2002 Annual Report

Australian Biological Control Laboratory

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AUSTRALIAN BIOLOGICAL CONTROL LABORATORY

2002 Annual Report

prepared by

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Cover Picture:
Front row (left to right) - John Goolsby, Tony Wright, Dalio Mira, Ryan Zonneveld
Back row (left to right) - Bradley Brown, Jeff Makinson, Matthew Purcell,
- Gio Fichera, Karryn Waterworth

January 1 – December 31, 2002

Dr John A. Goolsby - Research Entomologist & Director
Mr. Tony Wright - Experimental Scientist
Mr. Matthew Purcell - Experimental Scientist
Mr. Jeff Makinson - Research Officer
Mr. Ryan Zonneveld - Research Officer
Mr. Bradley Brown – Research Officer
Mr. Dalio Mira - Plant Culture (part-time)
Mr. Gio Fichera – Research Officer (part-time)
Ms. Karryn Waterworth – Research Officer (part-time)

CAUTION: The results in this report are preliminary and tentative. In order to prevent the spread of out-of-date or inaccurate information, this report should not be quoted or cited without verifying accuracy with the USDA-ARS Australian Biological Control Laboratory.

Table 1. List of acronyms used in this report

ABCL	- (USDA-ARS) Australian Biological Control Laboratory
ANIC	- Australian National Insect Collection
APHIS	- (USDA) Animal and Plant Health Inspection Service
ARS	- (USDA) Agricultural Research Service
cNSW	- Central New South Wales, Coffs Harbour to Wollongong
CSIRO	- Commonwealth Scientific and Industrial Research Organisation
nNSW	- Northern New South Wales, north of Coffs Harbour
NQ	- North Queensland, north of the Tropic of Capricorn
NSW	- New South Wales
OIRP	- (USDA-ARS) Office of International Research Programs
QLD	- Queensland
SEL	- (USDA-ARS) Systematic Entomology Laboratory
SNSW	- Southern New South Wales, south of Wollongong
SQ	- South Queensland, south of the Tropic of Capricorn
TAG	- (USDA-APHIS) Technical Advisory Group on the Biological Control of Weeds
USDA	- United States Department of Agriculture

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Executive Summary

This was a very productive year for the Australian Biological Control Laboratory (ABCL), and our stateside research partners at the Invasive Plant Research Laboratory, Ft. Lauderdale, FL. Our research programs for the broad-leaved paperbark tree, *Melaleuca quinquenervia,* Old World climbing fern, *Lygodium microphyllum,* and hydrilla, *Hydrilla verticillata,* all reached important milestones. Two agents, in the pipeline from ABCL to stateside collaborators, have now been field released and four more are in the final stages of quarantine screening and permitting. The melaleuca psyllid, *Boreioglycaspis melaleucae,* was permitted this year with the first official release made on April 22, 2003 by Gail Norton, Secretary of the Interior. Petitions for release of the bud-gall fly, *Fergusonina turneri,* for *M . quinquenervia*, and leaf defoliating *Cataclysta camptozonale* for *L. microphyllum* have been submitted to USDA-APHIS, Technical Advisory Group (TAG) and are pending approval. For *M. quinquenervia*, preliminary host-range testing has been completed for *Poliopaschia lithochlora* and *Lophodiplosis trifida*. Both are available as the next candidate in the 'pipeline' for *M. quinquenervia*. For *L. microphyllum*, final host-range testing has been completed for the leaf defoliator *Neomusotima conspurcatalis* and a petition for release will be submitted in 2003. Full host-range testing is underway at ABCL for the eriophyid mite, *Floracarus perrepae*. A new initiative to explore Southeast Asia for natural enemies of the aquatic weed *Hydrilla verticillata* is underway, with cooperators in Thailand and Indonesia culturing several herbivore species for evaluation. Finally, a preliminary investigation into the potential for biological control of downy rose myrtle, *Rhodomyrtus tomentosa,* was completed by our collaborator, Dr Amporn Winotai of the Thai Department of Agriculture. A suite of herbivores was collected, which included a flower-feeding moth that caused significant damage to the plant in its native habitat.

Research on the biological control of *M. quinquenervia* continued to focus on developing priority candidates while surveying new regions for more insect agents. Exploratory surveys by ABCL scientists were conducted for the first time in Cape York, QLD and sites in New Caledonia. Several new insects were discovered and will be further evaluated. Preliminary host-range studies of the tube-dwelling moth, *Poliopaschia lithochlora*, in both the field and laboratory were completed during 2002. This insect is available for shipment to the Gainesville quarantine facility for final quarantine screening. The flower-feeding weevil, *Haplonyx multicolor,* was collected from *M. quinquenervia* flowers and buds in north Queensland and, for the first time, from buds in southeast Queensland. Intensive research is focused on developing rearing methods for this high priority agent. Its ability to kill flower buds could have a major impact on the reproductive potential of *M. quinquenervia*. Evaluation of the stem-galler, *Lophodiplosis trifida,* has revealed it is highly specific and has a significant impact on the growth of *M. quinquenervia* seedlings. A new potential agent, the leaf binder *Eochrois leiochroa,* was collected from *M. quinquenervia* in north Queensland.

Exploration for new agents for the biological control of *L. microphyllum* continued in Australia and Asia. New areas in India, Sri Lanka and Cape York (far north Queensland) were explored for the first time. Collections of *L. microphyllum* from across its distribution were compared using molecular diagnostics. The population from the Iron Range in Cape York appears to be a match for the invasive population in Florida. Although this comparison is made using a single gene sequence, the match has led us to a population of the eriophyid mite, *Floracarus perrepae,* which readily feeds and develops on the Florida form of *L. microphyllum*. *Floracarus perrepae* was the primary focus of our research in 2002. Populations of the mite were characterized using molecular techniques. Six unique genotypes of the mite were identified which appear to be geographic host races adapted to local genotypes of *L. microphyllum*. Each of the mite genotypes were tested for their ability to feed and develop on the invasive form of *L. microphyllum*. Only the genotypes from Thailand and the Iron Range developed normally on the Florida *L. microphyllum*. Both genotypes are now in culture at ABCL and full host-range testing will soon be initiated. A two-year study of *F. perrepae* field populations in southeast Queensland has been completed. The mite is active year round but peaks following periods of high humidity. Predator mites have a significant effect on populations of the mite, but significant damage to the fern is still incurred. Chemical exclusion tests, to measure the impact of the Queensland population of *F. perrepae* on biomass production, show a 50% suppression of plant growth over an 18-month period. We estimate that this type of suppression is typical of the mite across its native range. Our research indicates that the combination of extremely narrow host specificity and potentially significant biomass suppression make *F. perrepae* an excellent candidate for biological control of *L. microphyllum*. Progress was made in developing an artificial diet for the stem-borer, *Ambia* sp. Stem-borer larvae collected from *L. flexuosum* completed development on the diet. A rearing system based on the artificial diet will enable us to rear large numbers of the moth for host-range testing. Discussions are underway with biologists in Singapore to assist with studies of a second species of the moth, which feeds exclusively on *L. microphyllum*. Collaborative studies were initiated with Dr A. Jesudasan of Madras Christian College, Chennai, India. A graduate student will make regular surveys in southern India and determine the biology of the noctuid moth, *Callopistria* sp. A., and the thrips, *Octothrips lygodii*.

Preliminary surveys for herbivores of *Hydrilla verticillata* were conducted in Indonesia, Malaysia and Singapore. On-going collaborative studies have been arranged in with Dr Manop Siriworakul of the Royal Thailand Irrigation Department and with Dr Desmier de Chenon, based at the Marihut Research Station, in Indonesia. Dr de Chenon has been supervising a student to study the aquatic herbivores of hydrilla in Sumatra. The surveys have revealed a suite of aquatic weevils and nymphuline moths, several of which have been collected and are now in culture. Selected herbivores will be imported to ABCL in 2003 for further study.

Overview

The staff of the Australian Biological Control Laboratory (ABCL) actively search the natural areas of Australia and Southeast Asia for insects and other organisms that feed on pest insects and plant species that are invasive in the USA. Based in Brisbane, Queensland, the ABCL is operated by the U.S. Department of Agriculture, Agricultural Research Service (USDA-ARS), hosted by the Commonwealth Scientific and Industrial Research Organisation (CSIRO). We collaborate closely with stateside scientists, including those at the USDA-ARS Invasive Plant Research Laboratory in Ft. Lauderdale and at Gainesville, Florida.

Many invasive weeds in the USA such as the broad-leaved paperbark tree (*Melaleuca quinquenervia*), Old World climbing fern (*Lygodium microphyllum*), carrotwood (*Cupaniopsis anacardioides*) and Australian pine (*Casuarina* spp.) are native to this area of Australia. However, the native distribution of many of the weed species in this region continues northward from Australia into tropical and subtropical Southeast Asia, including Indonesia, Malaysia, Thailand, Vietnam, Papua New Guinea, New Caledonia, and southern China. ABCL scientists have the capability to explore this entire region to find the most promising biological control agents.

Research conducted at ABCL follows a sequence of events involving: determination of the native distribution of a weedy plant species, exploration for natural enemies, DNA fingerprinting of newly discovered species, ecology of the agents and their weed hosts, field host-range surveys, and ultimately preliminary host-range screening of candidate agents. Our research attempts to determine what regulates the plant in its native environment, which brings to light the full array of potential biological control agents. Organisms with a narrow host range and good regulatory potential are intensively investigated. The data we gather on potential agents is combined with information about the ecology of the weed where it is invasive. Our stateside USDA-ARS collaborators use a science-based process to make the final decision on which organisms are best suited to be biological control agents. This dual-country approach ensures the most successful outcome.

Environmentally adapted flora and fauna coupled with globalization of trade and travel between Australasia and the USA is now, and will continue to be, the cause of many serious weed and pest invasions. The ABCL is committed to research and development of biological control solutions for U.S. weeds and insects of Australian and Southeast Asian origin. Our research is critical, not only because biological control offers the safest and most cost-effective approach to long-term management of widespread invasive weeds and pests, but also because in some instances it is the only viable control option.

Administration and Support

The ABCL is a research unit within the USDA-ARS, Office of International Research Programs under the direction of Arlyne Meyers (Assistant Administrator) and Dr Richard Greene (Deputy Director). The personnel and facilities of the ABCL in Australia are provided through a co-operative agreement with CSIRO Entomology. The United States Embassy in Canberra provides the administrative support. Mr. Andrew Burst assumed duties as Agricultural Counselor in December 2002.

A coalition of federal, state and local agencies fund the overseas research on biological control of *M. quinquenervia* and *L. microphyllum*. South Florida Water Management District, Jacksonville District of

United States Army Corps of Engineers, and Florida Department of Environmental Protection contributed substantially to the research during 2002.

ABCL works closely with the following project leaders to co-ordinate the research: Dr Ted Center (Melaleuca & Hydrilla), Dr Bob Pemberton (Lygodium), and ARS National Program leaders Dr Ernest Delfosse (Weeds) and Dr Kevin Hackett (Biological Control).

Staff and Facilities

Dr John Goolsby is Laboratory Director and Research Entomologist. Five CSIRO personnel are employed full-time by ABCL: Mr. Tony Wright, Mr. Matthew Purcell, Mr. Jeffrey Makinson, Mr. Ryan Zonneveld and Mr. Bradley Brown. Mr. Dalio Mira, Mr. Gio Fichera, and Ms. Karryn Waterworth work part-time for ABCL in greenhouse culture of test plants.

New equipment was purchased for the laboratory with funds from OIRP. These acquisitions include two 120 X dissecting scopes and funds to refurbish the high security quarantine to allow for importation of the mite, *Floracarus perrepae*, from Thailand.

Travel and Visitors

In December, John Goolsby attended the Entomological Society of America Annual Meeting in Ft. Lauderdale, and made an invited presentation: *In country pre-release evaluation of agents.*

Dr Bart Drees (Texas A&M University) was hosted in June by ABCL as consultant to the Queensland Department of Primary Industries, Fire Ant Eradication Program.

Andrew Oles and Lara Vallely each spent three months (June - November) at ABCL as USDA student interns from Berea College, Berea, Kentucky. Both students took time out from their studies in agriculture to assist in the research at ABCL. We appreciate the support of Dr Panciera (Berea College) and OIRP staff: Arlyne Meyers, Rich Greene and Heather Phelps for arranging the internships.

Biological Control of Melaleuca

The Australian broadleaf paperbark tree, *Melaleuca quinquenervia*, was introduced into Florida in the early 1900's as an ornamental and as a means to dry up sections of the Everglades for development (Laroche and Ferriter, 1992; Bodle et al., 1994; Laroche, 1994). Since its introduction, the spread of *M. quinquenervia* has been explosive; with the rate still accelerating (Bodle et al., 1994), and in the process it has caused extensive environmental and economic damage (Laroche, 1994; Balciunas et al., 1995). Impacts include: a reduction in the native wildlife populations, a loss of native vegetation, additional stress placed onto the survival of rare, threatened and endangered species, increased fire hazard (Balciunas and Center, 1991; Timmer and Teague, 1991), increased skin and respiratory allergic reactions (Diamond et al. 1991) and reduced fishing, hunting and air boating activities (Balciunas and Center, 1991).

There are up to 250 species in the genus *Melaleuca* in Australia, though most are shrubs with needle-like leaves and do not resemble *M. quinquenervia*. *Melaleuca quinquenervia* is placed with 14 other closely related *Melaleuca* species in the *M. leucadendra* complex. These 15 species have different habitat requirements, and can be distinguished by differences in fruits, flowers, and leaf proportions. Saplings, as well as sterile material from older trees, can be taxonomically difficult even for botanists familiar with the group.

Melaleuca quinquenervia is widespread along the eastern coast of Australia, usually occurring in swamps and other wetlands (inset left). The Australian range is roughly from Sydney to the tip of Cape York Peninsula. The most extensive stands are located in southeast Queensland (SQ) and northern New South Wales (nNSW) near the border with Queensland (QLD).

Melaleuca Field Collections and Exploratory Surveys in 2002

During 2002, field surveys were conducted to collect priority insects for laboratory testing and evaluation. A complete breakdown of collections relating to the *Melaleuca* project is outlined in Table 1 while a list of all collection records is supplied in Appendix 1.

The laboratory culture of *Poliopaschia lithochlora* (Lepidoptera: Pyralidae) collapsed due to disease. Therefore, many collections were made in SQ and nNSW to establish a new colony. Regular surveys were also conducted in north Queensland (NQ) to collect flower-feeding weevils that have become priority insects for our research, and to make observations of the field populations of *Lophyrotoma zonalis* (Hymenoptera: Pergidae). Collections were also made in this region of *Fergusonina* spp. (Diptera: Fergusoninidae) galls in a collaborative project with Dr Kerrie Davies (University of Adelaide) and Prof. Robin Giblin-Davis (University of Florida) to finish studies on this fly genus that has been a priority for several years.

A survey was conducted in Iron Range in June (inset right) in conjunction with the *Lygodium* project. The Cape York Region has never been surveyed for *Melaleuca* biological control agents. Several new species of *Melaleuca* were observed and herbarium collections were made for identification. Only a few small surveys of stands were performed due to a tight time schedule. Many of the existing agents under evaluation were recorded. Several new insects were collected, though they appear to have minimal potential as biological control agents. However, due to the wide diversity of *Melaleuca* spp. encountered, we believe that this region should be surveyed in more detail in future.

Two surveys were also conducted in New Caledonia. In February, extensive collections concentrated on flower feeding insects of *M. quinquenervia* in both the south and central regions. Tortricid and pyralid larvae were abundant, severely damaging the flowers (inset left). Many adult specimens were reared at the quarantine facilities in Brisbane but are yet to be identified. One predominant pyralid species is very similar to *Syntonarcha* found in Australia. Small weevils that attack and deform the branch ends of *M. quinquenervia* were also collected but failed to breed in quarantine. These weevils, as well as a psyllid, *Ctenarytaina* sp., have not been found in Australia and warrant further investigation due to the damage they inflict on young foliage. Severe die back was also observed in a localized area on the west coast. We observed a very high population of cicadas on these trees (inset right) and noticed that the dead branches were riddled with their eggs. This type of damage has not been observed in Australia. A second smaller survey was conducted in July, in conjunction with the *Lygodium* project, which mainly involved the collection of Lepidoptera tip-binders as well as weevils attacking young buds and foliage. Due to the limited nature of our surveys in New Caledonia, more intensive surveys are planned for 2003.

Table 1. Summary of field search collections made during 2002 in north Queensland (NQ), southeast Queensland (SQ), northern NSW (nNSW), central NSW (cNSW), and New Caledonia (NC) relating to *Melaleuca* research.

Plant		Number Collections	Number Sites	Regions
Family	**Species**			
Myrtaceae – *M .leucadendra* complex	*M. argentea*	1	1	NQ
	M. cajuputi	3	3	NQ
	M. dealbata	1	1	NQ
	M. fluviatilis	2	1	NQ
	M. leucadendra	7	5	NQ
	M. nervosa	2	2	NQ
	M. quinquenervia	138	69	NQ/SQ/nNSW /cNSW/NC
	M. sp.	5	5	NQ
	M. stenostachya	2	2	NQ
	M. viridiflora	17	10	NQ/SQ
Other Myrtaceae	*Acacia* sp.	2	2	SQ
	Callistemon sp.	2	2	nNSW/SQ
	Eucalyptus sp.	1	1	SQ
	Leptospermum polygalifolium	1	1	SQ
	Leptospermum sp.	3	3	SQ
	Eucalyptus seeana	1	1	SQ
Other Plants	? Species	1	1	NQ

Exploration in Australia and New Caledonia.

4-7 February 2002: Surveys between Cairns and Townsville in NQ to search for *Lophyrotoma zonalis* sawflies and to collect *Haplonyx multicolor* (Coleoptera: Curculionidae), *Storeini* sp. (Coleoptera: Curculionidae) and *Eochrois leiochroa* (Lepidoptera: Pyralidae) larvae from Edmund Kennedy NP.

25-28 February 2002: Surveys in New Caledonia to identify insects attacking terminal growth of *M. quinquenervia* as well as flower feeding insects.

18-21 March 2002: Surveys between Daintree NP and Townsville to search for *L. zonalis* for toxicity studies and to collect *H. multicolor* weevils and *E. leiochroa* moth larvae from Edmund Kennedy NP.

30 April – 2 May 2002: Surveys between Cairns and Townsville to check sites and collect *H. multicolor* and *Storeini* sp. weevils from Edmund Kennedy NP.

6-9 May 2002: Surveys for *Fergusonina* spp. galls throughout NQ with Dr Kerrie Davies.

23-28 June 2002: *Melaleuca* surveys in the Cape York region including Iron Range National Park.

1-15 July 2002: General surveys in New Caledonia.

22-24 July 2002: Surveys between Cairns and Townsville to search for *L. zonalis* sawflies and to collect *H. multicolor* and *Storeini* sp., as well as *E. leiochroa* larvae from Edmund Kennedy NP.

19-22 November 2002: Surveys between Townsville and Cairns to search for *L. zonalis* sawflies and to collect *H. multicolor, Storeini* sp. (Coleoptera: Curculionidae) and *E. leiochroa* larvae from Edmund Kennedy NP.

Tube-dwelling moth – *Poliopaschia lithochlora* (Lepidoptera: Pyralidae)

Poliopaschia lithochlora (Fig. 1) has been a high priority agent at ABCL since 1996. The biology, seasonality and field host specificity of this insect has been studied intensively, however laboratory host specificity tests are yet to be completed. *Poliopaschia lithochlora* can be found at most field sites in all regions where broad-leaved *Melaleuca* trees grow in Australia. They appear to have a strong preference for low-lying sites (usually seasonally inundated) with a grassy understorey and high humidity. The grass understorey may provide higher humidity with dew formation during the early morning periods. Larvae live in tubes and feed on both young and old leaves. Although found on older trees, saplings and suckers are preferred. For these reasons it may be an excellent agent for the wetland areas in Florida, especially where extensive stands of seedlings predominate.

Fig. 1. *Poliopaschia lithochlora* adult (left) and larval tubes surrounded by protective silken threads (right).

During 2002, extensive drought conditions existed right across Australia. During this dry period, the field populations of *P. lithochlora* were extremely low, and field collection was exceedingly difficult. The few colonies that could be found were located at sites where permanent water existed and the larvae were present close to the ground in grassy low lying areas where humidity was high (inset left). Roadside ditches are favoured habitats. As in previous years, larvae and pupae were frequently parasitised by *Carcelia* sp (Diptera: Tachinidae) and an Ichneumonidae (Hymenoptera) species. During 2002, parasites were reared from eggs collected in the Daintree region of tropical NQ. These micro-hymenoptera will be sent to taxonomists for identification.

In the laboratory, females oviposit lines of cream coloured eggs along the margins of *M. quinquenervia* leaves. Lining the roof of oviposition cages with moist towelling can dramatically increase oviposition. Eggs are usually laid in confined spaces (eg. on leaves pushed against the gauze cage covering) or on damaged sections of leaves. Adult longevity and fecundity are reduced under dry conditions.

The larvae hatch and form tubes attached side-by-side to the leaf surface. The tubes are made of frass-like material that is covered and bound together with silken threads. If a leaf is unsuitable, or the surrounding branch is defoliated, larvae will drop on silken threads to other branches or plants (inset right). As larvae become larger, the colonies will often split forming smaller groups or occasionally a single larva. The tubes become convoluted and are no longer attached to the leaf surface, often suspended between stems, branches and leaves by a mass of silken threads. The larvae move from these tubes to feed on surrounding foliage. In the prepupal stage, a small bulb is formed at the open end of tubes. The bulb is eventually sealed and pupation occurs within. Adults are active on dusk and most oviposition occurs at night. Development from egg to adult is approximately 72 days and adults survive for approximately seven days.

We continued our extensive laboratory studies of the *P. lithochlora* during 2002. Initially, a large laboratory colony was being maintained. Unfortunately, in April, an outbreak of a cytoplasmic polyhidrosis virus devastated the colony and ruined many long-term no-choice tests. A decision was made to terminate the colony to eradicate the disease and start a new culture from field-collected insects. An infestation by a predatory mirid also increased mortality.

A cytoplasmic polyhedrosis virus (CPV) often does not kill but debilitates (delayed or no pupation, non-fecund adults etc.). It forms polyhedra in the cytoplasm of the gut cells. EM work was needed to confirm the CPV. Pre-pupae within the colony of *P. lithochlora* at ABCL were not pupating and there was a high larval mortality. Immatures, both in the colony and in the host-range tests, failed to thrive or develop past the larval stage. This has never occurred in the many previous years of research on this moth. This was a major setback that necessitated re-establishing and rebuilding a disease free laboratory colony to continue our testing program and to prepare a culture for shipment to US quarantine facilities when needed.

The mirids were observed feeding on larvae (inset left). Many of these immatures were still alive during the attack, however, many more were found dead within their tubes. Specimens were collected and sent to Dr Gerry Cassis, an expert taxonomist on Hemiptera at the Australian Museum. He determined that there were two species of mirid, both new to the genus *Kundakimuka*. There are only three other known species of this genus in Australia, and very little is known about their biology.

Laboratory host-range testing of *P. lithochlora* was initiated in September 2001. The tests include closely related Myrtaceae species that are grown as ornamentals in Florida. Tests are being performed on eight plant species with *M. quinquenervia* as the control. The design is a no-choice oviposition test with two pairs of adults released into a small gauze cage containing one potted plant. The adults are provided with a 10% honey solution for feeding. The cages are also misted with water and a moist cloth is placed on the roof lining to increase humidity. In each test, two plant species are tested against a *M. quinquenervia* control. The test is completed when both females have died. The longevity of the adults is recorded. Eggs are then counted on each plant and the resultant survival to the adult stage is recorded. Results are summarised in Table 2.

Due to the long development time from egg to adult (72 d), these tests take some time to complete. Many tests started in 2001/2002 had to be abandoned due to the outbreak of the virus. Larvae on the plants tested were not developing even on the controls (as in the colony). Additionally, due to the extremely hot and dry conditions, the survival of the adults was low in many trials and eggs were not oviposited on the control plants in many tests; these tests were disregarded. Eggs have only been laid on *M. quinquenervia* and four test plant species, *M. alternifolia*, *Callistemon citrinus*, *C. rigidus* and *C. viminalis*. Oviposition on the four test species is much lower than on *M. quinquenervia*. Survival was also lower for all other test species except *C. viminalis*, though oviposition was extremely low on this species. Of concern was oviposition that occurred on *Eucalyptus citriodora* in two invalid trials, where no eggs were laid on the *M. quinquenervia* control. Twelve eggs were laid on *E. citriodora* in one trial (survival egg to adult 91.7%) and five eggs in another (test ongoing). More replicates will be conducted in 2003.

Table 2. Results of 2001/2002 no-choice oviposition/survival tests of *P. lithochlora*. Eight plant species were tested with *M. quinquenervia* as a control.

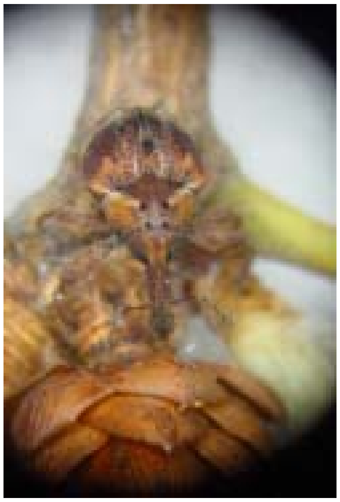
Plant Family	Plant Species	No. Tests	Mean Oviposition (eggs/test)	Mean Oviposition (% control)	Mean Survival Egg to Adult
Myrtaceae	*Melaleuca quinquenervia*	13	112.7	100	37.4
	M. alternifolia	2	13	18.8	11.5
	M. linariifolia	4	0	0	-
	M. styphelioides	2	0	0	-
	Callistemon citrinus	2	5	2.3	23.1
	C. rigidus	4	12.8	9.7	31.4
	C. viminalis	4	1.3	1	100.0
	Eucalyptus camaldulensis	4	0	0	-
	E. citriodora	2	0	0	-

To further test specificity, plants on which oviposition occurred in no-choice tests were subjected to choice tests. A *M. quinquenervia* control and a test plant species were placed in opposing diagonal corners of a large screened cage (2m x 1m x 1m high). In each test four pairs of adults were released into the cage and maintained in the same way as for the no-choice tests. Only results on oviposition are given in Table 3 as the tests conducted during 2002 were affected by the outbreak of the virus, impacting on survival results. Oviposition has only occurred on *M. quinquenervia* and not on any test plant.

Table 3. Results of 2002 choice oviposition tests of *P. lithochlora.* Four plant species were tested with *M. quinquenervia* as a control.

Plant Family	Plant Species	No. Tests	Mean Oviposition (eggs/test)
Myrtaceae	*Melaleuca quinquenervia*	5	27.2
	Callistemon citrinus	1	0
	C. rigidus	1	0
	C. viminalis	2	0
	E. citriodora	1	0

Flower-Feeding Weevil - *Haplonyx multicolor* (Coleoptera: Curculionidae)



Haplonyx multicolor is one of four species of *Haplonyx* weevils that have been collected during the *Melaleuca* project. It occurs in NQ, SQ and nNSW. The adults can live for several months, feeding on leaf and flowers buds as well as young foliage (inset left). Adults often feed by tunneling out sections of plant tissue resulting in the death of buds or young tips. Larvae have been collected and reared from flowers, buds and young tips.

In February 2002, adults were found feeding on young *M. quinquenervia* saplings in NQ. These adults fed on the stem bases of young tips causing them to fracture and die. Adults were also found within *M. viridiflora* flower buds. Buds from this species that were collected in December 2001 also contained larvae and pupae. In January 2002, one adult emerged from one of these buds and a second was found within another, as observed in the field. These buds would have formed during the flowering season in winter 2001, meaning that immatures (and possibly adults) could be in diapause awaiting the formation of flower buds in the following season. This mechanism for surviving between seasons could have positive prospects for its survival and effectiveness in Florida if released as a biological control agent. Additionally, the dual mode of feeding on both flowers and leaves would also assist populations persisting in the field. Its ability to kill flower buds could have a major impact on the reproductive potential of trees growing in Florida. In February, as buds were forming on many of the *M. viridiflora* saplings, *H. multicolor* adults could be seen crowding around branch ends near the newly formed buds, apparently waiting to feed and oviposit.

Many adults were collected from Edmund Kennedy National Park in NQ during 2002 and brought back to the laboratory to evaluate techniques for rearing this insect, and for biology studies. The field-collected adults were set up on cut bouquets of flower buds, with their bases immersed in a florists tube. All leaf material was removed from the branches to reduce evapotranspiration. Forty-two adults were collected in NQ in February and set up on bouquets of flower buds, leaf buds and cut branches. No immatures developed on the material. More success was obtained after field collections of adults during surveys conducted in March, April, July, and November. Eggs, larvae and pupae have been found in the buds and young leaf tips from both bouquets and live saplings (Fig. 2). Transferring larvae to young leaf tips sandwiched between moist filter paper in a petrii dish proved to be the most successful method for rearing them to the adult stage. Attempts to make larvae pupate in sand and vermiculite failed. Further investigations in the field in NQ in November 2002 revealed that larvae could also be found enclosed within the leaves of young tips. We believe that pruning young saplings to induce production of large buds will greatly assist our attempts to colonise this high priority insect.

