1	8.7
<i>Larinus curtus</i>											
% of YST heads with <i>Lr. curtus</i> damage/infestation	5.4	1.5	1.2	4.2	0	1.5	0.2	0.6	51.4	32.3	27.8
% of YST heads with exclusive <i>Lr. curtus</i> damage/ infestation	3.5	1.1	1.2	4.2	-	1.5	0.2	0.6	51.4	32.3	27.8
Avg. width of <i>Lr. curtus</i> damaged/infested heads (mm)	9.34	7.95	5.00	6.33	-	7.50	6.43	9.94	7.23	9.48	7.69
Avg. no. of seeds in exclusively <i>Lr. curtus</i> damaged/infested heads	5.1	5.3	0.0	0.7	-	7.8	2.0	0.0	4.5	14.6	6.4
% seed reduction attributed to <i>Lr. curtus</i>	65.9	69.4	100	87.8	-	55.4	66.1	100	50.5	3.3	26.4

Lr. curtus seems best established in Idaho with 27.8 to 51.4% of the YST heads being damaged or infested by this weevil. At the sites surveyed in the other three states, *Lr. curtus* is poorly established, with 0 to 5.4% of the YST heads damaged or infested by this weevil. Although we attribute a high percentage of YST seed reduction to *Lr. curtus* in CA, OR, and WA, because the infestation rate is so low, we conclude that this weevil is not effective in areas where it is poorly established and marginally effective in areas where its established (Idaho).

The most prevalent biocontrol agents found at the *Lr. curtus* release sites were the seed head flies *Ch. succinea* and *Ch. australis*. Since we split the YST heads and counted the larvae or damage of *Chaetorellia* we were unable to differentiate between the two species of flies. We suspect that all *Chaetorellia* recovered from Idaho were *Ch. australis*, and those in California were all *Ch. succinea*. Flies in Washington and Oregon were probably predominantly *Ch. australis* with some *Ch. succinea* present. Table 12 shows the *Chaetorellia* statistics at each *Larinus* release site.

Table 12: Summary of *Chaetorellia* sp. impact at *Larinus curtus* release sites.

Statistics	CA		OR				WA		ID		
	Castle Oaks	Sutter Butte	Copper Butte	Kelly ridge	Myrtle Creek	Pendleton Airport Dump	Colfax	Union Flat	Dworshak	Harriston	Schembeckler
<i>Chaetorellia succinea</i>											
% of YST heads <i>Chaetorellia</i> spp. damage/ infestation	39.1	50.7	17.3	16.7	2.6	23.2	13.2	0.3	7.0	5.6	18.3
% of YST heads with exclusive <i>Chaetorellia</i> spp.	26.9	48.1	17.3	16.7	2.6	16.6	10.5	0.0	7.0	5.6	16.7
Avg. width of <i>Chaetorellia</i> spp. damaged/infested heads (mm)	7.75	7.70	6.57	5.83	5.50	6.21	6.58	8.70	7.17	9.78	7.65
Avg. no. seeds in excl. <i>Chaetorellia</i> spp. damaged/infested heads	5.0	4.2	2.1	3.6	1.0	1.1	1.7	-	4.5	7.3	7.9
% seed reduction attributed to <i>Chaetorellia</i> spp.	66.9	75.8	81.0	34.9	89.2	93.7	71.2	-	50.5	51.7	9.2

Chaetorellia spp. was the most effective biocontrol agent at the *Larinus* release sites sampled. It was present and reduced the number of YST seeds at 10 of the 11 sites. Infestation rates ranged from 0.3 to 50.7% of YST heads being or having been infested by *Ch.* flies. The percentage of YST seeds reduced ranged from 9.2 to 81%.

Of all the YST biocontrol agents found at the *Larinus* release sites we checked, *Chaetorellia* spp. and *Lr. curtus* (because it had been released at the site) were the most ubiquitous. We recovered the other three agents at various sites. Generally, these agents were found well established at some sites while absent at others (Table 13).

Table 13: Summary of the impact of other YST agents at *Larinus curtus* release sites.

Statistics	CA		OR				WA		ID		
	Castle Oaks	Sutter Butte	Copper Butte	Kelly ridge	Myrtle Creek	Pendleton Airport Dump	Colfax	Union Flat	Dworshak	Harriston	Schembeckler
<i>Eustenopus villosus</i>											
% of YST heads with <i>Eu. villosus</i> damage/infestation	0	0	1.2	4.2	0	7.7	49.9	59.0	0	0	10.3
% of YST heads with exclusive <i>Eu. villosus</i> damage/infestation			1.2	4.2		6.6	44.4	54.4	-	-	10.3
Avg. width of <i>Eu. villosus</i> damaged/infested heads (mm)	-	-	7.00	4.67	-	7.30	6.44	9.48	-	-	7.83
Avg. no. seeds in exclusively <i>Eu. villosus</i> damaged/infested heads	-	-	10.0	0.3	-	2.7	2.1	2.0	-	-	7.7
% seed reduction attributed to <i>Eu. villosus</i>	-	-	11.5	94.5	-	84.6	64.4	82.5	-	-	11.5
<i>Urophora sirunaseva</i>											
% of YST heads with <i>Ur. sirunaseva</i> damage/infestation	21.8	2.3	0	5.6	0	28.6	0	0	0	0	0
% of YST heads with exclusive <i>Ur. sirunaseva</i> damage/infestation	12.1	0.9	-	5.6	-	21.2	-	-	-	-	-
Avg. width of <i>Ur. sirunaseva</i> damaged/infested heads (mm)	8.16	7.97	-	6.75	-	6.58	-	-	-	-	-
Avg. no. seeds in excl. <i>Ur. sirunaseva</i> damaged/infested heads	11.3	16.2	-	10.5	-	17.4	-	-	-	-	-
% seed reduction attributed to <i>Ur. sirunaseva</i>	24.7	6.4	-	-90.9	-	0.6	-	-	-	-	-
<i>Bangasternus orientalis</i>											
% of YST heads with <i>Bg. orientalis</i> damage/infestation	0	0	0	0	0	-	20.1	20.8	16.8	32.5	39.7
% of YST heads with exclusive <i>Bg. orientalis</i> damage/infestation	-	-	-	-	-	-	16.9	16.5	16.8	31.0	38.1
Avg. width of <i>Bg. orientalis</i> damaged/infested heads (mm)	-	-	-	-	-	-	5.65	8.12	7.22	9.48	7.69
Avg. no. seeds in exclusively <i>Bg. orientalis</i> damaged/infested heads	-	-	-	-	-	-	1.8	6.5	8.3	19.9	11.5
% seed reduction attributed to <i>Bg. orientalis</i>	-	-	-	-	-	-	69.5	43.0	8.8	-31.8	-32.2

Eu. villosus, when found in larger numbers- like the two sites in Washington- reduced numbers of YST seeds, while the other two agents *Ur. sirunaseva* and *Bg. orientalis* reduced YST seeds in some cases (sites) and at other sites (sites) had no effect at all.

Unlike what we had anticipated, it seemed that *Lr. curtus* was only marginally established at sites in California, Oregon and Washington. The percent of YST infested with *Lr. curtus* ranged from 0-5.4 at the 18 sites we sampled in these three states. With such low infestation rates it seems that they are contributing little towards controlling YST populations. However, *Lr. curtus* is well established at the three sites in Idaho; one of which *Nosema* sp. was detected, one where it wasn't detected, and one where it wasn't checked for. Because of these ambiguous results, we suspect that *Nosema* sp. has little to do with the establishment and success of various populations.

G. Yellow Starthistle Research in Eurasia

1. Introduction

Although six imported biocontrol agents, all attacking the seed heads of YST, have become established in the USA, YST remains a serious problem and continues to spread. Additional agents, especially those attacking the vegetative portions of YST are needed. In 1996, shortly after assuming the leadership of the YST project, Joe Balciunas began a series of overseas projects and trips to reopen the "biocontrol pipeline" (see our previous report - Balciunas and Mehelis 1999 - for further details on overseas studies during 1996-1998).

2. Cooperative Research on Yellow Starthistle in Turkey

In August 1998, we established a cooperative agreement with the University of Çukurova in Turkey. The purpose of this agreement was to provide us with more information about the biology of YST, in its native range, and to learn more about the biology and ecology of the apionid weevil, *Ceratapion* sp., as well as other potential biological control agents for this weed.

YST is one of the most important of the 172 *Centaurea* species in Turkey. Within *Ce. solstitialis* are three subspecies: *Ce. solstitialis* subsp. *solstitialis* which is non-endemic and is a noxious weed in the U.S., *Ce. solstitialis* subsp. *pyracantha* and *Ce. solstitialis* subsp. *carneola*, both which are endemic. Studies from 1998 to 2000 sought to determine and develop insect natural enemies, as well as elucidate the population dynamics of YST around the vicinity of Çukurova, Turkey.

The following results are extracted from Dr. Sibel Uygur's 2000 Report to us. Trap plants were used to evaluate a promising agent - the apionid weevil *Ceratapion* (*Cp.*) (described in detail in section 4). Nine plants representing eight genera from the family Asteraceae (including YST, and Cardueae thistles: *Cynara scolymus*, *Crt. tinctorius*, and *Silybum marianum*) were harvested from a plot in mid-summer and assessed for *Cp.* infestations. Only YST and *Silybum marianum* were infested with *Cp.* weevils, the rates of infestation were high - 90 and 88% respectively. We are unsure of the species identification of these weevils, but are encouraged that none of the plants in the plot that are grown agriculturally in the U.S. (*Cynara scolymus* and *Crt. tinctorius*) were infested with these weevils.

Population dynamic studies were initiated at three roadside sites. Site one located near Catalan and Adana, had *Ce. solstitialis* subsp. *carneola*. Two other sites (Camardi/Pozanti and Goreme/Nevsehir) had *Ce. solstitialis* subsp. *solstitialis*.

Soil cores were taken at these sites in the summer and then approximately six months later to determine the "minimum" and "maximum" YST seed bank. Twenty samples were taken from plots using a soil corer. For the minimum sample, the site with *Ce. solstitialis* subsp. *carneola* had the least number of seeds per m² - 640, the other two sites had more seeds - 960 and 1480

seeds m². The maximum ranged from 2,760 to 14,120 seeds per m². This number is similar to the maximum sample at the Wildcat Canyon study site (section 2, subsection C) of 15,000 seeds per m². The *Ce. solstitialis* subsp. *carneola* sample had 5,160 seeds m².

YST density studies were conducted at the three sites in southeastern Turkey in late July or early August when YST plants had completed their flowering and shed their seeds. Ten 0.25m² frames were placed on the soil at each site. Mature plants and thistle heads were counted in each frame. The site at Camardi/Pozanti had the smallest number of plants - 5.6 and heads - 238.6 per m². Site one (Catalan/Adana) with *Ce. solstitialis* subsp. *carneola* had slightly more plants - 7.2 and heads - 255.8 per m². The site at Goreme/Nevsehir had 26.4 plants and 69.7 heads per m², much greater than the other two sites.

Once 95% of the YST plants at each site had completed flowering, a study evaluating the damage of *Ceratapion* spp. was initiated. Ten plants were collected at each site along a transect: by collecting a plant, taking two steps, then collecting another plant, until 10 plants had been collected. Plants were examined for damage and this was classified as either none, light, medium or heavy. Site one had the most damage with all plants being damaged and three with heavy, six with medium, and one with light damage. The site at Camardi/Pozanti was similar, one had heavy, six had medium, and three had light damage. The site at Goreme/Nevsehir was affected the least, five plants showed no damage, and one had heavy, two had medium, and one had light damage.

Finally, a survey of the Middle and South parts of Anatolia were conducted to examine the insects on YST and related plant species. Twenty sites were visited in late May and early June. Nine of the sites had *Ceratapion* sp. infestations. *Bangasternus* sp. were recovered at three other sites, and two Dipteran species were recovered at another site. Two more sites had aphids on YST plants and one had a species of *Tetranychus* mites. YST plants collected at seven sites were clean of insects.

We are hoping that this research will lead to a greater understanding of YST population dynamics and possibly contribute another insect for quarantine studies and possible release for the biological control of YST in California. In 1999, we received a shipment of *Ceratapion* sp. from our Turkish cooperators (see section 4). These cooperators will be relied on for more shipments in the future, if this insect is chosen to be studied further.

3. Joe's 1999 Asia Minor Trip

In May of 1999, the Project Leader - Joe Balciunas, along with cooperators from Sacramento [CDFA] and Rome [ENEA], spent several days at the EBCL substation in northern Greece, and five days at University of Çukurova in Adana, Turkey. They were able to visit many of the sites in northern Greece that had been the source of the YST agents shipped to Albany, and eventually released throughout western USA. In Turkey, Joe reviewed the YST research by our cooperator,

Dr. Sibel Uygur, and prepared protocols for seed bank and YST population dynamics studies to be conducted by our Greek and Turkish cooperators [see above].

Joe then traveled to Armenia where he joined a multi-national scientific team surveying for leafy spurge insects. Armenia is slowly recovering from a tragic war, and economic conditions there are still very difficult. The team spent 10 days, usually camping, traveling around the country. Although YST was sparse in Armenia, Joe was able to find some on most days, and found an apionid weevil boring into the root crowns at most YST sites.

