Biological Control of Cape-ivy Project
2005-2006 Biennial Research Report

prepared by Joe Balciunas and Chris Mehelis

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

Executive Summary

by Dr. Joe Balciunas

This is our second 'electronic' report, and most of you will receive our Biennial Report for 2005 and 2006 as PDF attachment to an email. We hope that this will make our report more easily accessible, since you may chose to store it on your hard disk. There are also a 58 pages of Appendices that supplement this report. They can be viewed / downloaded from our FTP dropbox at ftp://147.49.50.52/dropbox/Balciunas/.

The good news is that by the end of 2006, we had reached the milestone of completing our host range testing of our two most promising potential biological control agents for Cape ivy, the gall fly, *Parafreutreta regalis*, and the stem-boring moth, *Digitivalva delaireae*. We have tested more than 80 species of plants, and neither of our candidate agents was able to complete development on anything other than their Cape ivy host.{See sections II & III]

We have collated our results, and are preparing a formal 'petition' seeking permission to release both of these agents in the field. This 'petition' will be submitted to TAG during the second quarter of 2007. We hope to receive a positive recommendation from TAG within 6 months. The outlook for receiving a release permit from USDA-APHIS-PPQ has improved, but is still not clear. The main problem is that PPQ is only now filling the posts vacated when most of their staff was transferred to the new agency, Homeland Security. In the meantime requests for release have been piling up and not acted on. The complex and lengthy approval process for obtaining permission to release a herbivorous agent is outlined in Section V.B of this report.

Another major accomplishment over the past two years was 'catching up' with my scientific publications. My administrators made it clear that this needed to be my first priority. A lengthy list of publications that were in various stages of preparation have now been published, or are well on their way to appearing in print [see List of Publications, Section VI.A].

As always, if you have any questions or comments, don't hesitate to contact me.

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 Aug. 2005)
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

Cooperators:

Academy of Sciences – St. Petersburg, Russia
Bureau of Land Management – Fort Ord
California Dept. of Food & Agriculture (CDFA)
California Invasive Plant Council (Cal-IPC, formerly, California Exotic Pest Plant Council)
California Native Plant Society (CNPS)
California Polytechnic State University, San Luis Obispo
Colorado State University, Ft. Collins, Colorado
East Bay Regional Park District (EBRPD)
New Mexico State University, Las Cruces, New Mexico
Plant Protection Research Institute – Pretoria, SOUTH AFRICA
Rio Pedros Reserve, Carmel, CA
Stansell, Veva – Forest Service (retired)
Univ. of California – Berkeley
Univ. of California – Davis
Univ. of California – Santa Barbara
USDA-ARS, Frederick, Maryland
USDA-ARS, Sidney, Montana
USDA-Forest Service, Siskiyou National Forest

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

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

Ch.	*Chaetorellia* flies
Cnt.	*Centaurea* plants
Del.	*Delairea* ivy
Di.	*Digitivalva* moths
Eu.	*Eustenopus* weevils
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 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, Robison 2006) 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, DiTomaso and Healy 2006).

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 2006)

Dr. Balciunas made his first trip to South Africa, the native home of Cape-ivy, early in 1996, to attend an international symposium. After the symposium ended, he visited five South African herbaria, and collated the collection records from the pressed Cape-ivy specimens at these institutions. 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).

The Cape-ivy Biocontrol Project began in 1998, and since then, Dr. Balciunas, the project leader, has made four additional visit to South Africa. On each visit, he spent 4-5 weeks with our South African cooperators, reviewing their results, participating in field studies, and jointly planning the research for the following year. During the first two years, our South African cooperators, Beth Grobbelaar and Stefan Neser, collected over 230 species of plant-injuring insects from Cape-ivy (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.

By mid-2000, three of these six insects had been dropped from further consideration, and the focus of the last five years of research in South Africa has been to assist us in a collaborative effort to evaluate the host range of the three most promising insects: *Digitivalva delaireae*, *Diota rostrata* and *Parafreutreta regalis*. 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 these three Cape-ivy insects, and have compiled valuable information on the biology and life history of these three insects, and developed rearing techniques. They have also nearly completed their portion of the host range evaluations of our top three candidate biocontrol agents. They confirmed that the moth *Diota rostrata*, whose caterpillars sometimes spectacularly defoliate Cape-ivy patches, has several other hosts, and will not be safe enough for release here. They have also confirmed the safety of *Digitivalva delaireae*, and *Parafreutreta regalis*.