Three larvae were collected from buds of *M. quinquenervia* at Peregian in SQ in July, one of which developed into an adult. The second was used in DNA analysis and the third died in rearing. In December, two pupae were found within dead buds but both were parasitised by micro-hymenoptera (Fig. 2). This could explain the low populations at field sites in SQ. Several adults were also collected from *M. quinquenervia* at Woodburn in nNSW. More intensive surveys of these two sites close to ABCL will be conducted in 2003.

Fig. 2. A pupa (left) and a parasitised immature (right) found within flower buds of *M. quinquenervia.*

Two *Haplonyx* adults collected from Edmund Kennedy NP in NQ from *M. quinquenervia* and *M. viridiflora*, as well as a larva collected from *M. quinquenervia* at Peregian in SQ, were compared using D2 genetic analysis by staff at CSIRO Entomology in Canberra. The specimens only differed by four base pairs. Interestingly, the specimen from *M. viridiflora* in NQ was an exact match to the larva collected from *M. quinquenervia* in SQ.

Seven specimens of *Haplonyx* were sent to the ANIC for identification, five collected from *M. quinquenervia* and two from M. *viridiflora*, all collected from Edmund Kennedy National Park in December 2001. Dr Rolf Oberprieler, a weevil taxonomist, thought that the weevils from each plant species might have been different due to the large colour variations, with the specimens from *M. quinquenervia* being *H. multicolor*. He passed these specimens onto Dr Elwood Zimmerman, the Australian weevil expert (now retired), who believed they were all the same species as he was more familiar with the colour variations within the group. This seems to concur with the DNA (D2) analysis. Therefore, we will now call all specimens *H. multicolor*. However, we will keep the specimens from each *Melaleuca* species separate as a precaution.

Flower Feeding Weevils - Curculioninae sp. A and sp. B (Coleoptera: Curculionidae)

Two species of Curculioninae weevils were collected in all surveys conducted at Edmund Kennedy National Park and nearby sites during 2002. Adults were observed feeding on the young foliage of *M. quinquenervia,* while larvae were collected feeding on the flowers of both *M. quinquenervia* and *M. viridiflora*. Adults from both species were present on *M. quinquenervia* saplings/suckers, though only Curculioninae sp. A was reared from larvae collected on *M. quinquenervia* flowers, not Curculioninae sp. B. However, subsequent D2 analysis confirmed that larvae of both weevil species feed on *M. quinquenervia* flowers. External characters readily separate the adults of each species. Species A is smaller and has small protrusions on the elytra. The antennal clubs on Species A are joined together while those on species B are quite separate. A collar exists at the anterior end of the pronotum on species B, absent in species A.

The larvae of these two weevil species are found in very high populations at Edmund Kennedy NP in NQ and inflict heavy damage on the flowers of both *M. quinquenervia* and *M*. *viridiflora.* Attempts are being made to rear cultures of all flower-feeding weevils at ABCL in Brisbane. It is likely live flowering plants will be needed for cultures as cut plant material appears to be unacceptable.

A series of specimens from both species were sent to Dr Rolf Oberprieler at the ANIC who verified his findings with Dr Elwood Zimmerman. He thought that both species belong to the Storeini/Tychiini complex of Curculioninae. It is one of the most taxonomically difficult groupings in Australia. He also believes there may be no true Tychiini in Australia, but the literature does not follow this path. Neither species is identified in the ANIC collection, and it's likely that both are undescribed. Dr Zimmerman confirmed that both these species are undescribed and they belong to two different, undescribed genera. Dr Zimmerman also agrees that they are not true Tychiini (i.e. not related to the largely northern-hemisphere group including *Tychius*, *Sibinia* and several other genera) but belong to the amorphous Australian "flower weevils" placed in the large subfamily Curculioninae.

Stem-Boring Cerambycidae (Coleoptera)

Larvae of a stem-boring cerambycid (species A) were observed at most sites, mainly during the spring and summer months. These larvae feed within the stems of *M. quinquenervia*. They cause extensive damage and even large branches are killed (inset left). Larvae tunnel the stems in a spiralling motion toward live tissue. They mostly consume tissue of the phloem, effectively ring-barking the tree. These beetle larvae are long lived and we suspect that there may only be one generation per year. A second species also severely damages the stems of *M. quinquenervia,* but tunnels directly through the centre of the branch. Their damage is distinctive, having exit holes in the branch to remove frass.

In 2002, we renewed our efforts to obtain adults by rearing larvae on artificial diet. Previous rearing attempts, using cut stems and transferring larvae to live branches, have failed. Once disturbed, the larvae fail to feed and die either immediately or within a short period of time. In 2002, a new diet containing blended extracts of freshly collected leaves and young stems of *M. quinquenervia* was tested. The diet, which is based on a meridic diet (Harley and Willson 1968), was placed in black and clear tubing and held vertically to simulate a branch. Like in the field, larvae have been feeding in a spiralling fashion down the tubes (inset right). However, the material often became contaminated by fungal pathogens that resulted in high mortality of the larvae. Both Cerambycidae species feed on the diet. One adult was reared on this diet, and two other adults were reared by storing damaged stem material from the field in large sealed containers. These three specimens, as well as an adult collected from Coffs Harbour in cNSW, were sent to the CSIRO ANIC for identification.

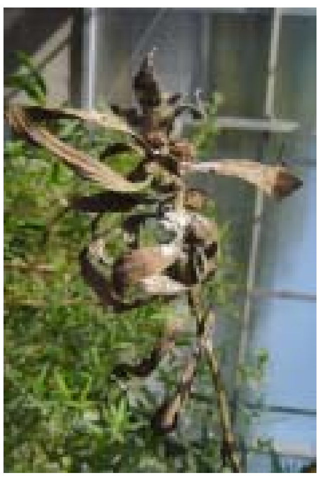
Six Cerambycidae sp. A larvae sent to Canberra for D2 genetic analysis were collected in the field from a variety of plant species. Two specimens were collected from *M. nervosa* (NQ), and one from each of *M. quinquenervia* (SQ), *M. viridiflora* (NQ), *Callistemon* sp (SQ) and *Leptospermum* sp (SQ). Except for the specimens from *Leptospermum,* the results indicate that the remainder are all likely to be from one species, with only two base pair differences between the specimens. Interestingly, the specimen from *M. quinquenervia* was more closely aligned to the specimen from *Callistemon*.

Four Cerambycidae sp. B larvae were also analysed using the D2 technique, one specimen collected from each of *M. quinquenervia*, *Acacia* sp., *Eucalyptus* sp. and *Leptospermum* sp. Results indicated that they are all distinctly different species.

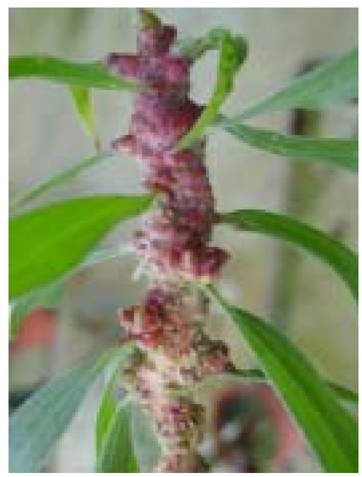
Once the adult specimens have been named, we will be able to determine if they are known herbivores of other plant species through literature searches and host records on specimens held in major collections. As Cerambycidae sp. A has already been collected in the field on *Callistemon*, our future research will concentrate on sp. B. Due to the slow development time of these insects, and difficulties of laboratory rearing and host-testing, research on this stem-borer will be opportunistic throughout 2003.

Cecidomyiid Stem Galler - *Lophodiplosis trifida* (Diptera: Cecidomyiidae)

Small stem galls are frequently seen in very low numbers on young tips of *M. quinquenervia*, both in the field and on laboratory potted saplings. Early in 2002, many of our laboratory saplings held on benches within a glasshouse at ABCL became infested with stem galls. Cecidomyiid adults were reared from these galls and sent to Dr Raymond Gagné at the Systematic Entomology Laboratory in Washington. They were identified as *Lophodiplosis trifida* (Diptera: Cecidomyiidae), a species previously recorded as inquilines in other Cecidomyiidae gall types on *M. quinquenervia* (Gagné et al. 1997). Within an enclosed bush house, the population of gallflies exploded, probably due to the humid conditions and a



reduction of parasite populations. The plants were severely damaged, and some died while others suffered severe dieback (inset left). If the galls are large enough, it appears that they can sever vascular connections. The galls on the terminal ends of branches were very large and severely deformed the growth of the plant (inset right). As the galls develop, we assume that the plant must produce more lignin because the galls ultimately become woody. This could drain the plant's resources at the expense of stem elongation, foliage production, and the production of reproductive tissue on older plants.



The sexes of *L. trifida* are easily distinguished. The female (inset right) is larger and has a bright red/orange abdomen due to the mass of eggs she is carrying. The male is smaller and has a thinner abdomen with two distinctive hooks on the posterior end. The adults are short lived, surviving for one to two days, though females can lay hundreds of eggs during that period. The red eggs are oviposited on both the leaves and stems of young foliage. The eggs are similar in size and shape to the eggs of the melaleuca psyllid, *Boreioglycaspis melaleucae*, but have no pedicel attached to the plant tissue. The neonate larvae emerge and burrow into the stem.

Virtually all branches on every *M. quinquenervia* plant were galled in the cecidomyiid-infested glasshouse, and it appeared the population had become resource limited, and oviposition sites were severely limited. Therefore, we initiated a choice host-range test within the building using four replicates of 14 plant species (all Myrtaceae), set up on benches surrounded on all sides by the heavily infested plants (inset right). All of the plants selected had young growth and buds available to the flies for oviposition. The plant species used, and a summary of gall formation, are listed in Table 4.

Table 4. Results of glasshouse plot choice test of *Lophodiplosis trifida*. Twelve Myrtaceae species were tested and *M. quinquenervia* was used as a control.

Plant Species	Mean Galls per Plant (Mean % Tips Galled) (n=4)		
	4 weeks	**8 Weeks**	**12 Weeks**
Melaleuca quinquenervia	7.3 (35.0)	15.5 (48.8)	27.0 (69.06)
M. cajuputi	6.0 (24.4)	18.3 (38.8)	16.5 (33.99)
M. dealbata	0.0 (0.0)	0.0 (0.0)	9.5 (33.58)
M. alternifolia	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
M. stenostachya	0.0 (0.0)	0.0 (0.0)	3.8 (2.25)
M. viridiflora	3.0 (22.5)	3.0 (17.9)	7.5 (100.0)
M. argentea	8.5 (34.9)	8.5 (11.6)	49.8 (90.28)
M. linariifolia	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Callistemon rigidus	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
C. viminalis	0.5 (8.3)	0.5 (3.9)	3.0 (37.5)
C. citrinus	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Eucalyptus citriodora	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
E. camaldulensis	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

After four weeks, all of the plants were checked and the number of galls on each was counted. This was repeated at 8 and 12 weeks, after which the test was terminated. *Lophodiplosis trifida* heavily attacked the broad-leaved *Melaleuca* species; some of these plants were severely stunted with all branches galled (inset left). A very small number of galls formed on other small-leaved *Melaleuca* species and *Callistemon viminalis*, though development of adults within these galls has not been confirmed. Galls were dissected from at least one plant of each of the broad-leaved *Melaleuca* species, and for all galls on the remaining *Melaleuca* and *Callistemon* species. Numerous immatures ranging from early instar larvae to pupae were extracted from the broad-leaved *Melaleuca* species. However, no evidence of any insect life stages could be found in the galls on other plant, though empty chambers were located in galled tissue of *C. viminalis*. Only one of the four *C. viminalis* plants was attacked, yet the undamaged plants also had multiple tips available for oviposition.

The population in this glasshouse was devastated by parasites following the choice test. Efforts then concentrated on achieving a clean, parasite-free colony. Once this was achieved, we started an experiment to determine the impact of these gallflies on the growth of *M. quinquenervia*. A large cage (2m x 1m) was covered in black gauze and plastic, and divided into two sections, separated by a plastic sheet. Three hundred and sixty *M. quinquenervia* seedlings were sorted into pairs by height, and then divided into two matched groups of 160 seedlings (inset right). Girths were also measured. There was no significant difference between the heights or girths of seedlings between the two groups. We assumed that the average biomass was also the same since all the seedlings were unbranched and of the same age. Each group of 160 plants was placed on either side of the divided cage, with the matched plants placed in the same position as its pair on either side of the enclosure. Two hundred and sixty-four males and 429 females of *L. trifida* were released onto the treatment plants over a one-week period while the control plants were isolated from any insect attack. Both the treatment and control plants were watered daily and fertilised weekly (equally) with a liquid fertiliser.

After emergence of the F1 generation (30 days), 40 plants were removed from each enclosure (40 original matched pairs). These plants were measured for height, girth, stem weight, leaf weight, gall weight (treatment) and root weight. Both wet weight and dry weight was measured. Statistical analysis of the height, girth and wet weights has been completed. After one generation of galls there was a highly significant difference between the heights of the control and test plants, though there were no differences in any other parameters. Breakdown of the remaining plants will occur in 2003.

In August we began to monitor the field abundance of *L. trifida* galls at Woodburn in nNSW and at Roys Road in SQ. At each site 30 *M. quinquenervia* plants along a transect were searched for galls. The trees were all less than 2.5 m in height. Each site was to be surveyed on a four weekly basis. After only one survey at Roys Road, the site was cleared. In October, surveys began at a third site, Ewan Maddock Dam in SQ, and Roys Road surveys were terminated. Results thus far are given in Figure 3. Gall populations have dropped dramatically at Woodburn over the last quarter. As the flush of young growth occurs mainly during the winter months, available oviposition sites would have decreased during spring.

As this insect has become one of our top priority agents, no-choice host-range tests and biology studies will be initiated in 2003. Seasonality studies will continue.

<u>Leaf Binder - *Eochrois leiochroa* (Lepidoptera: Oecophoridae)</u>

A new species of leaf-feeding Lepidoptera larva was located at two sites in NQ, Edmund Kennedy National Park and Lake Mitchell. The larvae web the leaves together, binding them to the stems on saplings/suckers. Unlike *Poliopaschia lithochlora*, the leaves are plastered together face against face with the larva/larvae between them. The larval feeding causes skeletonising of the leaf tissue. Many larvae can be found together on one small plant forming small colonies. Larvae were collected from both *M. quinquenervia* and *M. viridiflora* at Edmund Kennedy National Park and Lake Mitchell respectively. The larvae were reared to the adult stage in Brisbane and released onto caged saplings and a small colony persisted for nearly six months.

The specimens from both *M. quinquenervia* and *M. viridiflora* were identified by Dr Ted Edwards (ANIC) as *Eochrois leiochroa* (Lepidoptera: Oecophoridae). The adults reared from both *Melaleuca* species were compared using D2 genetic analysis and both are the same species (identical over 540 base pairs). A check of 14 other specimens at the ANIC revealed that these moths have only been collected from broad-leaved *Melaleuca* spp. or at night from light traps.

Fig. 3. Seasonal populations of Lophodiplosis trifida at Ewan Maddock Dam and Boys Road in SQ and Woodburn in nNSW.

A further collection of larvae was made from NQ in November and a small colony has been established at ABCL. Host testing and biology studies will begin in 2003.

<u>Melaleuca Sawfly – *Lophyrotoma zonalis* (Hymenoptera: Pergidae)</u>

Intensive biology, life history and host specificity studies have been conducted in Australia on the sawfly *Lophyrotoma zonalis*; this insect was shipped to quarantine in Florida in 1992. All quarantine screening has been completed, and the insect is known to be highly specific to *Melaleuca* spp. and severely

the papery bark and therefore this insect completes its whole life cycle on the tree. Adults are very mobile, especially males which swarm around trees preparing to mate with emerging females. For these reasons, *L. zonalis* could be an effective biocontrol agent, especially in the remote wetland areas of southern Florida.

Research conducted in Australia through the ABCL liasing with the National Research Center for Environmental Toxicology (NRCET) in Brisbane, determined that *L. zonalis* larvae contain two toxins, lophyrotomin and pergidin. These peptides are known to occur in related sawflies in Australia and in countries from two other continents, Europe and South America (Oelrichs et al. 1999). Lophyrotomin was isolated from *L. interrupta*, a sawfly that feeds on *Eucalyptus melanophloia*, and poisoned cattle in Queensland (Oelrichs 1982; McKenzie et al. 1984).

Extractions of the toxins from *Lophyrotoma zonalis* revealed high concentrations of pergidin and lower levels of lophyrotomin (Oelrichs et al. 2001). Larvae of *L. interrupta* have a higher proportion of lophyrotomin. Since lophyrotomin is known to be more toxic than pergidin, toxicity tests conducted for *L. interrupta* can be no guide to the toxicity of *L. zonalis*. Additionally, there are differences between the larval habits of *L. zonalis* and that of other sawflies that have been responsible for animal poisonings around the world. *Lophyrotoma zonalis* larvae pupate individually in the papery bark of *Melaleuca* trees, inaccessible to many animals, while those of the other species move on mass to the soil where they pupate in large gregarious masses. Furthermore, the larvae of *L. zonalis* regurgitate fluid that possibly contains, or has been derived from, high concentrations of essential oils found in the leaves of *Melaleuca*. Therefore, animals may reject them as a food source due to their unpalatability.

Because *L. zonalis* was a high priority candidate for biological control of *M. quinquenervia*, in 2001 we requested that the NRCET undertake toxicity studies of large animals to determine the potential of *L. zonalis* to poison animals. After agreement to do these studies, progress has been limited due to the retirement of the only staff with expertise in this area. We will continue to pursue the completion of these tests, however it may be necessary to find alternative laboratories either in Australia or in the US that could conduct such studies. During 2002, further collections of larvae from field sites in NQ were made (inset right) and the larvae frozen to supplement material collected in 2001. In the event that toxicity testing proceeds, there should be sufficient stocks of larvae for testing. However should more material be required, *Melaleuca* sites in NQ are constantly being monitored. The field release of this insect has been given a lower priority due to the difficulties of toxicity studies, and research is now focusing on other biological control agents.

Melaleuca Psyllid – *Boreioglycaspis melaleucae* (Hemiptera: Psyllidae)

The Melaleuca Psyllid, *B. melaleucae,* severely damages the young growth of *M. quinquenervia*, especially when its parasites are eliminated. At ABCL, young potted saplings were killed by this sap-sucking bug when population levels were high. After proving to be highly specific in host-range testing both in Australia and in the US, this insect was released in Florida early in 2002. *Boreioglycaspis melaleucae* appears to be establishing well at release sites (Fig. 4). The insects are easily located in the field as the young nymphs excrete white flocculant material, which can become quite abundant when populations are high.

Fig. 4. Release site near Estero, Florida (left) where the weevil, *Oxyops vitiosa*, and the psyllid, *Boreioglycaspis melaleucae*, (right) are now established.

Future Plans

Our highest priority for 2003 is to restart and complete the host-range testing program for *Poliopaschia lithochlora*. We will also maintain a disease and pest free colony of this moth so that it can be shipped to the quarantine facility in Gainesville at any time.

As we have some concerns over the specificity of *P. lithochlora*, we will accelerate research on the stem cecidomyiid, *Lophodiplosis trifida*. As it stands we have limited data on its host specificity, though it looks promising, and very little data on its biology and life history. This insect also has a severe impact on plant growth and we will quantify this effect through the completion of the impact experiment. Seasonality field surveys will continue.

The flower feeding weevils will also be a high priority, with efforts focusing on *Haplonyx multicolor*. The ability of this weevil to damage buds and flowers, yet persist at field sites between seasons through diapause, makes it a very attractive proposition for release in Florida. However, mass rearing is difficult so we will continue to fine-tune techniques so that sufficient insects are available for further research and testing.

Opportunistic studies will be performed on the stem-boring Cerambycids and on the leaf-binding moth, *Eochrois leiochroa.*

Biological Control of Lygodium

Old World climbing fern, *Lygodium microphyllum*, is an invasive weed in south Florida where it threatens many wetland communities in the Everglades ecosystem. *Lygodium microphyllum* is native to wet areas in the Old World tropics and subtropics including Africa, India, Southeast Asia, Australia and the South Pacific. The fern entered Florida as a commercial ornamental plant and was first documented to have become naturalized in 1965. However, its explosive growth and rapid spread is now causing concern because of its dominance over native vegetation in many communities. Amy Ferriter, of the South Florida Water Management District, reports that since the 1999 survey, over 1,000 acres of *L. microphyllum* was discovered in the backcountry Ten Thousand Islands area of Everglades National Park, and on scattered tree islands in Southern Miami-Dade County. The *L. microphyllum* is concentrated in short hydro-period coastal marshes dominated by low elevation woody vegetation (predominantly wax myrtle) adjacent to buttonwood/mangrove communities. The populations appear to be newly established, as no rachis material was present. Although newly established, the plant is extremely widespread and covers more than 107,000 acres. Old World climbing fern density and frequency is also increasing rapidly in Central Florida, with major infestations popping up along the Kissimmee River and in many bay swamps throughout the region, although there are no comprehensive regional surveys conducted outside South Florida. Old World climbing fern is now the number one invasive weed species in Florida.

The biological control program was initiated for *L. microphyllum* due to strong demand by stakeholders in south Florida for a biological control solution. Dr Robert Pemberton (ARS-Ft. Lauderdale) is the project leader. The overseas exploration program is being conducted by Dr John A. Goolsby and Mr. Tony Wright at the ABCL. *Lygodium microphyllum* is considered to be a good target for biological control. First, it belongs to a taxonomically isolated group, not closely related to native or economic plants in Florida. Second, the plant is not known to be a weed in its native range. Third, non-biological control methods are environmentally damaging and too expensive to use on the scale required to control the plant.

After preliminary surveys were conducted around southeast Queensland in 1996 and 1997, extensive areas of Australia and Asia have been searched, between 1998 and 2002, for biocontrol agents for *L. microphyllum* (Fig. 5). During 2002, 271 collections of herbivores and field data were made at 101 sites in Australia, China, Fiji, India, Indonesia, Malaysia, New Caledonia, Singapore, Thailand, and Sri Lanka. Twenty insect species, and two mite species, have now been collected feeding on *L. microphyllum* (Table 5).

Intensive field surveys were conducted in Singapore and Thailand to study and collect the stem-boring pyralid moth, *Ambia* sp. Considerable progress has been made in collecting this moth, which occurs at extremely low density. Artificial diets are being developed and tested with our cooperators in Thailand.

Laboratory and field evaluation of the eriophyid mite, *Floracarus perrepae* continued in 2002. We were aided by the continued collaboration of Dr Sebahat Ozman, a visiting scientist from Trakya University in Turkey and the University of Queensland Tropical Pathology CRC. In addition, Dr Thomas Freeman of North

Dakota State University, through his affiliation with Dr Dennis Nelson of the ARS Insect Genetics and Biochemistry Unit, Fargo, ND, visited ABCL to investigate the ultrastructure of *F. perrepae* with scanning electron microscopy (SEM). The SEM research revealed that the inability of selected *F. perrepae* genotypes to feed on the invasive Florida form of *L. microphyllum* was not due to mechanical constraints of its mouthparts or differences in cell wall thickness. Biochemical interactions are likely to be responsible for the extremely narrow host specificity of mite genotypes. The biochemical interactions are manifested in drastic changes in cell morphology. During 2002 we embarked on an ambitious plan to host test each of the unique *F. perrepae* genotypes to determine if they could feed and develop on the Florida genotype of *L. microphyllum*. We traveled to China, New Caledonia, Thailand, and India/Sri Lanka to test field populations of the mite. Populations from Thailand and the Iron Range of Cape York, Queensland, fed and developed on the Florida genotype of the fern. Both populations are in culture at ABCL pending full host-range testing. Field studies of the local southeast Queensland population of *F. perrepae* continued in 2002. The mite is active year round, peaking in the cooler, winter months. Chemical exclusion tests to measure the impact of the mite on biomass production show a 50% suppression of plant growth over an eighteen-month period. These two-year studies will be completed in early 2003. The information will be used to support our prediction that *F. perrepae* has the potential to have a significant impact on *L. microphyllum* in Florida.

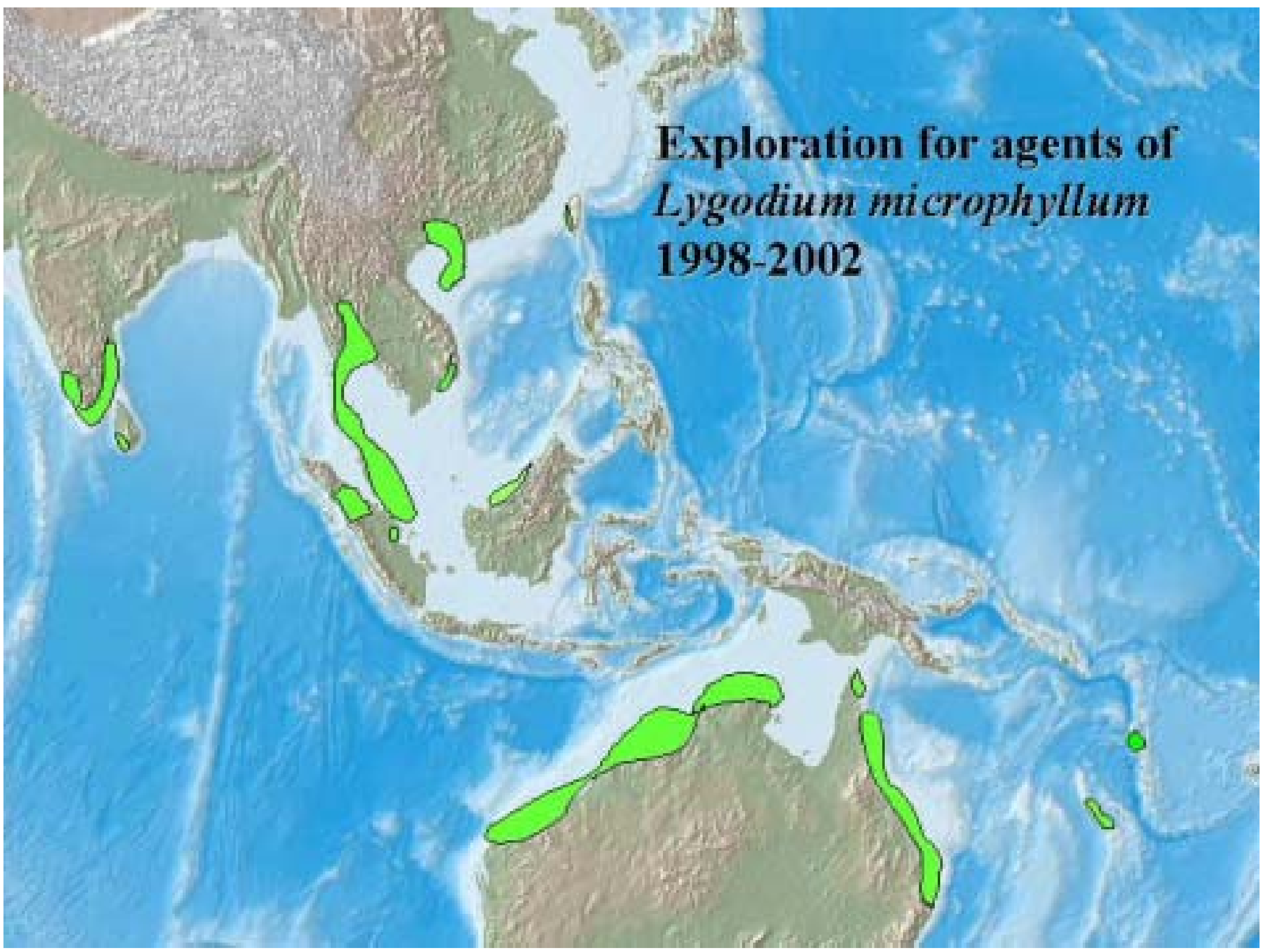


Fig. 5. Areas explored for agents of *Lygodium microphyllum* from 1998-2002.

