Biological Control of Cape ivy Project
2003 Annual Research Report

prepared by Joe Balciunas, Chris Mehelis, and Maxwell Chau, with contributions from Liamé van der Westhuizen and Stefan Neser

Dr. Joe Balciunas inspecting Cape ivy infesting an area near Melbourne, Australia (Photo courtesy of Eligio Bruzzese)

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Executive Summary

by Dr. Joe Balciunas

We made significant progress during 2003, our fifth year of research, in developing the first biological control agent for Cape ivy, *Delairea odorata*. Our research at our USDA-ARS quarantine facility in Albany, California, as well as at our collaborator's laboratory in Pretoria, South Africa, concentrated on verifying the safety of our top two candidate insects through host range tests to determine their host-specificity. Despite nearly losing our colony of the Cape ivy gall fly, *Parafreutreta regalis*, we are still nearly on schedule, and hope to complete our tests of this gall-maker by mid-2004 [see Section II]. Likewise, in addition to Cape ivy, we have completed 137 additional host range tests of the Cape ivy moth, *Digitivalva delaireae*, whose caterpillars mine the leaves and stems of Cape ivy, and frequently kill them [see Section III].

Both of these insects still appear to be safe for release, and during 2004, we anticipate taking the first steps in obtaining governmental approval for their release. This is a lengthy process [see Section VI. B] that is made less predictable, by the changes to the permitting process that are still underway. These yet-to-be finalized modifications are a response to biosecurity concerns raised by the events of Sept. 11, 2001. Although cautious, we remain hopeful that one or both of these insects will be approved for release in California during 2005.

Our collaborators in South Africa have now extensively tested a third insect, the arctiid moth *Diota rostrata* [see Section IV]. The large hairy caterpillars of this moth occasionally become very numerous in South Africa, and they then cause extensive damage to Cape ivy, completely defoliating large patches of this vine. During 2003, our collaborators confirmed that although this moth readily feeds and develops on Cape ivy, other relatives of Cape ivy are more preferred. Even more disturbing, when exposed to two California native species, this moth will oviposit and develop on one of them, *Senecio flaccidus*. We have therefore decided to suspend further research on this moth. While it is disappointing to rule out this damaging caterpillar as an agent, being able to do so in South Africa has saved us years of effort, and hundreds of thousands of dollars.

We anticipate a busy and productive 2004 [see Section VI]. Undoubtedly, our biggest hurdle will be to cope with the huge decrease in the external funds that supplement our USDA-ARS funds [see Section VI. C]. We have drastically scaled back research planned in South Africa, and are concerned about the level of effort that we will be able to sustain in California.

We thank our past collaborators and contributors for their efforts and support. We hope we can count on them again as we come ever closer to harvesting the rewards of this last half-decade of research.

List of Staff and Cooperators

USDA 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 Lednicky, Biological Science Technician (July 7, 1999 to present)

PPRI Staff at Reitondale (Pretoria) in South Africa:
Stefan Neser, Principal Investigator
Liamé van der Westhuizen, Agricultural Research Technician
Yogie Kistensamy, Nursery Manager
Lin Besaans, MS student (Rhodes University, Grahamstown)
Maryna Serdani (PPRI, Stellenbosch)

Cooperators:

Academy of Sciences – St. Petersburg, RUSSIA
Bonsell Reserve, Oceanside, CA
Bureau of Land Management – Fort Ord
California Dept. of Food & Agriculture (CDFA)
California Dept. of Transportation (CalTrans)
California Invasive Plant Council (formerly, California Exotic Pest Plant Council)
California Native Plant Society (CNPS)
California State Parks and Recreation
California Polytechnic State University, San Luis Obispo
East Bay Regional Park District (EBRPD)
Golden Gate National Recreation Area
National Park Service – Redwood National Park
Plant Protection Research Institute – Pretoria, SOUTH AFRICA
Rhodes University, Grahamstown, SOUTH AFRICA
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USDA-Forest Service, Siskiyou National Forest
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List of Acronyms and Abbreviations

List of Acronyms

APHIS	Animal and Plant Health Inspection Service (an agency of USDA)
ARS	Agricultural Research Service (an agency of USDA)
BCDC	Biological Control Documentation Center
CA	California
Cal- IPC	California Invasive Plant Council (formerly, California Exotic Pest Plant Council)
CNPS	California Native Plant Society
CSIRO	Commonwealth Scientific and Industrial Research Organization
EA	Environmental Assessment
EIS	Environmental Impact Statement
EIW	Exotic & Invasive Weed Research Unit, USDA-ARS, Albany, California
FWS	US Fish and Wildlife Service
FONSI	Finding Of No Significant Impact
GGNRA	Golden Gate National Recreation Area
PPRI	Plant Protection Research Institute (an agency of the Agricultural Research Council of the Republic of South Africa)
PPQ	Plant Protection and Quarantine (a section within APHIS)
SPRO	State Plant Regulatory Official
TAG	Technical Advisory Group for Biological Control of Weeds
T&E species	Threatened and Endangered species
USDA	United States Department of Agriculture

List of Generic Abbreviations

Del.	*Delairea* ivy
Di.	*Digitivalva* moths
Pa.	*Parafreutreta* flies
Sen.	*Senecio* plants

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I. Introduction

A. Cape ivy (*Delairea odorata*, prev. *Senecio mikanioides*)

Cape ivy (also known as German ivy), a vine native to 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 is now *Delairea odorata*. A recent survey in California (Robison *et al.* 2000) reports Cape ivy infestations from San Diego to southern coastal Oregon. Cape ivy is spreading in riparian forests, coastal scrubland, coastal bluff communities, and seasonal wetlands. Though it 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 (Bossard 2000).

Cape ivy has become the highest-ranked invasive species problem in the Golden Gate National Recreation Area (GGNRA). GGNRA spent a $600,000 grant over three years for Cape ivy control efforts. California State Parks along the coast, such as Big Basin, Hearst San Simeon, Mt. Tamalpias, Van Damme, and Jughandle, are heavily impacted as well. U.S. Forest Service lands along the Big Sur coast are also frequently heavily infested, as are other public and private lands along the coast.

Cape ivy 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 in the Hawaiian Islands this vine is restricted to the Big Island. However, Wagner *et al.* (1990) state that it is also sparingly naturalized on Maui.

B. Overview of collaborative research in South Africa (1996 through 2003)

On his first trip to South Africa during 1996, Dr. Balciunas completed a study of South African Cape ivy herbarium records. These records were used to locate Cape ivy sites for future surveys and to develop a distribution map of Cape ivy in South Africa (Balciunas *et al.*, in press). Since 1997, the California Invasive Plant Council (Cal-IPC [formerly, California Exotic Pest Plant Council]) and the California Native Plant Society (CNPS), have raised funds ($25,000-$65,000 annually) to assist our USDA-ARS project on the biological control of Cape ivy. We have used these contributions to support research in South Africa, the native home of Cape ivy. There, we have been fortunate enough to obtain the services of Dr. Stefan Neser, a world-renowned biological control specialist, as well as several talented, younger South African scientists.

Almost every year, Dr. Balciunas, the project leader, spends 4-5 weeks with our South African cooperators, reviewing their results, participating in field studies, and jointly planning the research for the following year. In Aug. of 1998, during the first year of collaborative research there, he joined our South African cooperators, Beth Grobbelaar and Stefan Neser, and participated in a 3000 km survey that visited most of the Cape ivy sites in the country, and collected the natural enemies that attacked it. During the next two years, over 230 species of plant-injuring insects were collected at these sites (Grobbelaar *et al*., 2003).

Six of the most promising of these insects were selected for further research. These included: *Diota rostrata* (Arctiidae) - a defoliating caterpillar; *Digitivalva delaireae* (referred to as *Acrolepia* new species in earlier reports) – 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 (April 1999 to March 2000), 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, the arctiid moth, *Diota rostrata*, was collected on anything other than Cape ivy, and so it appears that at least five insects are very host-specific to Cape ivy.

The focus of the last three years of research in South Africa (April 2000 through December 2003) has been to evaluate the host range of these promising insects. This phase of research has been led by Dr. Stefan Neser, and his assistant Liamé van der Westhuizen. They were able to establish laboratory colonies of three Cape ivy insects: *Digitivalva delaireae*, *Diota rostrata* and *Parafreutreta regalis*. Neser and van der Westhuizen have compiled valuable information on the biology and life history of these three insects, and developed rearing techniques.

In May of 2002, Dr. Balciunas made his fifth trip South Africa where he then accompanied Dr. Neser and Mrs. Lin Besaans on a two week trip to Kwazulu-Natal, and East and West Cape Provinces. The focus of this field trip was to investigate the insects that attack Cape ivy flowers. Our timing was good, and we found flowers at nearly every Cape ivy site we visited in these three provinces. The most widespread and consistently collected insect feeding on Cape ivy flowers were the larvae of a phalacrid beetle. Mrs. Besaans began research on this beetle, hoping to earn her Masters degree (at Rhodes University, Grahamstown) in the process. However, by mid-2003, she had determined that this beetle probably only has one generation per year, which means that comprehensive evaluation of this phalcrid beetle's host specificity and biology would require many years of research. This makes it unsuitable for a Master's project, and Mrs. Besaans has now chosen another topic for her MS.

The South African pathologist, Maryna Serdani, who was studying the pathogen that causes lesions on leaves of Cape ivy in South Africa, emigrated to the USA in early 2003. The pathogen, a variety of *Cercospora*, causes severe injury, even death, to Cape ivy plants in South Africa, and we still believe it deserves additional research. We are attempting to find collaborators who are capable and willing to evaluate this pathogen.

Our collaborators in Pretoria made good progress during 2003 in their host range evaluations of our top three candidate biocontrol agents [see Sections II-IV]. They confirmed that the moth *Diota rostrata*, whose caterpillars sometimes spectacularly defoliate Cape ivy patches, has several other hosts, and will <u>not</u> be safe enough for release here. The dreadful state of California's economy has caused a severe decline in contributions to the Cape ivy project) no additional funds were received this year from any California state agency. Accordingly, we scaled back research in South Africa during 2003, and anticipate a much-reduced research effort there during 2004.

II. The Cape ivy gall fly, *Parafreutreta regalis*

A. Observations, Biology, and Life History

Parafreutreta regalis (Diptera: Tephritidae) was described in 1943 by Munro, and identified as a potential agent for the biological control of Cape ivy during insect surveys in South Africa in 1998-99. An adult *Pa. regalis* is about the size of a housefly or slightly smaller. Females lay eggs in the nodes or growing tips of Cape ivy vines. The maggots that hatch cause Cape ivy to grow a spherical gall, about a ½-inch in diameter (Figure 1), within which they complete their life cycle, before adult flies emerge from the gall. These galls sometimes inhibit further elongation of that stem, although side shoots are usually produced.

Figure 1. *Parafreutreta regalis* adult on Cape ivy gall. Note emergence holes (windows) at bottom left

Dr. Balciunas brought back the first gall flies to the US from South Africa in January, 2001. We started our colony in our quarantine laboratory from a subsequent shipment of these flies in August 2001. Our colony produced one generation in late 2001, six in 2002 and another six in 2003.

During 2002 & 2003, the colony seemed to flourish during the cooler winter months and struggled during the summer. We suspected a pathogen, and in the summer of 2002 submitted samples to ARS insect pathologist Joel Siegel who ruled out microsporidia, bacteria, and fungi, but thought the colony might be infected with a virus. Fortunately, in Sept. 2002, the colony made a comeback.

During 2003, we had another problem. In June, most of our galls failed to produce adults. Typically, we remove galls from Cape ivy plants about ten days before we anticipate adult emergence, then place them in a shaded room with the stems embedded in oasis foam. However, these galls had been set up in moistened oasis foam in the greenhouse portion of the quarantine laboratory as a part of an experiment testing *Pa. regalis* oviposition preference on Cape ivy with and without stipules [see Table 3]. We speculate that the galls may have overheated while sitting in the glare of the early morning sun though the greenhouse glass. The colony foundered after this, with the few flies that emerged being smaller and sluggish. By Oct., we were left with only five females. Fortunately these five were able to produce another generation (the 13th) of 52 females and 65 males.

Although our colony has been used primarily for host range testing, we have observed and studied several aspects of its biology and life history as well. *Parafreutreta regalis* eggs are opaque and elongate. They are laid exclusively inside the nodes or growing tips of Cape ivy vines. Often, oviposition punctures are seen on the growing tips. The eggs can be laid singly, but are more commonly found in a tightly packed bundle of 3-8 eggs. Eggs appear to hatch in 4-7 days. We plan to study this in detail, but have been unable to hatch eggs outside of Cape ivy.

We presume *Pa. regalis,* like most other tephritid species, has three larval instars. The larvae are cylindrical and truncate at both ends. The first instar is extremely small (<2mm). Its size makes it distinct from the subsequent two instars. The second and third instars are very similar in structure, though the second instar is likely smaller. At a magnification of 50x under a dissecting microscope, we have been unable to identify structural differences between second and third instar larvae.

Shortly before pupation, late third instar larvae chew holes into the gall from the inside to the outer epidermis layer, but do not puncture the epidermis. These epidermis-covered holes appear like a clear circle or "window" on the outside of the gall. Usually, there is only one window on each gall. After emerging from their pupal cases, adult flies break through these windows and exit the gall head-first.

A preliminary life history of *Pa. regalis* was constructed by our cooperators in South Africa, then corroborated from observations made during our host range tests. The life cycle of *Pa. regalis* is summarized below.

Table 1. Time required during different stages of the *Parafreutreta regalis*' life cycle.

	Mean ± SE	Range	Median
South African tests			
Gall formation to window appearance (n= 37)	16.65 ± 0.70 days	7-24 days	17 days
Window appearance to adult emergence (n= 30)	21.30 ± 0.41 days	18-28 days	21 days
Flies per gall (n= 30)	5.00 ± 0.41 flies	1-9 flies	5 flies
California tests (data from 2001-2003 host range tests)			
Exposure (of flies to plant) to adult emergence (n= 23)	54.87 ± 1.01 days	48-64 days	54 days
Flies per gall (n= 120)	3.57 ± 0.22 flies	1-17 flies	3 flies

We now have studied longevity of adults in detail. Table 2 summarizes the results of two studies. The first study (Albany longevity tests) was completed in 2002. This study was run in a Conviron model No. E7 growth chamber at a constant temperature (20°C) with a consistent regime of light (16:8 light:dark). Male and female pairs of newly emerged (within one day) *Pa. regalis* adults were kept in plexiglass tubes (26cm height, 14.5cm diameter) covered with plastic mesh inside the growth chamber. A nutrient source -- 5% honey (with a small amount of yeast hydrolysate) and 95% water -- was supplied to the flies in the tube. Except for weekends and holidays, these longevity tests were checked daily.

In the second study (Albany oviposition), our results were taken from our oviposition studies. The flies in the oviposition studies were contained in a plexiglass tube with a source of sugar water (50% Mountain Dew® [a soda produced by Coca Cola Co.] and 50% water in a shell vial with a wick), and a Cape ivy vine stuck in moistened oasis foam. There were minor differences with the temperature and light regime between the two studies.

Table 2. Longevity of *Parafreutreta regalis* adults following their emergence from a gall.