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 ($10,000-$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. Future contributions will allow us a more extensive effort during the “Release and Establishment” phase.

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

Parafreutreta regalis (Diptera: Tephritidae) was described in 1940 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* (Figure 1) is about the size of a housefly or slightly smaller. Females lay eggs inside the nodes or growing tips of Cape-ivy vines. The maggots 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 has since produced six or seven generations in each of the last five years.

A. Host range evaluations

During the past six 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 1 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 2006. 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 and Pretoria.

Table 1. Plant species evaluated by USDA and PPRI for *Parafreutreta regali & Digitivalva delairea* oviposition and development (2001 through 2006).

Species tested		Region of endemism, notes	Location of tests	# of successful reps. with *Parafreutreta*	# of successful reps. with *Digitivalva*
Category 1 - Genetic types of the target weed species found in N. America					
Family Asteraceae	Subfamily Asteroideae				
Tribe Senecioneae	Subtribe Senecioninae				
	Delairea odorata stipulate variety	S. Africa, target weed	Albany	62	65
	Delairea odorata astipulate variety	S. Africa, target weed	Albany & Pretoria	6 69	4 57
Category 2 - N. American species in the same genus as the target weed (none)					
Category 3 - N. American & South African plants of other genera in the same family as the target weed, divided by tribe					
Family Asteraceae	Subfamily Asteroideae				
Tribe Senecioneae	Subtribe Senecioninae				
	Cineraria "butterfly" ornamental cultivar hybrid	S. Africa	Pretoria	8	5
	Cineraria deltoidea	S. Africa, coastal herb in S. Africa	Pretoria	5	5
	Cineraria saxifraga	S. Africa, ornamental in US	Pretoria	9	6
	Erechtites glomerata	Europe, West coast US weed	Albany	5	6
	Euryops chrysanthemoides	S. Africa, ornamental in US	Pretoria	9	6
	Euryops pectinatus	S. Africa, ornamental in US	Albany & Pretoria	5 5	5 5
	Euryops subcarnosus	S. Africa, weed in Arizona	Albany	6	5
	Mikaniopsis cissampelina	S. Africa, close relative of Cape-ivy	Pretoria	5	5
	Packera bolanderi	N. America, US west coast herb	Albany & Pretoria	5 5	5 -
	Packera breweri	N. America, US woodland herb	Albany	5	6
	Packera macounii	N. America, widespread in US	Albany	5	5

	Species tested	Region of endemism, notes	Location of tests	# of successful reps. with *Parafreutreta*	# of successful reps. with *Digitivalva*
	Pseudogynoxys chenopodioides	S. America, ornamental	Albany	5	5
	Senecio angulatus	S. Africa, ornamental	Pretoria	7	5
	Senecio articulatus	S. Africa	Pretoria	5	6
	Senecio blochmaniae	N. America, US west coast dune shrub	Albany	8	8
	Senecio brachypodus	S. Africa	Pretoria	6	5
	Senecio deltoideus	S. Africa	Pretoria	5	5
	Senecio flaccidus	N. America, widespread in southwest US	Albany & Pretoria	5 5	5 1
	Senecio gerrardii	S. Africa, widespread shrub	Pretoria	5	6
	Senecio glastifolius	S. Africa	Pretoria	5	-
	Senecio helminthioides	S. Africa, widespread shrub	Pretoria	6	5
	Senecio hybridus	Africa, ornamental in the US	Albany	5	5
	Senecio jacobaea	Africa, noxious weed in several US states	Albany	7	8
	Senecio macroglossus	S. Africa, ornamental	Pretoria	5	5
	Senecio oxyodontus (form A)	S. Africa	Pretoria	9	5
	Senecio oxyodontus (form B)	S. Africa	Pretoria	5	5
	Senecio oxyriifolius	S. Africa	Pretoria	6	6
	Senecio pleistocephalus	S. Africa	Pretoria	5	5
	Senecio serratuloides	S. Africa	Pretoria	-	5
	Senecio tamoides	S. Africa, widespread, ornamental	Pretoria	5	5
	Senecio triangularis	N. America, common in riparian areas	Albany	5	6
	Senecio vulgaris	Europe & N. Africa, widespread US weed	Albany	7	5
Tribe Senecioneae	Subtribe Blennospermatinae				
	Blennosperma nanum	N. America, uncommon west coast plant	Albany	5	5
Tribe Senecioneae	Subtribe Tussilagininae				
	Lepidospartum latisquamum	N. America, desert shrub	Albany	5	6