Table 5. Herbivores collected from *Lygodium* spp. in Asia and Australia (1998-2002)

Name	Collection Locations	Host Plant
Floracarus perrepae Knihinickiand Boczek Acarina: Eriophyidae	Australia, China, India, Indonesia, Malaysia, New Caledonia, Singapore, Sri Lanka, Thailand	*L. microphyllum* (Cav.) R. Br. *L. reticulatum* Schkuhr
Brevipalpis sp. Acarina: Tenuipalpidae	China, Singapore, New Caledonia	*L. microphyllum* (Cav.) R. Br. *L. japonicum* (Thunb.) Sw.
Neomusotima conspurcatalis Warren Lepidoptera: Pyralidae	Australia (Queensland, Northern Territory, Western Australia), China, Indonesia, Malaysia, Singapore, Thailand	*L. microphyllum* (Cav.) R. Br.
Cataclysta camptozonale Hampson Lepidoptera: Pyralidae	Australia (Queensland)	*L. microphyllum* (Cav.) R. Br.
Cataclysta sp. B Lepidoptera: Pyralidae	Australia (Queensland)	*L. microphyllum* (Cav.) R. Br. *L. reticulatum* Schkuhr
Musotima sp. Lepidoptera: Pyralidae	Malaysia, Singapore, Thailand	*L. microphyllum* (Cav.) R. Br.
Neomusotima fuscolinealis Yoshiyasu Lepidoptera: Pyralidae	Japan	*L. japonicum* (Thunb.) Sw.
Pyraustine sp. Lepidoptera: Pyralidae	New Caledonia	*L. microphyllum* (Cav.) R. Br.
Ambia sp. (Stem-borer) Lepidoptera: Pyralidae	Singapore, Thailand	*L. microphyllum* (Cav.) R. Br. *L. flexuosum* (L.) Sw.
Callopistria sp. A Lepidoptera: Noctuidae	Australia (Queensland), China, India, Thailand	*L. microphyllum* (Cav.) R. Br.
Callopistria sp. B Lepidoptera: Noctuidae	Australia (Northern Territory)	*L. microphyllum* (Cav.) R. Br.
Callopistria sp. C. Lepidoptera: Noctuidae	Thailand	*L. microphyllum* (Cav.) R. Br.
Spodoptera litura (F.) Lepidoptera: Noctuidae	Australia	*L. microphyllum* (Cav.) R. Br.
Archips machlopis Meyrick Lepidoptera: Tortricidae	Malaysia, Singapore, Thailand	*L. microphyllum* (Cav.) R. Br.
Neostromboceros albicomus (Konow) Hymenoptera: Tenthridinidae	Malaysia, Singapore, Thailand, Vietnam	*L. microphyllum* (Cav.) R. Br. *L. flexuosum* (L.) Sw.
Metriona sp. Coleoptera: Chrysomelidae	Australia (Northern Territory, Western Australia)	*L. microphyllum* (Cav.) R. Br.
Endelus bakerianus Obenberger Coleoptera: Buprestidae	Singapore, Thailand	*L. microphyllum* (Cav.) R. Br.
Manobia sp. Coleoptera: Chrysomelidae	Thailand	*L. flexuosum* (L.) Sw.
Lophothetes sp. Coleoptera: Apionidae	Palau (Arakabesang Is.)	*L. microphyllum* (Cav.) R. Br.
Acanthuchus trispinifer (Fairmaire) Homoptera: Membracidae	Australia (Queensland, Northern Territory)	*L. microphyllum* (Cav.) R. Br.
Pseudococcus longispinus (Targioni-Tozzetti) Homoptera: Pseudococcidae	Australia (Queensland)	*L. microphyllum* (Cav.) R. Br.
Octothrips lygodii Mound Thrips: Thysanoptera	China, Singapore, Thailand	*L. microphyllum* (Cav.) R. Br. *L. flexuosum* (L.) Sw. *L. japonicum* (Thunb.) Sw.

***Lygodium* Exploration in Australia, New Caledonia, India and Sri Lanka**

Exploration for natural enemies of *L. microphyllum* continued in 2002 with trips within Queensland (13), New South Wales (1), India (2), New Caledonia (2), and Sri Lanka (1) (Fig. 6). New survey methods were employed including night searches and visits to sites during the tropical monsoon season. Both methods proved to be successful and will be continued in 2003.

Cape York in the far north of Queensland (QLD) was explored for the first time by ABCL. There were no known records of *L. microphyllum* north of the Daintree in the wet tropics, but we predicted that it must occur in the rainforest patches of Cape York. A survey trip commenced on 24 June from Port Douglas north of Cairns headed as far north as the Iron Range National Park, 200 kilometers from Bamaga at the tip. June is the dry season and most roads are open in the Cape, but driving conditions are extremely difficult and require a four-wheel drive vehicle with winch. Multiple streams and rivers are forded which can only be crossed in the dry season when water levels are low and slow moving. We were fortunate to have the new Toyota Landcruiser with winch for this demanding trip. On the way north we encountered vast stands of *Melaleuca* spp., many of which had never been surveyed for herbivores by ABCL (see Melaleuca section). Late in the afternoon of 26 June we arrived at Iron Range National Park and camped at Chili Beach. Heavy winds, high tides and light rain did not dampen the enthusiasm of the two explorers (Goolsby & Purcell) despite only finding the related species *L. reticulatum*. On the morning of 27 June as we were crossing a stream, a small patch of *Lygodium* was spotted. Upon closer inspection it was confirmed to be *L. microphyllum* and a larger patch was located downstream. Several pyralid larvae were collected but surprisingly we did not find *Floracarus perrepae*. Another surprise from this trip came some time later after the genetic diagnostic work on the *Lygodium* samples had been completed. The sample from the Iron Range was an exact match with *L. microphyllum* from Florida. Could this area be the origin of the invasive form of Old World climbing fern? Additional exploration for agents of both *M. quinquenervia* and *L. microphyllum* is planned in 2003 for this remote and unspoiled tropical wilderness.

The central QLD coast between Mackay and Townsville was surveyed for the presence of *L. microphyllum*. Most of this area lies in a region called the dry tropics, an area not suited to *L. microphyllum*. In addition, herbarium records show a gap from the wet tropics near Tully in north Queensland (NQ) to Bundaberg in southeast Queensland (SQ), a span of 1200 km. Intensive searches were conducted in the wetlands around Proserpine and Airlie Beach. Only small patches of *L. reticulatum* were located at Cedar Creek Falls near Proserpine. It appears that a formidable biogeographical barrier occurs between SQ and the wet tropics to the north. This may account for the absence of selected *Lygodium* herbivores in SQ.

French New Caledonia was surveyed again this year. This island lies 900 miles east of Australia but has an ancient Gondwanan connection to NQ. New Caledonia split from Australia over 30 million years ago and took with it the rainforest species that were common during this time period, including *L. microphyllum*, *L. reticulatum* and what may be a hybrid between the two species. Dr Hervé Jourdan was contracted to conduct monthly surveys of *L. microphyllum* herbivores at three locations in Province Sud. Dr Jourdan also hosted Jeff Makinson (ABCL) for evaluation of the New Caledonian *F. perrepae* genotype.

Exploration in southern India and Sri Lanka was initiated this year. Collaborative links were established with Dr B.V. David and Dr Alex Jesudasan (Madras Christian College), both of whom had cooperated with Drs. Alan Kirk and Lerry Lacey (EBCL) during the exploration program for silverleaf whitefly parasitoids. Dr David obtained herbarium records with locations of *L. microphyllum* in southern India. Initial searches in Tamil Nadu, near Tirunvelli, in the southern Ghats, were delayed due to difficulties in accessing nature preserves of the Bengal tiger. We did enter these areas, but found only one patch of *L. microphyllum*. No insects or mites were observed. We did however meet American naturalist and photographer Ian Lockwood. He provided valuable knowledge about the biogeography of the area. In the second part of the trip we crossed over the southern Ghat Mountains into the state of Kerala. This is an area of much higher rainfall as it has a double monsoon. We observed both *L. flexuosum* and *L. microphyllum* in this area, with the former being much more common. Collections of *F. perrepae* were made from *L. microphyllum* in Quilon (Kollam) close to the southern tip of the subcontinent. Traveling north to Coimbatore, we crossed over the Central Ghats in the Nilgris district into an arid region near the state border with Karnataka. We gained permission to enter an elephant preserve and surveyed many kilometers of dry riverbeds for *L. microphyllum*. With the help of locals, we found *L. microphyllum* growing from a seep along the river. Several moth larvae were collected from this location, however elephants destroyed the site sometime later. Tragically, two local farmers were killed and the elephants were culled.

A second trip to India was made to collect *F. perrepae* for testing. Mites were collected for testing from southern Kerala near Quilon and from Nagercoil in Tamil Nadu. A new site was visited north of Chennai (Madras) near Kaambakkam in the state of Andra Pradesh. *Lygodium microphyllum* was very common along this rapidly moving rocky stream. Since this site is only 2 hours from Chennai, our cooperator, Dr Jesudasan, and his student plan quarterly night searches. While we were in Chennai, Dr Jesudasan made the short trip to Sri Lanka to survey for *L. microphyllum*. Several stands were located south of Colombo with *F. perrepae*.

Exploration Log

- Southeast Queensland - Monthly visits to five field sites to determine phenology of *Cataclysta camptozonale* and *Floracarus perrepae.*
- New Caledonia (Feb 24-29) - Surveyed Province Nord & Sud.
- China, Hong Kong (April 21-28) - In-country host testing of *F. perrepae* mite.
- India, Tamil Nadu, Kerala (April 29-May 10) - Initiated exploration in southern India for *L. microphyllum*. Set up collaborative program with Madras Christian College.
- New Caledonia (May 19-24) - Negotiated permits with French regulatory officials to import *L. microphyllum* (Florida) to New Caledonia to conduct evaluation of *F. perrepae* mite. Surveyed Province Sud and trained IRD cooperator, Hervé Jourdan.
- Queensland, Cape York, Iron Range (June 24-27), Surveyed unexplored regions of remote rainforest for presence of *Melaleuca quinquenervia* and *L. microphyllum* and new potential agents.
- Central Queensland, Townsville to Rockhampton (June 29-July 3) – Surveyed for presence of *Lygodium* spp. between the wet tropics and Southeast Queensland.
- Central Queensland, Mackay to Townsville (Sept 25-29) - Surveyed for presence of *Lygodium* species in this gap between wet areas in Southeast Queensland and the Wet Tropics.
- India, Tamil Nadu, Kerala, & Andra Pradesh (Oct 12-20) - In-country host testing of *F. perrepae* mite and survey of new *L. microphyllum* locations.
- USA, Florida, (Nov 15-22) - visited with colleagues at the ARS-Invasive Plant Research Laboratory.

Fig. 6. Lygodium exploration in Australia, India, and New Caledonia, and related developments in Ft. Lauderdale, Florida. Pp. 29-33.

Typical *L. microphyllum* stand at Carbrook Creek, Logan, Queensland (March 2001).

Typical *L. microphyllum* stand at Carbrook Creek, Logan (January 2003).

Goolsby in New Caledonia with local landowners washing roots of *L. microphyllum* looking for root borers.

New Caledonian cooperator, Dr Hervé Jourdan (IRD).

North Queensland sugar mill in operation.

The road into the Iron Range, Cape York.

Floracarus perrepae on *Lygodium reticulatum* at the Iron Range.

Lygodium microphyllum, Iron Range, Cape York.

Campsite at Chili Beach, Iron Range National Park.

Mt. Tozer cloaked in rainclouds Iron Range National Park, Queensland.

River crossings, Cape York.

Unknown python species, Cape York.

Goolsby collecting *Floracarus perrepae* in Hong Kong for use in the in-country host-range tests.

Dr Alexander Jesudasan, Reader of Entomology, Madras Christian College greeting John Goolsby.

Lygodium microphyllum growing below tea plantation in southern Tamil Nadu.


Dr B.V. David and J. A. Goolsby collecting insects from *L.microphyllum* near Quilon, Kerala, India.


Lygodium microphyllum habitat in the Ghat Mountains near Silent Valley, Kerala, India.


Locals lead ABCL explorers to *L. microphyllum* near Gudalur, Tamil Nadu-Kerala border.


Seeps along banks of this rocky creek near Gudalur support remote outcrops of *L. microphyllum.*


Rice planting near Kambakkam, Andra Pradesh near *L microphyllum* field sites.


Lygodium microphyllum near Nagercoil, Tamil Nadu, near the very southern tip of India.

The new biological control quarantine facility is under construction at the ARS Invasive Plant Research Laboratory, Ft. Lauderdale, FL.

Stands of *L. microphyllum* in Ft. Lauderdale, FL treated with herbicides.

Re-growth of *L. microphyllum* from spores and rhizomes under treated stands.

Lygodium Exploration in Southeast Asia

Over 100 site visits were made in four countries, with many additional stops made within these countries during travel between collecting sites (Fig. 7). Visits resulted in 64 collections, samples or data recorded. Other plants noted during visits included *Scaevola taccada, Thespesia populnea, Rhodomyrtus tomentosa,* and *Paederia foetida*.

Southeast Asian Exploratory Trips: Tony Wright

- Hong Kong (Feb 24 -Mar 1, Mar 4 - Mar 8) (Collaboration with A. Kirk, G. Mercadier, A. Tsang, D. O'Toole) - Meetings at City University with local workers, Prof. L. Vromoed and student T. Kwong to review pathogens on insects, and incorporation of ideas into CityU termite research. Field visits to sites at Kowloon, New Territories, Lantau Is. for *L. microphyllum*, *L. japonicum*, *R. tomentosa* and *Coptotermes* spp.
- Thailand (Mar 1 – 4) (Collaboration with A. Winotai) - Review of *R. tomentosa* herbivore project and visits to Nakhon Si Thammarat province for *L. microphyllum* and *R. tomentosa*.
- Singapore (Mar 31 – Apr 1, Apr 7–11) - Meeting at Raffles Collection with Mrs. Yang & lodgment of *Endelus bakerianus* det by S. Bílý, Prague Museum. *L. microphyllum* monitoring at Sungei Buloh, Bukit Timah and Pulau Ubin.
- Malaysia (Apr 1-7) (Collaboration with B.L. Tay) - Exploration in Sarawak, Borneo, was centered around Kuching districts where *L. microphyllum* is reasonably common and where both plant and herbivores appear the same as nearby Indonesia, Singapore and peninsula Malaysia.
- Thailand (May 30 – Jun 7) (Collaboration with A. Winotai, U. Chattrakul, A. Thagong) - Visited the northern Chiang Mai site where the Lygodium stem-borer is being collected and reviewed the laboratory rearing and research program. Also to Krabi and Nakhon Si Thammarat provinces in southern Thailand to explore for new sites for *L. microphyllum* and *R. tomentosa*.
- Hong Kong (Jul 29 – Aug 2) (Collaboration with A. Tsang, D. O'Toole) - Laboratory and field visits for *Coptotermes* spp., *L. microphyllum*, *L. japonicum*, and *R. tomentosa*.
- Singapore (Aug 13-16) (Collaboration with M. Purcell and R. Desmier de Chenon) - Visit to Sungei Buloh also covered hydrilla, while sites at Bukit Timah were for *L. microphyllum* only.
- Thailand (Aug 16-20) (Collaboration with A. Winotai, U. Chattrakul, A. Thagong) - Trat province in eastern Thailand to locate *Floracarus perrepae* in preparation for preliminary host testing in September by J. Makinson. Also to northern Thailand for meetings and inspection of stem-borer project.
- Thailand (Aug 29 Sep 16) (Collaboration with A. Winotai, J. Makinson) - Field collection of *F. perrepae* in eastern Thailand, Trat province. Host testing on *L. microphyllum* from Australia and Florida in DOA laboratories. Finalize *Rhodomyrtus* report.
- Hong Kong (Oct 21-24) (Collaboration with A. Tsang, D. O'Toole) - Termite project, laboratory and field visits.
- Thailand (Oct 24-27) (Collaboration with A. Winotai) - Revisit Trat province for further collection of *F. perrepae* for Brisbane quarantine and *Rhodomyrtus* borer insects for taxonomy.
- Hong Kong (Nov 26-28) (Collaboration with A. Tsang, D. O'Toole) - Visit New Territories & CityU for *Coptotermes* spp. and *L. microphyllum*.
- Thailand (Nov 28 – Dec 5) (Collaboration with A. Winotai) - To southern Thailand (provinces of Narathiwat, Songkhla, Nakhon Si Thammarat, Surat Thani) searching for stem-borer activity on *L. microphyllum*.

Fig. 7. *Lygodium* exploration in Southeast Asia.

Jeff Makinson and Amporn Winotai at Department of Agriculture laboratories, Bangkok.

Cycles are useful for sampling on Pulau Ubin and for getting sore legs.

Collaborator Roch Desmier de Chenon photographing *Paederia foetida* in Singapore.

Sungei Buloh Reserve, Singapore, where herbivores on both *L. microphyllum* and *H. verticillata* are studied.

Jeff Makinson and Amporn Winotai collect *F. perrepae* mites at Ban Klong Manao in eastern Thailand.

Amporn Winotai finds time in her schedule to show Jeff Makinson some of the vast history and culture of Thailand.

Characterization of *Lygodium microphyllum* Populations

Several genotypes of *Lygodium microphyllum* have been characterized using genetic diagnostic methods. A 990 base pair sequence of the chloroplast genome called trnF-trnL was sequenced to identify differences in the populations. Our goal was to determine which population or genotype was the most similar to the invasive Florida form of *L. microphyllum*. Finding the best match for Florida is critical to our work with the eriophyid mite, *Floracarus perrepae*. At the beginning of 2002 we had evidence that *F. perrepae* from QLD would not feed on the Florida form of *L. microphyllum*. Therefore, matching the Florida form with populations in the native range might lead us to the population of *F. perrepae* that has the most potential as a biological control agent. Populations of the fern from Africa (Ghana), Australia (Queensland- including Cape York, Western Australia), China (Hong Kong), India (Tamil Nadu, Kerala), New Caledonia (Province Sud), Thailand (Nakhon Si Thammarat) and the USA (Florida) were compared. The samples from Thailand and Australia (Cape York) were the most similar to Florida, with the Cape York population being an exact match (Fig. 8). In 2003, we intend to collect and sequence additional samples from Borneo, India, Malaysia, New Guinea, Thailand, and Singapore to better determine the distribution and variation in *L. microphyllum* genotypes across its native range.

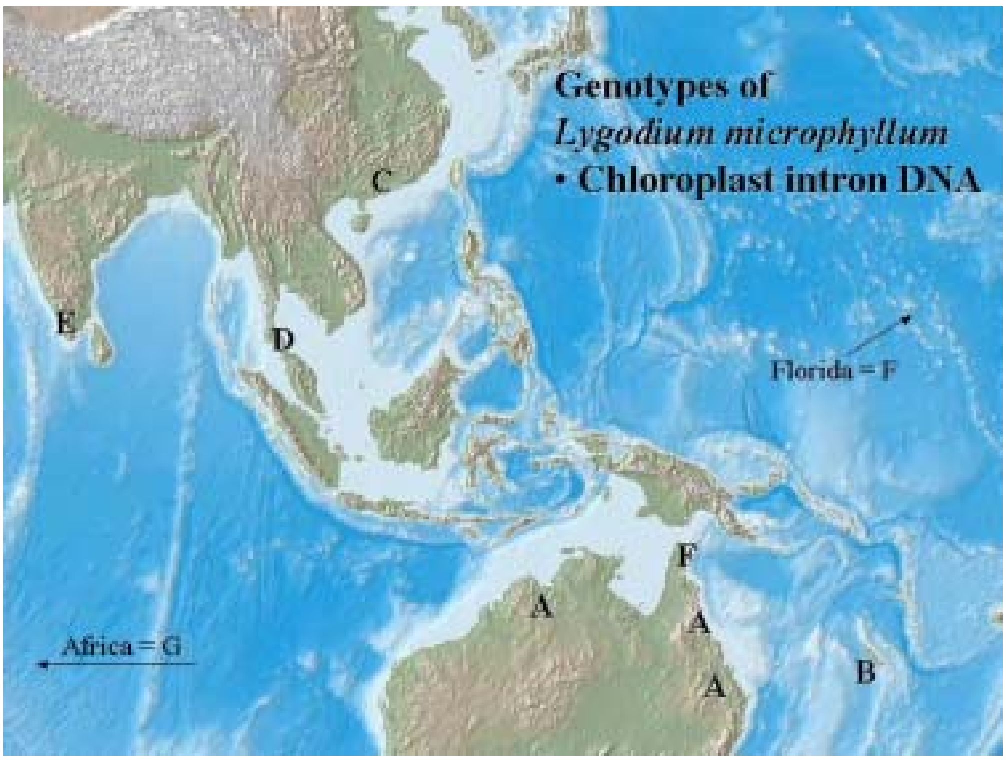


Fig. 8. Distribution of *Lygodium microphyllum* genotypes using comparison of the TrnF-TrnL chloroplast spacer gene sequence**.**

<u>Leaf Curling Mite - *Floracarus perrepae* (Acari: Eriophyidae)</u>

Floracarus perrepae (inset left) has excellent potential as a biological control agent for *L. microphyllum*. It appears to have a strong regulatory effect on the fern in its native habitat and very narrow host specificity. Excellent progress has been made this year on the biology of the mite due to collaborations with Dr Ozman, (Trakya University & Univ. of Queensland, CRC for Tropical Plant Pathology), Thomas Freeman (North Dakota State University), and Dennis Nelson (ARS-Fargo). We were also graciously hosted overseas by Des O'Toole (City University, Hong Kong), Jean Chazeau & Hervé Jourdan (IRD, New Caledonia), Amporn Winotai (Dept. of Agriculture, Thailand), and Alex Jesudasan (Madras Christian College, India) during our work screening the unique genotypes of *F. perrepae*.
The combination of people and laboratory facilities has resulted in excellent progress towards our goal of delivering an effective new agent for *L. microphyllum*.

Taxonomy and Distribution. *Floracarus perrepae* was recently described by Dr Danuta Knihinicki (NSW Agriculture) and Jan Boczek (Warsaw Agricultural University) (Knihinicki & Boczek 2002). *Floracarus perrepae* is the most widespread and common arthropod associated with *L. microphyllum*. It was present in 172 of the 513 field collections of *L. microphyllum* made from 1998 to 2002. It is distributed throughout Australia, Oceania and Asia and has been collected from locations in Australia, Borneo, China, India, Indonesia, Malaysia, New Caledonia, Thailand, Singapore, and Sri Lanka.

Characterization of Genotypes. Several genotypes of *F. perrepae* have been discovered using genetic diagnostic methods. The nuclear gene D2 expansion domain of the 28S rRNA gene (De Barro et al. 2000) was used to identify genotypes. Our prediction was that genotypic difference would relate to intrinsic biological attributes such as host range. Populations of the mite, from Australia (New South Wales, Queensland, Northern Territory, Western Australia), Australia (Cape York), China (Hong Kong, Hainan), India (Tamil Nadu, Kerala), Indonesia (Sumatra), Malaysia (Malacca), New Caledonia (Province Sud, Île de Pins), Thailand (Songkhla, Nakhon Si Thammarat), Singapore, and Sri Lanka (Colombo), were compared. We found a consistent gene sequence in all the Australian specimens, except those from Cape York. *Floracarus perrepae* from Borneo, Indonesia, Malaysia, Singapore, and Thailand were similar to the Australian population, with China, India/Sri Lanka and New Caledonia each with unique genotypes (Fig 9). The population from Australia (Cape York), genotype 'F', was only collected from *L. reticulatum*. *Lygodium microphyllum* in the same habitat did not have *F. perrepae* present at the time of collection. A second visit to Cape York is planned for the wet season to try to collect *F. perrepae* from *L. microphyllum*. This population may be useful since the plant genetics indicate a match with the invasive Florida form.

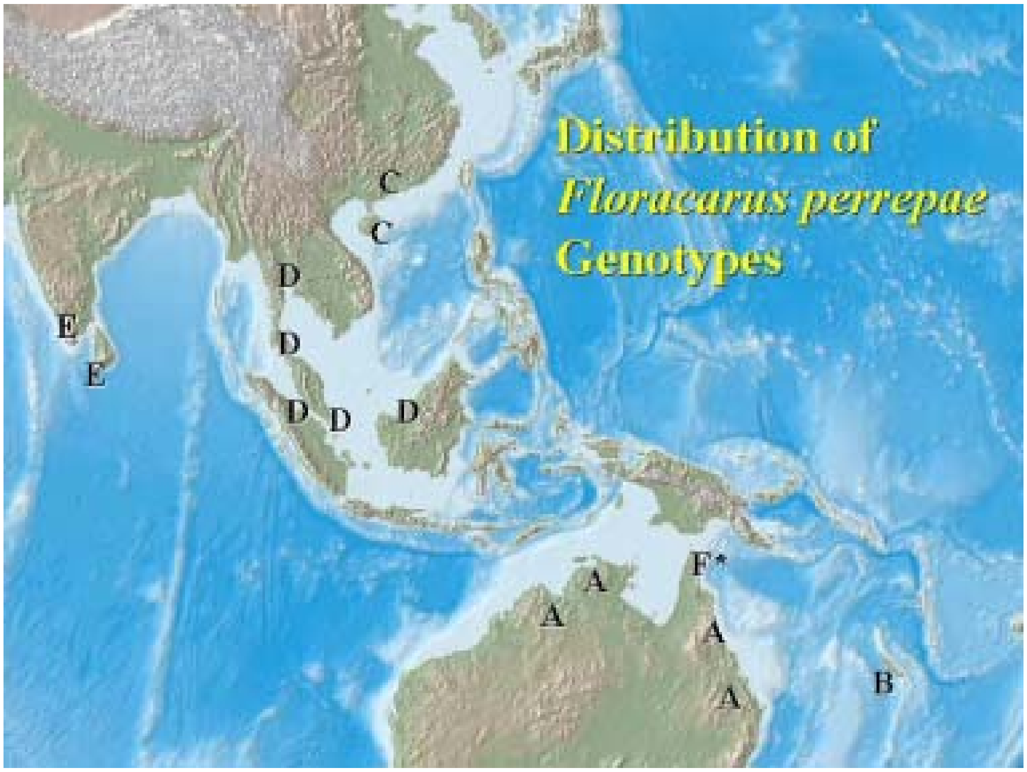


Fig. 9. Distribution of *F. perrepae* genotypes across native range of *L. microphyllum*. F* denotes that *F. perrepae was* collected from *Lygodium reticulatum* and may be specific to this plant species.

Biological Studies. Intensive studies of *Floracarus perrepae* were completed in 2002. *Floracarus perrepae* has a simple life cycle, consisting of an egg, larva, nymph, imago chrysalis, and adult. Adults prefer to feed on young, quickly expanding subpinnae, inducing the formation of swollen, curled margins (inset right). Eggs hatch within 5.0 days at 21 °C and 3.9 days at 26 °C and 70% RH. A quiescent or resting stage occurs between the larva and nymph, and again between the nymph and adult (imago). Development time from first instar to adult takes 4.0 days at 21 °C and 3.1 days at 26 °C. The mean fecundity is 59.0 and 35.4 progeny at 21 and 26 °C respectively. Adult longevity is 35.3 and 19.6 days at 21 and 26 °C respectively. Immatures are similar to adults in appearance, but smaller in size. Males are similar to females, but slightly smaller. Fertilization occurs in the leaf curl, and we have observed the male spermatophores. Figure 10C shows the opening to the female genitalia, the apodeme. The sex ratio of the mite colony is female biased. Eggs are laid in the leaf curling where the adult females feed. Eggs are very small, spherical and translucent and difficult to see when first laid. They change color within a few days of being laid, becoming more visible.

In preliminary host-range testing we found that the QLD ‘A’ genotype of the mite was not able to induce leaf curls and complete development on the Florida ‘F’ genotype of *L. microphyllum.* Additional collecting and genetic characterization revealed several distinct genotypes, or host races, of the mite from Australia, China, New Caledonia, Thailand and India. We predicted that one or more of the mite genotypes would be suited to the invasive Florida genotype of *L. microphyllum*. At this point we did not understand the mechanism for acceptance of the fern genotypes by the mite genotypes. Was the ability to feed, induce curls and reproduce based on a biochemical or mechanical interaction between mite and fern genotypes?

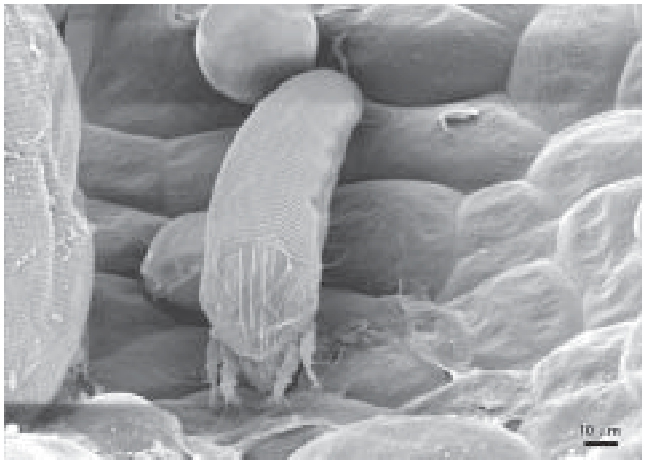
A collaborative project was set up with Dr Thomas Freeman (North Dakota State University) to take scanning electron micrographs (SEMs) of the mite ultrastructure and the epidermal layer of the fern. The objectives were to measure the length of the mite stylet and the thickness of the epidermal layer so that we could determine if there was a mechanical basis for the plant-mite interaction. Dr Freeman's expertise as a microscopist and plant morphologist also allowed us to learn more about the tissue of the leaf curl. This work was reported at the Entomological Society of America Annual Meeting in Ft. Lauderdale, FL in December 2002. A short summary of the research is presented below.