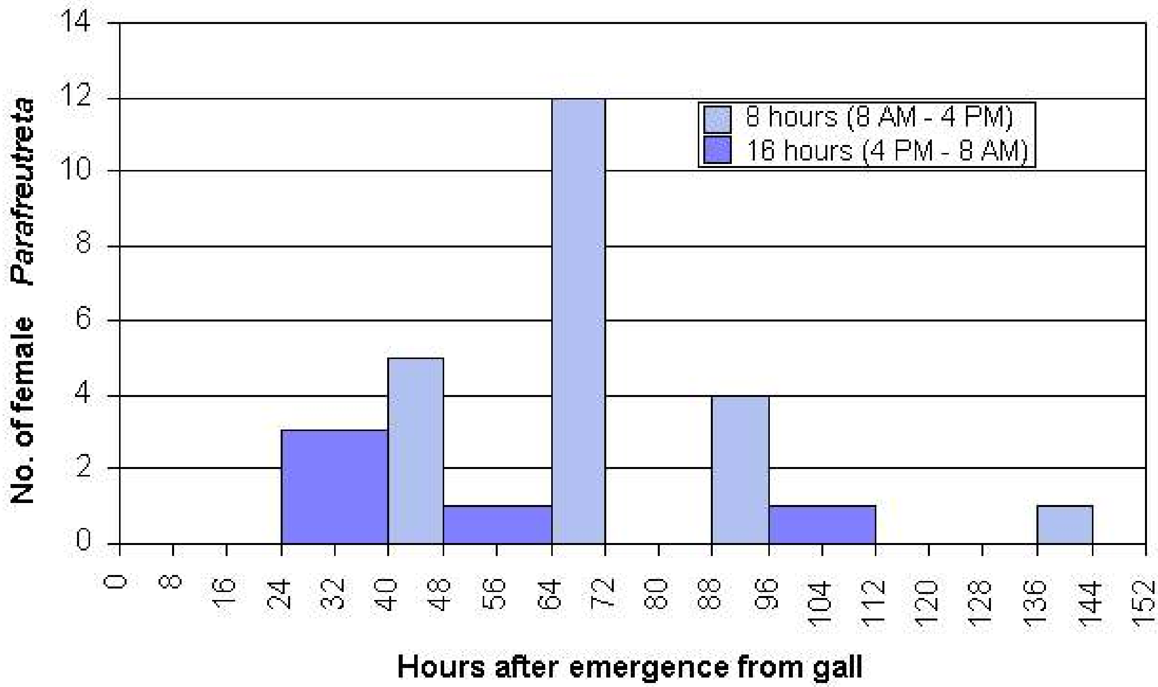
	Mean ± SE (days)	Range (days)	Median (days)
Albany longevity tests			
females (n=30)	14.5 ± 1.62	3-34	14
males (n=32)	12.1 ± 1.93	2-43	8
females & males (n=62)	13.3 ± 1.27	2-43	12
Albany oviposition tests			
females (n=32)	16.3 ± 0.98	9-28	15
males (n=15)	16.5 ± 1.55	7-28	16

Despite the different conditions, the results were similar and the differences in longevity between the sexes in each study were not significant. These results concur with preliminary observations by our cooperators in South Africa, who noted that adult flies live between 11 to 14 days.

In 2003, we studied two aspects of oviposition of *Pa. regalis* − the pre-oviposition period and net fecundity. To determine the pre-oviposition period − the time from when the female emerged from the gall to when it would first oviposit − we collected recently emerged females. This was done by checking our colony for emergence at 13:00 and then at 15:00. The females that emerged during this interval were placed with two males (sometimes slightly older) in a plexiglass tube (dimensions: 26 cm height, 14.5 cm diameter). The top and bottom of the tube were covered with mesh to prevent the flies from escaping and to allow air exchange. A 13-18 cm segment of Cape ivy vine, was placed in tube, with the bottom end of the vine embedded in moistened oasis foam. A source of sugar water (50% Mountain Dew®) was provided for the flies in the tube. We removed these vine segments after 24 hours (always in the late afternoon) and dissected them for eggs. If no eggs were found, we replaced the Cape ivy vine segment and continued dissections daily, at 8:00 and 16:00 hours, until eggs were discovered.

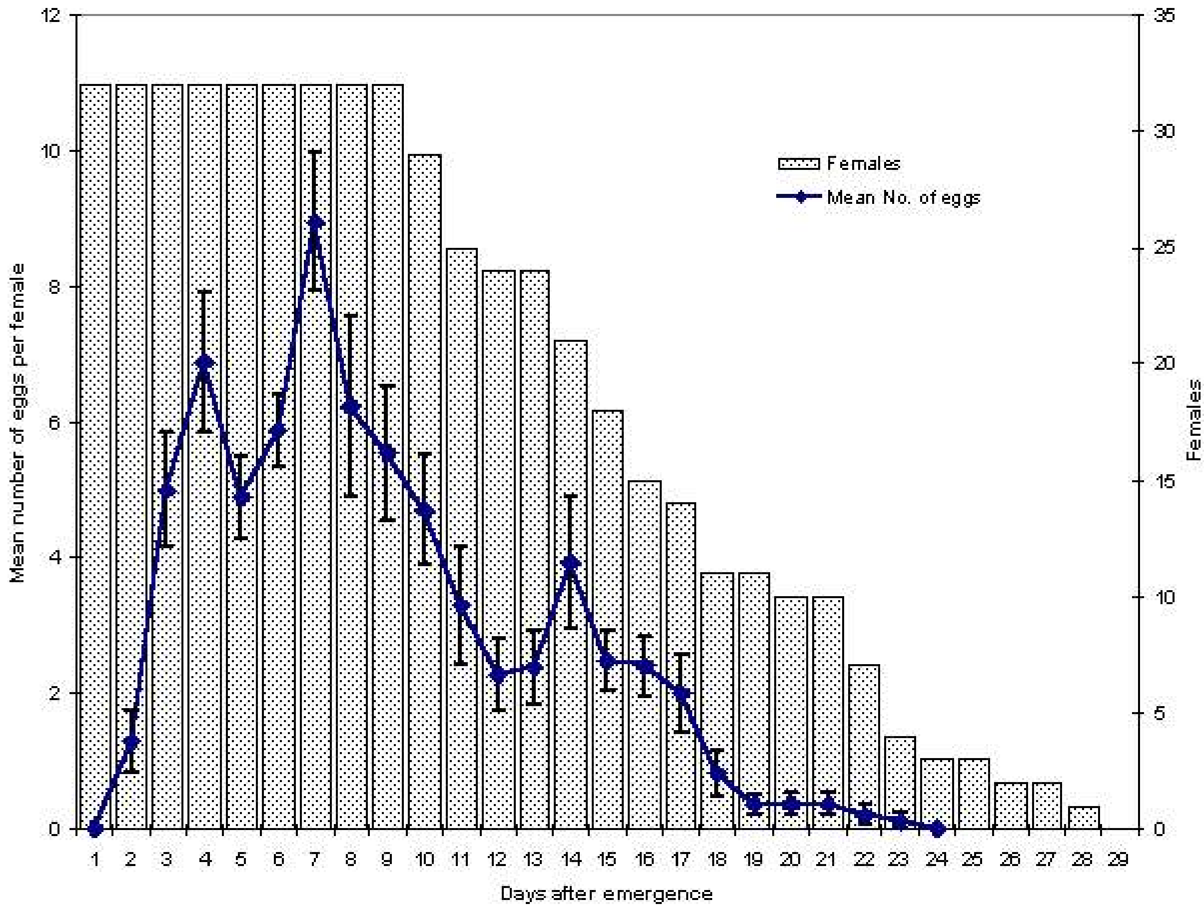
We completed 30 of these tests. Figure 2 shows the age of these females when initial oviposition was first observed. Since oviposition was not checked over the weekend, the results for three flies that oviposited during a weekend are not included in the graph, as we are not able to resolve their time of initial oviposition as accurately as for the other 27 females. None of the females oviposited within the first 24 hours after emergence. Most of the females (70%) oviposited between 24 to 72 hours. Furthermore, most of the females (82%) oviposited during daytime hours.

Figure 2. Age of *Parafreutreta regalis* females at initial oviposition.



After initial oviposition was discovered, we continued to dissect and replace the Cape ivy vines on an almost daily basis to determine the oviposition rate and net fecundity (total number of eggs oviposited by one female). We did this until the females. The number of eggs laid during a multi-day period was averaged to obtain a daily rate. If a female escaped or was damaged, her results were discarded, and another test was started. We completed 32 of these tests and determined the mean lifetime fecundity to be 61.5 eggs per female (SE ± 6.58, range: 5 to 138 eggs). The oviposition rate increased for a week, then decreased over the next two weeks (Figure 3). No eggs were laid after 24 days, although a few females lived for four weeks.

Figure 3. The mean number (± SE) of eggs oviposited daily by *Parafreutreta regalis* females during their lifetimes.



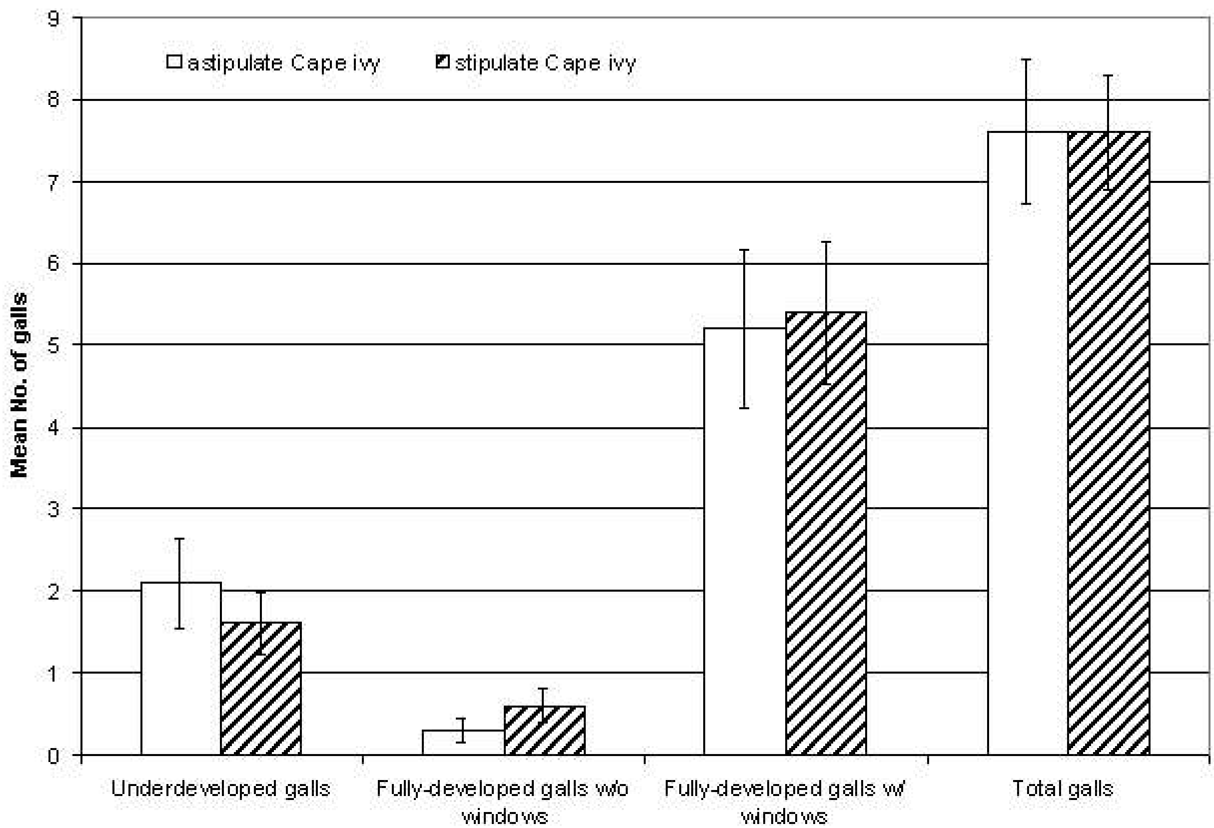
We also studied whether *Pa. regalis* preferred to oviposit on Cape ivy with or without stipules, and if there was a difference in gall development on either type of Cape ivy. Although we did not quantify *Pa. regalis* oviposition directly, we theorized that any differences, between the two types of Cape ivy, in the number of galls or the number of flies emerging from the galls would demonstrate an oviposition preference.

We ran five trials, each exposing four females and four males in a metal screen cage (dimensions: 122 x 91½ x 91½ cm) to four potted Cape ivy plants: two with stipules and two without stipules. A source of sugar water (50% Mountain Dew®) was provided for the flies in the cage. Six to seven days after the start of the trial we added an additional four females and four males. After 15 to 21 days, we removed the dead and living flies, then tracked the development of galls on each Cape ivy plant.

Eighty-five days after starting the trial, we counted and classified the galls by their development and dissected them to count immatures and dead adults. Since these galls had overheated [see page 3], few flies had emerged. "Underdeveloped galls" were swelling on the Cape ivy that were induced by *Pa. regalis* oviposition (confirmed by dissection) but the larvae had died early, and these galls never reached their typical size. "Fully-developed galls without windows" were normal-sized galls that had dead late instar larvae, but not pupae. "Fully-developed galls with windows" sometimes produced live adults, and when dissected contained mixtures of dead or live larvae, pupae, pupal cases, and/or dead adults.

Using a two-way ANOVA (SPSS, 1997), we analyzed for differences between the number of galls in each of the five trials, and for differences in gall numbers between stipulate and astipulate Cape ivy. In both comparisons there were no significant differences. Therefore, we pooled the results from the five trials (Figure 4).

Figure 4. The mean number (± SE) of *Parafreutreta regalis* galls (85 days after initial exposure to eight female-male pairs of flies) on 10 stipulate and 10 astipulate Cape ivy.



ANOVA results: Underdeveloped galls: difference between tests (df=4, P=0.577), difference between stipulate and astipulate Cape ivy (df=1, P=0.515), fully-developed galls without windows: difference between tests (df=4, P=0.737), difference between stipulate and astipulate Cape ivy (df=1, P=0.283), fully-developed galls with windows: difference between tests (df=4, P=0.247), difference between stipulate and astipulate Cape ivy (df=1, P=0.881), total galls (fully & underdeveloped galls): difference between tests (df=4, P=0.506), difference between stipulate and astipulate Cape ivy (df=1, P=1.0).

The number of flies that emerged was less then expected, since, as mentioned earlier, we believe the galls overheated. Using a two way ANOVA, we tested for differences in the number of total *Parafreutreta* (immatures and adults) between each of the five tests between stipulate and astipulate Cape ivy. In each case, we found no significant differences: difference between tests (df=4, P=0.175), difference between stipulate and astipulate Cape ivy (df=1, P=0.447).

B. Host range evaluations

For the past three years, the research at our Albany facility, as well as in Pretoria, has concentrated on evaluating the safety of some of the insects discovered during surveys in South Africa. Safety is the primary concern for those involved in releasing herbivorous insects from overseas. It is in everyone's best interest that the insects are narrowly host-specific – that once released and established, they will not cause significant damage to native, cultivated, or desirable ornamental plants. The host-specificity of candidate insects is typically determined by exposing the insects, in cages in the laboratory, to an array of potential host plants, then noting which of these (if any) are suitable as hosts. Traditionally, these laboratory host range evaluations are comprised of "no-choice tests" (sometimes called "starvation tests) where the known host (in this case, Cape ivy) is not present in the cage, and of "choice tests" where the target host is present.

Due to the short longevity of *Parafreutrata* adults, we designed another testing protocol. Essentially, these tests (that we call "no-choice/ host added") are a multi-plant, no-choice trial, to which, at the beginning of the fourth day, a Cape ivy plant is added. The procedures used in Albany (our collaborators in Pretoria used nearly identical protocols) are as follows: a metal screen cage (122 x 91½ x 91½ cm) was set up in our quarantine laboratory greenhouse with four different plant species, one in each corner. A source of sugar water (50% Mountain Dew®) was placed in the center of the cage. We then released four female-male pairs of flies into the cage. After 72 hours, we placed a Cape ivy plant into the center of the cage. Our initial oviposition studies showed that 70% of female *Parafreutreta* have begun to oviposit by this time. Seven to ten days after the start of the test (depending on the number of flies still alive after seven days), the test was ended and the remaining flies recovered. Plants were watered as necessary, and observed nearly daily for signs of gall formation. If no galls had formed after 60 days, or if the plant died earlier, we dissected the stems looking for signs of *Parafreutreta* damage, then disposed of the plants.

The host range tests of *Pa. regalis* conducted in Pretoria were also "no-choice/ host added" trials, and were very similar to those conducted in Albany. Three or four test plants of roughly similar size were placed in a cage (0.56m x 0.56m x 0.6m) with four pairs of newly emerged flies for three days. Flies were provided with a honey and yeast solution. On day four, the control, a Cape ivy plant of similar size, was added. After another three days of exposure, the flies were removed, while the plants were left in the cage and gall development monitored. At both locations, we attempted to test each plant species five times.

Table 3 summarizes the plants, number of repetitions, and galls formed on the "no-choice/ host added" tests that we and our cooperators in South Africa have completed through December 2003. Only the results from trials that produced galls on the control plant (Cape ivy) are included. Appendix A provides the complete, detailed results for each of these trials in Albany.