The team then flew to Krasnodar, in southern Russia for an additional week of surveys. Except near the Black Sea coast, YST was regularly encountered, and most sites had similar apionid weevils boring into the YST root crowns. Joe then spent several days in St. Petersburg, meeting with Russian Academy of Science officials and scientists. Due to Russia's current economic crisis, there are several world class biocontrol scientists available to assist in the YST project for very modest cost. Joe established an informal cooperative agreement with a Russian taxonomist - Dr. Boris Korotyaev - to identify our *Ceratapion* specimens (see section below).

4. *Ceratapion basicorne*

During his 1996 and 1997 trip overseas, Dr. Balciunas frequently encountered larvae of an apionid weevil, subsequently tentatively identified as *Cp. basicorne*, damaging the root crowns of YST growing in Greece and Turkey. In May of 1997, our quarantine received 33 apionid larvae collected by the EBCL from YST in Greece. In quarantine, these larvae were inserted into stems of YST and safflower. Adults emerged from both. Later, 50+ adults reared by Dr. Balciunas from YST in Turkey and Greece during his trip to Europe were also shipped to the Albany quarantine. As anticipated, these proved to be non-ovipositional, and were held for testing during the spring of 1998. Unfortunately by 1998, all the apionids in the quarantine had died.

On his 1996 and 1997 trips to Eurasia, Joe conducted extensive field surveys of apionid larvae present in YST and other Cardueae thistles. At every site where YST was present, Joe [and/or his collaborators] would gather 20 YST plants, then dissect them and count apionid larvae and make notes on their damage. The number of larvae in the root and in the stem were recorded separately for each plant. The results of these surveys can be found in the 1996-1998 Biological control of YST Tri-annual report (Balciunas and Mehelis, 1999).

In March of 2000, we sponsored a visit to our Albany quarantine by noted Russian weevil taxonomist, Boris Korotyaev. He examined our *Ceratapion* voucher specimens from previous shipments, as well as the apionids collected by Joe Balciunas on his 1996 and 1997 trips to Eurasia. He identified six species among them. Table 14 shows the species of *Ceratapion* weevils, their associated host plants and location of host.

Table 14. Summary of *Ceratapion* sp. reared from various thistles of the tribe Cardueae.

Host	Location*	No. of specimens of various <i>Ceratapion</i> species					
		<i>basicorne</i>	<i>carduorum</i>	<i>gibbiroste</i>	<i>onopordi</i>	<i>orientale</i>	<i>penetrans</i>
<i>Crd. pychnocephalus</i>	97GRC06 (nr. Kylline & Piryos)			7			
	97GRC09 (near Thesaloniki)			8			
	Prespa Lake, Greece			10			
<i>Cnt. cyanus</i>	97TUR12 (2 km E of Cay)	26					
	97TUR15 (near Doganhisar)	1					
	97TUR21 (3 km E of Tuz Golu)	2					
<i>Cnt. diffusa</i>	97GRC11 (Prespa Lake)						1
<i>Cnt. solstitialis</i>	97TUR08 (unknown site)	3					
	97TUR09 (unknown site)	3					
	97TUR11 (.2 m N of Yanuk)	9					
	97TUR12 (2 km E of Cay)	5					
	97TUR14 (E of Doganhisar)	11					
	97TUR15 (near Doganhisar)	12					
	97TUR18 (2.4 km S of Yarma)	6					
	97TUR21 (3 km E of Tuz Golu)	11					
	Kumisi Lake, Georgia	5					
	Saakadze, Georgia	2					
<i>Cnt. species</i>	Kozani and Ptolemaia, Greece	22					
	Knasnodar region, NW of Sochi						4
<i>Cir. species</i>	Prespa Lake, Greece				4		
<i>Cnicus benedictus</i>	97TUR12 (2 km E of Cay)	4				5	
<i>Crapina vulgaris</i>	Prespa Lake, Greece					12	
<i>Cynara cardunculus</i>	97GRC06 (nr. Kylline & Piryos)		2				
<i>Galactites tomentosa</i>	Kumisi Lake, Georgia				1		
<i>Ono. acanthium</i>	Osani, Georgia				1		
<i>Silybum marianum</i>	97GA02 (??)			4			
	Kumisi Lake, Georgia			2			

* TUR denotes a collection reared from Turkey

GRC denotes a collection reared from Greece

GA denotes a collection reared from Georgia

For more details of site locations consult 1996-1998 Tri-Annual Report - Appendix E (Balciunas and Mehelis 1999)

Cp. basicorne was reared only from YST, *Centaurea cyanus* and *Cnicus benedictus*. From this data, the field host range of *Cp. basicorne* appears specific enough that it merits consideration as a potential biological control agent for YST, and warrants further host range studies.

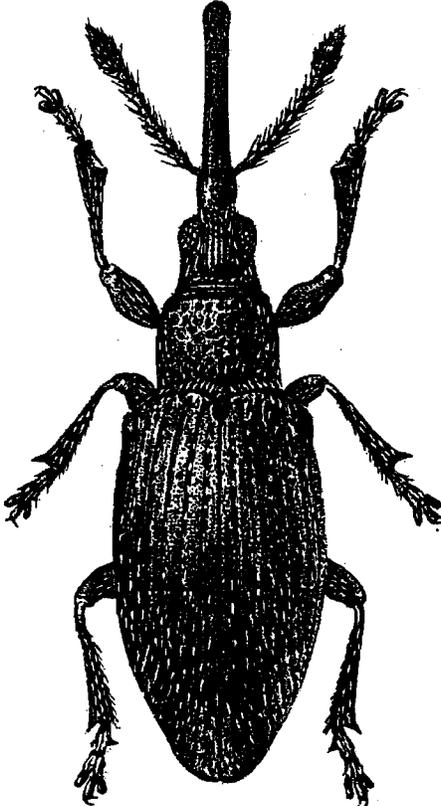
In 1999, we received three shipments of *Cp. basicorne* from cooperators in Greece and Turkey. Unfortunately, these shipments were collected late in the season, and many of the weevil larvae were parasitized. Using four survivors that emerged from the Greek shipment, and two groups consisting of seven and five *Cp. basicorne* respectively, that were sent as adults from Turkey, we measured the amount of feeding damage that these weevils inflicted to a YST. Generally these weevils will chew tiny holes through the leaf. We counted these feeding holes every morning and every evening for two weeks. We were able to show that the feeding damage they inflict on YST leaves doubles at night (Table 15). However, soon after that they all died.

Table 15. Results of *Ceratapion basicorne* day - night feeding studies.

	7 weevils from Turkey	5 weevils from Turkey	4 weevils from Greece	Total
	feeding damage / weevil / hour	feeding damage / weevil / hour	feeding damage / weevil / hour	
Day (8AM - 5:30 PM) percent of feeding damage	10	15	9	34
Night (5:30 PM - 8AM) percent of feeding damage	21	20	26	66

In 2000, we received three shipments of YST stems and root crowns infested with *Ceratapion* sp. larvae from Greece (see Appendix E for shipment details). From these shipments, 389 *Ceratapion* sp. adults emerged. These adults were placed on YST plants, however, these adults exhibited a high mortality rate, many dying a month after emergence. Dr. Balciunas sent some of the dead *Cp.* specimens to Dr. Joel Siegel, an insect pathologist at ARS- Fresno, CA., who determined that the *Cp.* samples were infected with *Nosema* sp. and *Bouveria* sp. fungus. Unfortunately, the numbers of surviving *Cp.* adults continued to decline, and all died before ovipositing.

Figure 15. *Ceratapion basicorne* illustration by an associate of Dr. Boris Korotyayev.



III. Scotch Thistle (*Onopordum acanthium*, Asteraceae)

A. Introduction

Scotch thistle (ScT) has been our other primary target. ScT is a native of Central Asia and has become an invasive noxious weed of ranges and pastures throughout a large portion of temperate North America (Hooper *et al.* 1970). Although, ScT infests wet meadows and pastures, it appears to be most invasive and damaging in arid western rangelands. Infestations of ScT inflict significant economic losses on the ranching industry due to this weed's ability to out-compete valuable forage plants, and to form dense stands, sometimes exceeding eight feet in height, which impede the movement of livestock. Applications of herbicides, with their associated economic and environmental costs, provide unsatisfactory long-term control options.

Successful importation into the United States of host-specific biological control agents from the weed's region of origin might provide sustainable, cost-effective, and environmentally sound control of ScT. Fortunately, a classical biological control program for ScT is well under way in Australia. The Australians have already released seven biological control agents on ScT, and several more are still under consideration (Table 16).

Table 16. Scotch thistle biocontrol agents released in Australia.

Biocontrol agent	Year Released	Status (March 2000)
<i>Larinus latus</i> Coleoptera: Curculionidae	1992	Well established. Slowly expanding out of release sites. High levels of seed destruction at some sites.
<i>Lixus</i> new species Coleoptera: Curculionidae	1993	Well established, sometimes at five times the European densities. Dispersing rapidly. Reduction in plant vigor observed at some sites.
<i>Tephritis postica</i> Diptera: Tephritidae	1994	No establishment.
<i>Trichosirocalus</i> new species Coleoptera: Curculionidae	1997	Establishment confirmed at two sites. Too early to assess impact.
<i>Tettigomettra sulfurea</i> Hemiptera: Fulgoridae	Rejected	Not released because feeding by this insect attracts ants, which may destroy other biocontrol agents.
<i>Eublemma amoena</i> [= <i>respersa</i>] Lepidoptera: Noctulidae	1999	Established at two sites. Too early to assess impact.
<i>Botanophila spinosa</i> Diptera: Anthophilidae	1999	Released in August of 1999. Establishment not confirmed.
<i>Urophora tenebrans</i> Diptera: Tephritidae	2000	Released late in 2000

With financial assistance from Oregon Dept. of Agriculture (ODA) and Bureau of Land Management, as well as substantial in-kind support from California Dept. Food & Agriculture (CDFA) and ODA, we were able to initiate research on this new target in 1996. To date, our quarantine evaluations have focused on insects already released in Australia. Interestingly, the only *Cirsium* species present in Australia are introduced weeds, therefore they do not have the same concerns as the United States does, in regards to threatening native *Cirsium* thistles with released biocontrol agents. Thus, our host range testing emphasizes native *Cirsium* species.

In the Spring of 1999, we met with scientists representing our cooperating agencies in Pendleton, Oregon. This meeting is summarized in Appendix C. With the exception of our quarantine laboratory host range testing, most of the North American ScT research is summarized here. We received *Lixus cardui* from Greece in our quarantine laboratory in 1996 and 1997 and *Lixus* new sp. from France in 1997. Both insects were tested under no choice and choice conditions. To our dismay, both species oviposited and developed on some of the native *Cirsium* species we tested. Upon this discovery we discontinued research on this insect due to the threat it poses for our native species.

B. *Trichosirocalus* new species

During his 1997 overseas trip, Dr. Balciunas spent three days traveling through Spain with David Briese, who had developed the *Onopordum* project for CSIRO. Joe learned how to distinguish over a half dozen species of *Onopordum*, and how to collect *Trichosirocalus* (*Tr.*) new species. The larvae of this weevil bores into the rosettes of ScT, sometimes killing the plant. Figure 16 shows *Tr.* adult and the damage the larvae cause to ScT rosettes. One variety was released in Australia (*Tr. horridus*) in 1997.

Figure 16. A *Trichosirocalus* new sp. adult, and its larval damage on ScT rosettes.



We first received this insect in our quarantine laboratory in 1998. We were able to run some preliminary feeding studies and establish a small colony for future research (cite 1996-98 triannual here).

More testing was needed and so, during June 1999, Dr. Balciunas collected another hundred of *Trichosirocalus* weevils in Spain to use for further quarantine evaluations. We separated these weevils into test groups and kept them on ScT rosettes for the remainder of the summer. Each test group consisted of 3 or 4 weevils, with each sex represented. In the fall (Oct. 1999) we confined each to a ScT plant by a 14.1 cm inner diameter, 25.3 cm length of plexiglass cylinder. The bottom end of the cylinder was buried in the soil of the pot about two cm deep. Perlite was poured over the soil at the base of the plant inside the cylinder, to facilitate weevil test group observation and recovery. The top end is covered by mesh, to contain the weevil test group and allow air exchange. This design is shown in Figure 17.

Figure 17. An example of no choice oviposition tests of *Trichosirocalus* new sp. on *Onopordum acanthium*.



ScT plants were exposed to *Trichosirocalus* test groups for two weeks, and then every leaf was removed from the plant and then examined and dissected to detect *Tr.* eggs. Once eggs were discovered on the ScT leaves of a test group, that test group would be exposed to a test plant to begin a no choice oviposition test.

We used 20 test groups of weevils and exposed each of them to one test plant once they had proved ovipositional. After two weeks, test groups were recovered and placed on an ScT plant confined with a plexiglass cylinder length which served as a ScT post test or control. Each test was replicated using other test groups of *Tr.* After two weeks, the ovipositional test groups were removed from ScT control plants and placed on different test plants. After each ScT control and test plant was exposed to *Tr.* test groups, the plants were dissected to determine numbers of eggs

and larvae. We were able to continue this cycle of exposing each ovipositional test pair to a ScT plant for two weeks, then a test plant for two weeks, and then another ScT plant for two more weeks, for several months. Table 17 summarizes the results of these no choice oviposition tests.