Mite feeding stimulates plant epidermal modifications resulting in curling of the subpinnae and eventual necrosis. Mature female mites prefer new sterile subpinnae for feeding and oviposition (Fig.10A). To feed, the mite attaches itself to the subpinnae surface by means of an anal sucker (Fig.10B) and then forces its stylets into the epidermal layer. Mouthparts of eriophyoid mites are complex (Fig.10D). The gnathosoma consists of a subcapitulum (rostrum), which encloses the cheliceral stylets, and oral stylet. Stylets are generally visible only during feeding.

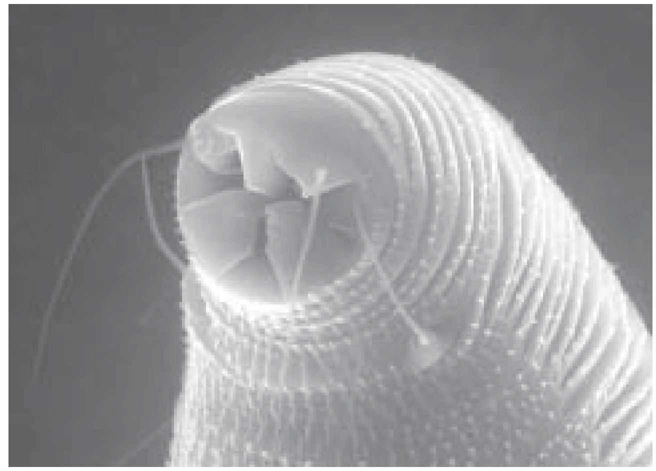
Newly formed subpinna curls were found to contain from one to five adult mites, although a single mite may cause the subpinna to curl. Subpinnae curl over, downward and inward (Fig.10E) rolling over themselves two to three times. Several hundred eggs can be laid inside the curl. The larvae and adults feed on the specialized leaf tissue in the leaf curls. The subpinna eventually dries and abscises as a result of the continued feeding, at which time the adult mites move to another young subpinna and repeat the cycle. In response to mite feeding the normal epidermal cells (Figs.10F&G) become significantly enlarged (Fig.10H), causing the subpinna to curl. Mature epidermal cells become meristematic, greatly increasing their cytoplasmic content and metabolic activity to form the nutritive tissue of the curl (Fig. 10I).

We concluded that interaction of mite and fern genotypes was not due to the inability of the mite to penetrate the epidermal layer of the host. The stylets of the mite were measured to be 10 microns, with the epidermal cell wall being 1-2 microns. Our observations also revealed the profound changes in the epidermal cells that are induced by feeding of the mite. A biochemical interaction between mite and the ferns of co-adapted genotypes must account for the formation of the nutritive tissue and leaf curls. The biochemical mechanism for this interaction is not known, but appears to be responsible for the high level of host specificity observed in the *F. perrepae* genotypes.

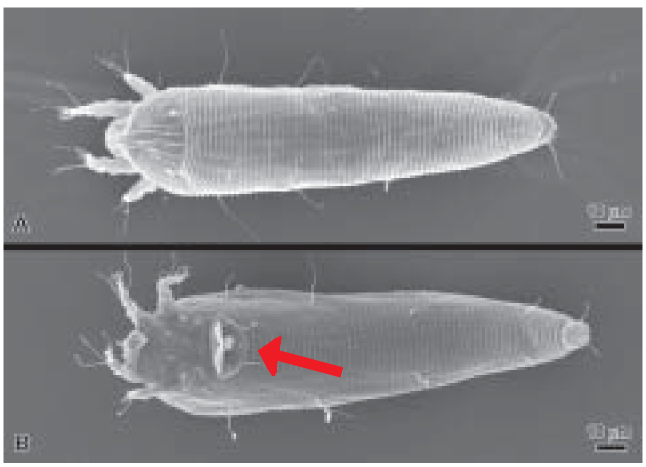
Fig. 10. SEM's of *F. perrepae* ultrastructure and epidermal layer of *L. microphyllum.*



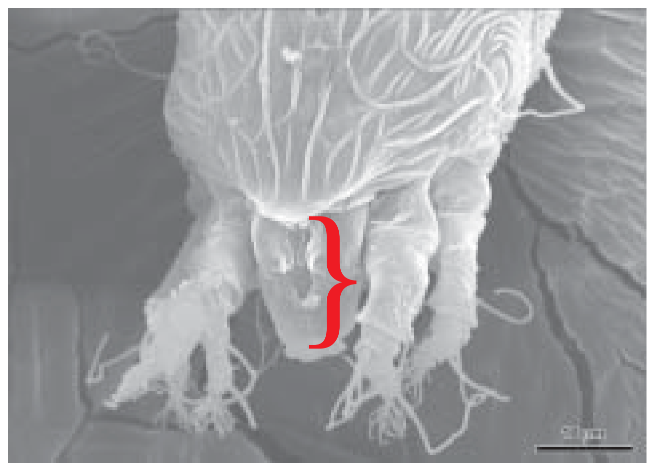
A. Mite in feeding position on *Lygodium microphyllum* epidermis.



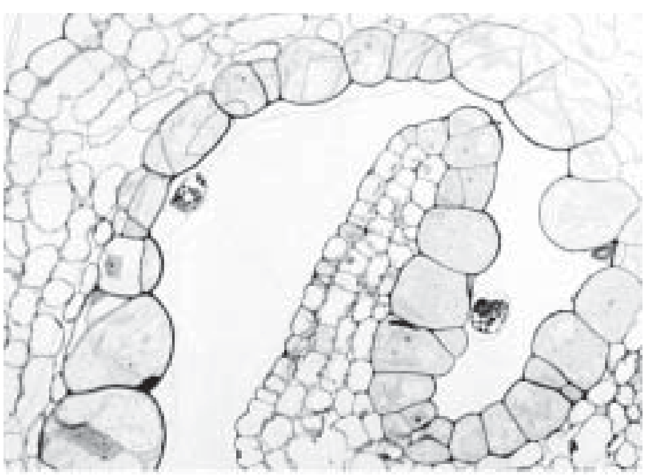
B. Anal sucker on distal end of mite used to hold mite in place during feeding and preparation for dispersal.



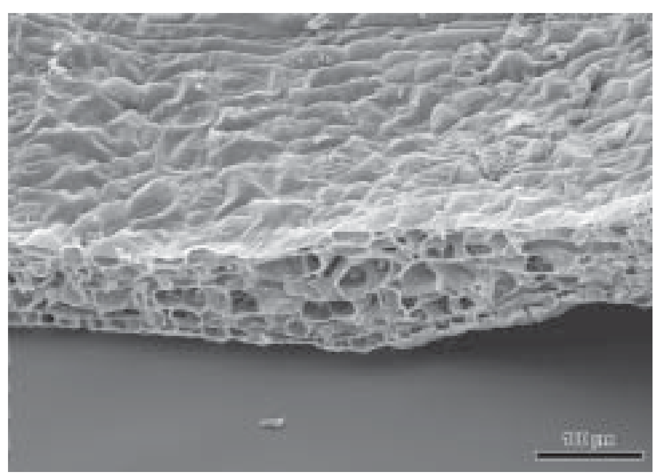
C. Dorsal and ventral view of adult mite, red arrow points to apodeme of female genitalia.



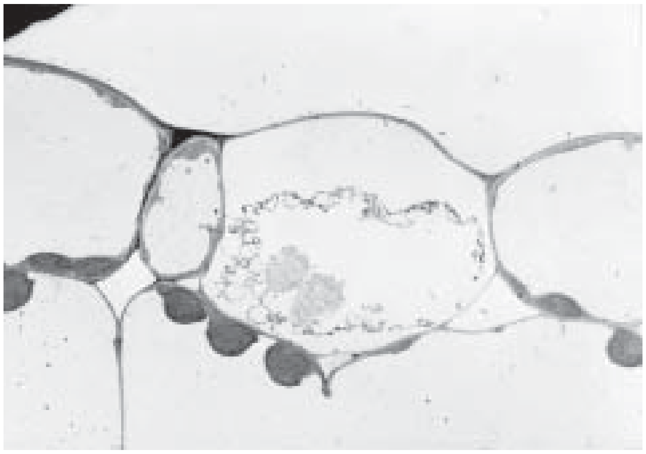
D. Dorsal view of the gnathosoma (mouthparts) in red and view of legs I and II.



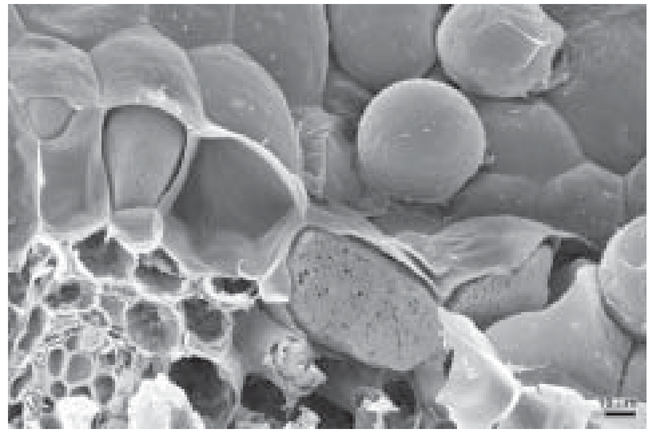
E. Nutritive enlarged abaxial (bottom) epidermal cells in *L. microphyllum* leaf curl.



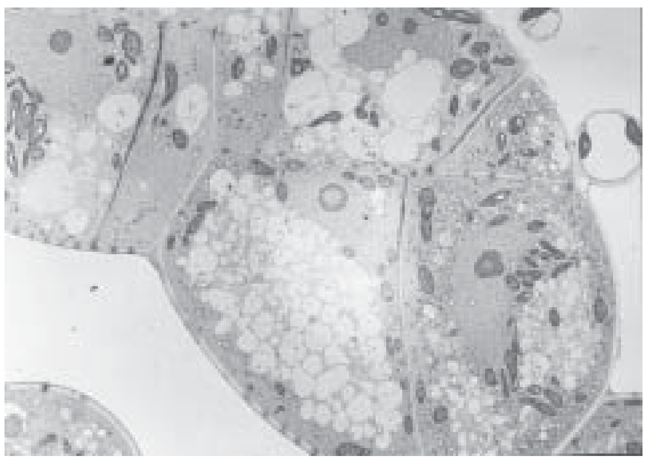
F. Surface and sectional view of a non-curled area of *L. microphyllum*.



G. Normal *L. microphyllum* epidermis in a non-curled area.



H. Curled area of *L. microphyllum* showing enlarged cells of nutritive epidermis.



I. Well developed nutritive tissue in leaf curl. Note size and shape of cells with numerous mitochondria.

Evaluation of *Floracarus perrepae* Genotypes. To determine which mite genotypes (A-E) found the invasive Florida genotype of the fern acceptable, we developed a standardized test for evaluation and went overseas to test each population (Fig. 11). *Floracarus perrepae* were collected from the field in each country and tested (Fig. 12). Sets of sporeling *L. microphyllum* from Florida and Australia were imported by permit into each country. Approximately, 10-25 individual plants, each with 3-5 true leaves of each type were used in each evaluation. Ten adult mites of mixed sexes were transferred from the field collected leaf curls to each leaf of the sporeling plants with a maximum of thirty per plant. Plants were held in a translucent sealed container and the mites were counted daily and signs of mortality, feeding and oviposition were recorded. Daily observations continued for two weeks to determine if the specific mite genotype was able to produce a healthy curl. At the end of the two-week period, curls were dissected and the numbers of eggs, nymphs and adults were counted. In one case, we opted to wait an additional two weeks to dissect the curls because they were slow in developing. Development of leaf curls and production of progeny for each genotype was compared to the standard, which was the Queensland *F. perrepae* on the Queensland *L. microphyllum*. The results of screening are shown in Table 6. Only the Thai 'D' genotype consistently produced normal curls and similar numbers of progeny to the QLD standard. All of the mite genotypes induced leaf curls and reproduced on the QLD fern genotype except the Thai mite.

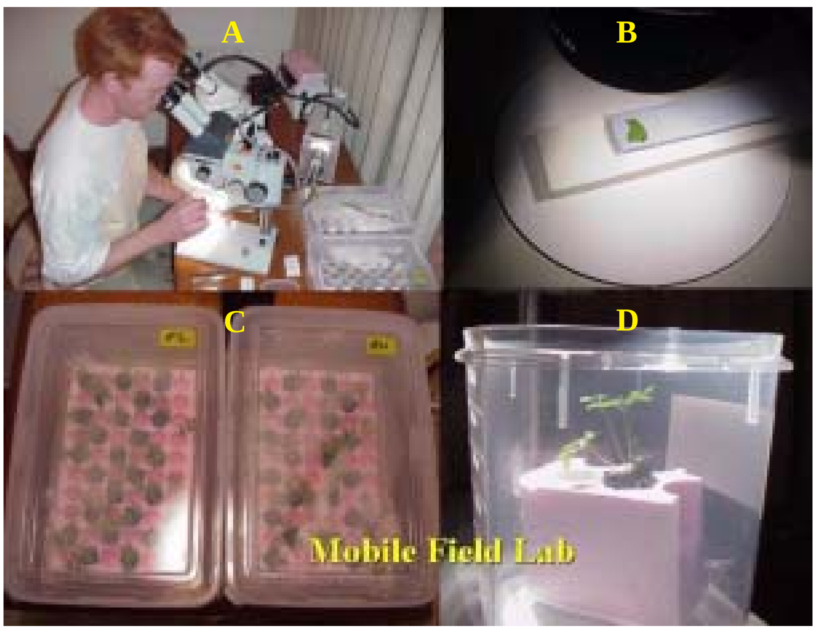


Fig. 11. Jeff Makinson is shown with mobile field laboratory with equipment and materials used to screen the *F. perrepae* at overseas locations. A) double microscope set-up for collection and transfer of mites from field collected leaf curl; B) field collected leaf curl on 'Blue Tac' to allow for uncurling of leaf curl and collection of adult mites; C) Double sets of sporeling *L. microphyllum* of the Florida and Australia genotypes held in sealed containers at 100% RH; D) sporeling on mite transfer stage showing age of leaves used in the test.

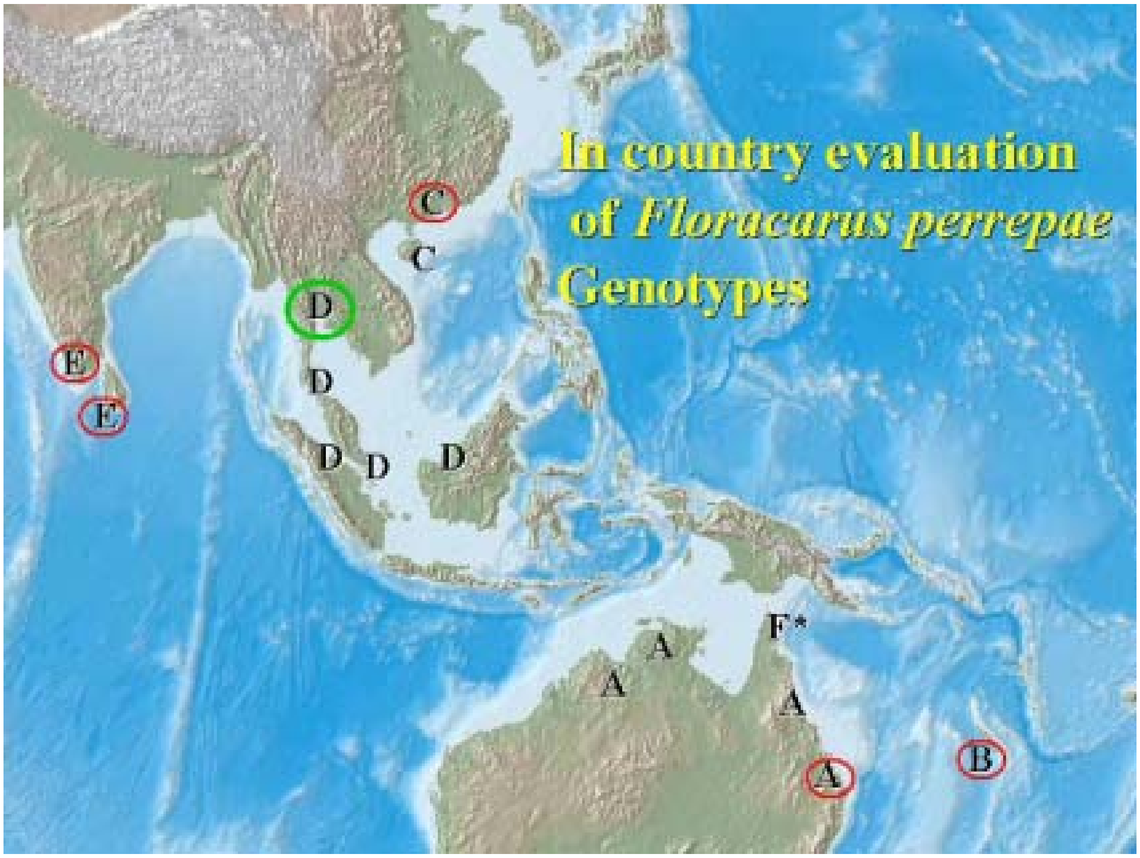


Fig. 12. Locations and identity of *F. perrepae* genotypes evaluated for acceptance of the FL *L. microphyllum* genotype. Locations circled in red are the source populations for each test. The 'D' genotype circled in green developed normally on the FL *L. microphyllum* and was exported to Australia for full host-range testing. The Cape York genotype 'F*' was collected from *Lygodium reticulatum*.

Table 6. Acceptance of Australian and Floridian genotypes of *L. microphyllum* by selected mite genotypes. The symbols are used as follows; (-) no leaf curling or reproduction, (+) poorly developed curls with limited reproduction, (++) moderately developed curls with reduced reproduction, and (+++) robust swollen curls with typical reproduction.

Mite	AUS	AUS (Cape York)	New Caledonia	China	Thailand	India/Sri Lanka
Fern	**A**	**F**	**B**	**C**	**D**	**E**
Australia	+++		++	++	-	++
Florida	-		+	-	+++	-

Host-Range Testing. *Floracarus perrepae* appears to have a very narrow host range. Field surveys have detected the mite on one other species, *Lygodium reticulatum,* which is sympatric with *L. microphyllum* in NQ and New Caledonia (NQ and New Caledonia shared an ancient Gondwanan connection, >30 million years ago). We have not observed the mite on other species, including: *L. japonicum*, *L. circinatum*, or *L. flexuosum*. It is probable that the *F. perrepae* genotype adapted only to *L. reticulatum*. Plans for host-range testing are discussed below.

Full host-range testing of *F. perrepae* will begin in early 2003. Through discussion with Dr Robert Pemberton (ARS Ft. Lauderdale) a host test list for *Floracarus perrepae* has been developed (Table 7). We will concentrate upon *Lygodium* species, close relatives in the environment where *L. microphyllum* is invasive, horticultural ferns, and known hosts of other *Floracarus* spp. Micro-cultured spore grown ferns will be used for the majority of the testing (inset left). A no-choice test design will be used. Five females and five males will be transferred to each test plant and held until death. Plants will be observed twice weekly to assess feeding, oviposition and leaf curling.

Host-range testing will be conducted in the new CSIRO high security Level 3 quarantine at Long Pocket Laboratory. Walk-in chambers have been refurbished which allow for control of temperature and day-length. Two separate chambers are dedicated to ABCL, which will provide ample space for the quarantine work.

Fern species grown from spores have proven to be ideal for studying *F. perrepae*. Individual mites can be transferred to the tiny plants and their biology observed. Spore grown plants provide actively growing plant tissue that is necessary for the female to feed, induce curling and oviposit. Gio Fichera and Karryn Waterworth (CSIRO Entomology) have developed the technique for growing the sporeling ferns. Most of the ferns on the test list, including the *Lygodium palmatum,* have now been successfully grown from spores. *Lygodium cubense* plants have been imported from Cuba via Ft. Lauderdale but have not produced fertile leaves with spores.

Table 7. Host test list for *Floracarus perrepae.*

Family	Species	Comments
Lygodiaceae	*Lygodium microphyllum* from Australia	Native host plant
Lygodiaceae	*Lygodium microphyllum* from Florida	Invasive phenotype
Lygodiaceae	*Lygodium reticulatum*	Related host from Australia
Lygodiaceae	*Lygodium japonicum*	Related invasive species from SE Asia
Lygodiaceae	*Lygodium flexuosum*	Closely related from Australia & SE Asia
Lygodiaceae	*Lygodium palmatum*	Closely related Caribbean endemic
Lygodiaceae	*Lygodium oligostachyum*	Closely related Caribbean endemic
Lygodiaceae	*Lygodium venuustrum*	Closely related Caribbean endemic
Lygodiaceae	*Lygodium cubense*	Closely related Cuban endemic
Lygodiaceae	*Lygodium volubile*	Closely related South American endemic
Schizaeaceae	*Anemia adiantifolia*	Allied family in Florida, where *L. microphyllum* is invasive
Pteridaceae	*Adiantum capillus-veneris*	Family representative
Blechnaceae	*Blechnum serrulatum*	Family representative
Dryopteridaceae	*Rumohra adiantifolis*	Family representative, horticultural plant
Gleicheniaceae	*Sticherus flabellatus*	Family representative, horticultural plant
Sterculiaceae	*Theobroma cacao*	Known host of related *Floracarus* sp.
Convolvulaceae	*Ipomoea alba*	Known host of related *Floracarus* sp.
Rutaceae	*Citrus paradisi*	Economic species
Poaceae	*Saccharum officinarum* (sugarcane)	Economic species

Seasonal Phenology. A two-year study at four sites in SQ to determine the seasonal phenology of *F. perrepae* on *L. microphyllum* was completed in 2002 (Fig. 13). Each month, fifty pinnae (leaflets) (approximately 300 subpinnae) were collected at each site. The numbers of curled sterile and fertile subpinnae were counted to estimate the level of infestation at each field site. We also dissected subpinnae and counted the numbers and stages of mites. From this count we could determine the actual numbers of mites responsible for the damaged subpinnae and look for the occurrence of predator mites. The purpose of this field research was to develop an understanding of what influences the population dynamics of *F. perrepae* in its native habitat; to assess its potential as a biological control agent. This information will be used to predict its impact in Florida.


Fig. 13. Field site, Bribie Island, Queensland.

Weather conditions in 2002 had a strong influence on *L. microphyllum* stands at the four study sites. Drought conditions continued through 2002. Average rainfall for the Brisbane area is 1200 mm per year (47 in.) with 667 mm (26 in.) recorded for 2002 (Fig. 14). The impact of the drought on *L. microphyllum* in the study locations was visually apparent in the change in the plant community. Soils around the plants are wet, but not water logged as they were for most of 1999 and 2000. Most stands continued to have new growth, but at a reduced level. Two sites, Gallagher's Point and McMahon Road are located at Bribie Island, 50 km north of Brisbane. At the Gallagher's Point site leaf necrosis associated with the mite was extremely prevalent (Fig. 15). Bush fires burned the McMahon Rd. site. The previously lush stands (Fig. 16) of *L. microphyllum* are regrowing but are hampered by lack of standing trees to climb on and competition from the fern, *Blechnum indicum* (Fig. 17). Plants are mite free at the McMahon Rd. site and we are monitoring for recolonization of the mite from the closest *L. microphyllum* stands that are 3 km distant.

The other two study sites, Carbrook Creek and Lagoon Road, are located 25 km south of Brisbane, near the Logan River. At the Carbrook Creek site (Fig. 18) *L. microphyllum* stands appeared to gradually lose dominance to another fern, *Hypolepis muelleri*. We do not know what is causing this shift, but salt-water intrusion from the Logan River may be responsible. The Lagoon Road site is approximately 2 km up the watershed from Carbrook and should be unaffected by salt intrusion in the water table. *Lygodium microphyllum* stands at Lagoon Road have grown slowly throughout the year (Fig. 19). The four study sites have provided a picture of *L. microphyllum* in a variety of settings. None of the stands have grown appreciably during the study, which is due to the combination of drought conditions, competition from other plants and continued attack by *F. perrepae* on new growth.

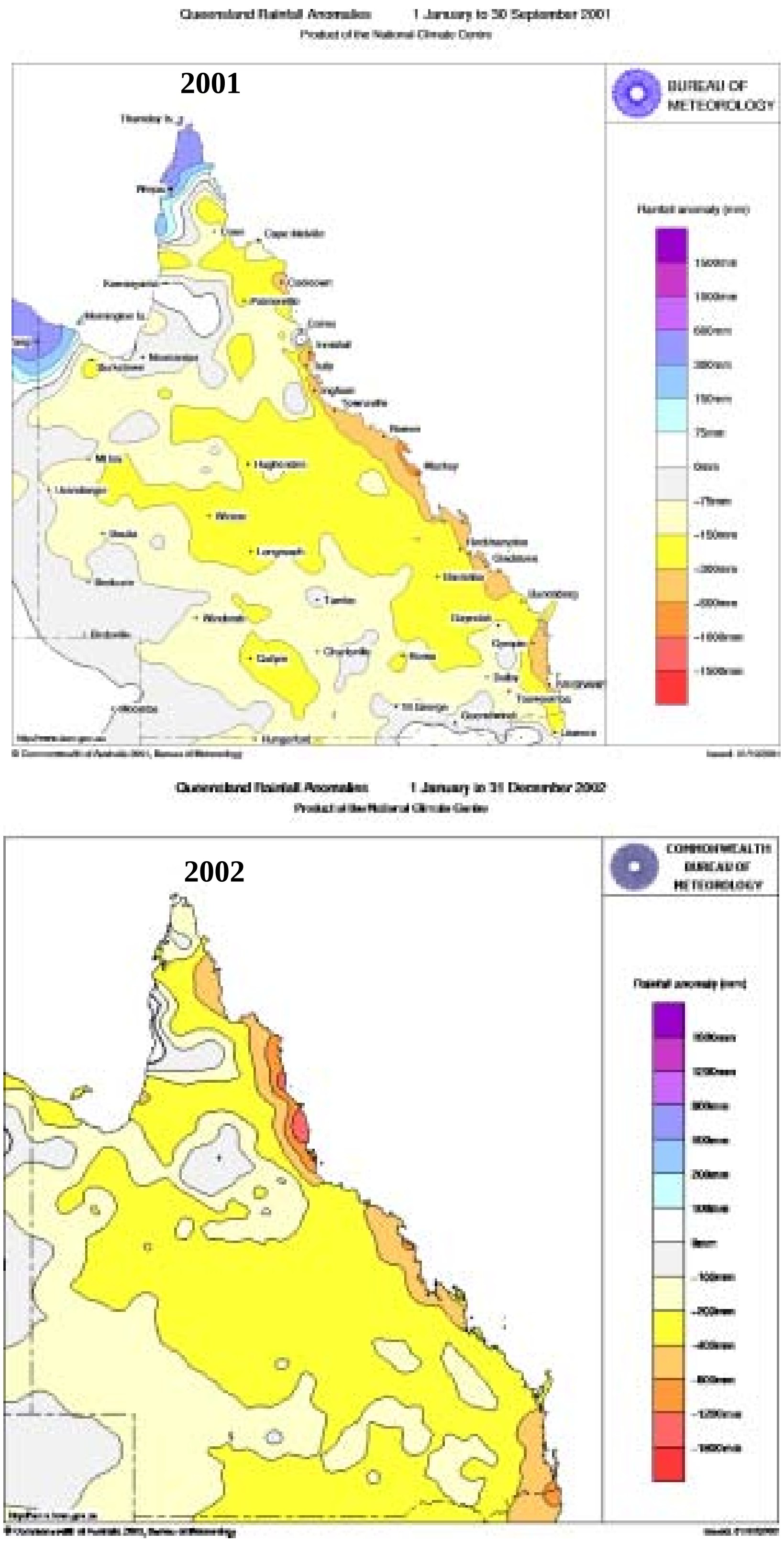


Fig. 14. Top figure shows rainfall deficits for 2001, bottom figure 2002 illustrating that the drought conditions have worsened. Field sites are primarily located between Brisbane and Gympie.

Fig. 15. Mite induced leaf necrosis reached high levels at Gallagher's Point, Bribie Island

Fig. 16. Lush stands of *L. microphyllum*, McMahon Road, Bribie Island.

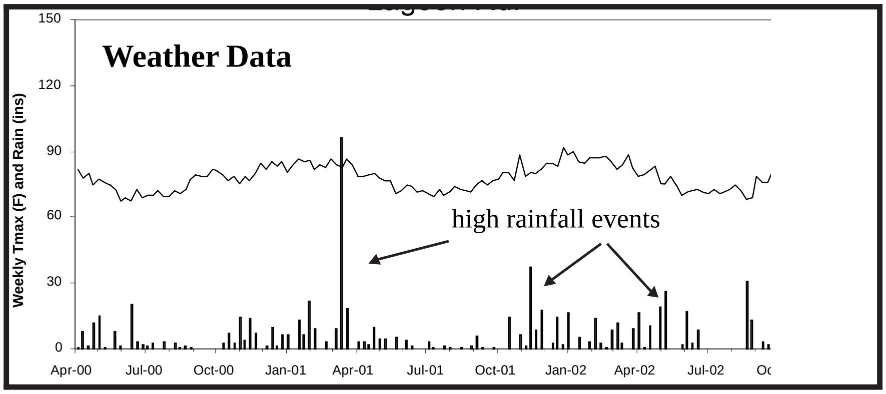
Fig. 17. Effect of bush fires on study site at McMahon Road. This site will be followed long-term to determine how long it takes *Floracarus perrepae* to recolonize from stands 3 km away.