Table 3. Plant species evaluated by USDA and PPRI for *Parafreutreta regalis* oviposition and development (2001 through 2003).

Tribe	Species tested (inc. Cape ivy)(73) 33 in CA (29 exclusively), 44 in SA (40 exclusively)	Location of test	# of reps.	Mean # of galls
Family Araliaceae				
	Hedera canariensis Willd.	Albany	5	0
	Hedera helix L.	Albany	5	0
Family Asteraceae				
Subfamily Asteroideae				
Anthemideae	*Schistostephium cf. heptalobum* (DC.) Oliv. & Hiern	Pretoria	4	0
Astereae	*Baccaris piluaris* DC	Albany	1	0
	Grindelia sp.	Albany	5	0
	Symphyotrichum chilense (Nees) G.L. Nesom	Albany	5	0
Calenduleae	*Calendula officinalis* L.	Albany	5	0
Eupatorieae	*Ageratina adenophora* (Spreng.) King & H.E. Robins	Pretoria	5	0
	Ageratina riparia (Regel) King & H.E. Robins	Pretoria	5	0
	Ageratum houstonianum Mill.	Pretoria	5	0
	Campuloclinium macrocephalum (Less.) DC.	Pretoria	5	0
	Chromolaena odorata (L.) King & H.E. Robins	Pretoria	1	0
	Mikania capensis DC.	Pretoria	4	0
Gnaphalieae	*Anaphalis margaritacea* (L.) Benth. ex. C.B. Clarke	Albany	5	0
Helenieae	*Eriophyllum staechadifolium* Lag.	Albany	5	0
	Tagetes sp.	Albany	5	0
	Tagetes minuta L.	Pretoria	4	0
Heliantheae	*Bidens formosa* (Bonato) Schultz-Bip.	Pretoria	5	0
	Coreopsis sp. *cv*.	Pretoria	5	0
	Dahlia pinnata cv. Cav.	Pretoria	4	0
	Galinsoga parviflora Cav.	Pretoria	4	0
	Helianthus annuus cv. 8751	Pretoria	1	0
	Helianthus annuus cv. 3037	Pretoria	4	0
	Helianthus tuberosus L.	Pretoria	5	0
	Rudbeckia sp. *cv*.	Pretoria	5	0
	Zinnia elegans cv. Jacq.	Pretoria	4	0
Senecioneae	Subtribe Blennospermatinae			
	Blennosperma nanum (Hook.) Blake	Albany	5	0
Senecioneae	Subtribe Senecioninae			
	Cineraria cv “butterfly”	Pretoria	4	0
	Cineraria deltoidea Sond.	Pretoria	5	0
	Cineraria saxifraga DC.	Pretoria	5	0
	Delairea odorata Lem.	Albany	38	5.7
		Pretoria	55	5.0
	Erechtites glomerata (Desf. ex Poir.) DC.	Albany	5	0
	Euryops pectinatus (L.) Cass.	Albany	5	0
		Pretoria	5	0
	Euryops chrysanthemoides (DC.) B. Nordenstam	Pretoria	5	0
	Euryops subcarnosus DC.	Albany	5	0
	Mikaniopsis cissampelina C. Jeffrey	Pretoria	5	0

Table 3 (continued)

	Packera bolanderi (Gray) W.A. Weber & A. Löve	Albany	5	0
		Pretoria	4	0
	Packera breweri (Burtt-Davy) W.A. Weber & A. Löve	Albany	5	0
	Packera ganderi (T.M. Barkl. & Beauchamp) W.A. Weber & A. Löve	Albany	3	0
	Packera macounii (Greene) W.A. Weber & A. Löve	Albany	5	0
	Pseudogynoxys chenopodioides Kunth	Albany	5	0
	Senecio angulatus L. f.	Pretoria	5	0
	Senecio articulatus (L.) Sch. Bip	Pretoria	2	0
	Senecio blochmaniae Greene	Albany	5	0
	Senecio brachypodus DC.	Pretoria	5	0
	Senecio deltoideus Less.	Pretoria	5	0
	Senecio flaccidus Less.	Albany	5	0
		Pretoria	5	0
	Senecio glastifolius L. f.	Pretoria	1	0
	Senecio helminthioides (Schultz-Bip.) Hilliard	Pretoria	5	0
	Senecio hybridus Regel	Albany	5	0
	Senecio jacobaea L.	Albany	5	0
	Senecio macroglossus DC.	Pretoria	5	0
	Senecio oxyodontus DC.	Pretoria	5	0
	Senecio oxyriifolius DC.	Pretoria	5	0
	Senecio pleistocephalus S. Moore	Pretoria	5	0
	Senecio tamoides DC.	Pretoria	5	0
	Senecio triangularis Hook.	Albany	5	0
	Senecio vulgaris L.	Albany	5	0
	Senecio sp. (unidentified)	Pretoria	5	0
Senecioneae	Subtribe Tussilagininae			
	Lepidospartum latisquamum S. Wats.	Albany	5	0
	Luina hypoleuca Benth.	Albany	5	0
	Petasites frigidus (L.) Fries	Albany	5	0
Subfamily Cichorioideae				
Arctoteae	*Arctotheca calendula* (L.) Levyns	Pretoria	5	0
Cardueae	*Carthamus tinctorius* L.	Albany	6	0
	Centaurea melitensis L.	Albany	1	0
	Cynara scolymus L.	Pretoria	2	0
Lactuceae	*Lactuca sativa* L.	Pretoria	4	0
Mutisieae	*Adenocaulon bicolor* Hook.	Albany	5	0
Vernoneae	*Vernonia missurica* Raf.	Albany	6	0
Family Brassiceae				
	Brassica oleracea L.	Pretoria	4	0
	Raphanus sativus L.	Pretoria	4	0
Family Chenopodiaceae				
	Beta vulgaris subsp. *cicla* (L.) Koch	Pretoria	4	0
Family Cucurbitaceae				
	Zehneria scabra (L. f.) Sond.	Pretoria	5	0
Family Rosaceae				
	Frageria chiloensis (L.) P. Mill.	Albany	5	0

In Albany, we've conducted 38 trials (each with four test plants) so far, that showed a positive control (galls formed on Cape ivy), while in Pretoria, 55 trials (each with 3-4 test plants) have showed a positive control. Between the two locations, we have tested 73 species, and have not found any sign of gall development or *Pa. regalis* damage to any species other than *Delairea odorata*, thereby confirming this fly's exclusive preference to Cape ivy.

During the first half of 2004, we plan to conduct more "no-choice/ host added" trials on a few additional related plant species, as well as species already tested, so that each species is tested five times. We will then compile our results and begin the lengthy process of obtaining federal and state approval to release this fly in California [see Section VI. B].

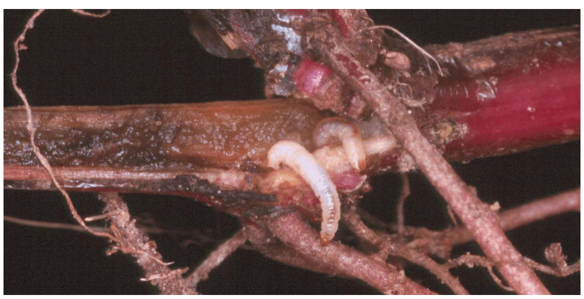
III. The Cape ivy stem boring/leaf-mining moth, *Digitivalva delaireae*

A. Observations, Biology, and Life History

The Cape ivy stem boring moth (initially identified as *Acrolepia* new species) was discovered during our surveys in South Africa, and is new to science. This moth was described in 2002 by Gaedike and Kruger as *Digitivalva delaireae*. It is one of the most widely distributed of Cape ivy natural enemies, and it has been collected at nearly all our Cape ivy sites in South Africa.

Digitivalva delaireae is a small moth (usually about ¼-inch in length). Adults (Figure 5, right) seem to be quiescent during daylight hours, but appear quite active at dusk. We have seldom observed moths mating. Females oviposit single opaque eggs on both sides of Cape ivy leaves, on stems, and stipules, and sometimes on the petiole. Tiny caterpillars (Figure 5, left) hatch out and tunnel within the leaves and stems, leaving distinctive "mines" in the leaves. Newly hatched caterpillars on the leaves usually 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. Mature larvae exit the stems and leaf mines, and crawl around on the ground, before pupating in small, flattened, silken pupal cases. It is during this stage that we collect the mature larvae (also called pre-pupae) and pupal cases from the floor of our cages, then use the emerging adults for our tests and colonies.

Figure 5. *Digitivalva delaireae* larvae (left) and adult (right). (Photos by E. Grobbelaar)



In 2002, our South African cooperators studied the longevity of *Di. delaireae*. The following is a summary of these preliminary tests: A single pair of moths was released into a ventilated honey jar that was placed over a small, newly potted *Delairea* cutting (6-8 leaves per plant). The adults were supplied with a 5% honey solution. Each trial was replicated 8 times. Three days after exposure, the adults were removed and the plants closely examined for eggs or any signs of mining. Average male and female longevity was found to be 8.2 days (n=8, range: 4-10 days) and 7.3 days (n=8, range: 3-10 days) respectively, eggs started hatching after 14 days and 50% of the females oviposited within 72 hours.

Dr. Balciunas hand-carried the first *Digitivalva delaireae* to our quarantine in Jan. 2001. From subsequent shipments, we started a colony in Oct. 2001. In 2002, we had seven generations of this multivoltine moth, and another six generations in 2003. Unlike the *Pa. regalis* colony, we have not had any problems with our *Di. delaireae* colony.

In 2003, we studied how soon after emergence female moths began to oviposit. We collected newly emerged females (within 1-2 hours of emergence) and placed them with two males (usually slightly older) in a plexiglass tube (dimensions: 26 cm height, 14.5 cm diameter). The top and bottom of the tube were covered with mesh to contain the flies and allow air exchange. A 13-18 cm cutting of a Cape ivy vine, was placed in tube with its end embedded in moistened oasis foam. After 24 hours (always in the late afternoon), we examined the cutting for eggs. If no eggs were found, we replaced the Cape ivy vine segment and continued dissections daily, at 8:00 and 16:00 hrs., until the first eggs were discovered.

Figure 6. Age of *Digitivalva delaireae* at initial oviposition (n= 28).

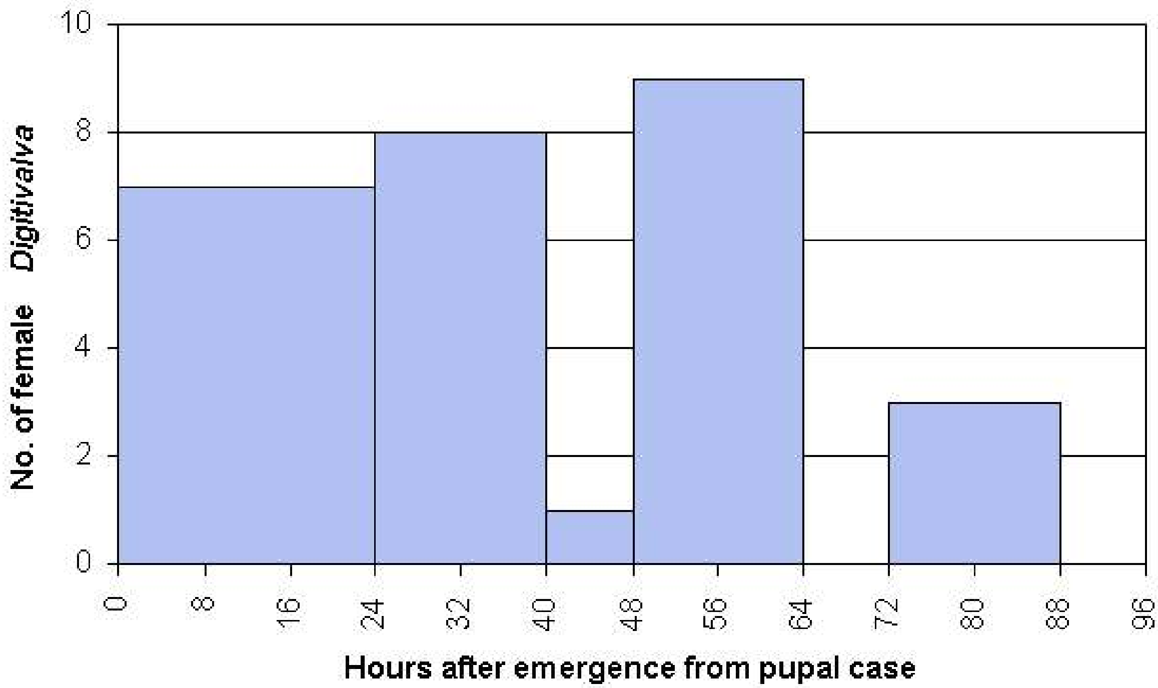


Figure 6 shows the age intervals when initial oviposition first occurred. We observed that these moths usually emerge from the pupal cases in the afternoon, and the females begin ovipositing shortly thereafter. Seven females (23%) oviposited within 24 hours of emergence (usually in the afternoon), and 25 (83%) oviposited within 64 hours. As in our *Parafreutreta* studies, oviposition was not checked on the weekend. Two moths that oviposited during the weekend are not included in the graph since we are not able to accurately resolve its time of initial oviposition.

Like our *Parafreutreta* studies, we also ran tests to determine the net fecundity of *Di. delaireae*. Our net fecundity study is not yet complete, but so far we have counted 974 *Di. delaireae* eggs oviposited by 22 females. Of these, 71% were oviposited on leaves, 23% on stems and stipules, and 6% on petioles.

B. Host range evaluations

We continued host range testing *Di. delaireae* in Albany and Pretoria through 2003. Some new species were tested, and other trials were run to test each plant five times. The protocols for the *Digitivalva* "no-choice/ host added" tests are identical to those for the gall fly, *Pa. regalis*.

The results of the successful "no-choice/ host added" trials completed in Albany and in South Africa are summarized in Table 4, while Appendix B provides detailed results of each trial conducted in Albany.