Table 17: *Trichosirocalus* new sp. no choice oviposition tests, Fall-Winter 1999-2000.

Test plant species	No. of replicates	Pre-test control (ScT)	Test plant		Post-test control (ScT)
		Mean no. of eggs/ female/ leaf	No. of replicates w/ eggs	Mean no. of eggs/ female/ leaf	Mean no. of eggs/ female/ leaf
<i>Carduus nutans</i>	3	0.27	2	0.04	0.36
<i>Cirsium brevistylum</i>	3	0.04	1	0.01	0.26
<i>Cirsium douglasii</i>	3	0.13	2	0.05	0.05
<i>Cirsium fontinale</i> var. <i>fontinale</i>	3	0.27	1	0.01	0.87
<i>Cirsium hydrophilum</i> var. <i>vaseyi</i>	3	1.31	2	0.14	2.04
<i>Cirsium occidentale</i> var. <i>candidissimum</i>	1	0.03	1	0.03	0.32
<i>Cirsium occidentale</i> var. <i>occidentale</i>	5	0.20	3	0.10	0.29
<i>Cirsium scariosum</i>	1	0.01	1	0.01	-
<i>Cirsium undulatum</i>	3	0.24	3	0.03	0.10
<i>Centaurea calcitrapa</i>	1	0.05	1	0.01	-
<i>Cynara scolymus</i> (Artichoke)	2	0.51	2	0.18	0.07
<i>Silybum marianum</i>	1	0.12	0	0.00	-

Trichosirocalus new species oviposited on every plant tested except *Silybum marianum*. In all cases the mean no of eggs per female was greater on ScT control tests than on thistles tested.

Though we were able to detect oviposition on almost all test plants, we wanted to see whether or not *Tr.* weevils would develop into adults on the *Cirsium* thistles we were testing. Therefore after ending the no choice oviposition tests (once oviposition began to taper off), we continued the two week cycle of testing, exposing each test group to a ScT plant for two weeks, then a test plant for two weeks, followed by another ScT plant for two more weeks. Rather than dissecting the leaves from each plant after two weeks, we saved each plant and examined it on a weekly basis, looking for signs of *Tr.* destruction or emerging adults. Table 18 shows the results of these development tests.

Table 18: *Trichosiocalus* new sp. no choice development tests, Winter 2000-2001.

Test plant species	n	Percent with late larval instars or adults emerging	Condition of test plant			
			Bolted	Good condition, no apparent <i>Trichosiocalus</i> damage	Dead or dying plants	No information on condition
<i>Carduus nutans</i>	6	0	0	4	1	1
<i>Cirsium brevistylum</i>	5	0	0	0	5	0
<i>Cirsium douglasii</i>	7	0	0	1	5	1
<i>Cirsium fontinale</i> var. <i>fontinale</i>	8	0	0	2	6	0
<i>Cirsium hydrophilum</i> var. <i>vaseyi</i>	7	0	0	0	7	0
<i>Cirsium occidentale</i> var. <i>candidissimum</i>	4	25	1	1	2	0
<i>Cirsium occidentale</i> var. <i>occidentale</i>	12	25	1	7	2	2
<i>Cirsium ochrocentrum</i>	2	0	0	1	1	0
<i>Cirsium scariosum</i>	1	0	0	0	1	0
<i>Cirsium vulgare</i>	1	0	0	1	0	0
<i>Centaurea calcitrapa</i>	1	0	0	1	0	0
<i>Cynara scolymus</i> (Artichoke)	3	0	0	3	0	0
<i>Silybum marianum</i>	1	0	0	0	0	1
<i>Onopordum acanthium</i>	95	25	5	46	15	28

14 different thistles from the tribe Cardueae were tested. Only three of the types of plants in the development tests had adults emerge, one being ScT - its host in its native range. The percent of each plant having adults was the same for ScT as it was for the native thistles *Cir. occidentale* varieties *occidentale* and *candidissimum* in these laboratory no choice conditions.

We ended these tests in early March when some of the *Tr.* weevils began to die. We kept the remaining weevils and the ones from the newest generation on ScT rosettes (ones that emerged from the *Tr.* no. choice development tests) for the rest of spring and summer. In the fall, we organized these weevils into test groups of four weevils, and began dissecting the ScT plants

every two weeks after they had been exposed, looking for oviposition. Once oviposition was detected from that particular test group a choice test was initiated. Choice tests were conducted similar to the no choice tests of the year prior, except we fit a test plant and a ScT plant inside the plexiglass sleeve (shown in Figure 17). Efforts were made to make each plant similar to the other in size, by removing leaves if necessary. The tests were run for a length of two weeks, then the test group was moved to another test plant - ScT combination, for another test. If within the two week time period a *Tr.* expired it was not counted in the test. We ended these tests in mid February after *Tr.* oviposition tapered off.

We divided the number of eggs we found on all of the replicates of test plants in a particular species by the total numbers of female weevils used in the number of tests. We divided this number by total number of leaves of all the test plants in each particular test plant species to give the no. of eggs per female per leaf, or the number of eggs you would find on one leaf exposed to one female for the two week period. We also did this for the ScT plants used in each test plant species. The results of this are shown in Table 19.

Table 19: *Trichosirocalus* new sp. choice oviposition tests, Fall-Winter 2000-2001.

Test plant species	No. of replicates	ScT	Test plant
		eggs/ female/ leaf	eggs/ female/ leaf
<i>Cirsium crassicaule</i>	4	0.15	0.01
<i>Cirsium douglasii</i>	5	0.13	0.01
<i>Cirsium fontinale</i> var. <i>fontinale</i>	5	0.20	0.00
<i>Cirsium hydrophilum</i> var. <i>vaseyi</i>	6	0.24	0.01
<i>Cirsium occidentale</i> var. <i>occidentale</i>	7	0.09	0.06
<i>Cirsium ochrocentrum</i>	1	0.38	0.39
<i>Cirsium undulatum</i>	1	0.88	0.00
<i>Cynara scolymus</i> (Artichoke)	5	0.18	0.00

In all tests the no. of eggs per female per leaf was greater on the ScT plant with the exception of the choice test of *Cir. ochrocentrum*. However, this plant was only tested once, and we suspect with more replicates, a *Tr.* preference for oviposition on the ScT. The *Cirsium occidentale* var. *occidentale* - ScT choice tests showed only a slight *Tr.* oviposition preference for ScT. This plant was tested several times.

We cannot discount these results. We will discontinue our research on *Trichosirocalus* new sp. since they pose a considerable threat to our native species.

IV. Cape Ivy (*Delairea odorata*, Asteraceae)

A. Introduction

Cape ivy (also known as German ivy), a native of South Africa, has recently become one of the most pervasive and alarming non-native plants to invade the coastal areas of the western United States. Botanically, this plant is a member of the sunflower family (Asteraceae), and, in the U.S., is still frequently referred to by its old name, *Senecio mikanioides*. However, its accepted scientific name in most other countries is *Delairea odorata*. A recent survey (Robison et al 2000) reports Cape ivy infestations from San Diego to southern coastal Oregon. Cape ivy is spreading in riparian forests, coastal scrubland, grassland, Monterey pine forest, coastal bluff communities, and seasonal wetlands (Figure 18). Though the species prefers moist, shady environments along the coast, there are increasing reports of infestations from inland riparian locations. This vine has the potential to cause serious environmental problems by overgrowing riparian and coastal vegetation, including endangered plant species, and is potentially poisonous to aquatic organisms.

Cape ivy has become the highest-ranked invasive species problem in the Golden Gate National Recreation Area (NRA). Golden Gate NRA has received a \$600,000 grant to spend over the next several years for Cape ivy control efforts. California State parks on the coast are heavily impacted as well. These include Big Basin, Hearst San Simeon, Mt. Tamalpais, Van Damme and Jughandle. U.S. Forest Service lands along the Big Sur coast are heavily impacted, as are lands of many other agencies on the coast.

It was introduced into the Big Island of Hawaii around 1909 and has become a serious weed in a variety of upland habitats there, between 200 and 3000 meters elevation. (Jacobi and Warshauer 1992). Two reports (Haselwood and Motter 1983, Jacobi and Warshauer 1992) state that this vine, in the Hawaiian Islands is restricted to the Big Island. However, Wagner et al. (1990) state that it is also sparingly naturalized on Maui.

Figure 18. Cape ivy growing in some of its diverse habitats: A. On a stream bank in South Africa. B. In the Manuka Forest in Hawaii. C. On Mt. Sutro in San Francisco, CA.



Unfortunately, Cape ivy on the West Coast prefers and grows vigorously in physically challenging environments such as streamside thickets, willows, and poison oak. Its growing habits in California make it extremely difficult to control by conventional mechanical and chemical means. Therefore, development of a safe and effective biocontrol program for Cape ivy has become an urgent priority. If successfully developed for Cape ivy, biocontrol will give land managers an extremely cost-effective tool, and it will bring the added benefit of minimizing the use of herbicides in the environment. Biocontrol offers the best chance, within a framework of integrated pest management, to protect coastal habitats from extensive degradation due to Cape ivy.

B. Cooperative Research in South Africa – the Native Home of Cape Ivy

Since 1997, CalEPPC, in partnership with California Native Plant Society (CNPS), has spearheaded a campaign to raise funds to assist our USDA-ARS project to develop biological control agents for Cape ivy, *Delairea odorata* (synonym, *Senecio mikanioides*). USDA-ARS does not receive sufficient funds from Congress to fully fund the research on Cape ivy, and we were unable to finance the necessary overseas research. In each of the past four years, CalEPPC and CNPS have been successful in raising \$45,000-65,000 annually, which was then contributed to the Cape Ivy Project at the USDA quarantine in Albany, CA. As leader of this project, I then used the CalEPPC/CNPS contributions to contract research in South Africa, the native home of Cape ivy, to locate and develop potential biological control agents. I was fortunate enough to obtain the services of several talented South African scientists for this project. Each year, I provide a research plan to these cooperating scientists, then spend 4-5 weeks with them in South Africa, assisting in the research, reviewing their results, and jointly planning the research for the following year.

The third year of Cape ivy research in South Africa was completed in March 2001. During the first year, the South African team, led by Beth Grobbelaar, located Cape ivy populations throughout South Africa, and collected the natural enemies that attacked it. This was not an easy task, since, in South Africa, Cape ivy is a very uncommon plant, and even expert botanists had never seen a plant in the wild. Nevertheless, our team was eventually successful in locating Cape ivy at several dozen sites in South Africa. Over 200 species of plant-injuring insects were collected on Cape ivy at these sites (Grobbelaar et al., in press). I visited South Africa in August of 1998, and after a 3000 kilometer survey that visited most of the Cape ivy sites in the country, we selected the six most promising of these insects for further research. These included: *Diota rostrata* (Arctiidae) - a defoliating caterpillar; *Acrolepia* new species (Plutellidae) – a stem boring/leaf mining moth caterpillar; *Parafreutreta regalis* (Tephritidae) - a stem galling fly; an unidentified leaf mining Agromyzid fly; and two species of Galerucine leaf beetles (Chrysomelidae) - which feed on leaves as adults or larvae.

During the second year, our South African team tried to collect these six insects on relatives of Cape ivy growing at these sites. More than a dozen close relatives of Cape ivy were repeatedly

examined, but only one of the six insects, an arctiid moth - *Diota rostrata*, was ever collected on anything but Cape ivy. However, during my 1999 visit to South Africa, we were able to conduct a field assessment that showed that this moth greatly prefers Cape ivy [see following section on *Diota*] Thus, it would appear that at least five insects are very host-specific to Cape ivy. These insects are likely to survive further intensive testing of their host range (the plants on which each insect species will feed and develop). And even the sixth, the moth *Diota*, may eventually prove to be sufficiently safe for release.

***Diota rostrata* – 1999 Open cage test**

During his 1999 visit to South Africa (see Appendix D for details), Joe Balciunas had an opportunity to conduct a quantitative assessment of the field host range of one of these agents. On Saturday, 4 December 1999, after returning from a week-long field trip, Beth Grobbelaar and Joe Balciunas noticed that the Cape ivy planted outside the laboratory in central Pretoria was heavily damaged by caterpillars of the moth *Diota rostrata* (Lepidoptera: Arctiidae). Some 10 days earlier, (Wed., 23 Nov. 1999), we had examined this Cape ivy and related *Senecio*'s planted in the same garden, and had not noted any *Diota* damage to Cape ivy, or to the other plants. During our subsequent examination, we were impressed not only by the extent of damage to Cape ivy, but the relatively light damage to its relatives. We felt that we had a fortuitous opportunity for an open-cage host range evaluation, and decided to quantitatively assess the damage to these various plants.