Fig. 18. At Carbrook Creek, Logan, QLD, soil moisture levels have lowered, due to the drought, and *L. microphyllum* stands show signs of defoliation.

Fig. 19. Soil moisture at nearby Lagoon Road, Logan, QLD, has remained at adequate levels, which allowed stands of the fern to keep producing new growth during 2002.

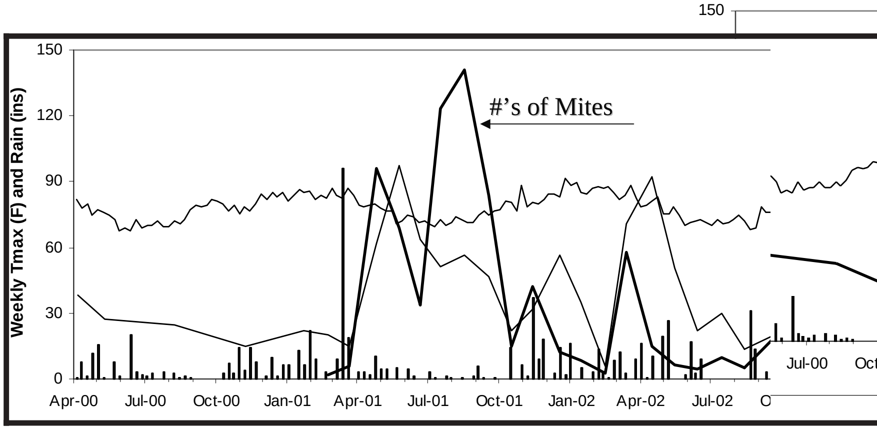
Despite the backdrop of drought conditions mite populations were persistent and damaging to *L. microphyllum* at all sites during the two-year study. All four sites followed the same trends in 2002 as they had in 2001, reaching the highest levels in the fall following periods of rainfall (Fig. 20A). Increases in mite populations were negatively correlated with hot dry weather and positively correlated with rainfall. Since the mites are active year round they benefit from rainfall whenever it occurs during the year. It appears that the benefit comes in terms of lowered mortality to the dispersing females. Dispersing females must spend a minimum of 3 days outside the leaf curl before a new protective curl can be produced on newly colonized new growth. Unfavorable conditions such as hot, dry, windy weather must cause high levels of mortality to the dispersing females. Moderate weather conditions with high humidity provide a more favorable environment for dispersal and colonization. Conditions in Florida should be equal to SQ, or perhaps even more favorable since Florida has a higher average RH and more rainfall in the summer months.

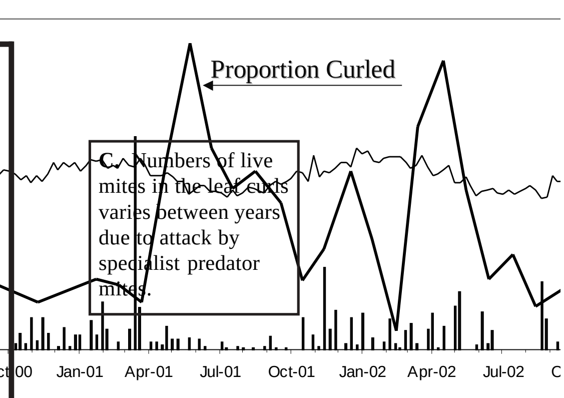
Natural enemies, primarily predator mites, were collected and identified from the field studies. Predator mites were collected from leaf curls observed to be feeding on the *F. perrepae* adults, nymphs and eggs. The first four, of the following six species, were commonly encountered: *Tarsonemus* sp., *Tydeid* sp. 1, *Tydeid* sp. 2, *Agistemus* sp., Cheyletidae sp., and Ascidae sp. A pathogenic fungus, *Hirsutellea thompsonii* (?), was common in the winter months at some locations as well as syrphid larvae. In 2002, populations of predator mites were higher than in 2001 (Fig. 20D). This correlated with lower numbers of *F. perrepae* in the leaf curls (Fig. 20C). *Tarsonemus* sp. was not as common as it was in 2001 and most predation was caused by the *Tydeid* sp. 2. Despite the higher level of predation, the proportion of leaf curls remained the same between years (Fig. 20B). This is significant because the proportion of leaf curls represents the level of damage to the plant. We can conclude that *F. perrepae* populations cause consistent damage to *L. microphyllum* plants despite a high level of mortality caused by natural enemies.



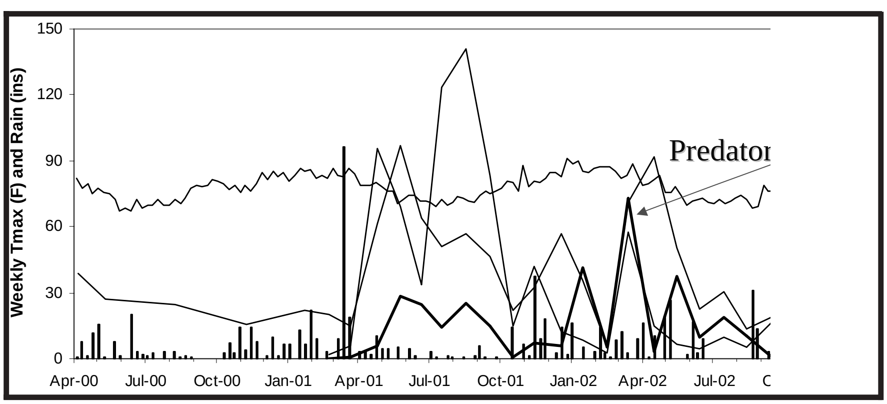
A. Rainfall stimulates mite populations.

B. Damage to plant (curled pinnae) peaks in the fall following rains.





C. Numbers of live mites in the leaf curls varies between years due to attack by specialist predator mites.



D. Predator mites (Tydeidae) are high in year two of the study, which depresses the *F. perrepae* population.

Fig. 20. Seasonal Phenology of *Floracarus perrepae.*

Similar studies of the phenology of *F. perrepae* are being conducted in New Caledonia, India and Indonesia. Dr Hervé Jourdan (IRD-Noumeá, New Caledonia) is conducting monthly surveys of the mite in three locations in Province Sud. The climate of New Caledonia is very similar to Florida (Climex ranking 71%). This study will aid our understanding of the influence of 'Florida-like' climatic factors on the mite's population dynamics. In India, Dr Alexander Jesudasan (Madras Christian College, Chennai) is supervising a graduate student who is surveying the mite in five locations in Tamil Nadu, Kerala and Andra Pradesh. The climate in these locations is quite different from each other, with Kerala being much wetter than Tamil Nadu or Andra Pradesh. Summer temperatures are higher in India than Florida, which will help us understand the upper thermal limit of *F. perrepae* activity. In Indonesia, Dr Roch Desmier de Chenon is supervising staff from the Indonesian Oil Palm Research Station in Marihut, Sumatra, to survey the mite in the local area. In this area of Sumatra, *L. microphyllum* grows in a cline from the lowland tropical rainforests up to high altitude cool tropical mist forests. This dramatic difference in altitude provides unique opportunities to document the seasonal phenology of the mite. All of the data gathered in the collaborative overseas studies, including those in QLD, will be combined to determine if each genotype responds differently to climatic forces such as temperature, rainfall, and humidity. This multi-continent study will help us select the best-adapted genotype for release in subtropical south Florida.

***Floracarus* Impact Studies.** Field populations of *F. perrepae* cause considerable damage to *L. microphyllum*. However, in field settings it is difficult to quantify the effect of the mite. Mite damage, which includes induction of leaf curling and deformation of growing tips, leads to necrosis and early senescence of subpinnae, and a reduction in photosynthetic ability. A field experiment was designed to measure the impact of this subtle debilitation of the plant over time in terms of biomass production.

In the experiment, one half of the *L. microphyllum* AU plants are treated with Vertimec® miticide to control the *Floracarus* mites. The other half is untreated, allowing mites to reach natural densities. By measuring the difference in biomass production between the two treatments it is possible to measure the impact of the mite. Each quarter, four pairs of plants are removed, washed clean of soil, dried, and weighed (Fig. 21). The plot has 64 plants, allowing us to harvest four pairs of plants each quarter, for two years. On a monthly basis, we are measuring other indicators of plant health, including the longevity of pinnae and numbers of infested subpinnae.

The *F. perrepae* impact study has run for 21 months and is nearing completion in March 2003 (Fig. 22). All seven harvests of plants from the impact study have shown significant differences in biomass production (Fig. 23). The untreated plants with *F. perrepae* have approximately 50% less biomass than the treated mite-free plants. In the final two harvests (June & Sept 2002) the treated plants have maintained similar levels of biomass. This may be due to the confining 'bonsai' type of effect of the pots. The roots of most of the plants have now completely filled the pots. However, the effect of the mite on growth of the plant is readily apparent. Mite infested plants have grown very slowly and only recently reached the top of the trellis. The treated plants reached the tops of the trellis at the end of 12 months. Longevity of the pinnae is also significantly different with treated pinnae living 9 months as compared to 3 months in untreated plants.

Digital imaging of the plants (Fig. 24) was used to estimate biomass (Fig. 25).

The impact study also provided valuable insights into the host-range of *F. perrepae*. Attack was not observed on plants added nearby the plot: *L. japonicum*, *L. flexuosum* or *L. reticulatum*. In addition, only minor attack was measured on the Florida genotype of *L. microphyllum*. Leaf curls of the FL type *L. microphyllum*, also added to the plot, had significantly fewer *F. perrepae* eggs, nymphs and adults. Because of this, all FL genotype plants were removed from the field plot before an abundance of spore bearing fertile pinnae were produced. After the impact study concludes, we intend to reconfigure the plot into a randomized block design with four types of *Lygodium* spp. to observe the field host-range of *F. perrepae* and other herbivores over the long term.

Fig. 21. Berea College interns, Lara Vallely and Andrew Oles, provided valuable assistance with the *F. perrepae* impact studies.

Fig. 22. *Floracarus perrepae* impact studies are nearly completed. The two-year study will be completed in March 2003. In the background, Ryan Zonneveld prepares *L. microphyllum* plants for harvest and measurement of dry weight.

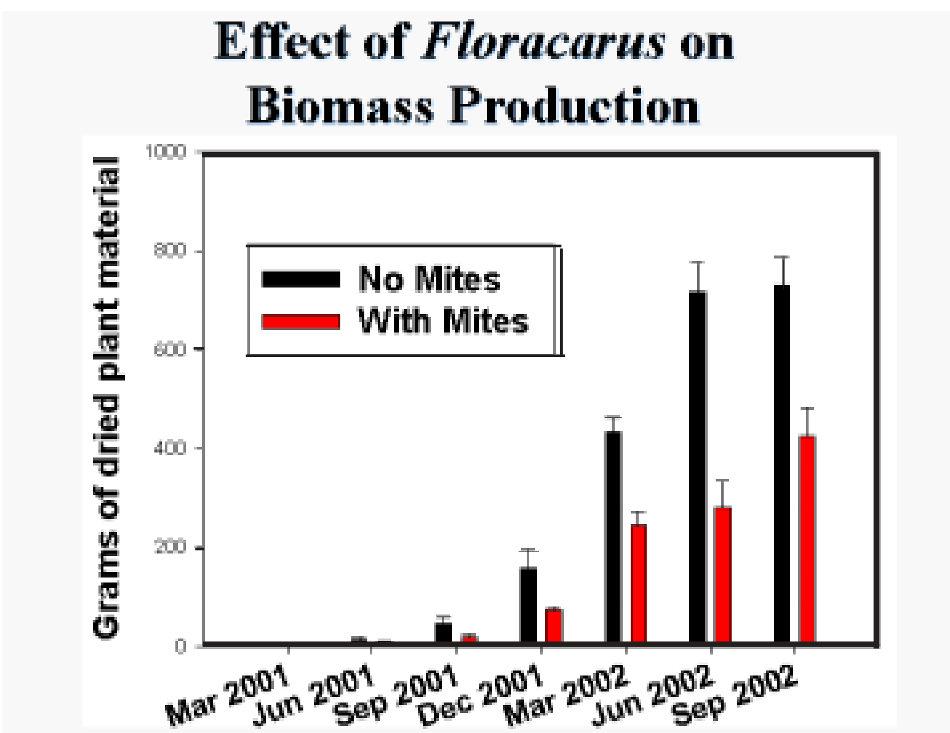


Fig. 23. Plants with *F. perrepae* mites have less than 50% biomass as compared to the treated 'mite free' plants.

Digital Images to Estimate Biomass

Fig. 24. SigmaScan[R] is used to count numbers of green … *microphyllum* plant. The program converts the green dots to red and ca… tructive sampling method correlates well with the actual measurements of …

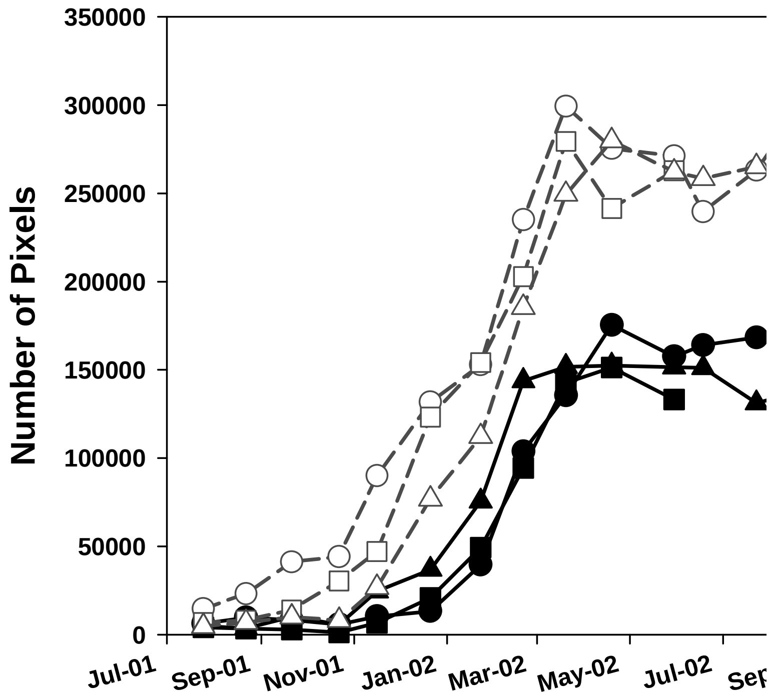


Fig. 25. Lines in **DASHED RED** plot the number of pixels from mite free plants. Lines in **SOLID BLACK** are untreated plants infested with *F. perrepae*.

Leaf Feeding Moth - *Cataclysta camptozonale* (Lepidoptera: Pyralidae) (Exported to Florida)

Dr Alma Solis (ARS SEL, Washington, D.C.) and Dr Shen-Horn Yen (British Museum of Natural History, London, UK) have recently revised the genera in the subfamily of Musotominae. *Cataclysta camptozonale* (inset left) will be moved into a new genus created for Australian fern feeding Musotominae. The new genus name should be published in 2003. *Cataclysta camptozonale* will be split into two species, one distributed in southeast Queensland (SQ) and another with a distribution which extends from north Queensland (NQ) to New Guinea and the Malaccas.

Populations of *C. camptozonale* were monitored monthly at four locations near Brisbane. Populations remained below detectable levels throughout 2002. The drought may have had an impact on the prevalence of this species.

Cataclysta camptozonale is narrowly specific, feeding and developing on a small subset of *Lygodium* species. For more information on this species see reports by G. Buckingham (USDA-ARS, Gainesville). Host-range testing has been completed and a petition to USDA-APHIS-TAG is forthcoming.

Leaf Feeding Moth – *Neomusotima conspurcatalis* (Lepidoptera: Pyralidae) (Exported to Florida)

Neomusotima conspurcatalis (inset left) was shipped to quarantine facilities in Gainesville in August 2002. Colonies have been established and final host-range testing is nearly complete. Preliminary host-range testing at ABCL indicated that it was a genus level specialist. Dr Gary Buckingham and Chris Bennett will test additional Florida native ferns and *Lygodium* species collected in the Caribbean and South America. The population of the moth shipped to Florida was originally collected in Litchfield National Park in the Northern Territory (NT), Australia, and from Kununurra, Western Australia (WA).

The distribution of this species includes, Australia (NQ, NT, WA), China, Indonesia, Malaysia, Singapore, Thailand, and possibly southern India.

Leaf Feeding Moth – *Musotima* sp. (Lepidoptera: Pyralidae)

In their revision of the Musotiminae, which should be published in 2003, Dr Alma Solis and Dr Shen-Horn Yen describe this moth as a new species. *Musotima* sp. has been collected in the lowland tropical parts of Malaysia and Thailand.

Leaf Feeding Moth – (Lepidoptera: Pyralidae)

Drs John Goolsby and Robert Pemberton collected this moth (inset left) on *L. microphyllum* in New Caledonia, in Province Sud near the capital, Noumeá. New Caledonia is an island 900 miles to the northeast of Brisbane and is an overseas territory of France.

The identity of this species is unknown. Specimens have been forwarded to Dr Alma Solis (SEL) for identification and description. Dr Solis has identified this species as a member of the Pyraustinae subfamily of Pyralidae. The pyralid species listed above are in the Musotominae subfamily.

The larvae feed on *L. microphyllum,* skeletonizing foliage as early instars and consuming entire pinnules as they mature. Larvae are nocturnal, pupating on the foliage. We did not observe the larvae on any other fern species in the *Melaleuca quinquenervia* swamp in which it was found, which may indicate that it is a specialist. At one location near Plum the entire patch of *L. microphyllum* (1 square kilometer) was heavily damaged. This may have been the largest single defoliation event we have observed in all of the exploration efforts.

We planned to conduct preliminary host-range tests on this species in 2002, however despite repeated attempts we were unable to establish a colony in quarantine.

Leaf Feeding Moths - *Callopistria* spp. A (QLD & China), B (NT & WA), & C (Thailand)

Callopistria sp. A (Noctuidae) was collected from *L. microphyllum* near Cairns in tropical NQ. As the larvae mature they consume whole leaves. *Callopistria* sp. B has been collected from the Kimberly Region near Kununurra in WA and from Litchfield National Park in NT. *Callopistria* sp. C has been collected in southern Thailand. Colonies of *Callopistria* spp. (inset left) have been terminated in order to shift resources to higher priority agents. Several larvae were collected from *L. microphyllum* in southern India. Adults will be forwarded to ABCL for genetic characterization. Representatives of all three species have been forwarded to The British Museum of Natural History for identification.

Lygodium Sawfly – *Neostromboceros albicomus* (Hymenoptera: Tenthridinidae)

Although the Lygodium sawfly was collected in Singapore early last century, we have been unable to locate it recently and efforts are continuing. However the insect has been found at sites in peninsula Malaysia, Vietnam and it is common in Thailand. Further research on this insect will therefore center on Thailand, and aim to learn if genetic diversity is necessary for establishing a viable breeding population.

Neostromboceros albicomus occurs on both *L. microphyllum* and *L. flexuosum* (Fig. 26) in Thailand and testing indicates a small genetic difference between insects from the different plants.

Fig. 26. Sawfly eggs on *L. flexuosum* shoot.

Stem-Borer (Lepidoptera: Pyralidae)

Larvae and pupae of the stem-boring pyralid have been collected from *L. microphyllum* in Singapore (Fig. 27) and *L. flexuosum* in Thailand. Comparative molecular D2 sequences are considerably different, indicating a likely two species. Although we have not found it again recently in Singapore, several successful collections this year in northern Thailand have enabled us to attempt rearing in paper straws filled with artificial diet. Adults of the Thai moth have white wings with dark lines resembling the legs of a spider, which it may mimic. Taxonomic studies of the moth, tentatively called *Ambia* new species, are currently underway at the British Museum.

Research in Thailand. In conjunction with Dr Amporn Winotai, our collaborators in Chiang Mai, Mrs. Usanee Chattrakul and Ms Amara Thagong, have visited field sites weekly and investigated rearing of the stem-borer in the laboratory.

In the field, larvae tunnel in *Lygodium* stems close to or below ground level (Fig. 28), including in the rhizome, causing death of the shoot (larvae found in stems more than 1m above ground level were all dead). It appears more larvae are found below ground level during the dry season. Larvae exit stems (Fig. 29) and pupate on the stem (Fig. 30). Pupae are always above ground level, not far from the emergence hole. Adults are active at night and are attracted to light. Larval duration is unknown.

In the laboratory, the stem-borer can be reared to adult in artificial diet (see Appendix 2 for preparation of this diet). Field-collected larvae are cut from the stems and placed into paper straws filled with diet (Fig. 31). The larva will feed within the straw, then emerge and pupate on it, as occurs in the field (Fig. 32). Further research is concentrating on obtaining enough pupae so that several adults are available at the same time for mating and oviposition. Because larvae in the field vary greatly in age and the pupation period in diet varies from 6-8 days, many larvae must be collected in order to obtain several adults at the same time.

Fig. 27. The very small *L. microphyllum* plant at Sungei Buloh, Singapore, on which was found a stem-borer pupa.

Fig. 28. Field, stem-borer larva in stem.

Fig. 29. Field, larval emergence hole.

Fig. 30. Field, pupa.

Fig. 31. Lab, artificial stems.

Fig. 32. Lab, pupa.

Voucher Specimens

Plant voucher specimens of *Lygodium microphyllum* from New Caledonia were lodged with the Queensland Herbarium. Dr Peter Bostock, fern specialist and Senior Botanist at the Queensland Herbarium, Brisbane, identified all specimens.

Voucher specimens of *L. microphyllum* and *L. reticulatum* collected in New Caledonia were deposited with Dr Tongi Faman, (IRD, Noumeá). Insect voucher specimens were deposited with Dr Jean Chazeau, (IRD, Noumeá).

Vouchers deposited at the Raffles Museum of Biodiversity Research, Singapore include specimens of *Neomusotima conspurcatalis* (collected Sumatra, Indonesia) and *Endelus bakerianus* (with determination labels from Dr Svatopluk Bílý). *Endelus bakerianus* specimens were also deposited with the Department of Agriculture, Thailand. Some of these specimens also bear determination labels from Dr Svatopluk Bílý.

Research Plans for 2003

Our research plans for 2003 include additional foreign exploration, biological studies of agents, and host-range testing. Exploration in Asia will focus on Thailand, Sri Lanka and India. Biological studies will be undertaken on the sawfly *N. albicomus* in Bangkok, Thailand, probably necessitating the employment of an additional collaborator.

We will also revisit Singapore and Thailand to recover and culture the stem-boring moth. We will investigate the possibility of collaborating, on a full or part-time basis, in research on biological studies of the stem-borer.

Additional methods will be tested and developed for culturing these agents. In Australia, new parts of the range of *L. microphyllum* in NQ, near the Iron Range, will be explored. The fauna of Iron Range, at the tip of Cape York, is similar to New Guinea. Host-range testing of the mite, *Floracarus perrepae,* will begin in 2003.

Biological Control of Hydrilla, *Hydrilla verticillata*

Hydrilla, *Hydrilla verticillata* (Hydrocharitaceae) (inset left), was first introduced into the United States through the aquarium trade in the early 1950's and since that time it has greatly expanded its range from Florida to Delaware on the East Coast and westward to Texas and California (Steward and Van 1987). Current control measures are very expensive and economic losses are excessive. In south Texas during drought, hydrilla infestations clog the Rio Grande River impeding water flow and distribution to cities and farms. Hydrilla is present in the U.S. in both the monoecious and dioecious biotypes, probably as a result of two separate introductions. The dioecious biotype has been found in 13 states (Steward et al. 1984).

The origin of hydrilla is unclear, but genetic evidence indicates that monoecious hydrilla closely matches material from Korea, and the more prevalent dioecious type is closely related to material from Bangalore, India, however literature records indicate Sri Lanka as the origin (Madeira et al. 2000)

Management of hydrilla through chemical and mechanical control is ineffective in the long term, environmentally damaging, and costly. Biological control using aquatic invertebrates is considered to be the safest, most cost-effective and sustainable long-term solution to controlling hydrilla. However, biological control of hydrilla has not yet been realized with the existing agents that have been found during extensive worldwide surveys. Global surveys were undertaken to compile lists of the natural enemies of hydrilla throughout its native range. Foreign scientists were contracted to conduct most searches in conjunction with overseas trips by US scientists. Surveys of northern and eastern Australia (1984-1988), China (1989 and early 1990's), eastern Africa (1976, 1981-1984), India (late 1960's, 1982) and Pakistan (1971-1976) were extensive, though trips to Panama (late 1970's, 1980), the Philippines (1982) and Southeast Asia - including Indonesia (1982), Malaysia (early 1970's and 1982), Thailand (1982 and 1996) and Vietnam (1996) - involved only brief surveys. Many phytophagous insects were found during these surveys, though few were selected as potential agents due to their specificity, availability and impact. Two *Bagous* weevils (Coleoptera: Curculionidae) and two *Hydrellia* flies (Diptera: Ephydridae) were released in the U.S. but have either not established or have had limited impact on the growth of hydrilla. New agents are needed.

Additional foreign exploration is needed to find new agents that are adapted to the range of environments where hydrilla is invasive, in particular, constant level rivers and lakes. After small surveys in southern Thailand in 1999, ABCL intended to canvas co-operators across the US to develop new support for exploration in Southeast Asia. In July 2001, a meeting (phone link) was held between parties interested in the further control of hydrilla. Representatives from the USDA-ARS in Beltsville, Tucson, Fort Lauderdale and Brisbane, as well as from the US Army Corps of Engineers, U.S. Bureau of Reclamation, and the Lower Rio Grande Valley Development Council, participated in the meeting. After an update by all participants about the current status of hydrilla and its biocontrol agents, it was generally agreed that more research was needed and the go-ahead was given for ABCL to conduct preliminary exploration in the poorly surveyed areas of Southeast Asia and Western Australia.

Thailand

After preliminary surveys in 2001 in Indonesia, Malaysia, Thailand and Singapore a follow up survey was undertaken in Thailand in May 2002. The main purpose of this trip was to finalize a collaborative program with the Royal Irrigation Department (RID) at Prachinburi near Bangkok. Dr Manop Siriworakul (inset left) agreed to supervise surveys of hydrilla at a number of field sites on a monthly basis. They have made berlese funnels for processing large amounts of hydrilla.

During surveys in May, *Bagous* weevils were collected from two sites east of Bangkok, at Prapong Reservoir, Sakaeo Province, and at Lam Taklong Dam, Nakhon Ratchasima Province. These insects will be forwarded to Doctor Charles O'Brien at the Florida A&M for identification. Dr O'Brien already has three weevil species collected in Thailand during 2001. Small amounts of hydrilla were observed in many natural locations (inset right), very distinctive from its introduced range in the US. These areas will be targeted in future surveys. *Hydrellia* flies, chironomids and nymphuline moths were also collected and will be investigated further.

A second trip to Thailand was conducted in September. Matthew Purcell instructed Dr Siriworakul on the most efficient use of berlese funnels to ensure maximum extraction of herbivorous insects. Sites were visited in the north and northeast of Bangkok. Small amounts of hydrilla were located in Phayao Lake near Chang Rai. At Nong Han Dam (inset left and below) at Sakon Nakhon small amounts of hydrilla were growing amongst many other aquatic plant species. Only in one area where mechanical harvesters constantly remove hydrilla does this plant form dense mats. This site will be a priority for further collections. *Bagous* weevils were also collected from shore material at Lam Pao Dam. Heavy rains at this site raised the water level significantly, and no submerged hydrilla could be found.

The moth specimens collected from the RID in Thailand during 2001 were identified by Dr Alma Solis (SEL) as *Parapoynx diminutalis*, the moth commonly found on hydrilla in many places throughout its native range. This moth was also present on hydrilla infestations in Florida, though the source of introduction is unknown.

Indonesia

Throughout 2002, surveys were also being conducted in North Sumatra, Indonesia. Dr Roch Desmier DeChenon (formerly with CIRAD) at the Marihut Research Station has kindly assisted in arranging and supervising a student to work on hydrilla in this region (inset left). They have reared at least four nymphuline aquatic moth species and *Hydrellia* leaf-mining flies. The most common moth species present in North Sumatra in the rivers where collecting has occurred is *P. diminutalis*.

Matthew Purcell visited Sumatra in August to finalize the research protocols for further exploration, to visit new field sites, and retrieve the reared moth specimens for identification on return to Australia. He also trained local staff in the identification of aquatic insect herbivores. Sites were visited at high elevation on a volcanic plateau but no hydrilla could be located

(inset far left). Flowing streams near the Marihut Research Station contained small amounts of hydrilla (inset left) and collections were made from several sites.

Dr DeChenon and Matthew also traveled to Palau Burung in central South Sumatra where there were reports of hydrilla growing in a large coconut plantation on peat soils. This proved to be a misidentification as the ph was very low (3.9) and the only submerged weed found was *Utricularia* sp.

Representatives of all of the nymphuline moth species from Indonesia were sent to the CSIRO ANIC in Canberra and to the SEL in Washington for identification.