Table 4. Plant species evaluated by USDA and PPRI for *Digitivalva delaireae* oviposition and development (2001 through 2003)

Tribe: Subtribe	Species tested, including Cape ivy: 70 36 in CA (3 exclusively), 37 in SA (34 exclusively) 3 tested in both locales	Location of test	# of reps	# w/ *Di.* infestation or damage
Family Araliaceae				
	Hedera canariensis Willd.	Albany	5	0
	Hedera helix L.	Albany	5	0
Family Asteraceae				
Subfamily Asteroideae				
Anthemideae	*Achillea millefolium* L.	Albany	3	0
	Schistostephium cf. heptalobum (DC.) Oliv. & Hiern	Pretoria	5	0
Astereae	*Baccharis pilularis* DC.	Albany	1	0
	Bellis sp.	Pretoria	5	0
	Erigeron glaucus Ker-Gawl.	Albany	2	0
	Grindelia sp.	Albany	6	0
	Symphyotrichum chilense (Nees) G.L. Nesom	Albany	5	0
Calenduleae	*Calendula officinalis* L.	Albany	5	0
Eupatorieae	*Argentina adenophora* (Spreng.) King & H.E. Robins	Pretoria	5	0
	Ageratina riparia (Regel) King & H.E. Robins	Pretoria	6	0
	Ageratum houstonianum Mill.	Pretoria	5	0
	Campuloclinium macrocephalum (Less.) DC.	Pretoria	3	0
	Chromolaena odorata (L.) King & H.E. Robins	Pretoria	4	0
	Mikania capensis DC.	Pretoria	4	0
Gnaphalieeae	*Anaphalis margaritacea* (L.) Benth. ex C.B. Clarke	Albany	6	0
Heliantheae	*Bidens formosa* (Bonato) Schultz-Bip.	Pretoria	2	0
	Dahlia pinnata Cav.	Pretoria	4	0
	Helianthus annuus L.	Pretoria	1	0
	Helianthus tuberosus L.	Pretoria	3	0
	Rudbeckia sp. *cv*.	Pretoria	5	0
Helenieae	*Tagetes* sp.	Albany	6	0
	Eriophyllum staechadifolium Lag.	Albany	5	0
Senecioneae: Senecioninae	*Cineraria deltoidea* Sond.	Pretoria	2	0
	Cineraria saxifraga DC.	Pretoria	6	0
	Delairea odorata Lem.	Albany Pretoria	40 47	40 47

Table 4 (continued)

	Erechtites glomerata (Desf. ex Poir.) DC.	Albany	5	0
	Euryops chrysanthemoides (DC.) B. Nordenstam	Pretoria	6	0
	Euryops pectinatus (L.) Cass.	Albany	5	0
		Pretoria	5	0
	Euryops subcarnosus DC.	Albany	5	0
	Mikaniopsis cissampelina C. Jeffrey	Pretoria	5	0
	Packera bolanderi (Gray) W.A. Weber & A. Löve	Albany	5	0
	Packera breweri (Burtt-Davy) W.A. Weber & A. Löve	Albany	6	0
	Packera ganderi (T.M. Barkl. & Beauchamp) W.A. Weber & A. Löve	Albany	1	0
	Packera macounii (Greene) W.A. Weber & A. Löve	Albany	5	0
	Pseudogynoxys chenopioides Kunth	Albany	5	0
	Senecio angulatus L. f.	Pretoria	5	1
	Senecio articulatus (L.) Sch. Bip	Pretoria	6	0
	Senecio blochmaniae Greene	Albany	5	0
	Senecio brachypodus DC.	Pretoria	5	1
	Senecio deltoideus Less.	Pretoria	5	0
	Senecio flaccidus Less.	Albany	5	0
		Pretoria	1	0
	Senecio helminthioides (Schultz-Bip.) Hilliard	Pretoria	5	0
	Senecio hybridus Regel	Albany	5	0
	Senecio jacobaea L.	Albany	5	0
	Senecio macroglossus DC.	Pretoria	5	2
	Senecio oxydontus DC.	Pretoria	5	1
	Senecio oxyriifolius DC.	Pretoria	3	0
	Senecio pleistocephalus DC.	Pretoria	5	1
	Senecio serratuloides DC.	Pretoria	5	0
	Senecio tamoides DC.	Pretoria	5	1
	Senecio triangularis Hook.	Albany	5	0
	Senecio vulgaris L.	Albany	5	0
	Senecio sp. (unidentified)	Pretoria	5	0
Senecioneae: Tussilagininae	*Lepidospartum latisquamum* S. Wats	Albany	5	0
	Luina hypoleuca Benth	Albany	5	0
	Petasites frigidus (L.) Fries	Albany	5	0
Subfamily Cichorioideae				
Arctoteae	*Arctotheca calendula* (L.) Levyns	Pretoria	6	0
Cardueae	*Carthamus tinctorius* L.	Albany	5	0
	Cynara scolymus L.	Pretoria	2	0
Mutisieae	*Adenocaulon bicolor* Hook.	Albany	5	0
Vernoneae	*Vernonia missurica* Raf.	Albany	3	0
Family Brassicaceae				
	Brassica oleracea L.	Pretoria	6	0
	Lepidium latifolium L.	Albany	4	0

Table 4 (continued)

Family Chenopodiaceae				
Beteae	*Beta vulgaris* subsp. *cicla* (L.) Koch	Pretoria	5	0
Family Cucurbitaceae				
	Marah fabaceus (Naud.) Naud. ex Greene	Albany	2	0
	Zehneria scabra (L. f.) Sond.	Pretoria	5	0
Family Rosaceae				
	Frageria chiloensis (L.) P. Mill.	Albany	5	0
Family Campanulaceae				
	Campanula muralis	Albany	5	0

Out of the 75 "no-choice/ host added" trials completed in Albany so far, 40 showed a positive control (oviposition and development on Cape ivy) and their results are shown in Table 4. In these 40 trials, we have had 208 female and 213 male *Digitivalva* moths emerge from Cape ivy, but have found no development or signs of infestation on any of the other 36 species of test plants.

In South Africa, 37 plant species have been tested. A total of 52 trials have been completed: 47 showed a positive control (oviposition and development on Cape ivy), while five did not. Single leaves were found to have been mined on *Senecio angulatus*, *Sen. brachypodus*, *Sen. oxyodontus, Sen. pleistocephalus* and *Sen. tamoides*. The mines were very small and very short. It seems as though the larva left the leaf shortly after entry, and no further damage could be detected. In addition, two *Senecio macroglossus* test plants showed more damage.

Late in 2002, in preliminary tests to verify the feasibility of our *Di. delaireae* oviposition protocols [see Section III. A], after testing Cape ivy, we also tested eight other plant species. We replaced the Cape ivy vine segment with either a leaf, or a vine segment, of one the following plants: *Senecio confusus, Hedera canariensis, H. helix, Erechtites glomerata* (2)*, Packera bolanderi, Petasites frigidus, Carthamus tinctorius*, to see whether or not *Di. delaireae* would oviposit on them. Interestingly, under those no-choice conditions, we noted oviposition on *Petasites frigidis* and *Carthamus tinctorius*. We were unable to determine whether or not these eggs would have hatched, since the leaves always deteriorated within a day or two after oviposition was noted. In our "no-choice / host added" trials [see above], both of these plant species were tested five times, and we did not discover any signs of *Di. delaireae* development damage.

Despite some tunneling in non-host species *Digitivalva* is still regarded as a very promising biological control candidate. Host range tests will continue during 2004.

IV. *Diota rostrata* (Lepidoptera: Arctiidae) moths tests in South Africa

During the last three years, our South African colleagues have also extensively tested a third insect, the Cape ivy defoliating moth, *Diota rostrata* (Figure 7). The hairy caterpillars of this moth are voracious feeders, and in South Africa, we frequently encounter patches of Cape ivy where most of the leaves are either totally missing or only small tatters remain.

Figure 7. *Diota rostrata* larvae (left) and adult (right).

Almost all developmental stages of *Diota rostrata* are temperature dependent, with higher temperatures resulting in shorter developmental stages. The pre-oviposition period lasts for 2-3 days, after which the female deposits most of her eggs on the underside of leaves, but leaf petioles and plant stems can also be selected. Under laboratory conditions, females will deposit their eggs on the sides of plastic containers. Clusters of eggs can be found with numbers ranging from 1-56 per group. The eggs are small, round, shiny and yellow in color, and will hatch within 7-15 days. Five larval instars can be distinguished, and the larval stage ranges between 21-35 days, and the pupal stage between 9-25 days. Adult longevity is normally 14 days.

Our South African cooperators found that six *Diota* larvae, when confined in a small (9.5 x 7.5 x 4.5 cm) container that also held a leaf of a test plant, would feed and develop on Cape ivy and nine other related species [see our 2001 Annual Report (Balciunas, *et al*. 2002)]. For the past two years, our cooperators have been testing to see if *Diota* moths will oviposit on these 10 plant species (and a couple non-hosts). To simulate more natural conditions, these oviposition trials have been conducted in a large, walk-in cage (4mx4mx2m) made from "psylla screen". The cage was set up in a fiberglass tunnel with a wet wall on the southern end, and an extraction fan on the northern end. Fourteen pairs of adults were released evenly throughout the cage and egg groups as well as the number of eggs per group and the date of emergence were recorded. Test plants used included all 10 species that sustained the development of *Diota* larvae as well as 2 non-target plants. In three trials, using moths collected in Kirstenbosch Gardens in Cape Town that were feeding on *Senecio oxyodontus*, they observed oviposition on nine of these plant species (Table 5). In all three trials, more eggs were laid on *Senecio oxyodontus* than on Cape ivy. The *Diota* from Kirstenbosch, therefore, appear too risky to be considered for release in California.

However, a population of *Diota* whose larvae had always fed on Cape ivy might show a different, more restrictive feeding preference. In 2002, a very isolated population of *Delairea* *odorata* (no other relatives of Cape ivy are nearby) was located in Addo National Park. This created an ideal opportunity to collect a possible "biotype" of *Diota* that might be more specific to *Del. odorata*, as compared to the previous culture from Kirstenbosch (Cape Town) which convincingly favored *Senecio oxyodontus* as its host. The first consignment of *Diota rostrata* from Addo National Park in the Eastern Cape was received in October 2002, but died out in December, and could not be recollected until May 2003. These were tested in the walk-in cage, using protocols similar to those used for the oviposition trials with the Kirstenbosch biotype.

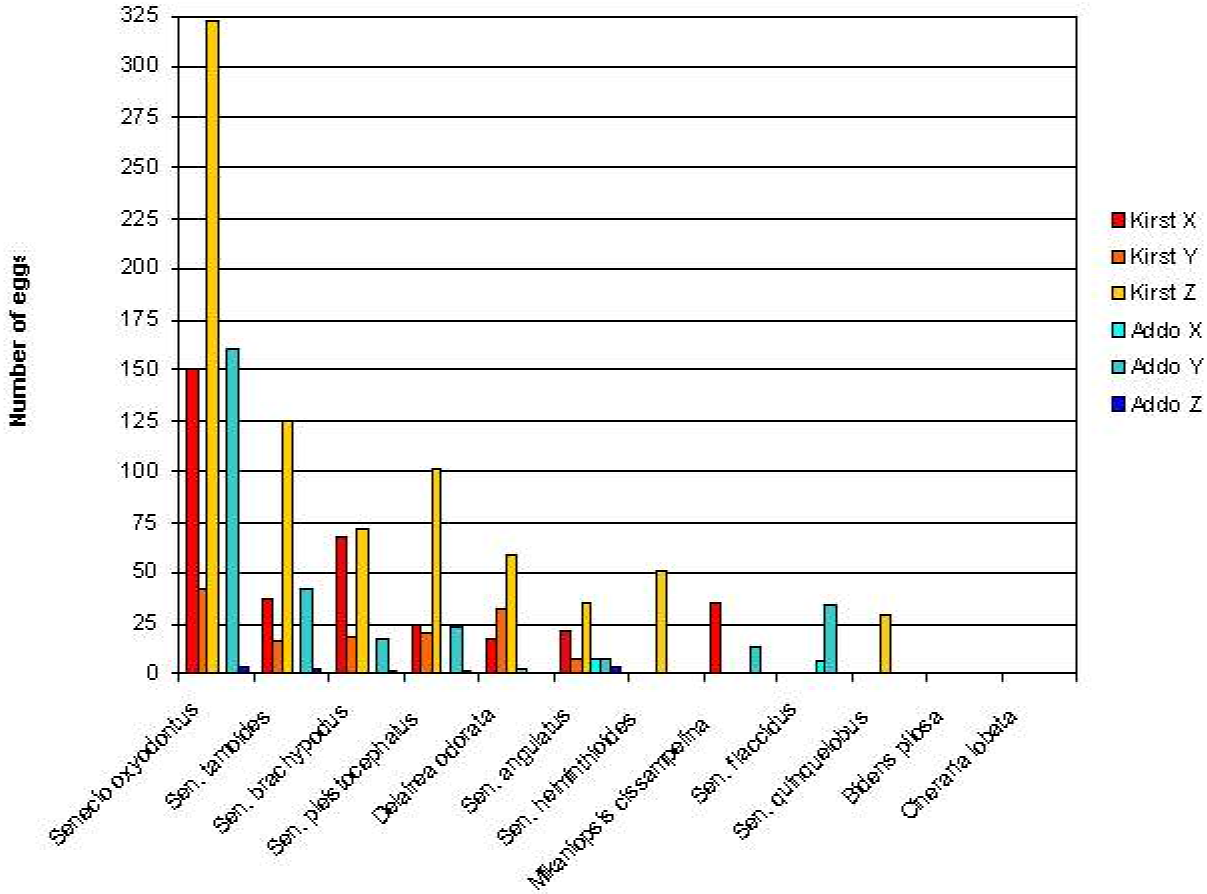
Table 5. The preliminary results of a *Diota rostrata* oviposition test in a walk-in cage that contained recorded host plants and hosts unsuitable(**) for larval development

Species	Kirst X		Kirst Y		Kirst Z		Addo X		Addo Y		Addo Z	
	# of eggs	% of total	# of eggs	% of total	# of eggs	% of total	# of eggs	% of total	# of eggs	% of total	# of eggs	% of total
*Bidens pilosa***	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
*Cineraria lobata***	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Delairea odorata	17	4.8	32	23.7	59	7.4	3	18.8	0	0.0	0	0.0
Mikaniopsis cissampelina	35	10.0	0	0.0	0	0.0	0	0.0	14	4.7	0	0.0
Senecio angulatus	21	6.0	7	5.2	35	4.4	7	43.8	7.0	2.4	4	28.6
Sen. brachypodus	67	19.1	18	13.3	72	9.1	0	0.0	17	5.7	2	14.3
Sen. flaccidus	0	0.0	0	0.0	0	0.0	6	37.5	34	11.4	0	0.0
Sen. helminthioides	0	0.0	0	0.0	51	6.4	0	0.0	0	0.0	0	0.0
Sen. oxyodontus	150	42.7	42	31.1	322	40.6	0	0.0	160	53.9	4	28.6
Sen. pleistocephalus	24	6.8	20	14.8	101	12.7	0	0.0	23.0	7.7	1	7.1
Sen. quinquelobus	0	0.0	0	0.0	29	3.7	0	0.0	0	0.0	0	0.0
Sen. tamoides	37	10.5	16	11.9	125	15.7	0	0.0	42	14.1	3	21.4
Total no of eggs	**351**	**100**	**135**	**100**	**794**	**100**	**16**	**100**	**297**	**100**	**14**	**100**

Thus far, we have three successful oviposition trials using *Diota rostrata* collected from the Addo site (Table 5 and Figure 8). The results were less predictable, possibly because several of the trials (Addo X and Addo Z) suffered from problems such as ant predation or overheating. Nevertheless, it appears that the *Diota rostrata* from Addo also prefer *Senecio oxyodontus* rather than Cape ivy. Even more disturbing, in two of these trials with the Addo moths, eggs were also found on *Sen. flaccidus*, which is native to California. *Diota*'s preference for hosts other than Cape ivy, along with the oviposition observed on a California native, *Senecio flaccidus*, have made this moth a poor candidate for introduction into the USA. Since we have two far more promising candidates, our efforts will now concentrate on getting them approved for release. No further testing of *Diota* is planned.

Although, unfortunately, this damaging moth has been ruled out from current consideration as an agent, this process has validated our approach of extensive testing of potential agents in South Africa. Testing this promising moth in our quarantine facility in Albany, under strict containment conditions, would have required a near full-time effort for many years. It was far quicker and cheaper to test this moth under non-containment conditions in South Africa.

Figure 8. The number of *Diota rostrata* eggs laid on each plant species



V. Other studies during 2003

A. Status of insects mentioned in earlier reports

In the previous sections of this report, we presented the results of the research we conducted, during 2003, on the three South African insects that show the most promise as biological control agents against Cape ivy infesting California. In our Annual Reports for 2001 and 2002 (Balciunas *et al.* 2002, Balciunas *et al.* 2003), we also discussed three other South African insects that received at least preliminary research into their suitability as possible agents for Cape ivy.

During 2002, we initiated studies on a small beetle (as yet unidentified, but in the family Phalacridae) whose larvae feed on developing flowers of Cape ivy. At our request, a graduate student in South Africa, Mrs. Lin Besaans, began to conduct research on this beetle. However, by early 2003, it was clear that this phalacrid beetle was probably univoltine– completes only one generation each year. Similar larvae were also found feeding on flowers of other plants, related to Cape ivy, growing in South Africa. It became apparent that the planned research would require many years and was inappropriate for a masters thesis. Accordingly, Mrs. Besaans has switched to a different topic for her graduate research. We are not very enthusiatic about the promise of this phalacrid beetle, especially in view of the many years of research that would still be required. We have shelved plans for additional research on this phalacrid, but continue to try to get it identified.

During 2001, our South African colleagues attempted to establish colonies of a chrysomelid beetle (*Luperodes* species) that severely damages Cape ivy. However, this beetle occurs sporadically in only a few locations in South Africa. Despite repeated attempts, our colleagues in South Africa have been unable to colonize this beetle.

This is also the case for the agromyzid fly, whose larvae mine the stems of Cape ivy. Given the ongoing funding shortfalls, it is unlikely that there will be further research on any of these interesting, but less promising insects.

B. *Cercospera* pathogen

Our colleagues in South Africa continue to see Cape ivy that is severely stressed by a plant pathogen, earlier idenitified as a species of the fungus *Cercospora*. Unfortunately, early in 2003, Dr. Maryna Serdani, the plant pathologist in South Africa who was working on identifying and testing *Cercospora*, emigrated to the USA. We are seeking assistance of other pathologists to conduct research in this promising pathogen.