We conducted our damage assessment on Dec 8, 1999. First, using a tape measure, we measured the dimensions of the garden, and the locations of the various plants. The garden consisted of a raised bed, retained by a 0.5 m high rock wall approx 5m long, along a pathway between two buildings at Biosystematics Division's main headquarters in Arcadia, Pretoria, South Africa. The garden bed was oriented on an East-West axis, and was broken into two unequal segments by a set of concrete steps. The western-most segment was shorter, but contained the greater number of Senecioneae species. The eastern segment was longer, but contained only two test plant species; *Cineraria deltoidea*, near the west end, and DODO+ near the eastern end. We assigned each species that we used in these and other tests an acronym as a label. The Cape ivy in this bed had ear-like "auricles" formed by the stipules at the base of the leaves. Our field observation over the past two years, indicate that this is the typical form in South Africa. In the western end, we grew the rarer - thus far found only at one location in South Africa - form of Cape ivy that lacks these auricles. Interestingly, in California, most Cape ivy plants lack auricles. The acronyms for the two forms are distinguished by adding a plus sign to the former, and a minus sign to the latter.

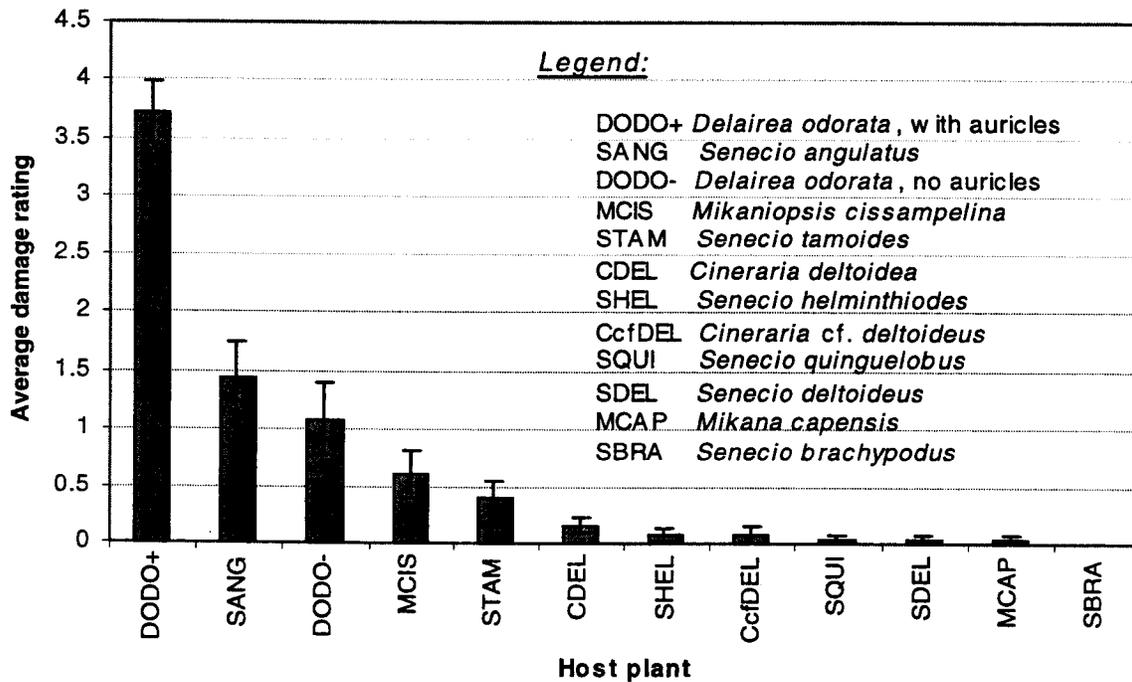
We then established a rating system for the damage. Since the area of the leaves consumed had not been measured prior to the attack, we could not determine the exact amount of leaf material consumed by the *Diota* caterpillars. However, we have extensive field experience with leaves of Cape ivy and its relatives, and we are confident we can categorize the extent of feeding damage. We established the endpoints for each damage class (see Figure 19). Both of us individually

scored the damage, and if our scores were not unanimous (in less than 5% of the cases) we would arrive at a consensus score. Leaves were selected with one of us standing with our back to the garden wall, and reaching backwards and selecting a stem. All leaves greater than 3.0 cm long, distal from the point where the evaluator's fingers encircled the stem were evaluated. This procedure was repeated for each plant species, until 25 leaves had been scored. We evaluated the data, using Excel 98 and SigmaPlot (ver. 4.0) software (figure 20).

Figure 19. *Diota rostrata* damage rating scale.



Figure 20: Average *Diota rostrata* damage rating per leaf for various host plants.



The Cape ivy with auricles was the most damaged, while *Senecio angulatus* and the Cape ivy without auricles were the next most preferred by *Diota*. Six of Cape ivy's close relatives were scarcely fed on by *Diota*. Since only Cape ivy occurs in the U.S., and it was the preferred host in this garden, further investigation into the safety of this damaging moth is warranted.

C. 2000 Research in South Africa

During the third year (2000-01), the research in South Africa focused on learning more about the biology, safety, and impact of some of the most promising insects. This phase of research was led by the eminent South African weed biocontrol specialist, Dr. Stefan Naser, and his assistant Liamé van der Westhuisen. They were eventually able to establish laboratory colonies of two Cape ivy insects: the stem-boring moth *Acrolepia* new species, and the gall-forming fly *Parafreutreta regalis*. Joanna Wing, a USDA-sponsored graduate student at Wits University in Johannesburg, has nearly completed her M.Sc. research, concentrating on the biology of the arctiid moth, *Diota rostrata*. And Beth Grobbelaar was able to temporarily maintain a colony of chrysomelid beetles that severely damage the leaves of Cape ivy - but this colony died out.

Dr. Naser made great progress in his studies of *Acrolepia* and *Parafreutreta*. I was convinced that not only would these two insects prove host-specific enough to release, but that we now knew enough about their biology to successfully rear them in our USDA quarantine in Albany, CA. As a result, prior to my visit to South Africa in December (see Appendix D for details), I obtained the necessary import permits, and when I returned from South Africa on Jan. 9, 2001, I hand-carried both these insects back to our quarantine. As these are the first Cape ivy insects to be imported into the United States, they represent a significant milestone.

The stem-boring moth (*Acrolepia* n. sp.) was discovered during our surveys and appears to be new to science and never before described. It is, however, one of the most widely distributed of Cape ivy natural enemies, and we collected it at nearly all our Cape ivy sites in South Africa. This tiny moth (less than a ¼ inch in length) lays eggs within the leaf of Cape ivy. Minute caterpillars hatch out and tunnel within the leaves, leaving distinctive, narrow "mines". Some of the caterpillars bore down through the leaf petiole, and then bore inside the stem of Cape ivy. In the lab, most of the mined leaves, and many of the bored stems die, and sometimes the entire Cape ivy plant is killed.

The gall fly, *Parafreutreta regalis*, is a fruit fly (family Tephritidae) that appears to specialize on Cape ivy. The female *Parafreutreta*, about the size of a large housefly, lays eggs inside the growing tip of Cape ivy. The little maggot that hatches out inside the tip causes Cape ivy to grow a spherical structure, about a ½ inch in diameter, within which the maggot completes its life cycle. These galls seem to inhibit further elongation of that stem, although side shoots are usually produced. The weight of the gall causes the stem to droop, and most galls are beneath a "mat" of Cape ivy. We theorize that "galled" Cape ivy plants will be less aggressive in clambering over native trees and shrubs.

Now that we have these first two Cape ivy insects in our quarantine, we will begin a lengthy investigation into their host specificity. We must be confident of the safety of any insect we seek to release to control Cape ivy. To speed up this process, our South African cooperators will conduct host-specificity tests of these two insects, in parallel with us in Albany. Several years of additional laboratory and field evaluations of their host range will be required. Then, if we still feel that the insect is safe, we will prepare a request for the release. Regulatory approval for release can easily take an year. Thus, it will probably be three to five more years before the first of these insects is released in California. In the meantime, we are assembling the test plants we will need for our host range tests.

D. Cape ivy Toxicity Tests

It is known that many *Senecio* species produce a variety of toxic compounds, among them pyrrolizidine alkaloids, that are potent mammalian hepato-toxins. Eleven different pyrrolizidine alkaloids have been detected in Cape ivy (Stelljes *et al.* 1991). Several preliminary, unpublished studies have shown that Cape ivy when floated in an aquarium, will kill fish (Carla Bossard, personal communication), and a variety of aquatic insects (Vincent Resh, personal comm.).

We wanted to confirm the toxic effects observed by Bossard and Resh. We initiated a series of tests on mosquito larvae (*Orthopodomyia signifera*) and mosquito fish (*Gambusia affinis*), by exposing them to differing concentrations of ground-up Cape ivy leaves in water, to see if they would succumb to Cape ivy exposure.

In one test, we exposed two male mosquito fish (collected from the quarantine laboratory moat) each in 225 mL of water they were captured from in a 250 mL Erlenmeyer flask with Cape ivy leaves and vines dangling into the water in the flask. A third fish was used as a control - kept in a similar set up as the previous two fish, except without Cape ivy. After five days, all three fish were alive.

In another test we used 30 late instar mosquito larvae which we collected from standing water beside the USDA-WRRC. We placed each larvae in an individual test tube with 10 mL of the stagnant water they were collected from. We crushed and liquefied Cape ivy leaves with a mortar and pestle. We pipetted five different concentrations of crushed Cape ivy leaves (0.2, 0.4, 0.6, 0.8 and 1 mL) into 10 mL of stagnant water, each with a mosquito larvae. The remaining five mosquito larvae were left in water without crushed Cape ivy (the control). We checked these larvae daily. This test was ended after seven days, when most (73%) of the mosquito larvae had emerged as adults or pupated. The remaining 27% of the larvae died. However, the highest rate of mortality was for the control, with four of the five mosquito larvae dying.

We repeated this experiment using a stronger concentration of crushed Cape ivy leaves. We started with 20 mid- to late instar larvae, each in an individual test tube with 5 mL of the stagnant water they were collected from. We classified the Cape ivy leaves we used, by separating them

into what we judged as young, middle aged, and old aged leaves, to test whether or not the age difference in the leaf would prove more lethal. The difference between the leaves was size, and position of growth on the plant. Leaf rust was present on almost all leaves. Older leaves had many more rust spots than the younger ones. Three solutions were made, crushing and liquefying 10 leaves from each age class (younger, middle-aged, older). We added 1 mL of crushed younger leaves to a test tube with one larvae, in 5 mL of stagnant water, for five larvae. We did the same for the middle aged leaves and older leaves. The five other larvae were kept in the water alone as the control.

We ended this test in five days after all the larvae had died or emerged as adults. All the control larvae emerged as adults. Interestingly, more larvae died in the younger solution (three) than survived and developed into adults (two). However the majority of mosquito larvae (60%) emerged as adults in the three solutions.

We also tested mosquito fish by exposing them to crushed Cape ivy or *Petasites frigidus* var. *palmatus* leaves. *Petasites frigidus* var. *palmatus* is a common riparian plant in the western US. We placed each fish (captured from the USDA-WRRC quarantine moat) in a 250 mL Erlenmeyer flask with 200 mL of moat water. We crushed one *Petasites* leaf in a mortar and pestle and added it to one flask, this was then repeated for another fish. We collected 40 Cape ivy leaves - 10 yellowing with stem, 10 yellowing without stem, 10 green with stem and 10 green without stem. Five Cape ivy leaves were crushed from each one of the leaf designations and added to a fish. We did this for the remaining eight fish so that each of the four leaf designations was repeated twice.

All fish died within seven days. Each of the two fish exposed to the *Petasites* control died almost immediately after adding the *Petasites* leaf to the flask. The two fish exposed to the green Cape ivy without stems, died within 2-3 days. Both fish exposed to the green Cape ivy with stems died after five days. Fish exposed to the yellow Cape ivy with stems died on the 6th and 7th day.

In all tests, the concentrations of Cape ivy was much stronger than would normally be encountered in nature. Essentially we were trying to kill these organisms, yet most survived. Furthermore *Petasites* - a plant more widespread than Cape ivy, proved to be lethal when exposed to mosquito fish in a matter of minutes, whereas Cape ivy took several days to kill fish or mosquito larvae. Although poisonous compounds may exist in Cape ivy, we doubt that these compounds are toxic at the level they would be found in nature to either of the organisms we tested. However, other organisms maybe far more susceptible, and thus the question of Cape ivy's reputed toxicity remains unsolved.

E. Senecioneae Germination and Test Plant Lists

Since Cape ivy is closely related to the genus *Senecio*, native plants (as well as some exotics) from this genus are essential for host range testing of potential Cape ivy biological control agents. In anticipation of the receipt of these agents in our quarantine laboratory in early 2001, we tested our current *Senecio* seed samples for germination, hoping to use them in future host range tests. We currently have nine species of *Senecio* as well as two other related plants important for host range testing. The germination rates are shown in Table 20.

Table 20: *Senecio* seeds tested for germination for future Cape ivy host range testing.