	Species tested	Region of endemism, notes	Location of tests	# of successful reps. with *Parafreutreta*	# of successful reps. with *Digitivalva*
	Luina hypoleuca Benthelot	N. America, Northwest shrub	Albany	5	5
	Petasites frigidus var. *palmatus*	N. America, common in riparian areas	Albany	5	5
Tribe Anthemideae	*Achillea millefolium*	N. America, common herbaceous plant	Albany	6	6
	Artemisia californica	N. America, common west coast shrub	Albany	6	7
Tribe Astereae	*Baccharis pilularis*	N. America, common west coast shrub	Albany	6	5
	Bellis sp.	Eurasia, ornamental	Pretoria	-	5
	Erigeron glaucus	N. America, common west coast shrub	Albany	5	8
	Grindelia stricta	N. America, common west coast shrub	Albany	5	6
	Symphyotrichum chilense	N. America, formerly *Aster chilensis*	Albany	6	8
Tribe Calenduleae	*Calendula officinalis*	N. Africa, ornamental	Albany	5	6
Tribe Eupatorieae	*Ageratina adenophora*	S. America, weed in S. Africa and US	Pretoria	6	6
	Ageratina riparia	S. America, noxious weed in HI	Pretoria	5	7
	Ageratum houstonianum	S. America, minor South African weed	Pretoria	5	5
	Campuloclinium macrocephalum	S. America, invasive in S. Africa	Pretoria	5	5
	Chromolaena odorata	S. America, weed in HI & S. Africa	Pretoria	5	5
	Mikania capensis	S. America, S. Africa, vine	Pretoria	9	4
Tribe Gnaphalieae	*Anaphalis margaritacea*	N. America, widespread in the US	Albany	5	6
	Gamochaeta purpurea	N. America, widespread weed	Albany	7	7
Tribe Helenieae	*Eriophyllum stoechadifolium*	N. America, common west coast shrub	Albany	6	5
	Madia elegans	N. America, common west coast plant	Albany	5	5
	Tagetes erecta	N. America, ornamental - marigold	Albany	5	5
	Tagetes minuta	N. America, noxious weed in CA	Pretoria	5	5
	Tagetes sp. cv.	N. America, ornamental	Pretoria	-	5
Tribe Heliantheae	*Bidens formosa*	N. & S. America, global weed	Pretoria	5	2
	Coreopsis cf. lanceolata (garden cultivar)	N. & S. America, ornamental in S. Africa	Pretoria	5	-
	Dahlia pinnata cv.	S. America, ornamental in US & S. Africa	Pretoria	6	5

	Species tested	Region of endemism, notes	Location of tests	# of successful reps. with *Parafreutreta*	# of successful reps. with *Digitivalva*
	Galinsoga parviflora	S. America, weed in S. Africa & US	Pretoria	5	7
	Helianthus annuus	N. America, sunflower - comm. crop	Pretoria	6	5
	Helianthus tuberosus	N. America, Jerusalem artichoke	Pretoria	9	6
	Rudbeckia hirta (garden cultivar)	N. America, ornamental - coneflower	Pretoria	5	5
	Zinnia violacea cv.	S. America, ornamental	Pretoria	5	5
Tribe Inuleae	*Dittrichia graveolens*	N. Africa, minor weed in the US	Albany	5	5
Tribe Plucheeae	*Pluchea odorata*	N. America, widespread in US wetlands	Albany	7	6
Subfamily Cichorioideae					
Tribe Arctoteae	*Arctotheca calendula*	S. Africa, noxious weed in California	Pretoria	5	6
Tribe Cardueae	*Carthamus tinctorius*	Eurasia, safflower - commercial crop	Albany	6	5
	Centaurea melitensis	Eurasia, weed in many states	Albany	5	-
	Cynara scolymus	Eurasia, artichoke - commercial crop	Pretoria	5	5
Tribe Lactuceae	*Cichorium intybus*	Europe, chicory - minor crop and weed	Albany	8	7
	Lactuca sativa	Europe, lettuce - commercial crop	Pretoria	6	-
	Picris echioides	Europe, widespread weed in the US	Albany	5	5
Tribe Mutisieae	*Adenocaulon bicolor*	N. America, common woodland herb	Albany	5	5
Tribe Vernonieae	*Vernonia missurica*	N. America, endangered in Ohio	Albany	6	7
Category 4 - Threatened and endangered species in the same family as the target weed divided by Tribe					
	Packera ganderi	N. America, California listed - Rare (Category 4)*	Albany	6	5
Category 5 - N. American species in other families in the same order that have some similiarity to the target.					
Family Campanulaceae					
	Campanula muralis	Europe, ornamental in the US	Albany	6	7
	Lobelia erinus	Europe, ornamental in the US	Albany	6	5
Category 6 - N. American species in other orders that have some similarities to the target weed.					
Family Araliaceae					