C. Studies on Cape ivy biology

In May of 2003, Dr. Balciunas attended the XI International Symposium on Biological Control of Weeds, held in Canberra, Australia. After the meeting, he had an opportunity to see Cape ivy in Australia. Dr. El Bruzzese took Joe to various infestations at Mornington Peninsula, SE of Melbourne (see photo on cover of this report). These are considered among the most damaging on the continent. Australian flowers were included in our studies into the phenology and seed production of Cape ivy.

The inflorescences ("heads") of most species of *Senecio*, along with its close relatives, are usually composed of two types of florets: outer ligulate ("ray") florets surrounding central discoid ("disk") florets. Cape ivy is different in that each head is composed entirely of disk florets. Our interest in quantifying the damage of flower-feeding insects led us to investigate how many seeds might be produced. Authoritative texts, both in USA (Barkley, 1993) and abroad (Blood, 2000) give the number of disk florets per head as 20-40, or 15-40. Each of these florets is capable of producing a seed. From our searches for seeds of Cape ivy, we began to suspect that this was far more florets per head than we were encountering.

Accordingly, during 2003, we began to quantify the number of florets per head of Cape ivy flowers from various regions. During this project, we have amassed Cape ivy seed heads from a variety of locations around the world. We dissected the inflorescences of *Delairea odorata* that had been collected at five locations in South Africa, one in Australia, one in Hawaii, and one in California. The florets in each head were counted (Table 6).

Table 6. Number of Cape ivy florets / inflorenscence collected from various sites.

Nation	Location (date collected)	No of inflorescences dissected	Mean florets / inflorescence (range)
USA	Hawaii, Manuka Forest (May 2000)	5	9.6 (9-10)
USA	Calif., Rodeo Beach (Feb. 15, 2000)	8	11.4 (8-14)
Australia	Victoria, Rye Beach (July 30, 2003)	5	11.2 (8-13)
South Africa	E. Cape, Kubusi St. Forest (June 8, 2000)	7	10.1 (9-11)
South Africa	W. Cape, Plettenburg Bay (Aug. 2002)	3	11.0 (10-13)
South Africa	E. Cape Hogsback (July 29, 2003)	5	10.4 (9-12)
South Africa	W. Cape Montago Pass (Aug. 14, 1999)	5	9.8 (9-10)
South Africa	Kwazulu-Natal (N. Vryheid, 1999)	5	11.0 (10-12)

In each inflorescence, the number of florets ranged from 8-14, with a mean number of 10.6 florets from all 43 inflorescences at the seven locations, far less than what has been reported in the literature. We plant to investigate this further, at more sites in California, after Cape ivy flowers in early 2004.

VI. Future Plans

A. Research planned for 2004

Our efforts, both in Albany and Pretoria, will be directed to completing the host range testing of *Parafreutreta regalis* and *Digitivalva delaireae*. We will then compile this data, and begin the lengthy process of obtaining regulatory approval for release [see Section B below]. We will also begin selecting our release sites, and establish relationships with agencies and individuals that might assist us in the pre-release and post-release evaluations at these sites.

No further research on other South African insects is planned at this time. Instead, we will concentrate on beginning research on the pathogen that kills leaves (and sometimes entire vines) in South Africa. Our ARS colleagues located at Foreign Plant Disease Laboratory in Ft. Detrick, Maryland have tentatively expressed interest in assisting us in a portion of the planned research for this *Cercospera* species.

We will also continue our studies into the basic biology of Cape ivy, especially flowering and seed viability. We are also still trying to find colleagues interested in assisting us in molecular studies into Cape ivy's origin and distribution.

B. The next step: obtaining approval for release

Barring unforeseen delays, during 2004, I anticipate beginning the process of obtaining permission for release for the Cape ivy gall fly, *Parafreutreta regalis*, and possibly also for the Cape ivy stem-boring moth, *Digitivalva delaireae*. There are substantial differences in the approval processes for biocontrol agents targeting insect pests, compared with those targeting weeds. Approval for release of an overseas insect (usually a parasitoid) to control <u>an insect pest</u> is primarily done at the state level, and is straightforward and relatively quick. However, releasing a herbivorous insect to control a weed has always been considered more risky. As a result, gaining approval for release of a new weed biological control agent is a complex and lengthy process (see diagram in Figure 9) that involves an advisory panel and an array of federal agencies. The entire approval process takes at least a year, and sometimes much, much longer.

By mid-2004, we plan to take the first step in this process and submit a "petition" requesting release of the Cape ivy gall fly to the Technical Advisory Group for Biological Control of Weeds (**TAG**). This advisory panel currently has 16 members (plus the Chair and Executive Secretary) from 12 federal agencies, as well as representatives from Canada, Mexico, the National Plant Board, and the Weed Science Society [for more information, visit http://www.aphis.usda.gov/ppq/permits/tag/].

The petition for TAG is prepared in a special format, and contains a summary of what is known about the proposed agent, as well as our research into its host range and safety. The taxonomy of the target weed, and a summary of its impact is also included in the petition. TAG members review the petition, and make their recommendation to the TAG chairman. Prior to making a recommendation, some TAG members may send the petition to internal and external experts for their comments. The TAG chair summarizes the comments from the TAG members, and then prepares a recommendation to USDA-APHIS Plant Protection and Quarantine Agency (**PPQ**). Not infrequently, TAG will indicate that additional information is required before it can recommend approval.

TAG's recommendation is not binding on PPQ, but, in practice, has great influence on PPQ's decision to issue a release permit. If TAG recommends release, I will then seek approval from the State (California) through the State Plant Regulatory Official (**SPRO**). If California also approves, PPQ prepares an Environmental Assessment (**EA**), using the information presented in our petition. This EA is circulated to other agencies, with the mandatory consultation with US Fish and Wildlife Service (**FWS**), being the most critical. FWS must provide their opinion if the release of the weed biocontrol agent might impact a federally-listed Threatened and Endangered (**T&E**) species. If they reach a finding of no significant impact (**FONSI**), PPQ will issue a release permit. As mentioned earlier, this complex approval process can easily require one year.

However, if FWS feels there might be an impact on a T&E species (and release of the agent is still desired), a full Environmental Impact Statement (**EIS**) must be prepared. After receiving the EIS, FWS must consent to allowing the impact to the T&E species. With FWS approval, PPQ then issues a release permit. Preparing the EIS, and securing approval from FWS is very time-consuming – 5 to 10 years might be required if the EIS process is triggered.

Another potential obstacle to approval is that this approval process is currently being overhauled. A large portion of the staff of USDA-APHIS, including PPQ, was transferred to the recently created Department of Homeland Security. Many critical vacancies were created in the PPQ staff that handles the approval process, and most have not yet been filled. In addition, a post- September 11, 2001 review of potential biosecurity threats, found PPQ oversight and monitoring of importation of overseas organisms to be problematic. As a result, PPQ is in the process of changing these procedures. New regulations covering importation of organisms were issued in November 2003. But many of these were almost immediately "postponed" pending further modification. At this point, we still don't have the final regulations.

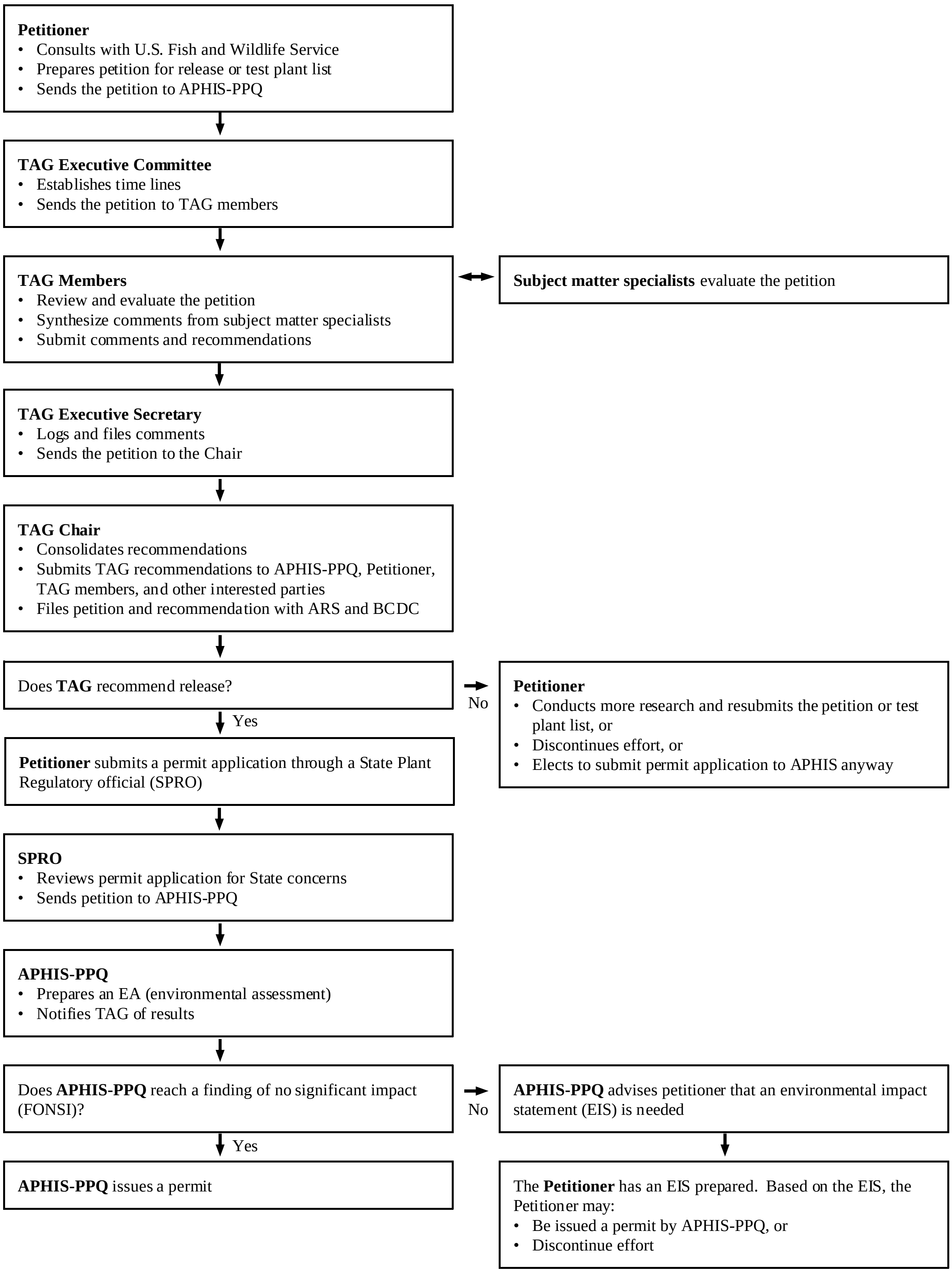
Although I remain hopeful that our thoroughly tested agents will be approved for release in 2005, it is possible that this complex and changing approval process will require more time.

C. Funding shortfalls

While USDA-ARS provides the bulk of the funds for our project, supplementary external funds have funded most of the research in South Africa. External funds also accelerated our research in Albany by providing most of the salary for an additional technician here, allowing us to test two foreign insect species simultaneously in our quarantine.

However, the enormous decrease in external funding that accelerated during 2003, will slow development and evaluation of Cape ivy agents. California's fiscal crisis has led every state agency to terminate their contributions to the Biocontrol of Cape ivy Project. This includes CalTrans' annual $25,000 that provided most of the support for one of my assistants here. The contributions ($5,000 - $15,000) from California State Parks & Recreation, as well as from individual parks, have also dried up. Fortunately, I continue to receive full support from USDA-ARS, but the decline in external funds has caused us to drastically scale back the supporting research in South Africa, and could slow down the evaluation of agents here in California. Since additional funds for research in South Africa during 2004 are likely to be minimal, we scaled back research there during 2003, in an attempt to stretch currently allocated funds into 2004. By doing so, we are hopeful that we will be able to maintain at least a part-time effort in South Africa during 2004.

Figure 9. Flowchart diagramming the approval process for release of weed biological control agents (from TAG Reviewer's Manual)



VII. Other activities and publications

A. Articles published or submitted since January 1st, 2003

Balciunas, J. 2002. Strategies for expanding and improving overseas research for biological control of weeds. pp. 1-7 *in:* Biological Control of Invasive Plants in Hawaiian Natural Ecosystems. Clifford W. Smith, Julie Denslow, and Stephen Hight (eds.). U.S. Forest Service, Honolulu, HI.

Pitcairn, M. J., J. A. Young, C. D. Clements, and J. K. Balciunas. 2002. Purple starthistle (*Centaurea calcitrapa*) seed germination. Weed Technology. 16: 452-456.

Grobbelaar, E., J. K. Balciunas, O. Neser, and S. Neser. 2003. South African insects for biological control of *Delairea odorata*. pp. 16-28 *in:* Proceedings, Cal-IPC Symposiums, 2000, 2001, 2002. M. Kelly (ed.), Concord, CA.

Uygur, S., L. Smith, M. Cristofaro, J. Balciunas, and M. Pitcairn. 2003. *Centaurea solstitialis* in Turkey with special emphasis on its biological control. pp. 11-12 *in:* 7th European Weed Research Society Mediterranean Symposium. Adana, Turkey.

Balciunas, J. (in press). Are mono-specific agents necessarily safe? The need for pre-release assessment of probable impact of candidate biocontrol agents, with some examples. pp. xx-xx *in:* XI International Symposium on Biological Control of Weeds. CSIRO Publishing, Melbourne, Australia.

Balciunas, J. (in press). Four years of 'Code of Best Practices': is biocontrol of weeds less risky, and receiving greater acceptance? pp. xx-xx *in:* XI International Symposium on Biological Control of Weeds. CSIRO Publishing, Melbourne, Australia.

Balciunas, J. (in press). *Delairea odorata* Lemaire. pp. xx-xx *in:* CABI Crop Protection Compendium.

Balciunas, J.K. (2004). Cape ivy, *Delairea odorata* (previously, *Senecio mikanioides*). pp. xx-xx *in*: E. M. Coombs, J. K. Clark, G. L. Piper, and A. F. Cofrancesco (eds.) Biological Control of Invasive Plants in the United States. Oregon State University Press, Corvallis, OR. (In press)

Balciunas, J.K., and E.M. Coombs. (2004). International code of best practices for biological control of weeds. pp. xx-xx *in*: E. M. Coombs, J. K. Clark, G. L. Piper, and A. F. Cofrancesco (eds.) Biological Control of Invasive Plants in the United States. Oregon State University Press, Corvallis, OR. (In press)

Uygur, S., L. Smith, F. Uygur, M. Cristofaro, and J. Balciunas. (submitted). Field assessment in land of origin of host specificity, infestation rate and impact of *Ceratapion basicorne* (Coleoptera: Apionidae), a prospective biological control agent of yellow starthistle. BioControl. xx: xx-xx.

Uygur, S., L. Smith, F. Uygur, M. Cristofaro, and J. Balciunas. (submitted). Population densities of yellow starthistle (*Centaurea solstitialis*) in Turkey. Weed Science. xx: xx-xx.