Scientific Name	Date collected	Location collected	Date planted	Seeds planted / % germinating
<i>Senecio blochmaniae</i>	10/15/98	Pismo State Beach, Oceano, San Luis Obispo Co., CA	3/5/99	? / 0.0%
			10/6/99	90 / 0.0%
	10/15/00	Guadalupe, CA	11/16/00	60 / 83.3%
	10/16/00	Oceano State Beach, CA	11/16/00	60 / 30.0%
	10/21/00	Lompoc, CA	11/16/00	60 / 80.0%
<i>Senecio bolanderi</i>	7/16/00	Brookings, OR	8/23/00	54 / 70.4%
<i>Senecio breweri</i>	5/1/98	Birdwell Ranch, Fresno Co., CA	2/14/00	72 / 36.1%
			12/13/00	27 / 48.1%
<i>Senecio flaccidus</i> var. <i>douglasii</i>	10/98	Rustic Canyon, Santa Monica Mtns., CA	10/27/99	18 / 33.3%
	10/99	"	10/27/99	18 / 48.1%
			12/29/99	150 / 73.3%
			12/13/00	36 / 58.3%
<i>Senecio ganderi</i>	6/16/00	Black Mountain, Ramona, CA	9/20/00	15 / 26.7%
			12/8/00	10 / 20.0%
<i>Senecio macounii</i>	8/3/99	Siskiyou National Forest, Curry Co., OR	9/13/99	54 / 40.7%
			10/6/99	180 / 37.8%
			12/29/99	120 / 50.0%
<i>Senecio triangularis</i>	8/31/00	Curry County, OR	9/13/00	36 / 5.6%
<i>Senecio jacobaea</i>	8/31/00	Curry County, OR	9/13/00	45 / 73.3%
			12/13/00	45 / 77.8%
<i>Senecio vulgaris</i>	10/2/00	Albany, CA	10/4/00	45 / 75.6%
	12/4/00	WRRC Greenhouse, Albany, CA	1/5/01	54 / 61.1%
Plants related to the genus <i>Senecio</i>, and deemed important for host range testing				
<i>Adenocaulon bicolor</i>	10/98	Muir Woods, Marin Co., CA	8/23/00	6 / 0.0%
	10/99	Big Basin State Park, Santa Cruz Co., CA	12/29/99	12 / 33.3%
			9/20/00	5 / 60.0%
			10/2/00	6 / 16.7%
			12/8/00	9 / 33.3%
<i>Luina hypoleuca</i>	7/22/00	Curry County, OR	12/8/00	45 / 37.8%
	8/30/00	"	8/23/00	18 / 22.2%
			9/13/00	36 / 5.6%
			12/8/00	45 / 24.4%

We have prepared a list of host plants on which we plan to test our biocontrol agents (Table 21). This is being circulated to our stakeholders and botanists for comment. We will then submit it to TAG for their approval. Recently, Cape ivy was removed from its former genus - *Senecio*, and placed in its own genus (Jeffery 1986, 1992). *Delairea* is a monotypic genus, and is thought to be closely related to plants of the genera *Mikaniopsis*, *Cissampelopsis*, and *Austrosynotis*, none of which are represented in the U.S (Jeffery 1986, 1992). Its closest relative in the US are plants from the genus *Senecio* of which it was once considered. *Senecio* is a very large and diverse genus with 36 species native to California and 6 other exotic introduced species (including *Delairea odorata* - formerly known as *Senecio mikanoides*)(Barkley 1993). These species are found in a very wide range of habitats and geographical locations, and vary greatly morphologically. Since Cape ivy is generally found in riparian forests, coastal scrubland, grassland, Monterey pine forest, coastal bluff communities, and seasonal wetlands in California and Oregon, we presume that these *Senecios* are the closest related to Cape ivy, and have selected them in our list. Nothing is known of the genetic variability of Cape ivy populations among different geographic locations.

Table 21. A list of host plants we wish to test Cape ivy biological control agents on.
* denotes an introduced exotic species.

Order: Family	Tribe: Subtribe	Plant species	Category	Notes
Asterales: Asteraceae	<u>Senecioneae:</u> Senecioninae	<i>Cineraria deltoidea</i> †	7	To be tested in S. Africa
		<i>Cineraria lobata</i> †	7	To be tested in S. Africa
		* <i>Delairea odorata</i>	1	Native of South Africa, the target weed
		<i>Senecio angulatus</i> †	7	To be tested in S. Africa
		<i>Senecio aphanactis</i>	3	
		<i>Senecio astephanus</i>	3	
		<i>Senecio blochmaniae</i>	3	
		<i>Senecio bolanderi</i>	3	
		<i>Senecio brachypodus</i> †	7	To be tested in S. Africa
		<i>Senecio breweri</i>	3	
		<i>Senecio californicus</i>	3	
		<i>Senecio deltoideus</i> †	7	To be tested in S. Africa
		* <i>Senecio elegans</i>		“Purple Ragwort” - and ornamental plant
		<i>Senecio flaccidus</i>	3	

Table 21. (continued)

Order: Family	Tribe: Subtribe	Plant species	Category	Notes		
Asterales: Asteraceae	<u>Senecioneae:</u> Senecioninae	<i>Senecio ganderi</i>	3	California listed - Rare		
		<i>Senecio helminthiodes</i> †	7	To be tested in S. Africa		
		* <i>Senecio hybridus</i>	3	“Cineraria” -an ornamental plant		
		<i>Senecio hydrophilus</i>	3			
		<i>Senecio integerrimus</i>	3			
		* <i>Senecio jacobaea</i>	3	Noxious weed “Tansy Ragwort”		
		<i>Senecio layneae</i>	4	CA listed - Rare, Federally listed - Threatened		
		<i>Senecio lyonii</i>	3			
		<i>Senecio macounii</i>	3	“Siskiyou Mtns. Ragwort”		
		<i>Senecio macroglossoides</i> †	7	To be tested in S. Africa		
		<i>Senecio pleistocephalus</i> †	7	To be tested in S. Africa		
		<i>Senecio quinquelobus</i> †	7	To be tested in S. Africa		
		<i>Senecio tamoides</i>	7	To be tested in S. Africa		
		<i>Senecio triangularis</i>	3			
		* <i>Senecio vulgaris</i>	3	“Groundsel”		
		* <i>Erechtites glomerata</i>	3			
		* <i>Erechtites hieracifolia</i>	3			
		* <i>Erechtites minima</i>	3			
		* <i>Euryops pectinatus</i>	3	Ornamental plant “Golden Scrub Daisy”		
		* <i>Euryops virgineus</i>	3	Ornamental plant		
		<i>Mikaniopsis cissampelina</i> †	7	To be tested in S. Africa		
		<u>Senecioneae:</u> Blennospermatinae		<i>Blennosperma bakeri</i>	4	“CA & Federally listed - Endangered
				<i>Blennosperma nanum</i>	3	California listed - Rare
		<i>Crocidium multicaule</i>	3			
<u>Senecioneae:</u> Tussilaginaceae		<i>Cacaliopsis nardosmia</i>	3			

Table 21. (continued)

Order: Family	Tribe: Subtribe	Plant species	Category	Notes
Asterales: Asteraceae	<u>Senecioneae:</u> Tussilaginaceae	<i>Lepidospartum squamatum</i>	3	
		<i>Luina hypoleuca</i>	3	
		<i>Petasites frigidus</i> var. <i>palmatum</i>	3	
		<i>Tetradymia argyraea</i>	3	
		<i>Tetradymia canescens</i>	3	
		<i>Tetradymia comosa</i>	3	
	<u>Eupatorieae</u>	<i>Mikania capensis</i> †	7	To be tested in S. Africa
	<u>Astereae</u>	<i>Aster</i> sp.	4	Several cultivated species
	<u>Helenieae</u>	<i>Arnica cernua</i>	4	CNPS List 2 or 4
	<u>Helenieae</u>	<i>Arnica spathulata</i>	4	CNPS List 2 or 4
	<u>Helenieae</u>	<i>Arnica fulgens</i>	4	CNPS List 2 or 4
	<u>Helenieae</u>	<i>Arnica venosa</i>	4	CNPS List 2 or 4
	<u>Helenieae</u>	<i>Arnica viscosa</i>	4	CNPS List 2 or 4
	<u>Cardueae</u>	<i>Helianthus annuus</i>	3	Agriculturally grown "Sunflower"
<u>Cardueae</u>	<i>Cynara scolymus</i>	3	Agriculturally grown "Artichoke"	
<u>Cardueae</u>	<i>Carthamus tinctorius</i>	3	Agriculturally grown "Safflower"	
<u>Mutisieae</u>	<i>Adenocaulon bicolor</i>	3	"Trail Plant"	
Apiales: Araliaceae		<i>Hedera helix</i>	6	Ornamental "English ivy"
Violales: Cucurbitaceae		<i>Marah fabaceus</i>	6	"California man-root"
Cornales: Vitaceae		<i>Vitis vinifera</i>	6	Agriculturally grown "Grapes"
Solanales: Convolvulaceae		<i>Convolvulus</i> sp.	6	Morning glory
		<i>Calystegia</i> sp.	6	Morning glory

† species not found in North America. Asteraceae Cladistics and Classifications from Bremer 1994.

Category 1 - Genetic types of the target weed species found in N. America

Category 2 - N. American species in the same genus as the target weed. (None)

Category 3 - N. American species of other genera in the same family as the target weed.

Category 4 - Threatened and endangered species in the same family as the target weed.

Category 5 - N. American species in other families (None)

Category 6 - N. American species in other orders that have some morphological or biochemical similarities to the target weed

Category 7 - Any plant on which the biological control agent or close relatives (within the same genus) have been previously found or recorded to feed and/or reproduce.

(from: Wapshere *et al.* 1974)

V. Code of Best Practices

The practice and theory of biological control is coming under increasing scrutiny and criticism, not only from members of the public, but from concerned ecologists and entomologists as well. However, many of these arguments do not apply to the classical biological control approach, though this does not mean that the criticisms lack merit. Classical biological control for weeds is an extremely powerful tool, and every practitioner must keep in mind that the release of even the safest organism is attended by a small, but not inconsequential, risk for causing harm. Therefore, a set of guidelines - a 'Code of Best Practices' - has been assembled to minimize risks to native plant populations and increase the overall effectiveness of classical biological control of weeds. These twelve guidelines apply not only to the introduction of new overseas agents, but also to the redistribution of established agents. It is hoped that this Code of Best Practices becomes widely adopted by those involved in biological control of weeds and supported and endorsed by a large array of ecologists.

Code of Best Practices for Classical Biological Control of Weeds

**(as approved July 9th, 1999, by the delegates to the
Xth International Symposium on Biological Control of Weeds,
Bozeman, Montana)**

- 1. Insure target weed's potential impact justifies release of non-endemic agents**
- 2. Obtain multi-agency approval for target**
- 3. Select agents with potential to control target**
- 4. Release safe and approved agents**
- 5. Ensure only the intended agent is released**
- 6. Use appropriate protocols for release and documentation**
- 7. Monitor impact on target**
- 8. Stop releases of ineffective agents, or when control is achieved**
- 9. Monitor impacts on potential non-targets**
- 10. Encourage assessment of changes in plant and animal communities**
- 11. Monitor interaction among agents**
- 12. Communicate results to public**

Resolution

**(ratified July 9th, 1999, by the delegates to the Xth International Symposium on Biological
Control of Weeds, Bozeman, Montana)**

Delegates and participants to the Xth International Symposium for Biological Control of Weeds, recognizing the need for professional standards in the subdiscipline of classical biological control of weeds, urge practitioners of the subdiscipline to voluntarily adopt the CODE OF BEST PRACTICES FOR BIOLOGICAL CONTROL OF WEEDS, as published in the proceedings of the Symposium, and adhere to the principals outlined in the code

VI. Seed Inventory

In the past few years, the seed collections of the Albany-based EIW unit have been maintained in a computerized Seed Inventory. This inventory, currently containing samples from locations around the world, is comprised of more than 1100 entries spanning over 200 species of plants. As seeds are collected they are assigned a collection number for the inventory and all collection data (date of collection, location, etc.) is entered with this number. These seed collections are currently stored in a refrigerator.

Though all seed specimens have already been entered, the USDA Seed Inventory is a work in progress; it is periodically revised with updated germination records, missing collection site information, and other pertinent pieces of knowledge. Since the entering of all the Albany-EIW seed collections into the seed inventory, efficiency in locating seeds and information on seed collections has been greatly increased. The USDA Seed Inventory will continue to be updated for new seed collections and site information.