Species tested	Region of endemism, notes	Location of tests	# of successful reps. with *Parafreutreta*	# of successful reps. with *Digitivalva*
Hedera canariensis	Europe, ornamental vine	Albany	5	5
Hedera helix	Europe, ornamental vine, weedy	Albany	7	7
Family Aristolochiaceae				
Aristolochia californica	N. America, California vine	Albany	5	6
Family Brassicaceae				
Brassica oleracea	Europe, cabbage - commercial crop	Pretoria	5	6
Lepidium latifolium	Europe, noxious weed in several states	Albany	5	6
Raphanus sativus	Europe, radish - commercial crop	Pretoria	5	-
Family Chenopodiaceae				
Beta vulgaris subsp. cicla	Europe, chard - commercial crop	Pretoria	5	5
Family Cucurbitaceae				
Marah fabaceus	N. America, California vine	Albany	6	7
Zehneria scabra subsp. scarba	S. Africa	Pretoria	5	5
Family Ranunculaceae				
Clematis ligusticifolia	N. America, common vine in western US	Albany	6	6
Family Rosaceae				
Fragaria chiloensis	N. America, common US west coast plant	Albany	8	8
Family Vitaceae				
Vitis californica	N. America, widespread western US vine	Albany	5	5
Category 7 - Any plant on which the biological control agent or its close relatives have previously found or recorded to feed on and/or reproduce (none)				

In Albany, we've conducted 68 trials (each with four test plants) that showed a positive control (galls formed on Cape-ivy), while in Pretoria, 69 trials (each with 3-5 test plants) have showed a positive control. Between the two locations, we have tested 89 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.

The host range testing for this insect is now complete, and we have begun the lengthy process of obtaining federal and state approval to release this fly in California [see Section V. 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 tiny moth (usually about ¼-inch in length). Adults (Figure 2, 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 2, 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 our laboratory, 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 2. *Digitivalva delaireae* larvae (left) (Photo by E. Grobbelaar), and newly-emerged adult on a Cape-ivy leaf with larvae tracks (right)(Photo by Joe Balciunas).

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, six generations in 2003, and another six generations in 2004. In Sept. of 2004, due to concerns about the lack of genetic diversity, we requested and received another shipment of *Digitivalva* from our cooperators. The shipment of 40 pupae arrived on Nov. 8th but unfortunately only 11 moths (five females and six males) emerged from these pupae. Most of these adults were feeble and died within a few days of emergence, so it is doubtful that they contributed to our colony.

B. Host range evaluations

We have now completed the host range testing *Di. delaireae* in Albany and Pretoria. 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 1, while Appendix B provides detailed results of each trial conducted in Albany and Pretoria.

Out of the 131 "no-choice/ host added" trials completed in Albany, 69 showed a positive control (oviposition and development on Cape-ivy). In these 69 trials, we have had hundreds of female and male *Digitivalva* moths emerge from Cape-ivy, but never found no development or signs of infestation on any of the other 47 species of test plants.

In South Africa, 41 plant species have been tested. A total of 69 trials have been completed: 57 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. Despite some tunneling in non-host species *Digitivalva* is still regarded as a very promising biological control candidate.

IV. Other studies during 2005

A. Origin of California Cape-ivy

Although Cape-ivy was being grown as a house plant and in many gardens in Europe during the first half of the nineteenth century (Walpers 1845), it was not described scientifically until 1844, when Lemaire placed it as the only species, *odorata*, in his newly described genus *Delairea*. Cape-ivy is still occasionally sold as an ornamental in North America, and this is the probable source of introduction and spread. The earliest known California specimen (accession # UC36003) was collected in 1892 at Strawberry Canyon, just east of the University of California campus at Berkeley. The geographic source of the Cape-ivy that has become so well established in California is not known, and it is likely that it was introduced many times from various sources. This makes it likely that some of California's Cape-ivy populations are distinct from each other genetically.