B. Selected meetings and travel by Dr. Joe Balciunas in 2003

Feb 10-14	Presented invited talks at the Weed Science Society of America, Annual Meeting in Jacksonville, FL
Mar 10	Presented invited talks at the North Coast Weed Management Area, Annual Meeting in Eureka, CA
Mar 20	Served as judge at the Bay Area Science Fair
Apr 27-May 2	Presented invited talks at the XI Symposium for Biological Control of Weeds in Canberra, Australia
Sep 9-12	Presented invited talks at the TAG Annual Meeting in Spokane, WA
Oct 1-3	Represented EIW and ARS at the Annual W-1185 Project Meeting, Asilomar, CA, and led the participants on a short hike to familiarize them with the plants of the central coast, and some of the invasives that are threatening them
Nov 3-8	Presented invited talk at the 7th International Conference on Ecology and Management of Alien Plant Invasions, Ft. Lauderdale, FL, and served as Abstract Reviewer, helping to select which talks and posters would be presented at the conference
2003	Served as “Activity Leader” for San Francisco Bay Chapter of the Sierra Club, and led nine hikes in the Bay Area. During these events, I helped familiarize participants with native plant communities and the impacts of invasive weeds

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Appendicies

Appendix A. USDA *Parafreutreta regalis* "no-choice/ host added" tests

Test No.	Non-target test plants	Dates	*Pa. regalis* developed on	Notes
PA-1-1004	*Euryops pectinatus* *Packera macounii* *Senecio blochmaniae* *Senecio triangularis*	4-26 to 5-3 2001	Cape ivy	3 females alive when CI added (4-30), 1 female alive at end of test. **CI had 3 galls**, 2 dissected 7-31: 1st had 1 pupal case (PC), 2nd had 1 dead pupa, 3rd had *Pa.* dmg
PA-2-1004	*Senecio blochmaniae* *Packera macounii* *Senecio hybridus* *Senecio triangularis*	4-27 to 5-4 2001	Cape ivy	3 females alive when CI added (5-1), 1 female alive at end of test. **CI had 2 galls**, 1 dissected 7-31: it had 2 PC
PA-3-1004	*Euryops pectinatus* *Packera bolanderi* *Senecio blochmaniae* *Senecio triangularis*	4-30 to 5-7 2001	nothing	2-3 females alive when CI added (5-3), no females alive at end of test.
PA-4-1004	*Euryops pectinatus* *Packera macounii* *Senecio blochmaniae* *Senecio hybridus*	4-30 to 5-7 2001	nothing	4 females alive when CI added (5-3), 2 females alive at end of test.
PA-5-1004	*Euryops pectinatus* *Packera bolanderi* *Packera breweri* *Senecio flaccidus*	5-8 to 5-15 2001	nothing (Rydin Rd.)	2 females alive when CI added (5-12), no females alive at end of test.
PA-6-1004	*Euryops pectinatus* *Euryops subcarnosus* *Packera bolanderi* *Sen triangularis*	7-9 to 7-16 2001	nothing (Rydin Rd.)	2 females alive when CI added (7-12), no females alive at end of test.
PA-7-1015	*Adenocaulon bicolor* *Hedera helix* *Packera bolanderi* *Senecio confusus*	8-22 to 8-29 2001	nothing (Rydin Rd.)	3 females alive when CI added (8-25), 1 or 2 females alive at end of test.
PA-8-1015	*Adenocaulon bicolor* *Erechtites glomerata* *Euryops subcarnosus* *Hedera helix*	8-22 to 8-29 2001	Cape ivy (Garrapata Ck.)	4 females alive when CI added (8-25), 2 or 3 females alive at end of test. **CI had 4 galls**, 3 dissected 11-5: 1st had 1 PC, 2nd had 3 PC, 3rd had 2 PC and 2 dead adults
PA-9-1015	*Adenocaulon bicolor* *Erechtites glomerata* *Packera bolanderi* *Senecio confusus*	8-22 to 8-28 2001	nothing	4 (?) females alive when CI added (8-25), all females escaped before end of test.
PA-10-1015	*Erechtites glomerata* *Euryops subcarnosus* *Senecio blochmaniae* *Senecio confusus*	8-22 to 8-27 2001	nothing (Rydin Rd.)	2 females alive when CI added (8-25), no females alive at end of test.

PA-11-1015	*Adenocaulon bicolor* *Erechtites glomerata* *Euryops subcarnosus* *Senecio confusus*	8-27 to 9-4 2001	Cape ivy (Rydin Rd.)	4 females alive when CI added (8-30), 2 females alive at end of test. **CI had 7 galls**, 5 split 11-5: 1st had 3 PC, 2nd had 1 dead adult, 3rd had 3 PC, 4th had 1 PC, 5th had *Pa.*dmg; 1 split 11-29: it had 5 PC and 1 dead pupa
PA-12-1015	*Erechtites glomerata* *Euryops subcarnosus* *Senecio flaccidus* *Senecio triangularis*	8-28 to 9-4 2001	Cape ivy (Garrapata Ck.)	4 females alive when CI added (8-31), 3 females alive at end of test. **CI had 7 galls**, 4 dissected 11-5: 1st had 8 PC, 2nd had 2 PC and 1 pupa, 3rd had 1 PC, 1 pupa and 1 dead adult, 4th had 4 PC
PA-13-1015	*Erechtites glomerata* *Hedera helix* *Packera bolanderi* *Senecio flaccidus*	8-29 to 9-5 2001	nothing (Rydin Rd.)	4 females alive when CI added (9-1), 2 females alive at end of test.
PA-14-1015	*Adenocaulon bicolor* *Euryops subcarnosus* *Packera macounii* *Senecio flaccidus*	8-29 to 9-5 2001	Cape ivy (Rydin Rd.)	4 females alive when CI added (9-1), 1 female alive at end of test. **CI had 6 galls**, 2 dissected 11-5: 1st had 3 PC, 2 pupae and 1 dead adult, 2nd had 5 PC, 1 pupa and 1 dead adult *Adenocaulon* had possible *Pa.* damage
PA-15-1015	*Adenocaulon bicolor* *Euryops subcarnosus* *Packera macounii* *Senecio flaccidus*	9-5 to 9-12 2001	nothing (Garrapata Ck.)	4 females alive when CI added (9-7), 3 females alive at end of test.
PA-16-1015	*Euryops subcarnosus* *Packera macounii* *Senecio confusus* *Senecio flaccidus*	9-12 to 9-19 2001	Cape ivy	4 females alive when CI added (9-14), 3 females alive at end of test. **CI had 1 gall**, 1 gall dissected 11-29: it had 2 PC
PA-17-1015	*Adenocaulon bicolor* *Senecio blochmaniae* *Senecio confusus* *Senecio flaccidus*	9-17 to 9-24 2001	Cape ivy (Garrapata Ck.)	4 females alive when CI added (9-20), 3 females alive at end of test. **CI had 4 galls**, 4 dissected 11-29: 1st had 2 PC, 2nd had 1 dead pupa, 2 live females and 1 live male, 3rd had 5 PC, 4th had 4 PC
PA-18-2015	*Euryops pectinatus* *Hedera helix* *Senecio blochmaniae* *Senecio confusus*	10-15 to 10-22 2001	Cape ivy (Rydin Rd.)	4 females alive when CI added (10-18), 2 females alive at end of test. **CI had 5 galls**
PA-1-3015	*Hedera canariensis* *Euryops pectinatus* *Packera bolanderi* *Senecio hybridus*	1-10 to 1-17 2002	Cape ivy (Rydin Rd.)	4 females alive when CI added (1-13), 2 females alive at end of test. **CI had 3 galls**, 1 dissected 4-10: it had 7 pupal cases, 2 dissected 4-24: 1st had 1 dead pupa, 2nd had 1 PC

PA-2-3015	*Hedera canariensis* *Euryops pectinatus* *Packera bolanderi* *Senecio hybridus*	1-14 to 1-22 2002	nothing (Garrapata Ck.)	4 females alive when CI added (1-17), 1 female alive at end of test.
PA-4-4015	*Packera breweri* *Packera ganderi* *Petasites frigidus* *Senecio blochmaniae*	3-4 to 3-11 2002	Cape ivy (Garrapata Ck.)	3 females alive when CI added (3-7), 1 females alive at end of test. **CI had 3 galls**, 2 dissected 6-17: 1st had 1 dead pupa and 2 PC, 1 dissected 6-27: it had 1 pupa
PA-5-4015	*Packera bolanderi* *Packera ganderi* *Packera ganderi* *Senecio hybridus*	3-7 to 3-14 2002	Cape ivy (Garrapata Ck.)	4 females alive when CI added (3-11), 2 females alive at end of test. **CI had 10 galls**, 10 dissected 6-3: 1st had 3 dead pupa, 2nd had 2 dead pupa and 15 PC, 3rd had 2 dead pupa, 4th had 1 dead pupa and 3 PC, 5th had *Pa.* damage, 6th had 3 dead pupa and 9 PC, 7th had 2 PC, 8th had 4 PC, 9th had 1 dead pupa and 1 PC, 10th had 1 dead pupa and 2 PC
PA-6-4015	*Luina hypoleuca* *Packera breweri* *Senecio vulgaris* *Senecio jacobaea*	3-11 to 3-18 2002	Cape ivy (Garrapata Ck.)	3 females alive when CI added (3-14), 3 females alive at end of test. **CI had 13 galls**, 13 dissected 6-3: 1st had 9 PC, 2nd had 4 PC, 3rd had 3 PC, 4th had 5 PC, 5th had 2 PC, 6th had 3 PC, 7th had 1 dead pupa and 3 PC, 8th had 3 PC, 9th had 5 PC, 10th had 4 PC, 11th had 3 PC, 12th had 1 PC, 13th had 2 PC
PA-8-4015	*Luina hypoleuca* *Packera breweri* *Senecio vulgaris* *Senecio jacobaea*	3-18 to 3-25 2002	Cape ivy (Garrapata Ck.)	3 females alive when CI added (3-21), 3 females alive at end of test. **CI had 11 galls**, 2 dissected 5-29: 1st had 9 PC, 2nd had 1 dead pupa and 7 PC; 9 dissected on 6-3: 1st had 5 PC, 2nd had 7 PC, 3rd had 2 PC, 4th had 5 PC, 5th had 1 dead pupa and 3 PC, 6th had 2 PC, 7th had 6 PC, 8th had 3 PC, 9th had 3 PC
PA-10-5015	*Hedera canariensis* *Luina hypoleuca* *Petasites frigidus* *Senecio jacobaea*	4-11 to 4-19 2002	Cape ivy (Garrapata Ck.)	2 females alive when CI added (4-15), 1 female alive at end of test. **CI had 4 galls**, 2 dissected 6-25: 1st had 1 dead pupa and 3 PC, 2nd had 1 PC; 2 dissected 6-28: 1st had 1 larvae, 2nd had 1 larva, 1 pupa
PA-11-5015	*Hedera helix* *Luina hypoleuca* *Petasites frigidus* *Senecio jacobaea*	4-15 to 4-22 2002	nothing (Garrapata Ck.)	3 females alive when CI added (4-18), 1 female alive at end of test.

PA-12-5015	*Euryops pectinatus* *Hedera helix* *Senecio hybridus* *Senecio triangularis*	4-18 to 4-29 2002	nothing (Garrapata Ck.)	1-3 females alive when CI added (4-22), no females alive at end of test.
PA-13-5015	*Carthamus tinctorius* *Hedera helix* *Petasites frigidus* *Senecio triangularis*	4-25 to 4-29 2002	n/a	test aborted due to high *Pa.* mortality rate
PA-14-5015	*Carthamus tinctorius* *Hedera helix* *Petasites frigidus* *Senecio triangularis*	4-29 to 5-6 2002	nothing (Garrapata Ck.)	3-4 females alive when CI added (5-2), no females alive at end of test.
PA-15-5015	*Carthamus tinctorius* *Hedera helix* *Petasites frigidus* *Senecio triangularis*	5-3 to 5-13 2002	Cape ivy (Garrapata Ck.)	2 females alive when CI added (5-7), 1 female alive at end of test. **CI had 6 galls**, 5 dissected 7-10: 1st had 2 PC, 2nd had 3 PC, 3rd had 1 dead pupa and 3 PC, 4th had 2 PC, 5th had 1 dead pupa and 2 PC; 1 dissected 8-26: it had 2 dead adults and 1 PC
PA-16-5015	*Carthamus tinctorius* *Packera bolanderi* *Packera ganderi* *Senecio hybridus*	5-6 to 5-14 2002	Cape ivy (Garrapata Ck.)	3 females alive when CI added (5-9), 3 females alive at end of test. **CI had 1 gall**, it was dissected on 8-12 and had 3 dead adults
PA-17-5015	*Carthamus tinctorius* *Erechtites glomerata* *Hedera helix* *Luina hypoleuca*	5-7 to 5-15 2002	Cape ivy (Garrapata Ck.)	4 females alive when CI added (5-10), 1 female alive at end of test. **CI had 10 galls**, 3 dissected on 6-28 only had *Pa.* dmg; 4 were dissected on 7-11: 1st had 3 PC, 2nd had 1 live female and 1 PC, 3rd had 4 pupa, 4th had 3 pupa and 2 PC; 2 were dissected 8-6: 1st had 8 PC, 2nd had 1 dead female and 2 dead pupa; 1 dissected 8-13: it had 1 pupa and 3 PC
PA-18-5015	*Carthamus tinctorius* *Erechtites glomerata* *Hedera helix* *Senecio blochmaniae*	5-16 to 5-24 2002	Cape ivy (Garrapata Ck.)	4 females alive when CI added (5-20), 1 female alive at end of test. **CI had 5 galls**, 2 were dissected 8-21: 1st had 6 PC, 2nd had 4 PC; 2 were dissected 8-27: 1st had 1 dead adult and 1 dead pupa, 2nd had *Pa.* dmg
PA-19-5015	*Euryops pectinatus* *Hedera helix* *Packera bolanderi* *Packera macounii*	5-17 to 5-24 2002	Cape ivy (Garrapata Ck.)	3 females alive when CI added (5-21), 1 female alive at end of test. **CI had 5 galls**, 1 dissected 8-26: it had 7 PC; 4 dissected 8-27: 1st had *Pa.* dmg, 2nd had 3 dead adults, 3rd had 5 dead adults, 4th had 2 dead pupa

PA-20-5015	*Carthamus tinctorius* *Euryops pectinatus* *Senecio triangularis* *Tagetes sp.*	5-28 to 6-6 2002	nothing (Garrapata Ck.)	4 females alive when CI added (5-31), no females alive at end of test.
PA-21-5015	*Hedera helix* *Packera bolanderi* *Packera breweri* *Tagetes sp.*	6-3 to 6-10 2002	Cape ivy (Garrapata Ck.)	3 females alive when CI added (6-6), no females alive at end of test. **CI had 3 galls**, 3 were dissected 8-13: 1st had 3 PC, 2nd had 3 PC, 3rd had 1 dead female and 6 PC
PA-22-5015	*Hedera helix* *Petasites frigidus* *Senecio flaccidus* *Tagetes sp.*	6-6 to 6-12 2002	nothing (Garrapata Ck.)	2 females alive when CI added (6-10), no females alive at end of test.
PA-23-5015	*Adenocaulon bicolor* *Luina hypoleuca* *Senecio jacobaea* *Tagetes sp.*	6-10 to 6-17 2002	nothing (Garrapata Ck.)	3 females alive when CI added (6-13), no females alive at end of test.
PA-24-5015	*Hedera helix* *Petasites frigidus* *Senecio flaccidus* *Tagetes sp.*	6-11 to 6-17 2002	nothing (Garrapata Ck.)	2 females alive when CI added (6-14), no females alive at end of test.
PA-25-5015	*Senecio confusus* *Senecio jacobaea* *Senecio vulgaris* *Tagetes sp.*	6-17 to 6-21 2002	Cape ivy (Garrapata Ck.)	4 females alive when CI added (6-17), no females alive at end of test. **CI had 3 galls**, 2 dissected 9-6: both had *Pa*. dmg
PA-26-5015	*Hedera helix* *Packera breweri* *Senecio flaccidus* *Tagetes sp.*	6-17 to 6-27 2002	nothing (Garrapata Ck.)	2 females alive when CI added (6-21), no females alive at end of test.
PA-27-5015	*Hedera helix* *Packera bolanderi* *Petasites frigidus* *Tagetes sp.*	6-18 to 7-1 2002	nothing (Garrapata Ck.)	3 females alive when CI added (6-21), no females alive at end of test.
PA-28-5015	*Carthamus tinctorius* *Euryops pectinatus* *Senecio triangularis* *Senecio vulgaris*	6-20 to 7-1 2002	nothing (Garrapata Ck.)	3 females alive when CI added (6-24), no females alive at end of test.
PA-29-6015	*Adenocaulon bicolor* *Senecio hybridus* *Senecio jacobaea* *Senecio vulgaris*	7-10 to 7-19 2002	nothing (Garrapata Ck.)	4 females alive when CI added (7-15), no females alive at end of test.