VII. Selected Meetings and Travel by Dr. Joe Balciunas

1999

Jan. 20	Meadowview	represent ARS at quarterly CINWCC meeting. Present update on saltcedar biocontrol project
Jan. 20	El Cerrito	host Ag. Canada officials (Rose DeClerk-Floate, Patrick Prue at dinner and discussions at my house
Jan. 21	Albany	host inspection of quarantine by Ag. Canada officials
Jan. 25	Albany	serve as host for CalEPPC board meeting; after meeting, provide tour of quarantine facilities
Feb. 1	Berkeley	attend meeting of UCB Biological Control Center meeting
Feb. 10	San Pablo	serve as judge at Contra Costa County Science Fair
Feb. 17	Meadowview	annual joint ARS/CDFA weed biocontrol meeting
Mar. 11	San Francisco	serve as judge at Bay Area Science Fair
Mar.31-Apr.1	Pendleton OR	organized meeting of scientists involved in research on Scotch thistle; arranged for discounted accommodations for participants, and for group supper; moderated morning discussion sessions; presented a) status of ScT biocontrol agents in Australia, b) summary of research at Albany quarantine on <i>Lixus</i> spp., c) status of CDFA ScT research in Modoc & Lassen Counties. Prepared 2 pp summary of meeting (see Appendix C).
Apr. 12	Meadowview	CalEPPC board meeting
Apr. 20	Meadowview	CINWCC meeting
May 9-13	Greece	inspect new EBCL substation at American Farm School; accompanied by Drs. Cristofaro & Woods, visit collection sites for previous shipments of YST agents.
May 14-20	Turkey	Adana - review cooperative research by Sybil Uygur, visit YST infestation at Pozanti and Goreme
May 20-30	Armenia	Yerevan, and camping in the countryside

May 30- 6/4	Russia	Krasnodar Region; Sochi
Jun. 5-7	Russia	St. Petersburg
Jun. 8-10	Spain	retrieve <i>Ceratapion</i> specimens from Dr. Zarazaga in Madrid; collect <i>Trichosirocalus</i> and hand carry back to Albany
Jun. 22	San Francisco	present invited talk , "Status of federal biocontrol of weeds research in California" at AAAS Pacific Branch Meeting
Jun. 24	Pt. Reyes	represent ARS at Marin County Area weed management meeting
Jul. 3-10	Bozeman	attend X International Symposium for Biological Control of Weeds; present invited talk "A Proposed Code of Best Practices for Classical Biological Control of Weeds": also present poster "The accidental introduction of <i>Chaetorellia succinea</i> into the USA – a 'lucky break' for biological control of yellow starthistle?"; organized and led meeting of taskforce (13 attendees) to review overseas yellow starthistle research; discussed progress on Cape ivy project with South African scientists
Jul. 22	Meadowview	attend quarterly CINWCC meeting
Aug. 6	Ione	organize and participate in television interview on biological control of YST by KCRA (Channel 3-Sacramento) at 2 sites near Ione
Aug. 10	Albany	interviewed about yellow starthistle by Elizabeth Ritter, Farm Bureau reporter
Oct. 15-17	Sacramento	CalEPPC Annual Symposium; present update on progress of Cape Ivy Biocontrol Project; moderate Cape ivy breakout session
Oct. 24-26	Albuquerque	W-185 Annual Meeting; invited to present short talk about the newly adopted "Code of Best Practices for Biological Control of Weeds"
Nov.11- Dec.17	South Africa	review PPRI research, and plan Year 3 research (see trip log)

2000

- Jan 19-21 Asilomar National Annual Ecological Farming Conference; at the request of organizers, assembled a panel of biocontrol researchers, and organized a workshop for this conference; presented lead talk "Theory and History of Weed BioControl" for this invited workshop. The workshop presentations were professionally tape recorded, and copies were sold during and after the conference.
- Feb 7-8 Ft. Cronkite Participated in 2-day retreat by present and former CalEPPC board members to develop action plan for CalEPPC
- Mar. 9 Meadowview represent ARS at Broom Taskforce meeting
- Mar. 21-28 Albany serve as host for Boris Korotyaev, Russian weevil specialist; arrange and travel with Boris to insect collections at Calif. Academy of Sciences, U.C. (Berkeley and Davis), and CDFCA (Sacramento); field collecting in Napa, Sonoma, and Marin counties; learn to identify different species of *Ceratapion* and *Lixus*; as coauthor, begin preparation of manuscript on hosts and identifications of seven *Ceratapion* species.
- Mar. 23 San Francisco Serve as judge at San Francisco Bay Area Regional Science Fair.
- Apr. 10 San Diego represent ARS at Cal EPPC Board meeting
- Apr. 18 Meadowview represent ARS at CINWCC meeting
- May 1-2 Kona, HI examine Cape ivy infestations on Big Island
- May 3-5 Honolulu, HI attend Biological Control of Weeds Workshop: present **invited** paper on "Strategies for Overseas Research"
- June 5 Pistol River examine Cape ivy and native *Senecio* sites in south-eastern Oregon with Veva Stansell and Eric Coombs; collect *S. bolanderi* and *S. triangularis* plants for transplanting at quarantine
- June 6 Arcata examine Cape ivy sites in NE California with Gordon Lepig
- June 12 Davis represent ARS at CalEPPC board meeting
- July 9 Dulles, VA pre-workshop meeting of Planning Committee for ARS Weed Science Workshop

July 10	Dulles, VA	received professional training to serve as “facilitator”
July 11-13	Dulles, VA	served as facilitator/recorder for three “breakout” sessions during ARS Weed Science Workshop; after the workshop, served as Co-coordinator for assembling and writing “Ecology” Component for new Weed Science National Program; also served on writing team for the “Evaluation” section of the “Biological Control” Component.
Aug. 17	Albany	served as host for CalEPPC board meeting
Sep. 8	Albany	served as video photographer at WRRC Open House
Oct. 6-8	Concord	CalEPPC Annual Symposium: served as moderator for Friday afternoon session, and Evening breakout session; presented talk, “Update on Biological Control of Cape Ivy” and poster [as coauthor with Jim Young] “Viability of Cape Ivy Seeds from California, Hawaii, and South Africa”
Oct. 18	San Diego	Overseas Yellow Starthistle Research Planning Meeting
Oct. 19-20	San Diego	Annual W-185 Meeting; present invited talk “Safety Considerations for Testing Weed Biocontrol Agents”

VIII. Publications Issued or Submitted

- Balciunas, J. K. 2000. Code of best practices for classical biological control of weeds. Pg. 435 *in: Proceedings of the X International Symposium on Biological Control of Weeds*, 4-14 July 1999, Bozeman, MT. Neal R. Spencer (ed.).
- Balciunas, J. 1999. The role of USDA-ARS quarantine in USDA-EIW-Albany, California in biological control of western weeds. Pp 15-16 *in: Extended Abstracts, Oregon Interagency Noxious Weed Symposium*, December 2-4, 1997, Corvallis, OR.
- Balciunas J. and G. Archbald. 1999. Cape ivy biological control effort enters year two. *Noxious Times* 2(2):8-9.
- Balciunas, Joe, and Chris Mehelis. 1999. Biological control of yellow starthistle project, Tri-Annual Report, 1996-1998. USDA-ARS, WRRC, Albany, CA.
- Balciunas, J. and B. Villegas. 1999. Two new seed head flies attack yellow starthistle. *California Agriculture* 53(2):8-11.
- Balciunas, J. and B. Villegas. (Submitted). The Unintentionally-released yellow starthistle seed-head fly, *Chaetorellia succinea* (Diptera: Tephritidae): a threat to safflower growers? *Environmental Entomology*.
- Balciunas, J. K., E. Grobbelaar, R. Robison, and S. Nesar. (In Press). Distribution of Cape ivy (*Delairea odorata* Lem.), a growing threat to western riparian ecosystems. *Journal of Aquatic Plant Management*.
- Burrows, D. W. and J. K. Balciunas. 1999. Host range and distribution of *Eucerochoris suspectus*, a potential biocontrol agent for the paperbark tree *Melaleuca quinquenervia*. *Environmental Entomology*. 28(2):290-299.
- Grobbelaar E., Balciunas, J. K., Nesar O., and S. Nesar. (In Press). South African Insects for Biological Control of *Delairea odorata*. Pp. x-x *in: Proceedings CalEPPC Symposium - Vol 6: 2000, 6-8 Oct 2000, Concord, CA*. Mike Kelly (ed.).
- Pitcairn, M. J., D. M. Woods, D. B. Joley, C. E. Turner, and J. K. Balciunas. 2000. Population buildup and combined impact of introduced insects on yellow starthistle. Pp. 747-761 *in: Proceedings of the X International Symposium on Biological Control of Weeds*, 4-14 July 1999, Bozeman, MT. Neal R. Spencer (ed.).

- Villegas, B., D. A. Mayhew, F. Hrusa, and J. Balciunas. 2000. Survey of *Chaetorellia* seedhead flies on *Cirsium* thistles in close proximity to *Centaurea* spp. in California. Pp. 62-63 in: Biological Control Program Annual Summary, 1999. D. M. Woods (ed.), California Department of Food and Agriculture, Plant Health and Pest Prevention Services, Sacramento, CA.
- Villegas, B., D. A. Mayhew, and J. Balciunas. 1999. Survey of *Chaetorellia* seedhead flies on commercial and non-commercial safflower in California. Pp. 85-87 in: Biological Control Program Annual Summary, 1998. D. M. Woods (ed.), California Department of Food and Agriculture, Plant Health and Pest Prevention Services, Sacramento, CA.
- Villegas, B., D. A. Mayhew, F. Hrusa, and J. Balciunas. 1999. Survey of *Chaetorellia* seedhead flies on *Cirsium* thistles in close proximity to yellow starthistle in California. Pp. 88-89 in: Biological Control Program Annual Summary, 1998. D. M. Woods (ed.), California Department of Food and Agriculture, Plant Health and Pest Prevention Services, Sacramento, CA.

IX. References Cited

- Balciunas, J. and B. Villegas. 1999. Two new seed head flies attack yellow starthistle. *California Agriculture* 53(2): 8-11.
- Balciunas, J. and B. Villegas. Submitted. The Unintentionally-released yellow starthistle seed-head fly, *Chaetorellia succinea* (Diptera: Tephritidae): a threat to safflower growers? *Environmental Entomology*.
- Balciunas, Joe, and Chris Mehelis. 1999. Biological control of yellow starthistle project, Tri-Annual Report, 1996-1998. USDA-ARS, WRRC, Albany, CA.
- Balciunas, J. K., E. Grobbelaar, R. Robison, and S. Nesar. (In Press). Distribution of Cape ivy (*Delairea odorata* Lem.), a growing threat to western riparian ecosystems. *Journal of Aquatic Plant Management*.
- Barkley, T. M. 1993. *Senecio*: groundsel, ragwort, butterweed. *In*: James C. Hickman (ed.) *The Jepson Manual: Higher Plants of California*. University of California Press, Berkeley, California. pp. 336-342.
- Bremer, K. 1994. *Asteraceae: Cladistics & Classification*. Timber Press, Portland, Oregon. 752 pp.
- Cordy, D. R. 1978. *Centaurea* species and equine nigropallidal encephalomalacia. *in*: Effects of poisonous plants on livestock. R. F. Keller, K. R. Van Kampen, and L. F. James (eds.), 327-36. New York, NY: Academic Press.
- Grobbelaar E., Balciunas, J. K., Nesar O., and S. Nesar. (In Press). South African Insects for Biological Control of *Delairea odorata*. Pp. x-x *in*: Proceedings CalEPPC Symposium - Vol 6: 2000, 6-8 Oct 2000, Concord, CA. Mike Kelly (ed.).
- Haselwood, E. L. and G. G. Motter, (eds.). 1983. *Handbook of Hawaiian weeds*, second edition. University of Hawaii Press, Honolulu, Hawaii. 491 pp.
- Hooper J. F., Young J. A., and R. A. Evans. 1970. Economic evaluation of Scotch thistle suppression. *Weed Science* 18(5): 583-86.
- Jacobi, J. D. and F. R. Warschauer. 1992. Distribution of six alien plant species in upland habitats on the island of Hawai'i. *In*: C. P. Stone, C. W. Smith, and J. T. Tunison (ed.) *Alien Plant Invasions in Native Ecosystems of Hawai'i: Management and Research*. University of Hawaii Cooperative National Park Resources Studies Unit, Honolulu, Hawaii. pp. 155-188.