Knowing how many different populations of Cape-ivy are represented in California, as well as their geographic origins, is of great interest to our Biological Control Project. After our insects are released, they may establish everywhere that Cape-ivy occurs in California, and build up to levels that reduce this invasive vine to levels where it causes little concern. However, perhaps a more likely scenario is that we will see huge populations of insects and effective control at some sites, but at others there may be little impact. The lack of control at some sites may be due to number of factors, including: site-specific environmental conditions, localized predators, or an unsuitable 'variety' of Cape-ivy. Recent advances in molecular techniques now allow comparison of the genetic material from different populations of plants and animals and determining which groups most probably share common ancestors or came from the same geographic area. We lack the equipment, skills, and time to conduct these molecular studies of Cape-ivy ourselves, so for several years we have been trying to find a qualified colleague who would be interested in cooperating in such studies.

In 2005, Dr. Ruth Hufbauer, a plant geneticist at Colorado State University, agreed to assist us, and we sent her several shipments of Cape-ivy from populations in California, Hawaii, and South Africa. Despite repeated attempts by her graduate students, they were unable to extract usable DNA from our samples. Apparently, unknown compounds in Cape-ivy leaves interfere with the standard extraction techniques.

In 2006, another colleague, Dr. John Gaskin, a USDA-ARS plant ecologist in Sidney, Montana, agreed to take on this task. We have sent Dr. Gaskin several shipments from eight different Cape-ivy populations. Despite having tried a variety of extraction techniques, Dr. Gaskin and his assistant have, as yet, been unable to extract 'good' DNA from the samples. However, Dr. Gaskin remains confident, that through trial-and-error, he will find some technique that will allow him to distinguish genetically distinct Cape-ivy populations. We hope to be successful in 2007 in this sub-project.

B. Cape-ivy floral biology

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. Each of these florets is capable of producing a seed. Our interest in quantifying the damage of flower-feeding insects led us to investigate how many seeds might be produced. We soon noted an anomaly in the literature. The original description of Cape-ivy by Lemaire (1844) noted 12 florets per head. However, several 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.

During 2003, we began to quantify the number of florets per head of Cape-ivy flowers from various regions by collecting 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. 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 recent literature.

In 2004, we continued these investigations by collecting heads at four sites in sites in California, after the Cape-ivy had flowered in early 2004. From each collection, 100 flowers were randomly selected and dissected. Our 2004 results confirm our 2003 results, with means between 10.95 and 11.67 florets at the four San Francisco region sites. In 2005, we examined Cape-ivy flowers from the same sites as we did in 2004 to determine if viable seed production varies from year to year.

Table 2. Number of Cape-ivy florets / inflorescence collected during 2004 & 2005 from sites near San Francisco, California.

Location	2004 No. of inflorences dissected	2004 Mean florets / inflorescence (range)	2005 No. of inflorences dissected	2005 Mean florets / inflorescence (range)
Tilden park	100	11.29 (9-13)	100	11.28 (7-13)
Bolinas	100	11.48 (10-13)	100	11.54 (9-14)
Wildcat Canyon	85	10.95 (7-13)	100	11.28 (8-13)
San Bruno Mtn.	100	11.67 (10-14)	100	12.34 (10-15)
Rydin Rd.	NA	NA	103	10.67 (8-13)
Mendocino Co.	NA	NA	100	11.58 (0-13)

The numbers of florets per inflorescence were nearly identical within each site where Cape-ivy was sampled between 2004 and 2005. Interestingly, none of the 4000+ florets examined from any of the sites contained viable seeds.

C. *Chaetorellia succinea* **"trap plant" studies**

Before Cape-ivy became my primary, full-time project in 2000, I spent four years working on biological control of yellow starthistle [*Centaurea solstitialis*], with considerable portion of my effort being devoted to potential non-target impacts of *Chaetorellia succinea*. This fly, whose larvae destroy the seeds of yellow starthistle, was not detected in a shipment of *Chaetorellia australis*, an approved biological control agent for yellow starthistle, that was released in Oregon in 1991. That shipment contained both species of *Chaetorellia*, and both species established there. *Ch. succinea*, but not *Ch. australis*, dispersed rapidly, and the former can now be expected at nearly every yellow starthistle site in California (Balciunas and Villegas 1999, Pitcairn et al. 2003), and is spreading its range in Idaho, Nevada, Oregon, and Washington (Balciunas, unpublished data).

After detecting this 'new' *Chaetorellia* in mid-1996, my California Department of Food and Agriculture [CDFA] colleague, Baldo Villegas, and I documented the establishment of *Ch. succinea*, and its rapid dispersal (Balciunas and Villegas 1999). We immediately curtailed further releases of *Chaetorellia* species, and began investigating the safety of the unintentionally introduced *Ch. succinea*. Despite concerns raised by earlier research in Europe, we demonstrated that this fly presents only a small risk to growers of safflower (*Carthamus tinctorius* L.) in California (Balciunas and Villegas 2001). More recently, we showed that native species of *Cirsium* thistles in California are not being attacked by this fly (Balciunas and Villegas, in press).