PA-30-6015	*Adenocaulon bicolor* *Hedera helix* *Senecio jacobaea* *Senecio vulgaris*	8-5 to 8-13 2002	Cape ivy (Garrapata Ck.)	3 females alive when CI added (8-8), no females alive at end of test. **CI had 5 galls**, 5 dissected 10-15: 1st had 3 PC, 2nd had 3 PC, 3rd had 3 PC, 4th had *Pa*. dmg, 5th had 5 PC
PA-31-8015	*Carthamus tinctorius* *Euryops pectinatus* *Petasites frigidus* *Senecio hybridus*	11-19 to 11-26 2002	Cape ivy (Garrapata Ck.)	2 females alive when CI added (11-22), no females alive at end of test. **CI had 10 galls**,
PA-32-8015	*Lepidospartum latisquamum* *Senecio triangularis* *Symphyotrichum chilense* *Tagetes sp.*	11-21 to 12-2 2002	Cape ivy	4 females alive when CI added (11-25), no females alive at end of test. **CI had 15 galls**, 7 dissected 1-27-03: 1st had 2 PC, 2nd had *Pa*. dmg, 3rd had 2 pupa, 4th had 1 pupa, 5th had 1 pupa, 6th had *Pa*. dmg, 7th had *Pa*. dmg
PA-33-8015	*Anaphalis margaritacea* *Lepidospartum latisquamum* *Symphyotrichum chilense* *Tagetes sp.*	11-26 to 12-3 2002	Cape ivy (Garrapata Ck.)	2 females alive when CI added (11-29), no females alive at end of test. **CI had 6 galls**,
PA-34-8015	*Anaphalis margaritacea* *Lepidospartum latisquamum* *Symphyotrichum chilense* *Tagetes sp.*	11-26 to 12-3 2002	nothing (Rydin Rd.)	2 females alive when CI added (11-29), 1 female alive at end of test.
PA-35-8015	*Anaphalis margaritacea* *Eriophyllum spicatum* *Lepidospartum latisquamum* *Symphyotrichum chilense*	11-29 to 12-6 2002	nothing (Garrapata Ck.)	4 females alive when CI added (12-2), 3 females alive at end of test.
PA-36-8015	*Anaphalis margaritacea* *Calendula officinalis* *Eriophyllum spicatum* *Lepidospartum latisquamum*	12-2 to 12-10 2002	Cape ivy (Garrapata Ck.)	3 females alive when CI added (12-5), 2 females alive at end of test. **CI had 1 gall**

PA-37-8015	*Anaphalis margaritacea* *Carthamus tinctorius* *Eriophyllum staechadifolium* *Symphyotrichum chilense*	12-2 to 12-10 2002	Cape ivy (Garrapata Ck.)	* test started w/ 3 females and 5 males 3 females alive when CI added (12-5), 2 females alive at end of test. **CI had 4 galls**
PA-1-9015	*Aster chilensis* *Calendula officinalis* *Senecio flaccidus* *Senecio vulgaris*	1-27 to 2-4 2003	nothing (Garrapata Ck.)	3 females alive when CI added (1-30), 1 female alive at end of test.
PA-2-9015	*Anaphalis margaritacea* *Aster chilensis* *Calendula officinalis* *Lepidospartum latisquamum*	1-28 to 2-5 2003	Cape ivy (Garrapata Ck.)	3 females alive when CI added (1-31), no females alive at end of test. **CI had 7 galls**, 7 dissected 4-8-03: 1st had 2 PC, 2nd had 11 PC, 3rd had 5 PC, 4th had 5 PC, 5th had 3 PC, 6th had 1 dead adult and 6 PC, 7th had 4 PC
PA-3-9015	*Aster chilensis* *Calendula officinalis* *Lepidospartum latisquamum* *Senecio flaccidus*	1-31 to 2-10 2003	Cape ivy (Garrapata Ck.)	3 females alive when CI added (2-4), no females alive at end of test. **CI had 6 galls**, 6 dissected 4-8-03: 1st had 2 PC, 2nd had 4 PC, 3rd had 7 PC, 4th had 1 pupae and 4 PC, 5th had 2 live adults, 1 pupae, and 2 PC, 6th had 2 dead adults and 2 dead pupa
PA-4-9015	*Calendula officinalis* *Eriophyllum staechadifolium* *Lepidospartum latisquamum* *Senecio vulgaris*	2-7 to 2-10 2003	n/a	test aborted due to high mortality and missing *Pa.* flies
PA-5-9015	*Anaphalis margaritacea* *Centaurea melitensis* *Eriophyllum staechadifolium* *Vernonia missurica*	3-14 to 3-21 2003	Cape ivy (Garrapata Ck.)	4 females alive when CI added, 2 females alive at end of test. **CI had 4 galls**, 3 dissected 5-27-03: 1st had 2 adults, 1 dead pupa, 2nd had 10 PC, 3rd had 5 PC; 1 dissected 6-11-03, it had 1 dead pupae
PA-6-9015	*Blennosperma nanum* *Calendula officinalis* *Grindelia sp.* *Vernonia missurica*	3-21 to 3-28 2003	Cape ivy (Garrapata Ck.)	4 females alive when CI added, 1 female alive at end of test. **CI had 6 galls**, 6 dissected 6-9-04: 1st had 7 PC, 2nd had 5 PC, 3rd had 4 PC, 4th had 8 PC, 5th had 1 pupae and 3 PC, 6th had 4 PC

PA-7-1015	*Blennosperma nanum* *Calendula officinalis* *Grindelia sp.* *Vernonia missurica*	3-25 to 4-1 2003	Cape ivy (Garrapata Ck.)	4 females alive when CI added, 1 female alive at end of test. **CI had 3 galls**, 3 dissected 6-2-03: 1st had 5 PC, 2nd had 6 PC, 3rd had 1 dead adult and 4 PC
PA-8-1015	*Blennosperma nanum* *Frageria chiloensis* *Grindelia sp.* *Vernonia missurica*	4-1 to 4-8 2003	Cape ivy (Garrapata Ck.)	4 females alive when CI added, 2 females alive at end of test. **CI had 9 galls**, 4 dissected 6-9-03: 1st had 5 PC, 2nd had 1 dead adult and 8 PC, 3rd had 4 PC, 4th had 3 dead larva; 5 dissected 6-19-03: 1st had 7 dead adults, 2nd had 5 dead adults, 3rd had 3 dead adults, 4th had 5 dead adults and 2 dead pupa, 5th had dmg
PA-9-1015	*Blennosperma nanum* *Eriophyllum staechadifolium* *Frageria chiloensis* *Vernonia missurica*	4-8 to 4-15 2003	Cape ivy (Garrapata Ck.)	4 females alive when CI added, 1 female alive at end of test. **CI had 8 galls**, 8 dissected 6-23-03: 1st had 5 PC, 2nd had 6 PC, 3rd had 4 dead adults, 4th had 4 dead adults and 1 dead pupa, 5th had 4 PC, 6th had 1 dead pupa and 1 PC, 7th had 1 dead pupa and 7 PC, 8th had 6 PC
PA-10-1015	*Blennosperma nanum* *Eriophyllum staechadifolium* *Tagates sp.* *Vernonia missurica*	4-15 to 4-22 2003	Cape ivy (Garrapata Ck.)	3 females alive when CI added, no females alive at end of test. **CI had 6 galls**, 6 dissected 6-25-03: 1st had 3 PC, 2nd had 1 dead pupa and 3 PC, 3rd had 4 PC, 4th had 2 dead adults and 2 PC, 5th had 4 dead adults, 6th had 3 PC
PA-16-1115	*(2x) Frageria chiloensis* *Grindelia sp.* *Senecio vulgaris*	5-13 to 5-22 2003	Cape ivy (Garrapata Ck.)	4 females alive when CI added, no females alive at end of test. **CI had 10 galls**, 10 dissected 7-25-03: 1st had 3 dead adults, 1 dead pupa, and 9 PC, 2nd had 2 dead pupa and 11 PC, 3rd had 6 PC, 4th had 5 PC, 5th had 2 dead adult and 6 PC, 6th had 2 dead adults and 3 dead pupa, 7th had 3 dead adults and 7 PC, 8th had 3 dead adults and 2 dead pupa, 9th had 1 dead adult, 2 dead pupa, and 8 PC, 10th had dmg
PA-17-1215	*Baccharis pilularis* *Frageria chiloensis* *Grindelia sp.* *Luina hypoleuca*	6-27 to 7-4 2003	Cape ivy (Garrapata Ck.)	4 females alive when CI added, no females alive at end of test. **CI had 2 galls**, 2 dissected 9-18-03: 1st had dmg, 2nd had dmg

Appendix B. USDA *Digitivalva delaireae* host range tests (C= choice, NC= no choice, NCHA= no choice/host-added)

Test No.	Test type	Non-target test plants	Dates	*Di. delaireae* developed on	Notes
DI-1-2001	C	*Packera bolanderi* *Packera macounii*	4-23 to 5-2 2001	Cape ivy	1 DI alive at end of test.
DI-2-2001	C	*Senecio triangularis*	4-24 to 5-7 2001	nothing	1 DI alive at end of test.
DI-3-3001	C	*Packera bolanderi*	6-14 to 6-21 2001	nothing	no DI alive at end of test.
DI-4-3001	NC	*Senecio triangularis*	6-15 to 6-22 2001	nothing	4 DI alive when CI added (6-19) 2 DI alive at end of test.
DI-5-3001	NC	*Senecio flaccidus*	6-18 to 6-25 2001	nothing	5 DI alive when CI added (6-22) 3 DI alive at end of test.
DI-6-3001	NC	*Senecio blochmaniae*	6-18 to 6-25 2001	Cape ivy	4 DI alive when CI added (6-22) 4 DI alive at end of test.
DI-7-3001	NC	*Petasites frigidus*	6-18 to 6-25 2001	nothing	4 DI alive when CI added (6-22) 2 DI alive at end of test.
DI-9-3001	NC	*Packera macounii*	6-20 to 6-28 2001	nothing	5 DI alive when CI added (6-25) 5 DI alive at end of test.
DI-10-1018	NCHA	*Euryops subcarnosus* *Hedera helix* *Senecio confusus* *Senecio triangularis*	11-13 to 11-26 2001	Cape ivy (Rydin Rd.)	7 DI alive when CI added (11-16) no DI alive at end of test. 2♀ & 2♂ adults emerged from CI
DI-11-1018	NCHA	*Euryops pectinatus* *Euryops subcarnosus* *Packera macounii* *Senecio hybridus*	11-13 to 11-26 2001	Cape ivy (Rydin Rd.)	7 DI alive when CI added (11-16) 4 DI alive at end of test. 4♀ & 3♂ adults emerged from CI
DI-12-1018	NCHA	*Euryops pectinatus* *Hedera helix* *Packera bolanderi* *Senecio confusus*	11-14 to 11-26 2001	Cape ivy (Rydin Rd.)	7 DI alive when CI added (11-20) 2 DI alive at end of test. 2♀ adults emerged from CI
DI-1-2018	NCHA	*Packera macounii* *Senecio hybridus* *Senecio jacobaea* *Senecio triangularis*	1-14 to 1-23 2002	Cape ivy (Garrapata Ck.)	8 DI alive when CI added (1-17) 1 DI alive at end of test. 4♀ + 5♂ adults emerged from CI
DI-2-2018	NCHA	*Packera breweri* *Packera macounii* *Senecio jacobaea* *Senecio triangularis*	1-28 to 1-31 2002	nothing (Garrapata Ck.)	5 DI alive when CI added (1-31) 3 DI alive at end of test.
DI-3-2018	NCHA	*Packera breweri* *Senecio confusus* *Senecio hybridus* *Senecio jacobaea*	2-5 to 2-13 2002	Cape ivy (Garrapata Ck.)	8 DI alive when CI added (2-8) 5 DI alive at end of test. 4♀ + 6♂ adults emerged from CI

DI-4-3018	NCHA	*Euryops pectinatus* *Euryops subcarnosus* *Packera breweri* *Packera macounii*	3-18 to 3-28 2002	Cape ivy (Garrapata Ck.)	8 DI alive when CI added (3-21) 4 DI alive at end of test. 17♀ + 14♂ adults emerged from CI
DI-5-3018	NCHA	*Hedera canariensis* *Luina hypoleuca* *Senecio jacobaea* *Senecio triangularis*	3-22 to 4-1 2002	nothing (Garrapata Ck.)	8 DI alive when CI added (3-26) 1 DI alive at end of test.
DI-6-3018	NCHA	*Luina hypoleuca* *Packera bolanderi* *Packera breweri* *Senecio vulgaris*	3-25 to 4-3 2002	nothing (Garrapata Ck.)	8 DI alive when CI added (3-29) 2 DI alive at end of test.
DI-7-3018	NCHA	*Luina hypoleuca* *Packera bolanderi* *Packera ganderi* *Senecio vulgaris*	3-28 to 4-5 2002	Cape ivy (Garrapata Ck.)	8 DI alive when CI added (4-1) 3 DI alive at end of test. 3♀ + 4♂ adults emerged from CI
DI-8-3018	NCHA	*Luina hypoleuca* *Senecio blochmaniae* *Senecio jacobaea* *Senecio vulgaris*	4-4 to 4-12 2002	nothing (Garrapata Ck.)	5 DI alive when CI added (4-8) 3 DI alive at end of test.
DI-9-3018	NCHA	*Luina hypoleuca* *Petasites frigidus* *Senecio blochmaniae* *Senecio jacobaea*	4-5 to 4-16 2002	nothing (Garrapata Ck.)	7 DI alive when CI added (4-9) 1 DI alive at end of test.
DI-10-3018	NCHA	*Carthamus tinctorius* *Erechtites glomerata* *Hedera canariensis* *Packera bolanderi*	5-13 to 5-21 2002	Cape ivy (Garrapata Ck.)	6 DI alive when CI added (5-16) 1 DI alive at end of test. 2♀ + 2♂ adults emerged from CI
DI-11-4018	NCHA	*Carthamus tinctorius* *Erechtites glomerata* *Hedera canariensis* *Packera bolanderi*	5-13 to 5-21 2002	nothing (Garrapata Ck.)	6 DI alive when CI added (5-16) 1 DI alive at end of test.
DI-12-4018	NCHA	*Carthamus tinctorius* *Packera macounii* *Senecio blochmaniae* *Senecio triangularis*	5-14 to 5-22 2002	nothing (Garrapata Ck.)	7 DI alive when CI added (5-17) 1 DI alive at end of test.
DI-13-4018	NCHA	*Carthamus tinctorius* *Euryops pectinatus* *Hedera helix* *Senecio blochmaniae*	5-21 to 5-28 2002	nothing	* CI not added to test 1 DI alive at end of test.