The moths were identified by the ANIC as follows: -

Species 1 is thought to be *Paracymoriza vagalis*. There does not appear to be any host information for the species, though the genus is known to feed on Podostemaceae. This species is particularly damaging in the rivers near Marihut, occasionally found in large numbers that cause extensive defoliation of hydrilla. This moth is in culture at Marihut and biology studies are underway.

Species 2 is *Parapoynx diminutalis*, which is already present in the United States, a generalist feeder.

Species 3 is *P. polydectalis*. There were many specimens (55) in the ANIC collection from Australia, so it has a widespread distribution. This species has been collected from tropical north Queensland and the Northern Territory right through to Victoria in southern Australia. Interestingly one specimen was reared from a larva on *Ludwidgea plepoides*, an emergent aquatic plant, indicating that its host range may be broad.

Species 4 is *P. villidalis* (= *Nymphula villidalis*), which has also been collected in Australia. There were 12 specimens in the ANIC collection, collected from Cairns in north Queensland through to central New South Wales. There were no host records on the labels.

The above identifications by ANIC were performed visually and the genitalia were not examined. If they are correct, then the *Parapoynx* species certainly have widespread distribution.

Dr DeChenon will check further still water sites in the region to verify if aquatic weevil species are present on hydrilla in the region.

Singapore

At least two species of nymphuline moth larvae were collected from Sungei Buloh Nature Reserve (inset left) during surveys conducted in conjunction with the trips to Thailand in May and September. Unfortunately we have no permits to import hydrilla insects into Australia and the larvae had to be preserved and could not be reared for identification. Collections were also conducted at the Singapore Botanic Gardens though few insects were extracted from this material. We suspect that this site may be frequently sprayed with insecticide for mosquito control. MacRitchie Reservoir and the Chinese Gardens were also visited, but no hydrilla could be found. During 2003, other reservoir sites will be explored to find additional hydrilla collection locations.

Vietnam

Dr Solis (SEL) also identified the nymphuline moth specimens that were collected from Vietnam in 1996. Two of the specimens were *P. diminutalis*, while two others were *Elophila orientalis*. The larvae had portable cases and severely damaged the stems of hydrilla. The life cycle of this moth in the field is unknown. However, larvae observed in Japan had cases made of *Phragmites* sp. and reared adults oviposited on *Potamogeton* sp. and the resultant larvae developed into adults (Yoshiyasu 1985).

Future Plans

During 2003, we will apply to Environment Australia and the Australian Quarantine Inspection Service to permit us to import hydrilla insects into Australia from Southeast Asia. Cultures of the weevils found in Thailand and moths from Indonesia will be established to provide insects for ABCL quarantine facility if permits are received.

We will continue field surveys throughout the region and import new insects into Australian quarantine for further evaluation. Insects with a fully aquatic lifecycle will be a priority.

Prospects for Biological Control of Downy Rose Myrtle, *Rhodomyrtus tomentosa*

The twelve-month project to review the plant distribution and herbivores on Downy Rose Myrtle in its native range in Thailand was completed in 2002 by Amporn Winotai of the Department of Agriculture, Bangkok, in collaboration with Tony Wright (Fig. 33). Although conditions during the period were often not favorable for plant growth and development of insect populations, much information on the plant and insect herbivores was collected, which should provide baseline information for a future biological control project on this weed.

One promising candidate is the leaf-feeding noctuid moth *Carea varipes* Walker (Fig. 34). It is widely distributed in Thailand and was not found on other plant species. It is also intermittently common on *R. tomentosa* in Hong Kong (Fig. 35). A second small moth species, as yet unidentified, is also widespread and the striped larvae are very active borers of fruit and young buds (Fig. 36).

For further information, the full report of the project is included as Appendix 3.

Fig. 33. Left, Amporn Winotai photographing herbivore damage on *R. tomentosa*. Right, Tony Wright with helpful landowners in southern Thailand.

Fig. 34. Larva of *Carea varipes.*

Fig. 35. *Rhodomyrtus tomentosa* on a hillside in HK's New Territories.

Fig. 36. Unknown larva boring in fruit of *R. tomentosa.*

Prospects for Biological Control of Carrotwood, *Cupaniopsis anacardioides*

Cupaniopsis anacardioides has invaded 14 southern and central counties in Florida (Lockhart et al. 1999). It was first recognized as a serious weed in 1989 about ten years after it became popular as an ornamental (Lockhart et al. 1999). In the late 1980's seed production was observed and it spread into natural areas, replacing native vegetation in variety of habitats (Oliver 1992). Birds spread the seed and are responsible for its rapid spread.

During 2002 only two collections were made on carrotwood in Queensland. Young foliage on ornamental plants at ABCL was attacked by a small weevil and moth larvae (Fig. 37). Adults of both have been curated and will be sent to the CSIRO ANIC for identification.

Fig. 37. Undetermined Lepidopteran larva feeding on young leaves of *C. anacardioides.*

Exploration in Hong Kong for Natural Enemies of the Formosan Subterranean Termite

The Formosan subterranean termite (FST), *Coptotermes formosanus* Shiraki, is thought to be native to East Asia, and was first discovered in the U.S. in 1965. Where it occurs, *C. formosanus* is the most destructive subterranean termite in the world. The present distribution of FST in the contiguous U.S. extends in the west to California, east to Florida, and northeast to North Carolina. The FST costs consumers over $1 billion/year, including repairs and termite control treatment. No parasites, pathogens, or predators of FST that provide population reduction or control are known in the U.S. or in the Far East. Our efforts are aimed at discovery, importation and establishment of one or more natural enemies to significantly reduce populations. This includes exploring known available agents and new indigenous organisms. Biological control organisms could provide sustained termite population reduction. Research is required to develop new microbes for improving baits as well as soil and wood treatments.

Following initial contact with Hong Kong Universities, an MPhil project was established at City University, Kowloon and research commenced by the selected student Ms Azura Tsang Wing Sze, supervised by Associate Professor Desmond K. O'Toole, Department of Biology and Chemistry. Drs Alan Kirk and Guy Mercadier, EBCL Montpellier, are providing valuable additional scientific and technical guidance to Ms Tsang as well as investigating their own samples at Montpellier (Fig. 38). As well as liaising with Ms Tsang and Dr O'Toole (four visits during 2002), ABCL is providing the DNA characterization services of CSIRO Canberra to compare termites collected in Hong Kong with known termite species recorded in GenBank. Discussions with the Hong Kong Agriculture, Fisheries and Conservation Department (AFCD) are underway in order to use AFCD facilities in the New Territories for holding colonies of FST under protected conditions, since there are restrictions on holding known serious pests such as FST on the University campus.

The project centers on the Special Economic Zone of Hong Kong including the New Territories and neighboring Hong Kong islands. Field research will provide an opportunity to study FST in relatively undisturbed natural settings as well as in the laboratory. Key components of the study include:

- screening termites for bacteria and entomopathogenic fungi by plating onto agar media (the natural flora of healthy termites is a part of this survey)
- field collection of termites encountered during field trips and from traps containing wood or paper baits at selected field sites
- rearing and identification of Diptera (possibly phorid flies parasitising termite soldiers) that offer interesting biological control potential.

Field collection of termites revealed some with a hard disk-like structure, possibly of bacterial origin and this is being investigated in the laboratory and by electron microscopy. Contamination of termites by mites is a continuing problem and has made some results of plating unclear as to the origin of pathogens developing on the media. In addition, surface treatment of the termites to kill mites affects the sensitive fungi associated with the termites. Few parasitised termites have been found in the field so far, however Ms Tsang has managed to film, probably for the first time, a full-grown larva leaving its host soldier.

Field sites for the study have been selected to reflect a range of environmental conditions and include Tai Po Kau and Tseun Wan (New Territories), Tai Tam and Pok Fu Lam (Hong Kong Is.), Lamma Is. and Lantau Is.

Alan, Azura & Guy on Lantau Island look for termites.

Azura handling agar plates in sterile cabinet.

Termite trap on forest floor at Pok Fu Lam.

Des O'Toole, Guy Mercadier, Azura Tsang and Alan Kirk at the City University laboratories, Hong Kong.

Fig. 38. Field and laboratory research on Formosan subterranean termite is being conducted in Hong Kong.

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Appendix 1: 2002 ABCL Field Explorations

HOST CODES

?cashew	? cashew
Alfalfa	alfalfa
ANON	No host searched
Asp	*Acacia sp.*
CEde	*Ceratophyllum demersum*
clover	clover
CPan	*Cupaniopsis anacardioides*
Csp	*Callistemon* sp.
Esp	*Eucalyptus* sp.
forest	Forest floor - termites
Gau	*Gossypium australe*
Hrs	*Hibiscus rosa-sinensis*
HYvr	*Hydrilla verticillata*
Lfl	*Lygodium flexuosum*
Ljp	*Lygodium japonicum*
Lmc	*Lygodium microphyllum*
Lpy	*Lygodium polystachyum*
Lrt	*Lygodium reticulatum*
Lsp	*Lygodium* sp.
LPpg	*Leptospermum polygalifolium*
LPsp	*Leptospermum* sp.
Mar	*Melaleuca argentea*
Mcj	*Melaleuca cajuputi*
Mdl	*Melaleuca dealbata*
Mfl	*Melaleuca fluviatilis*
Mlb	*Melaleuca leucadendra*
Mnv	*Melaleuca nervosa*
Mqn	*Melaleuca quinquenervia*
Msp	*Melaleuca* sp.
Mst	*Melaleuca stenostachya*
Mvr	*Melaleuca viridiflora*
Nsp	*Nephrolepis sp.*
Pfo	*Paederia foetida*
Psp	*Paederia* sp.
RHto	*Rhodomyrtus tomentosa*
SAsb	*Sapium sabiferum*
SVsp	*Scaevola* sp.
UNDET	Undet. plant

Collection #	Date	Host	Site	State/Province	Country
2002001	23-Jan-02	Mqn	Landsborough State Forest	QLD	Australia
2002002	23-Jan-02	HYvr	Traveston Crossing, Mary River	QLD	Australia
2002003	23-Jan-02	Mqn	Peregian Environmental Park	QLD	Australia
2002004	23-Jan-02	Mqn	Marcoola	QLD	Australia
2002005	31-Jan-02	Mqn	Nudgee Nature Reserve	QLD	Australia
2002006	31-Jan-02	Mqn	Nudgee Nature Reserve	QLD	Australia
2002007	4-Feb-02	Mqn	Roy's Road	QLD	Australia
2002008	4-Feb-02	Mqn	Ewan Maddock Dam	QLD	Australia
2002009	4-Feb-02	LPpg	Racecourse Rd	QLD	Australia
2002010	4-Feb-02	Mqn	Peregian Environmental Park	QLD	Australia
2002011	4-Feb-02	Mqn	Marcoola	QLD	Australia
2002012	4-Feb-02	Mqn	Marcoola	QLD	Australia
2002013	4-Feb-02	Mqn	Roy's Road	QLD	Australia
2002014	12-Feb-02	Mqn	Casuarina Beach	NSW	Australia
2002015	12-Feb-02	Mqn	Pottsville	NSW	Australia
2002016	12-Feb-02	Mqn	Pottsville, cnr Overalls and Taylor	NSW	Australia
2002017	6-Feb-02	Mqn	Pacific HWY	QLD	Australia
2002018	6-Feb-02	Mvr	Pacific HWY	QLD	Australia
2002019	6-Feb-02	Mvr	Pacific HWY	QLD	Australia

2002020	19-Feb-02	Mqn	Noosa-Eumundi Rd	QLD	Australia
2002021	19-Feb-02	Mqn	Silverwood Drive, Site 2	QLD	Australia
2002022	19-Feb-02	Mqn	Tewantin-Boreen Pt Rd	QLD	Australia
2002023	19-Feb-02	Mqn	Harrys Hut Rd	QLD	Australia
2002024	19-Feb-02	Mqn	Harrys Hut Rd, 0.4km from HH	QLD	Australia
2002025	19-Feb-02	LPsp	Cooloolah Way Rd	QLD	Australia
2002026	19-Feb-02	Mqn	Cooloola Way	QLD	Australia
2002027	22-Feb-02	Msp	Silverwood Drive, Site 2	QLD	Australia
2002028	6-Feb-02	Mlb	Centenary Lakes	QLD	Australia
2002029	4-Mar-02	Mqn	Hawthorne Park	QLD	Australia
2002030	4-Mar-02	Mqn	Morningside Park	QLD	Australia
2002031	5-Mar-02	Mqn	Nudgee Nature Reserve	QLD	Australia
2002032	5-Mar-02	Mqn	Nudgee Nature Reserve	QLD	Australia
2002033	12-Mar-02	Mqn	Roy’s Road	QLD	Australia
2002034	12-Mar-02	Mqn	Roys Road, East of HWY	QLD	Australia
2002035	12-Mar-02	Mqn	Roys Road, East of HWY	QLD	Australia
2002036	12-Mar-02	Mqn	Roys Road, East of HWY	QLD	Australia
2002037	12-Mar-02	Mqn	Peregian Environmental Park	QLD	Australia
2002038	12-Mar-02	Mqn	David Low Way, cnr Hewitt St	QLD	Australia
2002039	12-Mar-02	Mqn	David Low Way	QLD	Australia
2002040	12-Mar-02	Mqn	Mt. Coolum Chemist	QLD	Australia
2002041	12-Mar-02	Mqn	Tamarindus St, David Low Way	QLD	Australia
2002042	9-Apr-02	Mqn	Morningside Park	QLD	Australia
2002043	16-Apr-02	Mqn	Casuarina Beach	NSW	Australia
2002044	16-Apr-02	Mqn	Beech Drive Bike Path	NSW	Australia
2002045	16-Apr-02	Mqn	Tabbimobile Creek overflow	NSW	Australia
2002046	14-Apr-02	HYvr	Alipou Creek	NSW	Australia
2002047	23-Apr-02	Mqn	Casuarina Beach	NSW	Australia
2002048	30-Apr-02	Mqn	Flynn Rd, South Innisfail	QLD	Australia
2002049	30-Apr-02	Mqn	Pacific HWY	QLD	Australia
2002050	18-Mar-02	Lmc	McDowell Billabong	QLD	Australia
2002051	18-Mar-02	Mqn	Pandanus Road, Cape Trib. Rd	QLD	Australia
2002052	19-Mar-02	Mnv	Gilmore Rd.	QLD	Australia
2002053	19-Mar-02	Mnv	Agricultural College turnoff	QLD	Australia
2002054	19-Mar-02	Mvr	Agricultural College turnoff	QLD	Australia
2002055	19-Mar-02	Mvr	Lake Mitchell	QLD	Australia
2002056	19-Mar-02	Mst	Luster Creek, north Mount Molloy	QLD	Australia
2002057	19-Mar-02	Mst	Saltbag Creek	QLD	Australia
2002058	19-Mar-02	Lmc	Martyville	QLD	Australia
2002059	20-Mar-02	Mqn	Pacific HWY	QLD	Australia
2002060	20-Mar-02	Mvr	Pacific HWY	QLD	Australia
2002061	21-Mar-02	Mlb	Abbott St, Oonoonba	QLD	Australia
2002062	27-Mar-02	Mqn	CSIRO Long Pocket Labs	QLD	Australia
2002063	30-Apr-02	Mvr	Pacific HWY	QLD	Australia
2002064	30-Apr-02	Mqn	5km N Cardwell	QLD	Australia
2002065	30-Apr-02	Mvr	7km N Cardwell	QLD	Australia
2002066	30-Apr-02	Mqn	7km N Cardwell	QLD	Australia
2002067	30-Apr-02	Mvr	Pacific HWY	QLD	Australia
2002068	1-May-02	Mqn	Pacific HWY	QLD	Australia
2002069	1-May-02	Mvr	Pacific HWY	QLD	Australia
2002070	2-May-02	Mlb	Ross River Dam	QLD	Australia
2002071	7-May-02	Mfl	Ross River Dam	QLD	Australia
2002072	8-May-02	Mqn	Pacific HWY	QLD	Australia
2002073	8-May-02	Mqn	Poppies Rd	QLD	Australia
2002074	9-May-02	?cashew	Ellis Beach	QLD	Australia
2002075	9-May-02	Mcj	McDowell Billabong	QLD	Australia
2002076	9-May-02	Mqn	Pandanus Road	QLD	Australia
2002077	30-May-02	Mqn	Pacific HWY	QLD	Australia
2002078	30-May-02	Mvr	Poona National Park	QLD	Australia

2002079	30-May-02	Mqn	Peregian Environmental Park	QLD	Australia
2002080	13-May-02	HYvr	Left Main Canal	Kanchanburi	Thailand
2002081	13-May-02	HYvr	Left Main Canal	Kanchanburi	Thailand
2002082	14-May-02	HYvr	Rang Sai	Ayutthaya	Thailand
2002083	14-May-02	HYvr	Kabinburi Lake	Prachinburi	Thailand
2002084	14-May-02	HYvr	Klong Hard Reservoir	Sakaeo	Thailand
2002085	15-May-02	HYvr	Huay Chan Reservoir	Sakaeo	Thailand
2002086	15-May-02	HYvr	Prapong Reservoir	Sakaeo	Thailand
2002087	15-May-02	HYvr	Prapong Reservoir	Sakaeo	Thailand
2002088	15-May-02	HYvr	Sawannapa Reservoir	Surin	Thailand
2002089	15-May-02	HYvr	Huay Saneng Borrow Pond	Surin	Thailand
2002090	16-May-02	HYvr	Huay Chojorakae Mac Reservoir	Buri Rom	Thailand
2002091	17-May-02	HYvr	Lam Taklong Dam	Nakhon Ratchasima	Thailand
2002092	18-May-02	HYvr	Sungai Buloh Nature Park		Singapore
2002093	18-May-02	HYvr	Sungai Buloh Nature Park		Singapore
2002094	17-May-02	HYvr	Lam Taklong Dam	Nakhon Ratchasima	Thailand
2002095	6-Jun-02	Mqn	Brown Lake	QLD	Australia
2002096	6-Jun-02	Mqn	18 Mile Swamp	QLD	Australia
2002097	6-Jun-02	Mqn	Blue Lake Beach	QLD	Australia
2002098	6-Jun-02	LPsp	Keyholes	QLD	Australia
2002099	6-Jun-02	Mqn	Keyholes	QLD	Australia
2002100	6-Jun-02	Mqn	Adder Rock	QLD	Australia
2002101	6-Jun-02	Asp	William Creek Drive, Amity Point	QLD	Australia
2002102	12-Jun-02	Mqn	CSIRO Long Pocket Labs	QLD	Australia
2002103	14-Jun-02	Mqn	Nudgee Nature Reserve	QLD	Australia
2002104	14-Jun-02	Mqn	Nudgee Nature Reserve	QLD	Australia
2002105	19-Jun-02	Mqn	Roy’s Road	QLD	Australia
2002106	19-Jun-02	Mqn	Roys Road, East of HWY	QLD	Australia
2002107	19-Jun-02	Mqn	Roys Road, East of HWY	QLD	Australia
2002108	19-Jun-02	Mqn	Quinten Rd	QLD	Australia
2002109	19-Jun-02	Mqn	Peregian Environmental Park	QLD	Australia
2002110	19-Jun-02	Esp	Mount Coolum Village	QLD	Australia
2002111	19-Jun-02	Asp	Mount Coolum Village	QLD	Australia
2002112	19-Jun-02	Mqn	Mount Coolum Village	QLD	Australia
2002113	26-Jun-02	Mqn	Roys Road, East of HWY	QLD	Australia
2002114	26-Jun-02	Mqn	Roys Road, East of HWY	QLD	Australia
2002115	26-Jun-02	Mqn	Peregian Environmental Park	QLD	Australia
2002116	26-Jun-02	Mqn	Roy’s Road	QLD	Australia
2002117	2-Jul-02	Mqn	Sherwood	QLD	Australia
2002118	3-Jul-02	Mqn	Nudgee Nature Reserve	QLD	Australia
2002119	3-Jul-02	Mqn	Nudgee Nature Reserve	QLD	Australia
2002120	3-Jul-02	Mqn	Nudgee Nature Reserve	QLD	Australia
2002121	25-Jun-02	Mlb	Archer River, Iron Range Rd	QLD	Australia
2002122	25-Jun-02	Mvr	Headland North	QLD	Australia
2002123	26-Jun-02	Mcj	Chilli Beach Causeway	QLD	Australia
2002124	26-Jun-02	UNDET	Archer River-Iron Range Rd	QLD	Australia
2002125	26-Jun-02	Mqn	Archer River - Iron Range Rd	QLD	Australia
2002126	26-Jun-02	Msp	Archer River - Iron Range Rd	QLD	Australia
2002127	27-Jun-02	Msp	Coen - Archer River, Penninsula	QLD	Australia
2002128	24-Jun-02	Msp	North of Laura (Development Rd)	QLD	Australia
2002129	15-Jul-02	Mqn	Sherwood	QLD	Australia
2002130	16-Jul-02	Mqn	1km South of Roys Rd	QLD	Australia
2002131	16-Jul-02	Mqn	Peregian Environmental Park	QLD	Australia
2002132	16-Jul-02	Mqn	Havana Rd West, Peregian Beach	QLD	Australia
2002133	1-Jul-02	Mqn	la Coulee	Sud	New Caledonia
2002134	10-Jul-02	Mqn	la Coulee	Sud	New Caledonia

2002135	15-Jul-02	Mqn	Quen-Toro	Province Sud	New Caledonia
2002136	22-Jul-02	Msp	Pinnacle Village Holiday Park	QLD	Australia
2002137	22-Jul-02	Mcj	Mick Dow Way, East Daintree	QLD	Australia
2002138	22-Jul-02	Mlb	Supply Rd, Centenary Park	QLD	Australia
2002139	22-Jul-02	Mqn	Flynn Rd, South Innisfail	QLD	Australia
2002140	22-Jul-02	Mqn	Poppies Rd	QLD	Australia
2002141	22-Jul-02	Mqn	Pacific HWY	QLD	Australia
2002142	23-Jul-02	Mqn	Pacific HWY	QLD	Australia
2002143	23-Jul-02	Mvr	Pacific HWY	QLD	Australia
2002144	23-Jul-02	Mvr	Pacific HWY	QLD	Australia
2002145	23-Jul-02	Mqn	Pacific HWY	QLD	Australia
2002146	23-Jul-02	Mqn	near Merryburn Drive	QLD	Australia
2002147	24-Jul-02	Mqn	Wallace Vale Droughtmaster Stud	QLD	Australia
2002148	24-Jul-02	Mqn	Pacific HWY	QLD	Australia
2002149	24-Jul-02	Mlb	Ross River Dam	QLD	Australia
2002150	3-Aug-02	HYvr	Pabuta	North Sumatra	Indonesia
2002151	5-Aug-02	HYvr	Maning Kring	North Sumatra	Indonesia
2002152	5-Aug-02	HYvr	Bah HiLang	North Sumatra	Indonesia
2002153	5-Aug-02	HYvr	Sungei Rumah Sakit	North Sumatra	Indonesia
2002154	6-Aug-02	HYvr	Buntu Bayu Tondlian	North Sumatra	Indonesia
2002155	9-Aug-02	Lmc	Simarjarunjung	North Sumatra	Indonesia
2002156	9-Aug-02	Lmc	Sungei Tonduhan	North Sumatra	Indonesia
2002157	12-Aug-02	Lmc	Sungei Danai	North Sumatra	Indonesia
2002158	15-Aug-02	HYvr	Sungai Buloh Nature Park		Singapore
2002159	15-Aug-02	HYvr	Sungai Buloh Nature Park		Singapore
2002160	8-Aug-02	Mqn	McMahon Rd	QLD	Australia
2002161	8-Aug-02	Mqn	Tabbimobile Creek overflow	NSW	Australia
2002162	8-Aug-02	Mqn	Tyagarah	NSW	Australia
2002163	8-Aug-02	Mqn	Pottsville, cnr Overalls and Taylor	NSW	Australia
2002164	13-Aug-02	Mqn	CSIRO Long Pocket Labs	QLD	Australia
2002165	15-Aug-02	Mqn	Peregian Environmental Park	QLD	Australia
2002166	5-Aug-02	Csp	CSIRO Long Pocket Labs	QLD	Australia
2002167	29-Aug-02	Mqn	Ewan Maddock Dam	QLD	Australia
2002168	29-Aug-02	Mqn	Peregian Environmental Park	QLD	Australia
2002169	29-Aug-02	Mqn	Roys Rd, Boat Ramp	QLD	Australia
2002170	29-Aug-02	LPsp	Roys Rd, Boat Ramp	QLD	Australia
2002171	4-Sep-02	Mqn	Woodburn	NSW	Australia
2002172	4-Sep-02	Mqn	Byron Bay	NSW	Australia
2002173	4-Sep-02	Mqn	Casuarina Beach	NSW	Australia
2002174	12-Sep-02	Mqn	Nudgee Nature Reserve	QLD	Australia
2002175	12-Sep-02	Mqn	Nudgee Nature Reserve	QLD	Australia
2002176	12-Sep-02	Mqn	Nudgee Nature Reserve	QLD	Australia
2002177	30-Sep-02	Mqn	Woodburn	NSW	Australia
2002178	30-Sep-02	Csp	Woodburn	NSW	Australia
2002179	1-Oct-02	Mqn	Blue Lake Beach	QLD	Australia
2002180	3-Sep-02	HYvr	Kwan-Phayao Lake, 2km Muang	Pha-Yao	Thailand
2002181	4-Sep-02	HYvr	Lam Pao Dam	Kalasin	Thailand
2002182	5-Sep-02	HYvr	Nonq-Harn Lake, 2km Ta-Rare	Sakhon Nakhon	Thailand
2002183	8-Sep-02	HYvr	Fishery Station, 2km Muang	Sakhon-Nakhon	Thailand
2002184	5-Sep-02	HYvr	Nong-Harn Lake, 200m Muang	Sakhon-Nakhon	Thailand
2002185	1-Oct-02	Mqn	Adder Rock	QLD	Australia
2002186	9-Oct-02	Mqn	Nudgee Nature Reserve	QLD	Australia
2002187	9-Oct-02	Mqn	Ewan Maddock Dam	QLD	Australia
2002188	8-Oct-02	Mqn	Marcoola	QLD	Australia
2002189	9-Oct-02	Mqn	Delis Karissma Drive	QLD	Australia
2002190	15-Oct-02	Mqn	Cooloolah Way Rd	QLD	Australia
2002191	15-Oct-02	Mqn	Harrys Hut Rd	QLD	Australia
2002192	15-Oct-02	Mqn	200m S Illoura Place turnoff	QLD	Australia
2002193	31-Oct-02	Mqn	Airport	NSW	Australia