However, our research showed that several species of *Centaurea* could serve as hosts for *Ch. succinea*, and we found this fly attacking two introduced, weedy species of *Centaurea* in California: *Cnt. melitensis* (tocalote or Napa starthistle) and *Cnt. sulphurea* (Sicilian starthistle). Our laboratory tests also indicated that at least one of the two species of *Centaurea* native to North America might be susceptible to attack by *Ch. succinea*. Many botanists now consider that these two natives belong in their own genus, *Plectocephalus*. Neither *Cnt. americana* nor *Cnt. rothrockii* (known as American basketflower and Rothrock's basketflower, respectively) occur in California, and *Ch. succinea* is [as yet] not known from the regions where these two natives grow. We could not be certain if, under field conditions, these two natives might be attacked by *Ch. succinea*.

This fly is now widespread throughout California, and is destroying a large portion of the seeds produced by yellow starthistle here. Obviously, there is interest in getting this fly 'approved' by regulatory agencies, so that it can be used as a biological control agent for this weed. But the probability of attack on the native flora must be better quantified. Accordingly, during 2006, we set up a multi-site 'trap plant' study to see if basketflower plants, planted at sites where *Ch. succinea* flies were abundant, would be attacked. We grew two varieties of *Cnt. americana* at our greenhouse, and in May and June of 2006, transplanted a dozen of each of them at each of three sites: near Yreka, in Siskiyou County; in Meadowview, Sacramento County, and near Yountville, in Napa County. We also planted a dozen yellow starthistle plants at each site, to ensure that we could track the presence of *Ch. succinea* at the site. We visited each of these sites every few weeks, and clipped any 'old' flowers and saved them in a labeled, paper bag. This experiment ended in late October, when frosts had killed the last of our trap plants. We are now more than half-way through dissecting all of the thousands of heads that we collected during this study. We will present the data in next year's report.

D. *Centaurea rothrockii* & *Centaurea americana* seed genetic confirmation

Before being able to start the 'trap plant' study [see above], we first had to grow the trap plants. We used *Centaurea americana* seeds that I had obtained from a colleague [Dr. David Thompson, New Mexico State University]. We also planned to test *Cnt. rothrockii*, and used seeds that we had purchased from a commercial source [Seeds of the Southwest, Tucson, AZ]. We planted rosettes of *Cnt. americana* and the (purported) *Cnt. rothrockii* at our three study sites, and kept a large number in our green house. After the *Cnt. rothrockii* began flowering, we noticed that although they had consistent differences from *Cnt. americana*, they still would not 'key' to *Cnt. rothrockii*. A thistle specialist, Dean Kelch [CDFA], examined some of our *Cnt. rothrockii* specimens, and was of the opinion that they were, in fact *Cnt. americana*.

I, and my predecessors at the ARS quarantine lab in Albany, CA, had frequently tested both *Cnt. americana* and *Cnt. rothrockii,* in our studies to develop biological control agents for yellow starthistle and other thistles. Most of these tests used seeds obtained from several different commercial sources. I was concerned that perhaps some of these other commercial seeds might have also been misidentified. Lincoln Smith, my entomologist colleague here in Albany, and I enlisted the aid of Dr. John Gaskin, USDA-ARS, Sidney, Montana, to help in resolving this question using modern molecular techniques. We sprouted plants from all of the seed packets of both *Cnt. americana* and *Cnt. rothrockii* that we had available at our facility. We then sent leaf samples from these various seed sources to Dr. Gaskin. He and his assistant extracted the DNA from our samples and compared them to see which were different. As expected, the purported *Cnt. rothrockii* grown from seeds purchased in Tucson, AZ, clumped out with other *Cnt. americana*, confirming that these seeds had been misidentified. Fortunately, all the other seeds that we had used for earlier tests had been correctly labeled (see Table 3).

Table 3. Seeds that are similar, based on DNA analyses performed by Dr. J. Gaskin.