DI-14-4018 NCHA	*Carthamus tinctorius* *Erechtites glomerata* *Hedera canariensis* *Tagetes sp.*	5-21 to 5-29 2002	nothing (Garrapata Ck.)	6 DI alive when CI added (5-24) 1 DI alive at end of test.
DI-15-4018 NCHA	*Erechtites glomerata* *Hedera helix* *Senecio blochmaniae* *Tagetes sp.*	5-24 to 6-4 2002	Cape ivy (Garrapata Ck.)	7 DI alive when CI added (5-28) 1 DI alive at end of test. 6♀ + 5♂ adults emerged from CI
DI-16-4018 NCHA	*Euryops pectinatus* *Euryops subcarnosus* *Senecio triangularis* *Tagetes sp.*	5-24 to 6-3 2002	Cape ivy (Garrapata Ck.)	8 DI alive when CI added (5-28) 2 DI alive at end of test. 5♀ & 7♂ adults emerged from CI
DI-17-4018 NCHA	*Carthamus tinctorius* *Hedera helix* *Senecio blochmaniae* *Tagetes sp.*	6-4 to 6-10 2002	Cape ivy (Garrapata Ck.)	8 DI alive when CI added (6-7) no DI alive at end of test. 1♀ adult emerged from CI
DI-18-4018 NCHA	*Euryops pectinatus* *Euryops subcarnosus* *Petasites frigidus* *Senecio flaccidus*	6-10 to 6-17 2002	nothing (Garrapata Ck.)	7 DI alive when CI added (6-13) no DI alive at end of test.
DI-19-5018 NCHA	*Adenocaulon bicolor* *Hedera helix* *Packera breweri* *Senecio confusus*	7-9 to 7-17 2002	Cape ivy (Garrapata Ck.)	6 DI alive when CI added (7-12) 1 DI alive at end of test. 1♀ & 1♂ adults emerged from CI
DI-20-5018 NCHA	*Hedera helix* *Packera breweri* *Petasites frigidus* *Senecio flaccidus*	7-23 to 8-5 2002	Cape ivy (Garrapata Ck.)	8 DI alive when CI added (7-26) 1 DI alive at end of test. 2♀ adults emerged from CI
DI-21-5018 NCHA	*Adenocaulon bicolor* *Packera breweri* *Petasites frigidus* *Senecio flaccidus*	7-23 to 7-31 2002	Cape ivy (Garrapata Ck.)	8 DI alive when CI added (7-26) 1 DI alive at end of test. 1♀ & 2♂ adults emerged from CI
DI-22-5018 NCHA	*Adenocaulon bicolor* *Hedera helix* *Packera breweri* *Senecio flaccidus*	7-29 to 8-7 2002	Cape ivy (Garrapata Ck.)	7 DI alive when CI added (8-1) 1 DI alive at end of test. 2♀ & 5♂ adults emerged from CI
DI-23-6018 NCHA	*Carthamus tinctorius* *Euryops pectinatus* *Senecio hybridus* *Senecio vulgaris*	9-3 to 9-11 2002	Cape ivy (Garrapata Ck.)	8 DI alive when CI added (9-6) no DI alive at end of test. 1♀, 1♂, and 2 unsexed adults emerged from CI

DI-24-6018	NCHA	*Carthamus tinctorius* *Euryops subcarnosus* *Senecio flaccidus* *Senecio hybridus*	9-16 to 9-25 2002	Cape ivy (Garrapata Ck.)	8 DI alive when CI added (9-19) no DI alive at end of test. 1♀ & 3♂ adults emerged from CI
DI-25-6018	NCHA	*Hedera canariensis* *Packera bolanderi* *Senecio flaccidus* *Senecio vulgaris*	9-17 to 9-25 2002	Cape ivy (Garrapata Ck.)	8 DI alive when CI added (9-20) no DI alive at end of test. 7♀ & 3♂ adults emerged from CI
DI-26-6018	NCHA	*Packera bolanderi* *Packera macounii* *Senecio triangularis* *Senecio vulgaris*	9-23 to 10-1 2002	nothing (Garrapata Ck.)	8 DI alive when CI added (9-26) no DI alive at end of test.
DI-27-6018	NCHA	*Adenocaulon bicolor* *Packera macounii* *Senecio triangularis* *Senecio vulgaris*	9-26 to 10-7 2002	Cape ivy (Garrapata Ck.)	8 DI alive when CI added (9-30) 1 DI alive at end of test. no adults emerged from CI
DI-28-6018	NCHA	*Luina hypoleuca* *Packera macounii* *Senecio blochmaniae* *Senecio vulgaris*	9-20 to 10-8 2002	Cape ivy (Hawaii)	8 DI alive when CI added (10-3) no DI alive at end of test. 1♂ adult emerged from CI
DI-29-6018	NCHA	*Adenocaulon bicolor* *Hedera canariensis* *Petasites frigidus* *Senecio blochmaniae*	10-1 to 10-7 2002	nothing (South Africa)	4 DI alive when CI added (10-4) no DI alive at end of test.
DI-30-7018	NCHA	*Carthamus tinctorius* *Petasites frigidus* *Senecio blochmaniae* *Senecio confusus*	10-28 to 11-5 2002	Cape ivy (Garrapata Ck.)	* test started with 5♀ & 3♂ 8 DI alive when CI added (10-31) 2 DI alive at end of test. 3♀ & 4♂ adults emerged from CI
DI-31-7018	NCHA	*Packera bolanderi* *Petasites frigidus* *Senecio jacobaea* *Tagetes sp.*	10-29 to 11-7 2002	nothing (Garrapata Ck.)	8 DI alive when CI added (11-1) 2 DI alive at end of test.
DI-32-7018	NCHA	*Adenocaulon bicolor* *Petasites frigidus* *Senecio blochmaniae* *Tagetes sp.*	10-31 to 11-13 2002	Cape ivy (Garrapata Ck.)	7 DI alive when CI added (11-4) 2 DI alive at end of test. 6♀ & 8♂ adults emerged from CI
DI-33-7018	NCHA	*Erechtites glomerata* *Hedera canariensis* *Senecio jacobaea* *Senecio triangularis*	11-4 to 11-13 2002	Cape ivy (Garrapata Ck.)	8 DI alive when CI added (11-7) 2 DI alive at end of test. 8♀ & 8♂ adults emerged from CI

DI-34-7018 NCHA	*Erechtites glomerata* *Eriophyllum staechadifolium* *Senecio jacobaea* *Symphyotrichum chilense*	11-5 to 11-13 2002	nothing (Garrapata Ck.)	7 DI alive when CI added (11-8) 1 DI alive at end of test.
DI-35-7018 NCHA	*Anaphalis margaritacea* *Erechtites glomerata* *Eriophyllum staechadifolium* *Symphyotrichum chilense*	11-8 to 11-18 2002	Cape ivy (Garrapata Ck.)	7 DI alive when CI added (11-12) 1 DI alive at end of test. 8♀ & 6?♂ adults emerged from CI
DI-36-7018 NCHA	*Carthamus tinctorius* *Petasites frigidus* *Senecio confusus* *Symphyotrichum chilense*	11-13 to 11-19 2002	nothing (Garrapata Ck.)	7 DI alive when CI added (11-16) no DI alive at end of test.
DI-37-7018 NCHA	*Eriophyllum staechadifolium* *Petasites frigidus* *Packera bolanderi* *Symphyotrichum chilense*	11-13 to 11-20 2002	nothing (Garrapata Ck.)	8 DI alive when CI added (11-16) no DI alive at end of test.
DI-38-7018 NCHA	*Erechtites glomerata* *Eriophyllum staechadifolium* *Symphyotrichum chilense* *Tagetes sp.*	11-13 to 11-21 2002	Cape ivy (Garrapata Ck.)	7 DI alive when CI added (11-16) 1 DI alive at end of test. 9♀ & 11♂ adults emerged from CI
DI-39-7018 NCHA	*Anaphalis margaritacea* *Erechtites glomerata* *Hedera canariensis* *Senecio triangularis*	11-19 to 11-26 2002	Cape ivy (Rydin Rd.)	7 DI alive when CI added (11-22) 1 DI alive at end of test. 8♀ & 4♂ adults emerged from CI
DI-40-7018 NCHA	*Anaphalis margaritacea* *Eriophyllum staechadifolium* *Lepidospartum latisquamum* *Senecio jacobaea*	11-21 to 11-29 2002	Cape ivy (Garrapata Ck.)	8 DI alive when CI added (11-25) no DI alive at end of test. 1♀ & 1♂ adults emerged from CI

DI-41-7018	NCHA	*Anaphalis margaritacea* *Eriophyllum staechadifolium* *Lepidospartum latisquamum* *Senecio jacobaea*	11-21 to 11-29 2002	nothing (Garrapata Ck.)	5 DI alive when CI added (11-25) no DI alive at end of test.
DI-42-8018	NCHA	*Eriophyllum staechadifolium* *Lepidospartum latisquamum* *Senecio jacobaea* *Symphyotrichum chilense*	12-23 to 12-30 2002	nothing (Garrapata Ck.)	7 DI alive when CI added (12-26) no DI alive at end of test.
DI-43-8018	NCHA	*Lepidospartum latisquamum* *Senecio jacobaea* *Symphyotrichum chilense* *Vernonia missurica*	12-23 to 12-30 2002	nothing (Garrapata Ck.)	6 DI alive when CI added (12-26) 1 DI alive at end of test.
DI-1-8018	NCHA	*Calendula officinalis* *Eriophyllum staechadifolium* *Senecio jacobaea* *Symphyotrichum chilense*	1-10 to 1-21 2003	nothing (Garrapata Ck.)	8 DI alive when CI added (1-13) no DI alive at end of test.
DI-2-8018	NCHA	*Calendula officinalis* *Lepidospartum latisquamum* *Senecio jacobaea* *Symphyotrichum chilense*	1-10 to 1-21 2003	Cape ivy (Garrapata Ck.)	7 DI alive when CI added (1-13) no DI alive at end of test. 8♀ & 14♂ adults emerged from CI
DI-3-9018	NCHA	*Calendula officinalis* *Eriophyllum staechadifolium* *Lepidospartum latisquamum* *Symphyotrichum chilense*	2-14 to 2-21 2003	nothing (Garrapata Ck.)	8 DI alive when CI added (2-18) no DI alive at end of test.

DI-4-9018	NCHA	*Anaphalis margaritacea* *Calendula officinalis* *Lepidospartum latisquamum* *Symphyotrichum chilense*	2-20 to 2-28 2003	nothing (Garrapata Ck.)	4 DI alive when CI added (2-24) 1 DI alive at end of test.
DI-5-9018	NCHA	*Anaphalis margaritacea* *Calendula officinalis* *Eriophyllum staechadifolium* *Lepidospartum latisquamum*	2-25 to 3-4 2003	nothing (Garrapata Ck.)	7 DI alive when CI added (2-28) no DI alive at end of test.
DI-6-1018	NCHA	*Anaphalis margaritacea* *Calendula officinalis* *Eriophyllum staechadifolium* *Symphyotrichum chilense*	4-4 to 4-14 2003	Cape ivy (Garrapata Ck.)	8 DI alive when CI added (4-7) no DI alive at end of test. no emergence, but there was DI dmg on CI
DI-7-1018	NCHA	*Calendula officinalis* *Eriophyllum staechadifolium* *Lepidospartum latisquamum* *Symphyotrichum chilense*	4-11 to 4-18 2003	nothing (Garrapata Ck.)	5 DI alive when CI added (4-14) no DI alive at end of test
DI-8-1018	NCHA	*Blennosperma nanum* *Calendula officinalis* *Lepidospartum latisquamum* *Vernonia missurica*	4-15 to 4-21 2003	nothing (Garrapata Ck.)	4 DI alive when CI added (4-18) no DI alive at end of test
DI-9-1018	NCHA	*Calendula officinalis* *Eriophyllum staechadifolium* *Lepidospartum latisquamum* *Symphyotrichum chilense*	4-22 to 4-29 2003	nothing (Garrapata Ck.)	8 DI alive when CI added (4-25) 1 DI alive at end of test

DI-10-1118 NCHA	*Anaphalis margaritacea* *Calendula officinalis* *Frageria chiloensis* *Lepidospartum latisquamum*	6-3 to 6-16 2003	Cape ivy (Garrapata Ck.)	8 DI alive when CI added (6-6) 1 DI alive at end of test 13♀ & 10♂ adults emerged from CI
DI-11-1118 NCHA	*Calendula officinalis* *Frageria chiloensis* *Lepidospartum latisquamum* *Vernonia missurica*	6-3 to 6-11 2003	Cape ivy (Garrapata Ck.)	8 DI alive when CI added (6-6) 1 DI alive at end of test no emergence, but there was DI dmg on CI
DI-12-1118 NCHA	*Calendula officinalis* *Frageria chiloensis* *Grindelia sp.* *Vernonia missurica*	6-6 to 6-16 2003	Cape ivy (Garrapata Ck.)	7 DI alive when CI added (6-6) 1 DI alive at end of test 1♀ adult emerged from CI
DI-13-1118 NCHA	*Anaphalis margaritacea* *Frageria chiloensis* *Grindelia stricta* *Vitis californica*	6-10 to 6-16 2003	nothing (Garrapata Ck.)	8 DI alive when CI added (6-13) no DI alive at end of test
DI-14-1118 NCHA	*Anaphalis margaritacea* *Frageria chiloensis* *Grindelia stricta* *Vitis californica*	6-13 to 6-23 2003	nothing (Garrapata Ck.)	7 DI alive when CI added (6-16) no DI alive at end of test
DI-15-1118 NCHA	*Anaphalis margaritacea* *Frageria chiloensis* *Grindelia stricta* *Luina hypoleuca*	6-16 to 6-26 2003	Cape ivy (Garrapata Ck.)	8 DI alive when CI added (6-19) no DI alive at end of test 13♀ & 8♂ adults emerged from CI
DI-16-1118 NCHA	*Frageria chiloensis* *Grindelia sp.* *Luina hypoleuca* *Vitis californica*	6-16 to 6-25 2003	nothing (Garrapata Ck.)	6 DI alive when CI added (6-19) no DI alive at end of test
DI-17-1118 NCHA	*Eriophyllum staechadifolium* *Grindelia sp.* *Luina hypoleuca* *Symphyotrichum chilense*	6-17 to 6-24 2003	nothing (Garrapata Ck.)	8 DI alive when CI added (6-10) no DI alive at end of test

DI-18-1118 NCHA	*Eriophyllum staechadifolium* *Grindelia sp.* *Lepidospartum latisquamum* *Symphyotrichum chilense*	6-24 to 6-30 2003	nothing (Garrapata Ck.)	7 DI alive when CI added (6-27) no DI alive at end of test
DI-19-1218 NCHA	*Frageria chiloensis* *Leipidospartum latisquamum* *Luina hypoleuca* *Symphyotrichum chilense*	7-28 to 8-5 2003	nothing (Garrapata Ck.)	8 DI alive when CI added (7-31) 2 DI alive at end of test
DI-20-1218 NCHA	*Frageria chiloensis* *Grindelia sp.* *Luina hypoleuca* *Vernonia missurica*	7-29 to 8-5 2003	Cape ivy (Garrapata Ck.)	*test started with 4♀ & 3♂ 6 DI alive when CI added 1 DI alive at end of test 7♀ & 10♂ adults emerged from CI
DI-21-1318 NCHA	*Eriophyllum staechadifolium* *Frageria chiloensis* *Luina hypoleuca* *Symphyotrichum chilense*	9-26 to 10-6 2003	Cape ivy (Garrapata Ck.)	7 DI alive when CI added 1 DI alive at end of test 9♀ & 9♂ adults emerged from CI
DI-22-1318 NCHA	*Calendula officinalis* *Grindelia sp.* *Lepidospartum latisquamum* *Vernonia missurica*	9-30 to 10-10 2003	Cape ivy (Garrapata Ck.)	8 DI alive when CI added no DI alive at end of test 12♀ & 9♂ adults emerged from CI
DI-23-1418 NCHA	*Campanula muralis* *Grindelia sp.* *Lepidospartum latisquamum* *Marah fabaceus*	11-21 to 12-1 2003	Cape ivy (Garrapata Ck.)	7 DI alive when CI added no DI alive at end of test 10♀ & 13♂ adults emerged from CI adult emerged from CI
DI-24-1418 NCHA	*Achillea millefolium* *Campanula muralis* *Lepidospartum latisquamum* *Marah fabaceus*	11-24 to 12-1 2003	Cape ivy (Garrapata Ck.)	7 DI alive when CI added no DI alive at end of test 1♀ adult emerged from CI
DI-25-1418 NCHA	*Achillea millefolium* *Campanula muralis* *Lepidospartum latisquamum* *Marah fabaceus*	11-25 to 12-3 2003	Cape ivy (Garrapata Ck.)	7 DI alive when CI added no DI alive at end of test 11♀ & 15♂ adults emerged from CI

DI-26-1418	NCHA	*Achillea millefolium* *Campanula muralis* *Grindelia sp.* *Lepidospartum latisquamum*	12-2 to 12-11 2003	Cape ivy (Garrapata Ck.)	8 DI alive when CI added 6 DI alive at end of test 8♀ & 15♂ adults emerged from CI, and counting
DI-27-1418	NCHA	*Campanula muralis* *Clematis sp.* *Erigeron glaucus* *Lepidium latifolium*	12-12 to 12-19 2003	nothing (Garrapata Ck.)	6 DI alive when CI added 2 DI alive at end of test
DI-28-1418	NCHA	*Baccharis pilularis* *Campanula muralis* *Erigeron glaucus* *Lepidium latifolium*	12-12 to 12-19 2003	Cape ivy (Garrapata Ck.)	6 DI alive when CI added no DI alive at end of test 6♀ & 1♂ adults emerged from CI, and counting
DI-29-1418	NCHA	*Achillea millefolium* *Campanula muralis* *Erigeron glaucus* *Lepidium latifolium*	12-16 to 12-23 2003	n/a (Garrapata Ck.)	8 DI alive when CI added 4 DI alive at end of test 1♀ & 3♂ adults emerged from CI, and counting