2002194	1-Dec-02	Mqn	street betw York and Ocean Pde	NSW	Australia
2002195	1-Nov-02	Mqn	Richmond Drive	NSW	Australia
2002196	1-Nov-02	Mqn	Arrawarra	NSW	Australia
2002197	1-Nov-02	Mqn	Tyagarah	NSW	Australia
2002198	5-Nov-02	Mqn	Ewan Maddock Dam	QLD	Australia
2002199	5-Nov-02	Mqn	Roys Road, East of HWY	QLD	Australia
2002200	7-Jan-02	Lmc	McMahon Rd	QLD	Australia
2002201	7-Jan-02	Lmc	Bottom Swamp Crossing	QLD	Australia
2002202	7-Jan-02	Lmc	Gallaghers Point	QLD	Australia
2002203	7-Jan-02	Lmc	Dux Creek	QLD	Australia
2002204	14-Jan-02	Lmc	Carbrook Creek	QLD	Australia
2002205	14-Jan-02	Lmc	Native Dog Creek	QLD	Australia
2002206	23-Jan-02	Lmc	CSIRO Long Pocket Labs	QLD	Australia
2002207	7-Feb-02	Lmc	McMahon Rd	QLD	Australia
2002208	7-Feb-02	Lmc	Bottom Swamp Crossing	QLD	Australia
2002209	7-Feb-02	Lmc	Gallaghers Point	QLD	Australia
2002210	7-Feb-02	Lmc	Dux Creek	QLD	Australia
2002211	14-Feb-02	Lmc	Carbrook Creek	QLD	Australia
2002212	14-Feb-02	Lmc	Native Dog Creek	QLD	Australia
2002213	14-Feb-02	Lmc	Deep Swamp	QLD	Australia
2002214	21-Feb-02	Lmc	CSIRO Long Pocket Labs	QLD	Australia
2002215	8-Mar-02	Lmc	McMahon Rd	QLD	Australia
2002216	8-Mar-02	Lmc	Bottom Swamp Crossing	QLD	Australia
2002217	8-Mar-02	Lmc	Gallaghers Point	QLD	Australia
2002218	8-Mar-02	Lmc	Dux Creek	QLD	Australia
2002219	14-Mar-02	Lmc	Carbrook Creek	QLD	Australia
2002220	14-Mar-02	Lmc	Native Dog Creek	QLD	Australia
2002221	21-Mar-01	Lmc	CSIRO Long Pocket Labs	QLD	Australia
2002222	8-Apr-02	Lmc	Gallaghers Point	QLD	Australia
2002223	8-Apr-02	Lmc	McMahon Rd	QLD	Australia
2002224	8-Apr-02	Lmc	Dux Creek	QLD	Australia
2002225	15-Apr-02	Lmc	Carbrook Creek	QLD	Australia
2002226	15-Apr-02	Lmc	Native Dog Creek	QLD	Australia
2002227	15-Apr-02	Lmc	Deep Swamp	QLD	Australia
2002228	22-Apr-02	Lmc	CSIRO Long Pocket Labs	QLD	Australia
2002229	8-May-02	Lmc	McMahon Rd	QLD	Australia
2002230	8-May-02	Lmc	Bottom Swamp Crossing	QLD	Australia
2002231	8-May-02	Lmc	Gallaghers Point	QLD	Australia
2002232	8-May-02	Lmc	Dux Creek	QLD	Australia
2002233	15-May-02	Lmc	Carbrook Creek	QLD	Australia
2002234	15-May-02	Lmc	Deep Swamp	QLD	Australia
2002235	15-May-02	Lmc	Native Dog Creek	QLD	Australia
2002236	22-May-02	Lmc	CSIRO Long Pocket Labs	QLD	Australia
2002237	7-Jun-02	Lmc	Gallaghers Point	QLD	Australia
2002238	7-Jun-02	Lmc	Bottom Swamp Crossing	QLD	Australia
2002239	7-Jun-02	Lmc	McMahon Rd	QLD	Australia
2002240	7-Jun-02	Lmc	Dux Creek	QLD	Australia
2002241	14-Jun-02	Lmc	Carbrook Creek	QLD	Australia
2002242	14-Jun-02	Lmc	Native Dog Creek	QLD	Australia
2002243	19-Jun-02	Lmc	Peregian Environmental Park	QLD	Australia
2002244	20-Jun-02	Lmc	CSIRO Long Pocket Labs	QLD	Australia
2002245	21-Jun-02	SAsb	Sky Window, Eungella NP	QLD	Australia
2002246	23-Jun-02	Lmc	Edmund Kennedy National Park	QLD	Australia
2002247	23-Jun-02	Lrt	City Park, Tully	QLD	Australia
2002248	25-Jun-02	Lrt	near Iron Range	QLD	Australia
2002249	25-Jun-02	Lmc	W of campground, Iron Range	QLD	Australia
2002250	26-Jun-02	Lrt	Iron Range #1	QLD	Australia
2002251	26-Jun-02	Lrt	Iron Range #2	QLD	Australia
2002252	28-Jun-02	Lmc	McDowell Billabong	QLD	Australia

2002253	29-Jun-02	CEde	Paronella Park, near Ingham	QLD	Australia
2002254	29-Jun-02	Lmc	Martyville	QLD	Australia
2002255	8-Jul-02	Lmc	McMahon Rd	QLD	Australia
2002256	8-Jul-02	Lmc	Bottom Swamp Crossing	QLD	Australia
2002257	8-Jul-02	Lmc	Gallaghers Point	QLD	Australia
2002258	8-Jul-02	Lmc	Dux Creek	QLD	Australia
2002259	15-Jul-02	Lmc	Carbrook Creek	QLD	Australia
2002260	15-Jul-02	Lmc	Native Dog Creek	QLD	Australia
2002261	23-Jul-02	Lmc	CSIRO Long Pocket Labs	QLD	Australia
2002262	8-Aug-02	Lmc	McMahon Rd	QLD	Australia
2002263	8-Aug-02	Lmc	Bottom Swamp Crossing	QLD	Australia
2002264	8-Aug-02	Lmc	Gallaghers Point	QLD	Australia
2002265	8-Aug-02	Lmc	Dux Creek	QLD	Australia
2002266	13-Aug-02	Lmc	Carbrook Creek	QLD	Australia
2002267	13-Aug-02	Lmc	Native Dog Creek	QLD	Australia
2002268	26-Aug-02	Lmc	CSIRO Long Pocket Labs	QLD	Australia
2002269	29-Aug-02	Lmc	Gallaghers Point	QLD	Australia
2002270	29-Aug-02	Lmc	Bottom Swamp Crossing	QLD	Australia
2002271	29-Aug-02	Lmc	McMahon Rd	QLD	Australia
2002272	29-Aug-02	Lmc	Dux Creek	QLD	Australia
2002273	10-Sep-02	Lmc	Dux Creek	QLD	Australia
2002274	10-Sep-02	Lmc	Gallaghers Point	QLD	Australia
2002275	10-Sep-02	Lmc	Bottom Swamp Crossing	QLD	Australia
2002276	10-Sep-02	Lmc	McMahon Rd	QLD	Australia
2002277	12-Sep-02	Lmc	Kununura	WA	Australia
2002278	17-Sep-02	Lmc	Carbrook Creek	QLD	Australia
2002279	17-Sep-02	Lmc	Native Dog Creek	QLD	Australia
2002280	11-Oct-02	Lmc	McMahon Rd	QLD	Australia
2002281	11-Oct-02	Lmc	Bottom Swamp Crossing	QLD	Australia
2002282	11-Oct-02	Lmc	Gallaghers Point	QLD	Australia
2002283	11-Oct-02	Lmc	Dux Creek	QLD	Australia
2002284	17-Oct-02	Lmc	Carbrook Creek	QLD	Australia
2002285	17-Oct-02	Lmc	Native Dog Creek	QLD	Australia
2002286	24-Oct-02	Lmc	CSIRO Long Pocket Labs	QLD	Australia
2002287	11-Nov-02	Lmc	McMahon Rd	QLD	Australia
2002288	11-Nov-02	Lmc	Bottom Swamp Crossing	QLD	Australia
2002289	11-Nov-02	Lmc	Gallaghers Point	QLD	Australia
2002290	11-Nov-02	Lmc	Dux Creek	QLD	Australia
2002291	2-Nov-02	Lmc	Waidroka, 2.4km from Queens Rd		Fiji
2002292	26-Nov-02	Lmc	Carbrook Creek	QLD	Australia
2002293	26-Nov-02	Lmc	Native Dog Creek	QLD	Australia
2002294	27-Nov-02	Lmc	CSIRO Long Pocket Labs	QLD	Australia
2002295	11-Dec-02	Lmc	McMahon Rd	QLD	Australia
2002296	11-Dec-02	Lmc	Bottom Swamp Crossing	QLD	Australia
2002297	11-Dec-02	Lmc	Gallaghers Point	QLD	Australia
2002298	11-Dec-02	Lmc	Dux Creek	QLD	Australia
2002299	18-Dec-02	Lmc	Carbrook Creek	QLD	Australia
2002300	18-Dec-02	Lmc	Native Dog Creek	QLD	Australia
2002301	23-Dec-02	Lmc	CSIRO Long Pocket Labs	QLD	Australia
2002302	11-Oct-02	Lmc	Bukit Timah Nature Reserve		Singapore
2002303	25-Sep-02	Lrt	Cedar Creek Falls	QLD	Australia
2002304	26-Sep-02	Mlb	West Barratta Creek	QLD	Australia
2002305	25-Sep-02	Esp	Giru, road to Charters Towers	QLD	Australia
2002306	26-Sep-02	Gau	100km S Charters Towers	QLD	Australia
2002307	27-Sep-02	Asp	Clermont	QLD	Australia
2002308	27-Sep-02	Alfalfa	Dalby	QLD	Australia
2002309	27-Sep-02	clover	near Jondaryan	QLD	Australia
2002401	2-Mar-02	Lmc	Suan Ba Forest Park	NST	Thailand
2002402	2-Mar-02	Lmc	Wat Node, Tambol Photong	NST	Thailand

2002403	2-Mar-02	Lmc	Ban Pa Ni, Wat Node	NST	Thailand
2002404	2-Mar-02	Lmc	Ban Tan Ngen	NST	Thailand
2002405	2-Mar-02	RHto	Ban Yang Tia	NST	Thailand
2002406	2-Mar-02	RHto	Lung Ruangs	NST	Thailand
2002407	3-Mar-02	Lmc	Ban Tul	NST	Thailand
2002408	3-Mar-02	Psp	Suan Ba Forest Park	NST	Thailand
2002409	31-May-02	Lfl	Ban Pong Village	Chiang Mai	Thailand
2002410	31-May-02	Lfl	Ban Pong Village	Chiang Mai	Thailand
2002411	31-May-02	Lfl	Ban Pong Village	Chiang Mai	Thailand
2002412	31-May-02	Lfl	Ban Pong Village	Chiang Mai	Thailand
2002413	31-May-02	Lfl	Ban Pong Village	Chiang Mai	Thailand
2002414	31-May-02	Lfl	Ban Pong Village	Chiang Mai	Thailand
2002415	31-May-02	forest	Ban Pong Village	Chiang Mai	Thailand
2002416	2-Jun-02	Lfl	Toong Soong Au Luk	Krabi	Thailand
2002417	3-Jun-02	forest	Toong Soong Au Luk	Krabi	Thailand
2002418	3-Jun-02	RHto	Toong Soong Au Luk	Krabi	Thailand
2002419	3-Jun-02	Lmc	Wat Node, Tambol Photong	NST	Thailand
2002420	3-Jun-02	Lmc	Ban Pa Ni, Wat Node	NST	Thailand
2002421	4-Jun-02	RHto	Kuan Ngun	NST	Thailand
2002422	4-Jun-02	Lmc	Kuan Ngun	NST	Thailand
2002423	4-Jun-02	Lmc	Ban Tul	NST	Thailand
2002424	17-Aug-02	Lmc	1-2km S Hat Ploy Daeng	Trat	Thailand
2002425	17-Aug-02	Lmc	Ban Neun Payom	Trat	Thailand
2002426	30-Aug-02	Lmc	Ban Klong Manao	Trat	Thailand
2002427	30-Aug-02	Lmc	Ban Klong Manao	Trat	Thailand
2002429	31-Aug-02	Pfo	N of Klong Yai	Trat	Thailand
2002430	31-Aug-02	Lmc	N of Klong Yai	Trat	Thailand
2002431	31-Aug-02	RHto	Hat Ban Chuen	Trat	Thailand
2002432	31-Aug-02	Lmc	Hat Ban Chuen	Trat	Thailand
2002433	25-Oct-02	Lmc	Ban Klong Manao	Trat	Thailand
2002434	26-Oct-02	RHto	Hat Ban Chuen	Trat	Thailand
2002435	26-Oct-02	Lmc	Ban Mai Root	Trat	Thailand
2002436	29-Nov-02	Lmc	Lipao Demonstration Plot	Narathiwat	Thailand
2002437	30-Nov-02	Lmc	Roadside near Ban Sagom	Songkhla	Thailand
2002438	30-Nov-02	Lmc	5 mins from Ban Sagom site	Songkhla	Thailand
2002439	30-Nov-02	Lmc	near Ban Ta Ling Chan	Songkhla	Thailand
2002440	30-Nov-02	Lpy	Ban Kho Hong	Songkhla	Thailand
2002441	1-Dec-02	Lmc	near Hat Yai	Songkhla	Thailand
2002442	1-Dec-02	Lmc	Ban Bo Lo	NST	Thailand
2002443	1-Dec-02	Lmc	Ban Tul	NST	Thailand
2002444	2-Dec-02	Lmc	Wat Node, Tambol Photong	NST	Thailand
2002445	2-Dec-02	Lmc	Wat Node, Tambol Photong	NST	Thailand
2002446	2-Dec-02	Lmc	Suan Ba Forest Park	NST	Thailand
2002447	2-Dec-02	Lmc	opposite airport	NST	Thailand
2002448	2-Dec-02	Lmc	near Ban Don Sau-Thong Village	Surat Thani	Thailand
2002501	2-Apr-02	Lmc	near Kuching Ag.Research Station	Sarawak	Malaysia
2002502	3-Apr-02	Lmc	on side road, 17 miles from Kuching	Sarawak	Malaysia
2002503	3-Apr-02	Lmc	near Melon farm	Sarawak	Malaysia
2002504	3-Apr-02	Lmc	near Serian	Sarawak	Malaysia
2002505	3-Apr-02	Lmc	near Gedong	Sarawak	Malaysia
2002506	4-Apr-02	Lmc	6 1/2 mile road	Sarawak	Malaysia
2002507	4-Apr-02	Lmc	10 mile road (en route to Siburang)	Sarawak	Malaysia
2002508	4-Apr-02	Lmc	10 mile road (en route to Siburang)	Sarawak	Malaysia
2002509	5-Apr-02	Lmc	roadside near Damai Lagoon resort	Sarawak	Malaysia
2002510	5-Apr-02	Lmc	roadside near Damai Lagoon resort	Sarawak	Malaysia
2002511	5-Apr-02	Lmc	beside Kuching Golf and Country Club	Sarawak	Malaysia
2002512	6-Apr-02	Lmc	Bukit Jalil Park	Selangor	Malaysia
2002513	6-Apr-02	Lmc	near Berjaya Golf Resort	Selangor	Malaysia
2002550	22-May-02	Lmc	nr. Airport	Sud	New Caledonia

2002551	25-Feb-02	Lmc	Plum Swamp	Sud	New Caledonia
2002552	25-Feb-02	Lmc	Yate		New Caledonia
2002553	25-Feb-02	Lrt	Yate		New Caledonia
2002554	26-Feb-02	Lmc	near Ponerhouen		New Caledonia
2002555	27-Feb-02	Nsp	near Poindimie		New Caledonia
2002556	20-May-02	Lmc	la Coulee	Sud	New Caledonia
2002557	20-May-02	Lmc	Yate		New Caledonia
2002558	20-May-02	Lrt	near Inco mine	Sud	New Caledonia
2002559	20-May-02	Lmc	Riviere des Pirogues	Nord	New Caledonia
2002560	21-May-02	Lmc	la Coulee	Sud	New Caledonia
2002561	22-May-02	Lmc	nr. Kuto	Sud	New Caledonia
2002562	22-May-02	Lrt	Vao, Tribu de Vao	Sud	New Caledonia
2002563	22-May-02	Lmc	nr. Vao, Tribu de Waacia	Sud	New Caledonia
2002564	22-May-02	Mqn	nr. Yad, Tribu de Waacia	Sud	New Caledonia
2002565	23-Jun-02	Lmc	Yate		New Caledonia
2002566	24-Jun-02	Lmc	la Coulee	Sud	New Caledonia
2002567	24-Jun-02	Lmc	Riviere des Pirogues	Nord	New Caledonia
2002568	1-Jul-02	Lmc	la Coulee	Sud	New Caledonia
2002569	10-Jul-02	Lmc	la Coulee	Sud	New Caledonia
2002570	25-Jul-02	Lmc	la Coulee	Sud	New Caledonia
2002571	25-Jul-02	Lmc	near Yate		New Caledonia
2002572	25-Jul-02	Lmc	Riviere des Pirogues	Nord	New Caledonia
2002573	18-Aug-02	Lmc	la Coulee	Sud	New Caledonia
2002574	18-Aug-02	Lmc	near Yate		New Caledonia
2002575	18-Aug-02	Lmc	Riviere des Pirogues	Nord	New Caledonia
2002576	16-Sep-02	Lmc	near Yate		New Caledonia
2002577	16-Sep-02	Lmc	Riviere des Pirogues	Nord	New Caledonia
2002578	26-Sep-02	Lmc	la Coulee	Sud	New Caledonia
2002599	16-Sep-02	Lmc	la Coulee	Sud	New Caledonia
2002651	24-Feb-02	Lmc	near Yung Shue Wan	Hong Kong	China
2002652	26-Feb-02	Ljp	Kowloon Park, hill near wire fence	Hong Kong	China
2002653	27-Feb-02	Lmc	Ma On Shan trail	Hong Kong	China
2002654	27-Feb-02	RHto	Ma On Shan trail	Hong Kong	China
2002655	5-Mar-02	Ljp	hillside trail South of Mui Wo	Hong Kong	China
2002656	5-Mar-02	RHto	hillside trail South of Mui Wo	Hong Kong	China
2002657	6-Mar-02	Lmc	Shing Mun Country Park	Hong Kong	China
2002658	6-Mar-02	Lmc	Shing Mun Country Park	Hong Kong	China
2002659	6-Mar-02	Lmc	Shing Mun Country Park	Hong Kong	China
2002660	6-Mar-02	RHto	Shing Mun Country Park	Hong Kong	China
2002661	7-Mar-02	RHto	Tung Chung Valley trail	Hong Kong	China
2002662	7-Mar-02	Lmc	Tung Chung Valley trail	Hong Kong	China
2002663	30-Jul-02	Lmc	near Yung Shue Wan	Hong Kong	China
2002664	31-Jul-02	RHto	Victoria Peak	Hong Kong	China
2002665	31-Jul-02	Ljp	Victoria Peak	Hong Kong	China
2002666	27-Nov-02	Lmc	Shing Mun Country Park	Hong Kong	China
2002670	22-Apr-02	Lmc	near Yung Shue Wan	Hong Kong	China
2002671	22-Apr-02	Lmc	top of hill from Sok Kwu Wan	Hong Kong	China
2002672	23-Apr-02	Lmc	Tai Tam Tuk Reservoir	Hong Kong	China
2002673	23-Apr-02	Lmc	Tai Tam Tuk Reservoir	Hong Kong	China
2002674	25-Apr-02	Lmc	Tai Tam Tuk Reservoir	Hong Kong	China
2002675	25-Apr-02	Lfl	Tai Tam Tuk Reservoir	Hong Kong	China
2002676	25-Apr-02	Lmc	Tai Mo Shan, visitor centre	Hong Kong	China
2002677	1-May-02	Lmc	top of hill from Sok Kwu Wan	Hong Kong	China
2002678	1-May-02	Lmc	Tung O	Hong Kong	China
2002679	7-May-02	Lmc	Shing Mun Country Park	Hong Kong	China
2002701	8-Apr-02	Lmc	Sungei Buloh Nature Park		Singapore
2002702	8-Apr-02	Lmc	Sungei Buloh Nature Park		Singapore
2002703	8-Apr-02	Lmc	Sungei Buloh Nature Park		Singapore
2002704	8-Apr-02	Lmc	Sungei Buloh Nature Park		Singapore

2002705	9-Apr-02	Lmc	Pulau Ubin		Singapore
2002706	9-Apr-02	SVsp	Pulau Ubin		Singapore
2002707	9-Apr-02	SVsp	Pulau Ubin		Singapore
2002708	9-Apr-02	Pfo	Pulau Ubin		Singapore
2002709	9-Apr-02	Lmc	Pulau Ubin		Singapore
2002710	9-Apr-02	Lmc	Pulau Ubin		Singapore
2002711	10-Apr-02	Lmc	Bukit Timah Nature Reserve		Singapore
2002712	10-Apr-02	Lmc	Bukit Timah Nature Reserve		Singapore
2002713	9-Apr-02	Lmc	Pulau Ubin		Singapore
2002714	14-Aug-02	Lmc	Bukit Timah Nature Reserve		Singapore
2002715	14-Aug-02	Lmc	Bukit Timah Nature Reserve		Singapore
2002716	14-Aug-02	Lmc	Bukit Timah Nature Reserve		Singapore
2002717	14-Aug-02	Lmc	Bukit Timah Nature Reserve		Singapore
2002718	14-Aug-02	Lmc	Bukit Timah Nature Reserve		Singapore
2002719	15-Aug-02	Lmc	Sungei Buloh Nature Park		Singapore
2002720	15-Aug-02	Pfo	Tao Payoh Rise, Thompson Rd		Singapore
2002721	14-Aug-02	Lmc	Bukit Timah Nature Reserve		Singapore
2002722	19-May-02	Lfl	Tao Payoh Rise, Thompson Rd		Singapore
2002723	19-May-02	Lfl	Mount Pleasant Rd		Singapore
2002750	20-Jun-02	Lmc	River Ithikkara	Kerala	India
2002751	22-Jun-02	Lmc	Thomaiyarpuram	Tamil Nadu	India
2002752	22-Jun-02	Lmc	Thirunandhikara	Tamil Nadu	India
2002753	27-Jul-02	Lmc	Thomaiyarpuram	Tamil Nadu	India
2002754	27-Jul-02	Lmc	Thirunandhikara	Tamil Nadu	India
2002755	28-Jul-02	Lmc	River Ithikkara	Kerala	India
2002756	29-Jul-02	Lmc	Darala Road		India
2002757	20-Aug-02	Lmc	Thomaiyarpuram	Tamil Nadu	India
2002758	20-Aug-02	Lmc	Thirunandhikara	Tamil Nadu	India
2002759	21-Aug-02	Lmc	River Ithikkara	Kerala	India
2002760	22-Aug-02	Lmc	Nudukani, near Gudalur	Tamil Nadu	India
2002761	28-Sep-02	Lmc	Nudukani, near Gudalur	Tamil Nadu	India
2002762	29-Sep-02	Lmc	River Ithikkara	Kerala	India
2002763	30-Sep-02	Lmc	Thirunandhikara	Tamil Nadu	India
2002764	30-Sep-02	Lmc	Thomaiyarpuram	Tamil Nadu	India
2002765	13-Oct-02	Lmc	Thomaiyarpuram	Tamil Nadu	India
2002766	13-Oct-02	Lmc	Thirunandhikara	Tamil Nadu	India
2002767	14-Oct-02	Lmc	River Ithikkara	Kerala	India
2002768	14-Oct-02	Lmc	River Ithikkara	Kerala	India
2002769	18-Oct-02	Lmc	Boralegamura		Sri Lanka
2002770	18-Oct-02	Lmc	Bandaragama		Sri Lanka
2002771	19-Nov-02	Lmc	Nudukani, near Gudalur	Tamil Nadu	India
2002772	19-Nov-02	Lmc	Nudukani, near Gudalur	Tamil Nadu	India
2002773	20-Nov-02	Lmc	River Ithikkara	Kerala	India
2002774	20-Nov-02	Lmc	River Ithikkara	Kerala	India
2002775	21-Nov-02	Lmc	Thomaiyarpuram	Tamil Nadu	India
2002776	21-Nov-02	Lmc	Thirunandhikara	Tamil Nadu	India
2002777	29-Dec-02	Lmc	River Ithikkara	Kerala	India
2002778	29-Dec-02	Lmc	River Ithikkara	Kerala	India
2002779	30-Dec-02	Lmc	Thomaiyarpuram	Tamil Nadu	India
2002780	30-Dec-02	Lmc	Thomaiyarpuram	Tamil Nadu	India
2002781	30-Dec-02	Lmc	Thirunandhikara	Tamil Nadu	India
2002789	18-Oct-02	Lmc	Kambakkam Creek	Andra Pradesh	India
2002790	1-May-02	Lmc	Kothayar	Tamil Nadu	India
2002791	1-May-02	Lsp	Kothayar	Tamil Nadu	India
2002792	1-May-02	ANON	Sengottai	Tamil Nadu	India
2002793	2-May-02	Lmc	River Ithikkara	Kerala	India
2002794	2-May-02	Lfl	River Ithikkara	Kerala	India
2002795	3-May-02	Lfl	Bishop Moore College	Kerala	India
2002796	4-May-02	Lfl	Silent Valley	Kerala	India

2002797	4-May-02	ANON	Silent Valley	Kerala	India
2002798	6-May-02	Lmc	Nudukani, near Gudalur	Tamil Nadu	India
2002799	3-May-02	Lfl	Mavelikara, elephant saw mill	Kerala	India
2002800	24-Jan-02	Hrs	Sherwood	QLD	Australia
2002801	24-Jan-02	Hrs	Sherwood	QLD	Australia
2002802	24-Jan-02	Hrs	Sherwood	QLD	Australia
2002803	24-Jan-02	Hrs	Sherwood	QLD	Australia
2002804	24-Jan-02	Hrs	Sherwood	QLD	Australia
2002805	24-Jan-02	Hrs	Sherwood	QLD	Australia
2002806	24-Jan-02	Hrs	Indooroopilly	QLD	Australia
2002807	28-Feb-02	Hrs	Sherwood	QLD	Australia
2002808	28-Feb-02	Hrs	Sherwood	QLD	Australia
2002809	28-Feb-02	Hrs	Sherwood	QLD	Australia
2002810	28-Feb-02	Hrs	Sherwood	QLD	Australia
2002811	28-Feb-02	Hrs	Sherwood	QLD	Australia
2002812	28-Feb-02	Hrs	Sherwood	QLD	Australia
2002813	28-Feb-02	Hrs	Indooroopilly	QLD	Australia
2002814	28-Mar-02	Hrs	Sherwood	QLD	Australia
2002815	28-Mar-02	Hrs	Sherwood	QLD	Australia
2002816	28-Mar-02	Hrs	Sherwood	QLD	Australia
2002817	28-Mar-02	Hrs	Sherwood	QLD	Australia
2002818	28-Mar-02	Hrs	Sherwood	QLD	Australia
2002819	28-Mar-02	Hrs	Sherwood	QLD	Australia
2002820	28-Mar-02	Hrs	Indooroopilly	QLD	Australia
2002821	30-Apr-02	Hrs	Sherwood	QLD	Australia
2002822	30-Apr-02	Hrs	Sherwood	QLD	Australia
2002823	30-Apr-02	Hrs	Sherwood	QLD	Australia
2002824	30-Apr-02	Hrs	Sherwood	QLD	Australia
2002825	30-Apr-02	Hrs	Sherwood	QLD	Australia
2002826	30-Apr-02	Hrs	Sherwood	QLD	Australia
2002827	30-Apr-02	Hrs	Indooroopilly	QLD	Australia
2002851	14-Nov-02	Mqn	Ewan Maddock Dam	QLD	Australia
2002852	19-Nov-02	CPan	Rowes Bay Golf Club	QLD	Australia
2002853	19-Nov-02	Mfl	Ross River Dam	QLD	Australia
2002854	19-Nov-02	Mlb	Ross River Dam	QLD	Australia
2002855	20-Nov-02	Mqn	Pacific HWY	QLD	Australia
2002856	20-Nov-02	Mvr	Pacific HWY	QLD	Australia
2002857	20-Nov-02	Mqn	Wallace Vale Droughtmaster Stud	QLD	Australia
2002858	20-Nov-02	Mvr	Leumann Rd, 5km S Gordonvale	QLD	Australia
2002859	21-Nov-02	Mdl	Syndicate Rd	QLD	Australia
2002860	21-Nov-02	Mar	Saltbag Creek	QLD	Australia
2002861	21-Nov-02	Mvr	Lake Mitchell	QLD	Australia
2002862	22-Nov-02	CPan	CSIRO Long Pocket Labs	QLD	Australia
2002863	25-Feb-02	Mqn	West of Plum turnoff, off RP3		New Caledonia
2002864	25-Feb-02	Mqn	near Yate		New Caledonia
2002865	26-Feb-02	HYvr	5km NW Le Foa	Sud	New Caledonia
2002866	26-Feb-02	Mqn	RT1, South of Bouloupari		New Caledonia
2002867	26-Feb-02	Mqn	Fort Tremba Road off RT1		New Caledonia
2002868	26-Feb-02	Mqn	Moindou, RP1	Sud	New Caledonia
2002869	26-Feb-02	Mqn	RPN3 north of Houailou		New Caledonia
2002870	27-Feb-02	Mqn	Kon to Poindimie road		New Caledonia
2002871	16-Aug-02	HYvr	Singapore Botanic Gardens	Singapore	Singapore
2002872	17-Aug-02	Lmc	Chinese Gardens entrance		Singapore
2002873	5-Dec-02	Mqn	Peregian Environmental Park	QLD	Australia
2002874	5-Dec-02	Mqn	Coolum	QLD	Australia
2002875	5-Dec-02	Mvr	Caloundra Airport	QLD	Australia
2002876	18-Dec-02	Mqn	Tabbimobile Creek overflow	NSW	Australia
2002877	5-Nov-02	Mqn	Roy’s Road	QLD	Australia
2002878	29-Aug-02	Mqn	Gallaghers Point	QLD	Australia

Appendix 2: Artificial Diet for Lygodium Stem-Borer

(Collaboration with B. Brown and R. Chan, CSIRO Entomology)

Ingredients:

<u>Part A</u>

Water	300ml	
Pentavite (multi-vitamins)	0.262ml	(6 drops)
Ascorbic acid	2.80g	
Sorbic acid	0.66g	
Nipagin (methyl p-hydrox benzoate)	0.66g	
Lygodium dried & finely ground	58g	
Cannilini beans, dried & finely ground	24g	
Soluble starch (Corn flour)	24g	
Fibrous cellulose powder	24g	
Sucrose	24g	
Powdered brewer's yeast	28g	

<u>Part B</u>

Agar	18g
Water	280ml

Preparation of straws:
Commercially-available paper straws need the waxy coating removed by dipping or flushing with boiling water, then dried before filling with the diet preparation via an injection gun.

Diet preparation
- Weigh all ingredients, Part A blended until smooth and left in the blender
- Mix Part B separately in a clean beaker or saucepan and bring to the boil slowly. The mix must be stirred constantly to avoid charring. Remove from heat when the agar is boiling and the mixture is clear in color.
- Combine Part A ingredients from blender into beaker or saucepan with hot agar and mix well with a clean utensil. Make sure the mixture is combined together well.
- Set up diet gun with plunger retracted back and pour in hot diet to fill the gun's chamber. Attach the nozzle to the gun and you can now begin to inject straws with the diet. The remaining diet in breaker/saucepan can be kept warm on a hot plate until ready to refill the gun.