- Jeffrey, C. 1986. Notes on *Compositae* IV: The *Senecioneae* in East Tropical Africa. Kew Bull. 41: 873-943.
- Jeffrey, C. 1992. Notes on *Compositae*, VI: The tribe *Senecioneae* (*Compositae*) in the Mascarene Islands with an annotated world check-list of the genera of the tribe. Kew Bull. 47: 49-109.
- Maddox, D. M. 1981. Introduction, phenology, and density of yellow starthistle in coastal, intercoastal, and Central Valley situations in California. Agricultural Research results, ARR-W-20, USDA-ARS, Oakland, CA. 33 pp.
- Maddox, D. M. and A. Mayfield. 1985. Yellow starthistle infestations are on the increase. California Agriculture 39: 10-2.
- Pitcairn, M. J., O'Connell R.A., Gendron J. M. 1998. Yellow starthistle: Survey of statewide distribution. In: Woods D. M. (ed.). *Biological Control Program Annual Summary, 1997*. Sacramento: California Department of Food and Agriculture, Division of Plant Industry. pp. 64-5.
- Robison, R., E. Grotkopp, and R. Yacoub. 2000 (in press). Cape ivy (*Delairea odorata*) distribution in California and Oregon. In: M. Kelly, E. Wagner, and P. Warner (eds.) Proc., California Exotic Pest Plant Council Symp., October 15-17, 1999, Sacramento, California- Volume 5: 1999. pp. xx-xx.
- Sobhian R. and H. Zwölfer. 1985. Phytophagous insect species associated with flower heads of yellow starthistle (*Centaurea solstitialis* L.). Z ang Ent 99: 301-21.
- Stelljes, M. E., R. B. Kelley, R. J. Molyneaux, and J. N. Seiber. 1991. GC-MS determination of pyrrolizidine alkaloids in four Senecio species. J. Nat. Prod. 54: 759-773.
- Turner C. E., Piper G. L., Coombs E. M. 1996. *Chaetorellia australis* (Diptera: Tephritidae) for the biological control of yellow starthistle, *Centaurea solstitialis* (Compositae), in the western USA: establishment and seed destruction. Bull. Entomol. Res. 86: 177-82.
- Villegas, B., D. A. Mayhew, F. Hrusa, and J. Balciunas. 2000. Survey of *Chaetorellia* seedhead flies on *Cirsium* thistles in close proximity to *Centaurea* spp. in California. Pp. 62-3 in: *Biological Control Program Annual Summary, 1999*. California Dept. Food & Agriculture, Plant Health and Pest Prevention Services. Sacramento, CA. Dale Woods (ed.).
- Villegas, B., D. A. Mayhew, and J. Balciunas. 1999. Survey of *Chaetorellia* seedhead flies on Commercial and Non-Commercial Safflower in California. Pp. 85-7 in: *Biological*

- Control Program Annual Summary, 1998. California Dept. Food & Agriculture, Plant Health and Pest Prevention Services. Sacramento, CA. Dale Woods (ed.).
- Villegas, B., M. J. Pitcarin, and E. Coombs. 1998. Releases of Nosema-Free *Larinus curtus* for the Biological Control of Yellow Starthistle. Pp. 47-8 *in*: Biological Control Program Annual Summary, 1997. California Dept. Food & Agriculture, Plant Health and Pest Prevention Services. Sacramento, CA. Dale Woods (ed.).
- Wapshere, A. J. 1974. A Strategy for Evaluating the Safety of Organisms for Biological Weed Control. *Ann. appl. Biol.* 77: 201-211.
- Wagner, W. L., D. R. Herbst, and S. H. Sohmer. 1990. Manual of the flowering plants of Hawai'i, Volume 1. University of Hawaii Press, Honolulu, Hawaii. 988 pp.
- White, I. M. and Marquardt K. 1989. A revision of the genus *Chaetorellia* Hendel (Diptera: Tephritidae) including a new species associated with spotted knapweed, *Centaurea maculosa* Lam. (Asteraceae). *Bull. Entomol. Res.* 79: 453-87.
- Woods, D. M., M. J. Pitcairn, D. B. Joley, J. Balciunas, and C.E. Turner. 1998. Population buildup and impact of combined biological control insects on yellow starthistle. Pp. 52-4 *in*: Biological Control Program Annual Summary, 1997. California Dept. Food & Agriculture, Plant Health and Pest Prevention Services. Sacramento, CA. Dale Woods (ed.).

X. Appendices

A. Appendix

Protocols - 1998-1999 *Chaetorellia succinea* Surveys

1. Collect 10 YST plants/ 300 heads. Plants should be chosen randomly (*i.e.*, after every other step, sample YST plant closest to your toe). If 10 plants do not have 300 heads, continue sampling entire plants until more than 300 heads have been collected.
2. Record number of heads on each plant sampled. All heads from each site can then be combined into one bag/emergence cage.
3. Measure and record height of each plant sampled.
4. Using a quarter square meter frame (or other appropriate method), obtain 5-10 measurements of YST plant densities at the site. If this is not possible, at the minimum, record a "guesstimate" of order of magnitude of YST densities (*e.g.* more than 100 YST/square meter, but less than 1000).
5. Hold heads in emergence cages [or make arrangements with Joe to do so]. Record dates, sex, and # of flies emerging.
6. After flies finish emerging, hold heads for possible dissection to determine % of seeds destroyed, etc.

B. Appendix

Protocols - 1999 *Larinus curtus* Surveys

Old (1992-95) *L. curtus* release sites

1. After all YST flowers have wilted, but before seeds are beginning to be shed (approx. mid-Sept), chose transect line through YST infestation at which *L. curtus* is established. At one meter intervals (or every other step), place a frame (0.05 - 0.1 sq. meter) around the YST, and harvest all YST plants within frame. Obtain samples from 7 frames.
2. For each of the 7 frames, record:
 - A. frame size
 - B. the number of YST plants within the frame
 - C. the height of each YST plant
 - D. the number of intact heads on each YST plant
 - E. the number of *Eustenopus*-damaged wilted heads/branch "nubs" on each YST plant
3. Clip all heads from each frame, and combine into one bag - 7 bags per site.
4. At your laboratory (or make arrangements with Joe to have your sample processed at Albany), split each head, and for each damaged head record the following:
 - A. maximum head diameter
 - B. number of intact seeds remaining
 - C. species of agent(s) and life stage/damage in head; e.g., *Larinus* larva, *Eustenopus* pupal case, *Ch. succinea* larval damage; it is not easy to distinguish the pupal cases of *Eustenopus* from those of *Larinus* - remember that *Eustenopus* will always leave an oviposition puncture/scar on the side of the head.
5. For each site, also record the number of seeds in each of 30 undamaged heads.

Recent (1996-98) *Larinus* release sites

1. Record site name, county, nearest town, latitude & longitude, and elevation.
2. Record presence or absence of *Larinus* through direct observation of adults on flowers, sweep samples, and/or dissection of heads.
3. Estimate when *Eustenopus* became established at the site, and record the presence of other agents.
4. Remember to also record above for other sites examined for *Larinus*, even if *Larinus* was not present.

C. Appendix - Scotch Thistle Research and Planning Meeting, Pendleton, OR, April 1, 1999

Participants

Joe Balciunas	ARS - Albany, CA
Eric Coombs	ODA - Salem, OR
Christina Kuykendall	Nez Perce Tribe Bio-Control Center - Lapwai, ID
Chris Mehelis	ARS - Albany, CA
Gary Piper	WSU - Pullman, WA
Dan Sharratt	ODA - Union, OR
Linda Wilson	UI - Moscow, ID

The evening before the meeting, almost all the participants were able to attend a get-together dinner at Cimmiyotti's Restaurant. The research review and planning meeting began at 8:15 AM, on April 1st, 1999 in the County Extension Office in Pendleton, Oregon.

Joe Balciunas began the meeting by reviewing the status of 5 insects released in Australia for control of Scotch thistle (ScT), as well as a brief discussion of several other insects still under consideration there. Thus far, the Australian's have found *Lx. new sp.* to have the most impact on their infestations, but *Lr. latus* is also beginning to come on strong. *Tephritis postica* never established. *Tr. new sp.* and *Eublemma amoena* have only recently been released and their impacts cannot yet be determined. Joe and Dan Sharratt also noted that the ScT in Australia is different, usually being a hybrid with *Ono. illyricum*, and having larger heads than ScT in the USA.

Joe then presented the results of his quarantine research on *Lx. cardui* and *Lx. new sp.* - the former from Greece, the latter from France. After two years of host range testing, Joe found that both these weevils caused considerable damage to native thistles. Joe, therefore, suspended further evaluations of *Lixus* weevils. Joe and Chris Mehelis then talked about their just-initiated research on *Tr. new sp.* The larvae of these weevils bore into the roots of ScT rosettes, and frequently kill them.

The participants then reviewed the distribution and problems caused by ScT in their respective regions. Joe led off the discussions with maps of the distribution of *Onopordum* spp. in California. In addition to ScT, two other *Onopordum* spp. (*Ono. illyricum* and *Ono. tauricum*) are established in CA., but are found in only a few sites, and are under eradication efforts. ScT is much more widespread - Joe "guesstimated" about 10-15,000 acres in CA. It is a "Class A" weed, and is therefore mandated for eradication. The most severe infestations are in NE CA, especially in Modoc & Lassen counties. These two counties spent nearly \$200,000 in 1995 in trying to eradicate ScT.

After a ten minute break at 10AM, Linda Wilson and Chris Kuykendall discussed the distribution of ScT in Idaho. Only 9 of Idaho's 44 counties are free of this thistle. The most severe infestations are in the Snake River drainage areas. No estimates of acreages are available for the whole state. Chris K. did, however, present a map of the acreages for the watersheds in the Moscow- Orofino region.

Gary Piper presented a map which displayed the acres [usually a range of values] of ScT in each of Washington state's 39 counties. It has been reported from 23 counties, on both sides of the Cascades, with acreages ranging from 10 acres, to more than 1000 acres. The largest infestations were in Okanogan County, which borders Canada in North-central WA. Gary estimated the total infestation in WA at 8,000-9,000 acres.

Dan Sharratt and Eric Coombs presented the distribution data for Oregon. Although ScT is, for the most part, confined to east side of the Cascades, there is still 1.18 million gross acres [560,000 net acres] infested by this thistle -- most of it along streams and rivers draining into the Snake River. Unlike the other states, most of the infestations are on public lands.

Gary Piper presented the results of a master's. thesis survey of ScT herbivores by a former student of his, James Watts, conducted at 10 sites in 5 WA and ID counties. Sampling involved both sweeping and destructive examination of ScT plants. Although 30 spp. of insects in 17 families were found, only the aphid, *Brachycaudus cardui*, was common. Gary and Jim concluded that there was little impact on ScT from these insects, and that almost all feeding niches were available for introduced biocontrol agents.

Next, there was nearly an hour of discussion on the pre-release ScT population dynamics studies. All attendees agreed that there wasn't enough information on the biology of ScT. Joe handed out the protocols which ARS, CDFA, and ODA attempted to follow for the past 2-3 years. These protocols were an abbreviated version of the intensive studies being conducted on ScT in Australia by CSIRO. Dan Sharratt discussed the difficulties and time demands of studying ScT. Especially frustrating was the "jumping patch" nature of the ScT at his Oregon study sites, where, in some years, there would be nearly no ScT in his study plot, but a thriving patch would spring up a few yards away. Joe also presented the research done by Don Joley of the CDFA, who was unable to attend. CDFA is not allowing the ScT to produce seeds in their study plots. This should allow for measurement of seed depletion from the seed bank at their sites. Chris K. was optimistic about getting a grant to hire a couple of field technicians to focus on ScT biology research not only in Idaho, but perhaps even to assist Dan in his evaluations.

The meeting concluded with a group discussion on future research. There was strong support for Gary's view that ScT is a good biocontrol target, especially in view of its more limited, but expanding, geographic distribution. Formation of ScT task forces or consortia was discussed. The consensus appeared to be that such a consortia might best be launched when the release of an agent was imminent. The present participants would be the core "research arm" of this future

consortia. The group also supported organizing a larger meeting of land managers, administrators, and scientists interested in biocontrol of ScT in about ½ to 1 year's time.

The meeting adjourned at 1:30 after discussing and comparing personal research interests over lunch at Hal's Hamburgers.

D. Appendix - 1999 & 2000 South Africa Trip Logs

South Africa Travel Log, November - December 1999

On this trip Joe collected and processed samples from various South African sites, as well as consult with other prominent names in the South African biocontrol field. Joe also prepared an annual budget for Year Three research on Cape ivy.

11/11	Thursday	Fly to Capetown, via Miami
11/12	Friday	Arrive at Capetown
11/13	Saturday	Fly to Durban
11/14	Sunday	Investigate probable <i>Delairea</i> sites at Port Richards & St. Lucia
11/15	Monday	Meetings at Cedara at Drs. Jerry Olckers & Rob
11/16	Tuesday	Fly to George
11/17	Wednesday	Collect <i>Delairea</i> insects at Geroge/Wilderness area
11/18	Thursday	Drive to Tsitsikuma (Storms River) Nat. Park; collect en route at Alexandria Forest and Natures Valley
11/19	Friday	Collect at Storms River, then drive to Port Alfred
11/20	Saturday	Collect at Port Alfred, drive to Hogsback
11/21	Sunday	Collect at Hogsback, then drive to Kiusu Forest and collect there; then drive to Stutterheim
11/22	Monday	Drive 1000 km to Pretoria
11/23 - 11/24		Process samples & meetings in Pretoria
11/25 - 11/27		Free time
11/28	Sunday	Drive with Drs. Grobbelaar & Nesor to Umgeni Valley
11/29	Monday	Collect at Umgeni Valley, then drive to central Prakensberg
11/30	Tuesday	Collect at Umgeni Valley, then drive to Leopards Rock Preserve
12/1	Wednesday	Collect at Nahalalaze Reserve
12/2	Thursday	Process samples at leopards Reserve
12/3	Friday	Drive to Vryheid and collect at reserve; then drive to Pretoria
12/4	Saturday	Meetings with Drs. Helmut Zimmermann & Gerhard Prinsloo
12/5	Sunday	Meeting with Dr. Stefan Nesor
12/6	Monday	Drive to Johansburg Univ. with Dr. S. Nesor; participate in first M.S.C. Oversight committee for Joanna Wieg; review her proposal for <i>Delairea</i> research, suggest modifications.
12/7	Tuesday	Meet with Rick Holz, H.S. Agricultural Counselor; conduct quantitative assessment of <i>Diota</i> damage to <i>Senecio</i> spp. plants growing PPRI
12/8	Wednesday	Meet with Drs. Prinsloo & E. Grobbelaar and discuss upcoming Year 3 research by Biosystematics division then spend afternoon with Dr. Nesor

- discussing various scenarios for Weed Biocontrol Division evaluating some promising *Delairea* insects; choose most optimal plans and prepare annual budget
- 12/9 Thursday Fly to Cape town; meet with Dr. J. Hoffman and discuss his successful *Sesbania* biocontrol program
- 12/10 - 12/12 Free time
- 12/13 Monday Examine sites North of Cape Town for *Sesbania* and its biocontrol agents
- 12/14 - 12/15 Annual leave
- 12/16 Thursday Fly to Miami, then to San Francisco
- 12/17 Friday Arrive at San Francisco

South Africa Travel Log, December 2000 - January 2001

On this trip Joe collected two potential biological control candidates for Cape ivy and studied rearing techniques for these insects, as well as consult with other prominent names in the South African biocontrol field. Joe also prepared an annual budget for Year Three research on Cape ivy. In addition, Joe researched Cape ivy specimens in various South African herbariums.