Plant species and seed No.	Labelled as	Origin
Centaurea americana		
Seed 1161A	*Cnt. rothrockii*	"Plants of the Southwest"
Seed 1158	*Cnt. rothrockii*	"Plants of the Southwest"
Seed S074	*Cnt. americana*	Webb Co., TX
Seed 1157	*Cnt. americana*	Dave Thompson, AZ
Seed 1111	*Cnt. americana*	R. Sheety, TX
Seed 1110	*Cnt. americana*	R. Sheety, TX
Centaurea rothrockii		
Seed 1159	*Cnt. rothrockii*	Dave Thompson, AZ
Seed 1160	*Cnt. rothrockii*	Thompson & Morgan UK
Seed 78A	*Cnt. rothrockii*	Wildseed Co
Seed 76	*Cnt. rothrockii*	no information of origin

E. *New Mexico Centaurea studies*

Since the discovery of the accidental release of *Chaetorellia succinea* in 1997, we have monitored its dispersion and distribution (Balciunas and Villegas, 2001). In our previous host range studies of this biological control agent, we discovered that this fly not only developed on yellow starthistle, but also safflower, *Cnt. melitensis* and the native *Cnt. americana*. Though *Cnt. americana* is typically not found in the current range of yellow starthistle, our concerns that *Ch. succinea* had spread via *Cnt. melitensis* to populations of *Cnt. americana* further east of California.

In the summer of 2005, we received several shipments of heads of three different *Centaurea* spp. from cooperators in New Mexico. These heads were dissected to determine the presence or absence of *Ch. succinea* and any other YST biocontrol agents. Table four (below) shows the species and locations of the *Centaurea* collections. Dissection determined that all *Centaurea* sampled had no *Ch. succinea* present, but one collection of *Cnt. melitensis* may have had *Ch. succinea* and *Eustenopus villosus* larval damage.

Table 4. 2005 collections of various *Centaurea* species from collectors in New Mexico.

Centaurea species	Location collected	Coordinates	No. of heads	Date collected & Dissected	Notes	Results
C. solstitialis	Gila River	32°56.877 N 108°36.346W	150	7-2005 8/8/05	Heads nearly all F2s	No insect damage
C. americana	along Hwy.	32°57.214N 105°19.054W	256	7/28/05 8/8/05	½ of heads moldy, ½ OK	No insect damage
C. melitensis	Chapparal (town nr. El Paso)	NA	1269	6/22/05 6/29/05	Heads dry, mostly F1s and F2s	Four heads w/ possible *Ch. succinea* damage, no flies found. One head w/ possible *Eu. villosus* damage.
C. melitensis	Eddy County	32°56.839N 104°21.755W	670	6/15/05 6/20/05	Mostly Bu4s, some F1 & F2, fewer Bu1-Bu3s, all heads dry.	No insect damage

V. Future Plans

A. Research planned for 2007

Now that our host range testing efforts of the potential agents *Parafreutreta regalis* and *Digitivalva delaireae*, both in Albany and Pretoria, are complete, we have now compiled this data, and are beginning 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. We will also continue our studies into the basic biology of Cape-ivy and will continue assisting our colleagues in molecular studies into Cape-ivy's origin and distribution.

B. The next step: obtaining approval for release

During 2007, we will anticipate begin the process of obtaining permission for release for the Cape-ivy gall fly, *Parafreutreta regalis*, and also 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 an insect pest 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 4) 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.

Before mid-2007, 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.