Appendix 3: Full Report of *Rhodomyrtus tomentosa* Project

Biological Control Research Group
Entomology and Zoology Division
Department of Agriculture
Bangkok 10900 THAILAND

Rhodomyrtus tomentosa

Downy Rose Myrtle

Survey of the plant and its natural enemies in Thailand, 2001-2002

Amporn Winotai

Contents

Abbbreviations:

ABCL	USDA/ARS, Australian Biological Control Laboratory, Brisbane.
ANIC	Australian National Insect Collection
BMNH	British Museum of Natural History
CSIRO	Commonwealth Scientific and Industrial Research Organisation, Australia
DOA	Department of Agriculture, Bangkok, Thailand
EPPC	Florida Exotic Pest Plant Council
NST	Nakhon Si Thammarat
SEL	Systematic Entomology Laboratory, USDA

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Introduction

Project Background:
Several US weed species occur naturally in Thailand where they receive little attention because they are of only slight or no immediate economic, cultural or environmental interest. One of these is *Rhodomyrtus tomentosa*, a serious weed in Florida (EPPC category 1*). Information from Smitinand (1980) suggested *R. tomentosa* is plentiful in central and southern regions of Thailand within reasonable reach of the Bangkok laboratories. At the request of the USDA/ARS Australian Biological Control Laboratory (ABCL), a one-year survey of natural enemies commenced in April 2001. This report summarizes the results.

It was hoped that this study could form one part of a database of prospective targets for biological control with notes on distribution, biology and common insect herbivores. Such a database could potentially provide information to indicate the likelihood of success of biocontrol of the weeds in the US and perhaps also indicate potential costs of launching a full exploratory program. When combined with information on the spread and severity of problems caused in the USA, a basis for decisions relating to prioritizing and funding further research could be expected to result. Some of the information obtained, for example on insect identities, is likely to be of local interest for Thai biodiversity records.

Project Aim:
To assemble data on the distribution, biology and herbivores of *R. tomentosa* in the native range of the plant, which could provide base information on which a future biological control project in the USA can build.

Project Methods:
1. Searching herbarium records and distribution with Thai botanists to map and report distribution of *R. tomentosa.* Growing multiple specimens of *R. tomentosa* in the DOA laboratory plant nursery.
2. Information from field trips to include GPS locations, habitat and plant observations including insect damage.
3. Collection of live mature and immature insects to be taken to Bangkok laboratory and reared on nursery plants.
4. Insect herbivores pinned/preserved and sent to local taxonomists and SEL via ABCL for determination.
5. Insects encountered but not considered for further study included scales, mealybugs, insects apparently resting on the plant with no associated plant damage, and various flower beetles likely common to other plant species (see Notes and Recommendations).

* EPPC category 1: “Species that are invading and disrupting native plant communities in Florida. This definition does not rely on the economic severity or geographic range of the problem, but on the documented ecological damage caused.”

Scientific Name: *Rhodomyrtus tomentosa* (Aiton) Hassk.
Family: Myrtaceae
Synonyms: *Myrtus tomentosa* Aiton (1789)
Vernacular Names Smitinand 1980, Prosea 1992, Zheng, 1995

English:	- Downy myrtle
	- Rose myrtle
	- Ceylon hill cherry
Bahasa Indonesia	- Kemunting (Malay)
	- Harendong sabrang (Sundanese)
Bahasa Malaysia:	- Kemunting (Peninsular Malaysia)
	- Karamunting (Sabah, Sarawak)
Cambodia:	- pÛËch, sragan
China:	- Tojinniang
Vietnam:	- Sim
Thailand:	- Thoh (South)
	- Phruat (Trat)
	- Phruat kinluk (Prachin Buri)
	- Kaa thu (Chumphon)
	- Kaa muu ting kaa yuu (Malay Patani)
	- Ngaai (Suai)
	- Suat (Chanta Buri)
	- Pui (Khmer-Eastern)
	- Pruat Phee (Rayong)
	- Pruat Yai (Chon Buri)

Origin and geographic distribution

Downy myrtle grows wild as well as being cultivated in Southeast Asia, India, Sri Lanka, and south China (Prosea 1992). In Thailand *R. tomentosa* occurs most in coastal sandy soils on both coasts of the southern Peninsula, extending around to Trat Province and Klong Yai District in the east. It was introduced by the U.S. Department of Agriculture in the 1920's as a landscape plant and started invading and disrupting native plant communities in Florida. In Florida the plant has spread to several areas where it forms dense thickets that displace native vegetation.

Uses & Properties

The whole fruit is edible. It contains sugars, vitamins and minerals. The purple pulp is juicy and sweet when fully ripe, otherwise slightly astringent. Children in the native range compete with birds for the sweet fruits. In some areas jams, jelly, and wine used to be made from the fruits, which are only incidentally available in quantity. Old sources in Malaysia mentioned the use of fruits as a cure for dysentery and diarrhea. A decoction of the roots or leaves is drunk for diarrhea and stomach-ache, and as a protective medicine after birth. In Indonesia, the crushed leaves are used to dress wounds. The wood tar can serve as a black dye and has been used to blacken teeth, and eyebrows. In Australia, Indonesia, and Florida, where the species is cultivated in gardens, the shrub and flowers have ornamental value (modified after Prosea 1992).

Botanical characters

Prosea (1992) described botanical characters of this species as follows:
"Evergreen shrub or small tree, up to 4m tall; twigs, young leaves and inflorescences densely white or yellowish tomentose. Leaves elliptic to oblong-elliptic, 4.5-8 cm opposite, coriaceous, with 3 conspicuous longitudinal veins, upper surface glossy, glabrous, lower surface white or yellowish tomentose with raised nerves; petiole 3-5 mm long. Flowers solitary or in 3-flowered dichasia in upper axils; peduncles up to 1 cm, pedicels 0.5-2.5 cm long; bracts elliptic, leaf-like, 6-12 mm long, bracteoles elliptic or ovate, 2-3 mm long, persistent; calyx campanulate, 5-7 mm long, tomentose, 5-10 ribbed, 5 lobed, persistent; petals 5, broadly obovate, 15-18 mm x 9-13 mm, red or pink; stamens numerous, 10-15 mm long; ovary 3(-4)-locular. Fruit an oblongoid berry, 10-15mm x 8-10 mm, purplish-black, crowned by the calyx lobes, tomentose; wall 1 mm thick, pulp sweet. Seeds many in 6-8 pseudo-locules, divided by thin false septa, compressed-reniform, 1.5 mm in diameter."

Bangkok Herbarium records and distribution map of *R. tomentosa* in Thailand:
Southern Region
Chumporn, Hudsai Ree
Narathiwat, Amphoe Bacho
Narathiwat, Amphoe Waeng
Narathiwat, (several other specimens)
Phang-nga, Kao Pra-mee
Padang Besar
Prachuopkirikun
Surat, Kaw, Pa-ngan
Songkhla, Hat Yai
Songkhla, Tae Par
Trang, Amper Sikao

Eastern Region
Chantaburi, Muang district (several specimens)
Trat, Klong Yai district

North-eastern Region
Surin, Kapcherng
Ubol Ratchathani, Warinchumrap

Foreign Holdings at the Herbarium
1928, specimen ex Canton, Triang Ying, 2nd Agric. Stn.
1922 Amoy Is., hill slope E of Nanputo temple, Fukien Province
1960 Malaya, Kelantan, Kampong Gobek, Kerilla Estates

***Rhodomyrtus* in China (Hong Kong and Hainan Is.)**
R. tomentosa is listed as a very common plant of grassland and shrubland in Hong Kong (Corlett et al 2000). The sites below are examples of specific occurrence records
China, Hong Kong, HK Is., beside Tai Tam Tuk Reservoir
N 22º 14.89 : E 114º 12.90 *Rhodomyrtus* present as scattered plants
China, Hong Kong, New Territories, Ma On Shan trail, near cemetary
N 22º 23.12 : E 114º 15.52 *Rhodomyrtus* abundant on hillside
China, Hainan, nr Quanghai, roadside opposite piggery
N 18º 56.85 : E 110º 27.85 *Rhodomyrtus* present as scattered plants.

Distribution map of *R. tomentosa* study sites in Thailand:

Insects found associated with *Rhodomyrtus tomentosa* in Thailand

1. ***Carea varipes* Walker (Lepidoptera: Noctuiidae: Chloephorinae)**

The species is apparently widely distributed, having been collected at widely separated sites in the east (Trat) and the south (Nakhon Si Thammarat). Larvae are large and obvious leaf feeders but have not been observed in large numbers, perhaps because of their own natural enemies. Pupation occurs in rolled or between touching leaves. *C. varipes* has only been collected from *R. tomentosa*. [In Thailand, a related species, *Carea angulata* (F.), was found on rose apple and recorded as leaf eating caterpillar. There has been only one adult specimen of *C. angulata* collected in Bangkok and lodged in the DOA Insect Collection.]

Other countries:
The species has been recorded in Hong Kong, also on *R. tomentosa* (David L. Mohn).
A record from Australia published as an internet website noted C. varipes was collected on *Xanthostemon chrysanthus* (Myrtaceae) (Golden Penda), in Cairns, North Queensland. Investigations revealed two larvae were collected, reared to adult and sent to a hobby collector (also the author of the web page) who identified the emerged adults as *Carea varipes*. The identity of the specimens was checked by Dr Ted Edwards who confirmed they were not *C. varipes*, a species still not known from Australia.

Locations:
Thailand, Nakhon Si Thammarat Province, Muang district, Ban Yang Tia
N 8ºo 32.06 E 99º 58.16
Thailand, Trat Province, Klong Yai district, nr. Haad Sai Kaew
N 11º 54.83 E 102º 48.59
Thailand, Trat Province, Muang district, Mai Rood
N 11º 54.92 E 102º 48.37

2. Striped caterpillar (Lepidoptera) (Identity not yet received)

The striped caterpillar bores and feeds inside young flower buds and young fruit. This insect was found at all *R. tomentosa* study sites, often in numbers. The full grown larva is about 6-7 mm long, head pale, body creamy white with a red stripe on each abdominal segment. Although the larvae feed and develop in plant tissue, during laboratory studies full-grown larvae left the host and pupated outside on sides and lids of rearing tubes. The adult is a tiny moth, having brown fore wings with yellow bands at the posterior margins.

It is possible that more than one species is involved in producing the damage illustrated in the photos above. Early indications from Dr David Adamski, SEL, are that the insects are in the Superfamily Gelechioidea, and one species is possibly Fam. Amphitheridae, Genus *Agriothera*. This is very preliminary information as Dr Adamski has received the few adults available and few larvae, and the adults and larvae were not all collected at the same site.

Locations:

Thailand, Chantaburi Province, Klung district, Tambol Boh, near intersection
N 12º 25.74 E 102º 19.02

Thailand, NST Province, Chian Yai district, Ban Bo Lo sub district, just E of Ban Bo Lo
N 8º 05.88E 100º 06.56

Thailand, NST province, Muang district, nr Ban Pak Poon School
N 8º 31.41E 99º 58.37

Thailand, NST Province, Chien Yai district, Mae Chow Yuhua (W. of Bo Lo)
N 8º 06.06E 100º 05.81
Thailand, NST Province, Tha Sala district, Tambol Photong, Wat Node
N 8º 36.31E 99º 56.88
Thailand, NST Province, Muang district, nr Ban Pak Poon school
N 8º 32.06E 99º 58.16
Thailand, NST Province, Muang district, Ban Yang Tia
N 8º 32.06E 99º 58.16
Thailand, NST Province, Muang district, Lung Ruang's house, nr Ban Pak Poon
N 8º 31.93E 99º 58.52
Thailand, Songkhla Province, Chana district, nr Ban Sagom
N 6º 57.11E 100º 48.49
Thailand, Songkhla Province, Chana district, nr Ban Sagom, dirt road near #450
N 6º 57.38E 100º 48.13
Thailand, Surat Thani Province, Muang district, Bo Shaloek, nr. Klong Shaloek
N 9º 08.66E 99º 22.04
Thailand, Surat Thani Province, Phum Riang district.
N 9º 22.46E 99º 15.43
Thailand, Surat Thani Province, Don Sak district, nr Ban Don Sau Thong
N 9º 13.77E 99º 40.24
Thailand, Trang Province, Si Kao district, near Pak Meng
N 7º 27.43E 99º 20.51
Thailand, Trang Province, Si Kao district, Trang Horticultural Research Center
N 7º 31.83E 99º 23.86
Thailand, Trang Province, Si Kao district, Trang Hort.Res.Ctr., very close to previous site.
N 7º 31.21 E 99º 24.27
Thailand, Trat Province, Klong Yai district, 1km from Haad Sai Kaew
N 11º 54.83 E 102º 48.59
Thailand, Trat Province, Klong Yai district,
N 11º 50.03 E 102o 50.51
Thailand, Trat Province, Klong Yai district, Tambol Mai Rood, Ban Nong Muang
N 11º 52.94 E 102º 49.09
Thailand, Trat Province, Klong Yai district, Tambol Mai Rood
N 11º 55.78 E 102º 48.19

3. *Rhodomyrtus* thrips (Thysanoptera: Thripidae)

Host specific Thysanoptera have previously been used in programs on biological control of weeds, so thrips were also included in the survey. The first thrips collection was from the east of Thailand at Hat Ban Chuen, a small coastal area in Trat. These were identified as *Thrips hawaiiensis* Morgan (Thripidae), a common pest species.

Further thrips collections were made, but in the south of Thailand near Don Sak and at Ban Yang Tia in a neighbouring Province further south. These thrips were of a species not able to be identified and warranting further checking of material held in London. This thrips species is similar to, but not the same as, *Thrips coloratus* Schmutz (Thripidae), another common pest species. Initial collections of the *Rhodomyrtus* thrips revealed only females, so further collections were made including males and larvae to aid complete checking of reference material. Females are strikingly different to most thrips as they have very long ovipositors.

Females of the *Rhodomyrtus* thrips (photos by L. Mound).

All identifications were made by Dr Laurence Mound, Australian National Insect Collection (ANIC), CSIRO Entomology, Canberra, who also supplied the photographs. Mound suggested the new thrips is very possibly an undescribed species in which case probably specific to *Rhodomyrtus tomentosa*. The material is to be compared with reference material in London at the BMNH by Dr Mound in September 2002.

As a further check on specificity, thrips were collected from flowers of *Melastoma malabathricum* growing with *R. tomentosa* at the Ban Yang Tia site in southern Thailand. I reasoned that if the thrips on *R. tomentosa* was a non-specific species, it could also be on the flowers of *M. malabathricum* growing with and sometimes touching the *R. tomentosa*. The thrips from *M. malabathricum* were a different species and identified by Dr Mound as *Thrips melastomae* Priesner, associated with *Melastoma* between Thailand and the Philippines.

Locations:

- *Thrips hawaiiensis*

Thailand, Trat Province, Muang district, Tambol Laem klad, Hat Ban Chuen

N 11º 53.73 E 102º 47.20

- *Rhodomyrtus* thrips, near *T. coloratus*

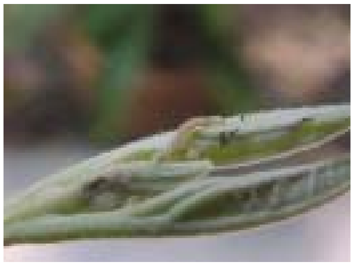
Thailand, Surat Thani Province, Don Sak district, Pang Na Chee

N 9º 13.57 E 99º 39.65

Thailand, NST Province, Muang district, Ban Yang Tia

N 8º 32.06 E 99º 58.16

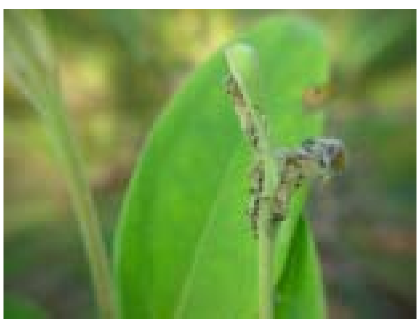
4. *Pingasa chlora* (Stoll) (Lepidoptera: Geometridae)

Young larvae bore inside folds and feed on young shoots. Large larvae feed on young flushes of *R. tomentosa*. Head and body are pale green covered with short dense white hairs; with a spiracular line and oblique lateral streaks of each segment. This species was reported as pest of rambutan and litchi.

Locations:
Thailand, NST Province, Muang district, Tambol Pak Poon, behind Ban Pak Poon School
N 08º 31.28 E 99º 58.56
Thailand, Trat Province, Klong Yai district, Tambol Mai Rood
N 11º 52 .78 E 02º 49.17
Thailand, NST Province, Muang district, Ban Pak Poon, nr. Lung Ruang's House
N 8º 31.93 E 99º 58.52

5. Tortricid (Lepidoptera: Tortricidae)

The larvae were found feeding on young shoots of *R. tomentosa*. Larvae clumped leaves together to form shelters in which they fed. The moths are about 2-2.5 cm long, and when the moth rests it looks bell-shaped. *Archips micaceana* Walker and *A. machlopis* Meyrick are common in Thailand. Their larvae are polyphagous, feeding on citrus, durian, rambutan, litchee and mango.

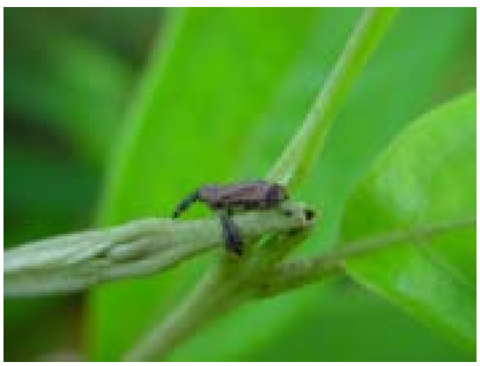
Locations:
Thailand, NST Province, Cha Uat district, Ban Tul
N 8º 02.89 E 99º 58.98
Thailand, NST Province, Muang district, Lung Ruang's House, nr Ban Pak Poon
N 8º 31.93 E 99º 58.52
Thailand, Surat Thani Province, Don Sak district, nr Ban Don Sau Thong
N 9º 13.77 E 99º 40.24

6. *Hyposidra infixaria* Walk (Lepidoptera: Geometridae)

Larvae cause minor damage to leaves of *R. tomentosa*. In Thailand the insect has been reported from castor bean and pomegranate. In other countries it is a pest of coffee and several other species.

Location:
Thailand, Trat Province, Klong Yai District, Tambol Mai Rood
N 11º 52 .78 E 102º 49.17

7. *Alcidodes* sp. (Coleoptera: Curculionidae)



The snout weevils were found causing shoot stem damage resulting in wilting and death of growing tips. Adults have only been observed on flushes of new growth. Eggs and larvae were not positively identified, however it is possible that 'shoot borer' activity (see 8.4) observed in the area is caused by larval activity. The weevil is possibly a species in the genus *Alcidodes*, which also has pest species. *Alcidodes buho* was reported as a pest of *Sessbanis sesban* (Papilionaceae), a plant widely distributed in Africa and used for firewood, leaves and pods fed to livestock, uses as windbreaks and hedge rows, as support for black pepper, cucurbits and betel vine, as shade tree for coffee and turmeric. *Alcidodes frenatus* Feisthamel was reported damaging leaf midribs and boring twigs of teak in Thailand.

Locations:
Thailand, NST Province, Muang district, Ban Yang Tia
N 8º 32.06 E 99º 58.16
Thailand, NST Province, Muang district, Lung Ruang's House, nr Ban Pak Poon
N 8º 31.93 E 99º 58.52

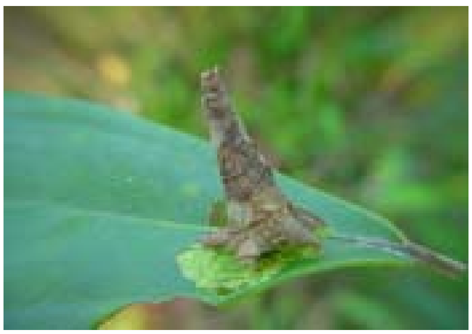
8. Other insects found feeding on *R. tomentosa* but not reared to adults

8.1 Leaf eating caterpillar (Lepidoptera: Noctuidae)

On one occasion at one site many larvae were found eating leaves of *R. tomentosa*. Larvae did not survive to adult under laboratory conditions.

Location:
Thailand, NST Province, Muang district, Tambol Pak Poon, Ban Yang Tia
N 8º 31.774 E 99º 58.377

8.2 Case worm or bagworm (Lepidoptera: Psychidae)



Minor damage to leaves of *R. tomentosa* was seen at three sites but larvae did not survive in the laboratory.

Locations:
Thailand, Chumphon Province, Muang district, Tambol Haad Sai Ree, Moo 6
N 10º 23.93 E 99º 16.72
Thailand, Krabi Province, Klong Tom district, Tambol Pae Hla, Ban Phu Teai
N 8º 05.90E 99º 08.65
Thailand, NST Province, Muang district, Lung Ruang's House, nr Ban Pak Poon
N 8º 31.93 E 99º 58.52

3.3 Ant Mimic caterpillar

These caterpillars feed on leaves and seem to mimic the common red ant in shape and manner. Larvae were about 2 cm long, slender, with head and body reddish brown, and a yellow streak on the 1st and 2nd thoracic segments. Abdominal segments 5 and 6 are dorsally swollen. The 8th and 9th abdominal segments are enlarged in a club-like shape. Some tactile setae on thoracic segments moved incessantly.

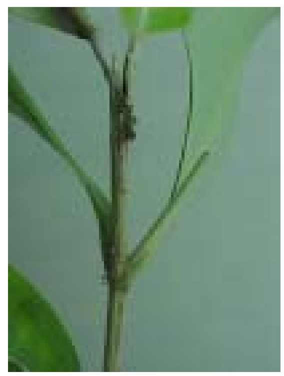
From examining pictures of larvae, the taxonomist suggested the genus *Homodes* (Lepidoptera: Noctuiidae). In Thailand two species of the genus *Homodes* are known, *H.*

bracteigutta (Walker), found on rambutan, longan and mango in Lamphun and Chumphon, and *H. perilitta* Hampson found on rambutan and Langsium in Chantaburi.

Locations:
Thailand, Narathiwat Province, Tak Bai district, Ga-looh-wor-nua village, roadside
N 6º 24.98 E 101º 51.03
Thailand, Trat Province, Muang district, near Hat Muk Resort
N 12º 02.03 E 102º 45.73

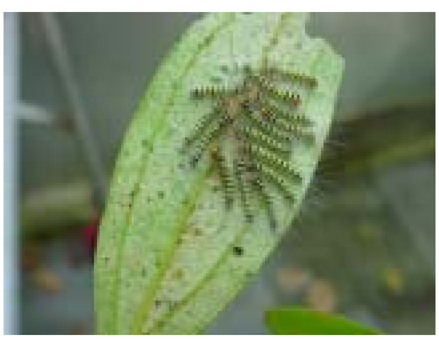
3.4 Unknown shoot borer:

Larval damage of a young shoot was found at one site near where adult snout weevils were found on another occasion. The shoots with damage were collected and reared in the laboratory in Bangkok. The larvae fed inside stem but all died before developing to adults.



Location:
Thailand, NST Province, Muang district, Tambol Pak Poon, behind Ban Pak Poon School
N 8º 31.28 E 99º 58.56

8.5 *Trabala vishnou* (Lefroy) (Lepidoptera: Lasiocampidae), Tent caterpillar



These caterpillars were found feeding on *R. tomentosa* growing in a small garden behind the laboratory at the Biological Control Research Group, Entomology and Zoology Division, DOA. The female moth lays eggs in masses covered with anal tufts. Newly hatched larvae are gregarious, the body is yellow with black stripes, and the last instar larva is about 6 cm long. The larval stages are considered to have poison on their bristles. Wingspan of the adult is about 5 cm. The body and wing coloration in males is pale green and yellow in females.

T. vishnou is distributed widely in Thailand. Larvae feed on many fruit crop plants, such as rose apple, sapodila plum, Rangoon creeper, Singapore almond (Terminalia catappa L.) Combretum quadrangulare (Kurz), etc. It has also been recorded on R. tomentosa in Hong Kong (David L. Mohn).

Location:
Thailand, Bangkok, Chatuchak, DOA plant garden nursery

***R. tomentosa*: soil pH values**

Soil pH testing indicated that *R. tomentosa* prefers acid soils. Two values obtained from Chumphon Province (pH 9 & pH 10, see below) appeared unusually high by comparison with all other values and retesting can be considered. All values were estimated with a field test kit (Inoculo Laboratories) using barium sulphate powder and pH indicator solution.

Thailand, Chumphon Prov., Tha Sae District, Tambol ThaKham, ThaKhamWittaya School
N 10º 34.65 E 099º 07.10' pH = 10
Thailand, Chumphon Province, Muang district, Tambol Haad Sai Ree, Moo 6
N 10º 23.93 E 099º 16.72 pH = 9
Thailand, Krabi Province, Muang district, Tambol PraNang, Moo 5
N 08º 02.56 E 098º 48.57 pH = 6
Thailand, Krabi Province, Klong Tom district, Tambol Pae Hla, Suan Pa (cultivated forest)
N 08º 09.17 E 099º 06.91 pH = 7
Thailand, NST Province, dirt road nr Ban Yang Tia site
N 8º 32.06 E 100º 58.15 pH 4.5
Thailand, NST Province, Sichon district, nr Ban Chai Thale, roadside on #401
N 8º 58.85 E 99º 53.60 pH 6.5 – 7 in roadside ditch, no *R.t.*
Thailand, NST Province, Tha Sala district, Tambol Photong, Wat Node
N 8º 36.31 E 99º 56.88 pH 5.5
Thailand, NST Province, Cha Uat district, Ban Tul
N 8º 02.89 E 99º 58.98 pH: low area 6.5, (no *Rt*)
high, grey sand, 4.0 – 4.5 (*Rt* present) high, red soil, 6.0 – 6.5 (no Rt)
Thailand, Surat Thani Province, Don Sak district, nr Ban Don Sau Thong
N 9º 13.77 E 99º 40.24 pH 4 - 4.5
Thailand, Surat Thani Province, Don Sak district, nr Ban Bor Woa
N 9º 09.47 E 99º 42.46 pH 5.5 – 6.0.
(No *R.t.* at soil sample site)
Thailand, Surat Thani Province, Tha Chang district, Tambol Tha kaui, Moo 5.
N 09º 14.77 E 099º 08.74 pH = 6.5
Thailand, Surat Thani Province, Tha Chana district, Tambol PraSong, Moo 12
N 09º 30.37 E 09º 908.01 pH = 6.5
Thailand, Trat Province, Klong Yai District, Tambol Mai Root.
N 11º 55.210 E 102º 48.47 pH = 6
Thailand, Trat Province, Klong Yai District, Tambol Mai Root.
N 11º 56.021 E 102º 48.05 pH = 5.5
Thailand, Trat Province, Klong Yai District, Tambol Mai Root, Ban Klong MaNao
N 11º 57.30 E 102º 47.41 pH = 5.5
Thailand, Trat Province, Klong Yai district, Tambol Mai Rood
N 11º 55.78 E 102º 48.19 pH = 7

Notes and Recommendations

1. Although many sites were visited and re-visited, the list of species presented here should not be considered complete. The survey was conducted over a period when Thailand and many other areas in Southeast Asia experienced weather drier than normal and conditions were less favorable for plant growth and insect populations. The USDA/ARS ABCL project exploring for potential biological control agents of *Lygodium microphyllum* during the period found that some insects previously collected were rare or absent in collections. Therefore it is likely that further collections from *R. tomentosa* in favorable seasons could result in additional species of interest.

2. An old record of an alternative plant host of *Carea varipes* (in Robinson et al. 2001) needs further investigation because the identity of the host has not been satisfactorily resolved.

3. Further study of the ‘striped caterpillar’ is recommended, including life-stage specimens required by SEL taxonomist, Dr David Adamski, to complete identification studies or a description of the insect. Because the damage to fruit can be severe, this insect may have the potential to reduce plant reproduction without killing plants, which could be an advantage if conflict between issues of the plant’s weediness and ornamental value arise.

4. Further study of the weevil is recommended, first to establish its identity, and second to indicate if ‘shoot borer’ damage observed is actually produced by larvae of the weevil.

5. Comparison of the DNA profiles of the ant-mimic larva with those similar species known as pests of crops could rapidly establish if further study is warranted.

6. Although the thrips could be host-specific, the damage to flower parts and effect on subsequent fruit production was not included in the scope of the survey and further study is required.

7. Flower beetles were collected from *R. tomentosa* but not studied or included in this report because they were visually very similar to species in flowers of *Melastoma malabathricum* commonly growing with *R. tomentosa*. Samples were collected in alcohol, so DNA comparisons could conveniently be used to indicate if the flowers of the two species share the same insect species.

8. Insects known to be non-specific are included in this report because the information may be of use to future researchers who are likely to encounter larval stages of these species.

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Discussions:

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Identifications:

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