- 12/6 Wednesday Depart San Francisco; Arrive in Atlanta
- 12/7 Thursday Depart Atlanta
- 12/8 Friday Arrive in Capetown, South Africa
- 12/11 Monday Fly to Johannesburg; accompanied by Dr. Stefan Naser and Ms. Liamé; visit Dr. Marcus Bryne and Ms. Joanna Wing at Wits University; review results of Ms. Wing's MS thesis research on *Diota rostrata*; drive to Pretoria
- 12/12 Tuesday Leave Pretoria, accompanied by S. Naser; drive to Lydenberg; examine *Acacia mearnsii* sites
- 12/13 Wednesday Drive to Long Tom Nature Reserve; collect Cape ivy and *Diota rostrata* at Reserve and at *Senecio tamoides* sites near Sabie
- 12/14 Thursday Drive to Songimvelo National Park; near Baadplaas; examine *Senecio angulatus* site by Komati River
- 12/15 Friday Return to Pretoria, via Barberton and Carolina; collect possible *Diota rostrata*
- 12/18 - 12/22 Pretoria- review Year 3 results with Stefan Naser; make video of rearing techniques for *Acrolepia* and *Parafreutreta*; plan Year 4 research, and prepare mutually agreeable budget; meet with PPRI administrators (H. Zimmerman and G. Prinsloo) and weed scientists (Carine and Martin); research at NBI Herbarium
- 12/23 - 12/24 Examine weed problems at Planesberg National Park; assemble field equipment
- 12/25 Monday Drive to Zuurberg, East Cape
- 12/26 Tuesday Collect Cape ivy and *Acrolepia* at Zuurberg Pass and Ado village; drive to Patensie
- 12/27 - 12/31 Annual Leave
- 1/1 Monday Drive from Patensie to Capetown; en route spend several hours searching (unsuccessfully) for Cape ivy in the Montagu region

1/2 - 1/5 Review and research label data of Cape ivy specimens at Bolus Herbarium (University of Capetown) and Compton Herbarium (Kirstenbosch); collect *Diota* caterpillars feeding on various *Senecio* spp. at Kirstenbosch gardens

1/7 Sunday Make final arrangements for obtaining live specimens of *Acrolepia* and *Parafreutreta* from Pretoria

1/8 Monday Leave Capetown

1/9 Tuesday Arrive in San Francisco; via Johannesburg, Cape Verde, and Atlanta (28 hrs)

E. Appendix - USDA-Albany Quarantine Laboratory Shipment Records for selected potential biological control agents.

1. *Ceratapion* shipments

Incoming file #	Location collected	Host collected from	Date received	Shipment of	Notes
BCW-WRRC-97-1001	Galani near Kozani / N. Greece	<i>Cnt. solstitialis</i>	5-6-97	58 YST roots & stems	Upon dissection retrieved 33 weevil larvae
BCW-WRRC-97-1004	Mauropigi Rd. Ptolemaida to Kozani / Macedonia, N. Greece	<i>Cnt. solstitialis</i>	6-11-97	142 adults + pupae, 54 YST roots	Not clear whether or not shipment was weevils + YST roots , or just roots that weevils emerged from . . .
BCW-WRRC-99-1005	Mauropigi, Kozani, NW Greece	<i>Cnt. solstitialis</i>	6-29-99	161 YST roots & stems	161 YST roots + stems received, 14 <i>Ceratapion</i> adults emerged.
BCW-WRRC-99-1006	Turkey	???	7-12-99	30 <i>Ceratapion</i> adults	Received 14 live adults, 16 dead, Need collection data from senders.
BCW-WRRC-00-1008	Galani near Kozani, Greece	<i>Cnt. solstitialis</i>	6/1/00	91 YST roots & stems	201 <i>Ceratapion</i> adults emerged.
BCW-WRRC-00-1009	Galani near Kozani, Greece	<i>Cnt. solstitialis</i>	6/16/00	287 YST roots & stems	92 <i>Ceratapion</i> adults emerged.
BCW-WRRC-00-1010	Mauropigi/ Ptolemaida, N. Greece Galani near Kozani, Greece	<i>Cnt. solstitialis</i>	6/23/00	344 YST roots & stems (266 from Mauropigi, 78 from Galani)	79 <i>Ceratapion</i> adults emerged from Mauropigi YST, 17 <i>Ceratapion</i> adults emerged from Ptolemaida.

2. *Chaetorellia* shipments

Incoming file #	Location collected	Host collected from	Outgoing file #	State	County	Release site	# of males	# of females	Total # released	# Specimens: <i>Jaceae</i> sp. grp.	# Specimens: <i>Loricata</i> sp. grp.
1986 ¹		<i>Cnt. cyanus</i>	host tests ²								
1987 ³		<i>Cnt. cyanus</i>	host tests ²								
BCWLA-88-1001	Mesimerion, nr Thessaloniki	<i>Cnt. solstitialis</i>	for Ursi								
BCWLA-88-1018	S. of Thermi, nr Thessaloniki	<i>Cnt. solstitialis</i>	for Ursi							9	
BCWLA-89-1010	Oreokastro, nr Thessaloniki	<i>Cnt. solstitialis</i>	BCWLA-89-26	CA	Napa	Gordon Valley Rd	60	60	120		

Incoming file #	Location collected	Host collected from	Outgoing file #	State	County	Release site	# of males	# of females	Total # released	# Specimens: <i>Jaceae</i> sp. grp.	# Specimens: <i>Loricata</i> sp. grp.
?			BCWLA-89-27	ID	Nez Perce	Central grade 6mi S Genesee	59	60	119	2	
?			BCWLA-89-28	WA	Whitman	Colfax	60	60	120		
?			BCWLA-89-29	CA	Nevada	Caltrans park & ride lot	100	100	200		
?			BCWLA-89-30	OR	Douglas	Hana Nickel Mine	58	59	117?		
?			BCWLA-89-31	CA	Plumas	Quincy	10	32	42		
BCWLA-90-1002	Thermi, nr Thessaloniki	<i>Cnt. solstitialis</i>	NR								
BCWLA-90-1008	Thessaloniki- Cereal Institute	<i>Cnt. solstitialis</i>	BCWLA-90-22 ⁴	CA	Nevada	Penn Valley	39	83	122	7	
BCWLA-90-1013	Thessaloniki- Cereal Institute	<i>Cnt. solstitialis</i>	BCWLA-90-22 ⁴	CA	Nevada	Penn Valley	39	83	122	13	
BCWLA-90-1015	6 km N. Thessaloniki, around Oreokastro	<i>Cnt. solstitialis</i>	NR								
BCWLA-91-1003	around Thessaloniki	<i>Cnt. solstitialis</i>	BCWLA-91-1	CA	Shasta	Fall River Mills	49	37	86		
BCWLA-91-1006	around Thessaloniki (Oreokastro, Mesmerion)	<i>Cnt. solstitialis</i>	BCWLA-91-10	OR	Josephine	Merlin			175?	2	10
BCWLA-91-1010	Kabani & Palino (8 km S Kilkis)	<i>Cnt. solstitialis</i>	BCWLA-91-12	WA	Whitman	Colfax			178?		

Incoming file #	Location collected	Host collected from	Outgoing file #	State	County	Release site	# of males	# of females	Total # released	# Specimens: <i>Jaceae</i> sp. grp.	# Specimens: <i>Loricata</i> sp. grp.
BCWLA-93-1001	Oreokastro	<i>Cnt. solstitialis</i>	BCWLA-93-3	ID	Latah	Kendrick	18	25	43		
?	?	<i>Cnt. solstitialis</i>	BCWLA-93-2	OR	Josephine	Merlin	50	53	103		
BCWLA-93-1002	Thermi	<i>Cnt. solstitialis</i>	BCWLA-93-1	OR	Josephine	Merlin	27	27	54		
BCWLA-94-1005	Thermi	<i>Cnt. solstitialis</i>	BCWLA-94-6	CA	Mariposa	El Portal	45	77	122		
BCWLA-94-1012	Thermi	<i>Cnt. solstitialis</i>	for bud stage expt								
BCWLA-94-1014	Thermi	<i>Cnt. solstitialis</i>	for bud stage expt								
Field Samples:			Date Collected								
			8-8-94	OR	Josephine	Merlin				1	6
			8-8-94	OR	Josephine	Rogue River Orchards				1	8
			11-8-95	CA	Humboldt	Willow Creek					9

¹ only documented shipment of YST seed heads is BCWLA-86-6, ? up to 50km around Salomika, ? received 4-18, for emergence of Ursi

² host specificity testing done using *Chaetorellia* emerged from Greek *Centaurea cyanus* seedheads

³ no documented shipments of YST seedheads or adult insects.

⁴ shipment made from more than 1 import

* from seedheads collected in Greece

? indicates specimens retained by receiver

3. *Larinus curtus* shipments

Incoming file No. BCWLA -	Location collected	Host from:	Outgoing file No.	State	County	Release site	No. of <i>Larinus</i> released	Date <i>Larinus</i> checked for <i>Nosema</i>	No. of <i>Larinus</i> checked for <i>Nosema</i>	No. of <i>Larinus</i> with <i>Nosema</i>
-7-89	Oreocastro, Greece	YST	Host range tests							
-11-89	Oreocastro, Greece	YST	Host range tests							
-16-90	Oreocastro, Greece	YST	Host range tests							
-19-90	Oreocastro, Greece - 6 Km N. of Thessaloniki	YST	Host range tests							
-24-90	Thoerri, Greece - 6 Km S. of Thessaloniki	YST	Host range tests							
-92-1002	Oreocastro, Greece	YST	Frozen at time of receipt for later laboratory analysis							
-92-1004	Oreocastro, Thessaloniki and on rd. between Kavala-Xanthi in N. Greece	YST	BCWLA-92-2	ID	Nez Perce	7 miles S. of Peck	205	9-2-95	6	1
			BCWLA-92-3	OR	Baker	Copper Butte	110	9-2-95	24	4
			BCWLA-92-3	OR	Douglas	Myrtle Creek Beacon	110			
			BCWLA-92-4	WA	Whitman	Colfax	210			
			BCWLA-92-5	CA	Sutter	Yuba City	210 or 270	7-13-95	10	4
-92-1005	Same shipment as -92-1004	YST	Destroyed due to presence of <i>Nosema</i>							
-93-1003	Oreocastro, Greece	YST	BCWLA-93-4	CA	Amador	Near Ione	200	7-26-98(?) & 7-31-99	60 / 72	0 / 0
			BCWLA-93-5	CA	Yolo	Rominger Ranch (?)	200			
			BCWLA-93-7	OR	Wasco	Kelly Ridge - The Dalles	148	7-26-98	30	0
			BCWLA-93-8	ID	Clearwater	SW of Dworshak Dam	143	9-2-95	9	0

Incoming file No. BCWLA -	Location collected	Host from:	Outgoing file No.	State	County	Release site	No. of <i>Larinus</i> released	Date <i>Larinus</i> checked for <i>Nosema</i>	No. of <i>Larinus</i> checked for <i>Nosema</i>	No. of <i>Larinus</i> with <i>Nosema</i>
			BCWLA-93-9	WA	Whitman	SW of Colfax - Union Flat	139	9-2-95	10	0
-93-1007	Oreocastro, Kavala	YST	Destroyed due to presence of <i>Nosema</i>							
-94-1011	Same as shipment -92-1004	YST	BCWLA-94-13	CA	Placer	Dry Creek	155	8-13-99	20	4
			BCWLA-94-14	CA	Sonoma	Sugar Loaf Ridge	214	8-4-99	15	1
-94-1013	Oreocastro, Kavala	YST	Destroyed due to presence of <i>Nosema</i>							
-94-1015	Oreocastro, Greece	YST	BCWLA-94-17	CA	Yolo	Rominger Ranch	201	8-16-99	8	0
-95-1003	Oreocastro / Thessaloniki and Kavala	YST	Destroyed due to presence of <i>Nosema</i>							

Notes:

BCWLA-92-4 – T15N, R43E, Sect. 14
 BCWLA-93-9 – T40N, R42E, Sect. 8 NW ¼

Site ???, Checked for *Nosema* on 9-6-95, 15 *Eustenopus* checked, none was found.