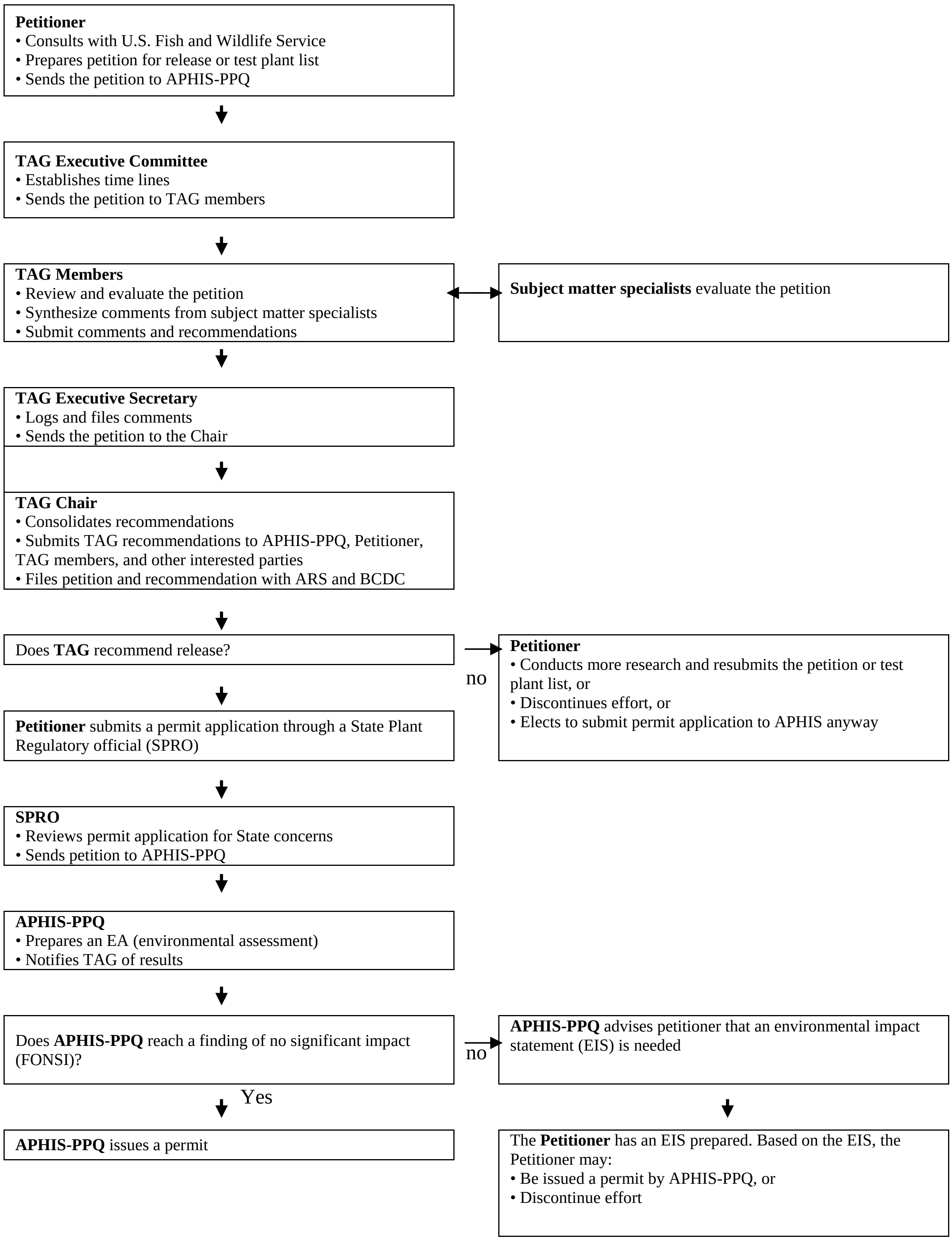
TAG's recommendation is not binding on PPQ, but, in practice, has great influence on PPQ's decision to issue a release permit. If TAG recommends release, I will then seek approval from the State (California) through the State Plant Regulatory Official (**SPRO**). If California also approves, PPQ prepares 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 <u>might be</u> 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 2007, it is possible that this complex and changing approval process will require more time.

Figure 3. Flowchart diagramming the approval process for release of weed biological control agents (from TAG Reviewer's Manual)



VI. Other activities and publications

A. Articles published or submitted since January 1st, 2005

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Balciunas, J.K., B. Grobbelaar and S. Neser. Submitted. Distribution of Cape ivy, in South Africa, with note about its taxonomy. Madrono.

Balciunas, J.K., C. Mehelis, L. van der Westhuizen and S. Neser. Submitted. Host range of *Parafreutreta regalis*; a candidate agent for Cape ivy. Biological Control.

B. Selected meetings and travel by Dr. Joe Balciunas

2005

- presented invited talk on the Code of Best Practices to the Toadflax Workshop in Idaho
- presented invited talks on biological control and Cape ivy to 1) Marin County Chapter, California Native Plant Society, and 2) Santa Barbara County Noxious Weed Meeting
- organized and moderated a session on "Reducing negative interactions between weed and insect biocontrol programs" at W-1185 Working Group annual meeting in Cloudcroft, NM
- attended the annual meeting of Entomological Society of America at Ft. Lauderdale, and presented "Impact of the Code of Best Practices on Biological Control Practitioners in the NorthWest"

2006

- presented invited talk on the Code of Best Practices to the Interagency Noxious Weed Symposium in Oregon.
- presented invited talk on Best Practices at a special session on "Ethics and Best Practices in Biological Control" at W-1185 Working Group annual meeting in Tucson, AZ

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Appendicies

Due to the length of the appendicies, they have been placed in separate files that can be downloaded from: ftp://147.49.50.52/dropbox/Balciunas/

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

Appendix B. ***Digitivalva delaireae*** **host range tests**