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2000 Annual Report

prepared by

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Mr. Tony Wright - Experimental Scientist
Mr. Matthew Purcell - Experimental Scientist
Mr. Jeff Makinson - Research Officer
Mr. Ryan Zonneveld - Research Officer
Mr. Dalio Mira - Plant Culture (part-time)
Mr. Gio Fichera – 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 In order to prevent the spread of out-of-date or inaccurate information, this report should not be quoted or cited without verifying accuracy with the USDA-ARS Australian Biological Control Laboratory.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 Organization
nNSW	- Northern New South Wales, north of Coffs Harbour
NQ	- North Queensland, north of the Tropic of Capricorn
NSW	- New South Wales
QLD	- Queensland
sNSW	- Southern New South Wales, south of Wollongong
SQ	- South Queensland, south of the Tropic of Capricorn
SEL	- (USDA-ARS) Systematic Entomology Laboratory
TAG	- (USDA-APHIS) Technical Advisory Group on the Biological Control of Weeds
USDA	- United States Department of Agriculture

Table of Contents

Executive Summary

Biological control research programs for the broad-leaved paper bark tree, *Melaleuca quinquenervia*, and Old World climbing fern, *Lygodium microphyllum,* progressed well during 2000. Several new insects show promise for biological control of *M. quinquenervia* including tip-boring moths and a leaf-defoliating beetle that can pupate in the tree bark. More than 10,000 galls of melaleuca gall fly, *Fergusonina* sp. were collected and shipped to collaborators in Gainesville, Florida to be used in quarantine screening. Plans to assess the toxicity of the melaleuca sawfly, *Lophyrotoma zonalis*, have been developed with veterinary pathologists in Brisbane, QLD. Over 30,000 *L. zonalis* larvae were reared and field collected for these studies.

Exploration for new agents for biological control of *L. microphyllum* continued in Australia and Southeast Asia. Several new colonies have been initiated, and we now have ten species/populations in culture at ABCL. Quarantine screening of the pyralid moth, *Cataclysta camptozonale* for 35 plant species was conducted at ABCL. Preliminary screening of two other pyralids, *Neomusotima conspurcatalis* and *Musotima* sp., was initiated. All three pyralids only completed development on *Lygodium* species, including the North American native *L. palmatum*. Cold temperature studies were initiated to determine the thermal tolerances of these tropical and subtropical moths. This data will be used to assess the risk these species may pose to *L. palmatum,* which grows in the cool temperate woodlands of the eastern North America. Field and laboratory studies have been started with an eriophyid mite, *Floracarus* sp. This mite appears to be associated with the seasonal decline of mature *L. microphyllum* stands in Australia and Southeast Asia.

Two insect biological control programs were started for *Pectinophora gossypiella* (Pink Bollworm) and *Maconellicoccus hirsutus* (Pink Hibiscus Mealybug). Both insects are native to Australia and their distribution is similar to Melaleuca and *L. microphyllum*. Added funding from these programs allows for more frequent travel to the Northern Territory (NT) and Western Australia (WA) for both insect and weed programs. Four parasitoid species were collected from *M. hirsutus* in QLD and WA. Pending identification, one or more species may be exported to Newark, DE for quarantine screening. Surveys were conducted in the NT and WA for parasitoids of *P. gossypiella*. Several species were recovered and are pending identification.

Overview

The staff of the Australian Biological Control Laboratory (ABCL) actively searches 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, QLD, 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 Australia). 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 paperbark tree (*M. quinquenervia*), Old World climbing fern (*L. 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 Australian Biological Control Laboratory 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 Arlyene Myers (Assistant Administrator) and Dr. Richard Greene (Deputy Director). The personnel and facilities of the ABCL in Australia are provided through a cooperative agreement with CSIRO Entomology. The United States Embassy in Canberra provides the administrative support.

A coalition of federal, state and local agencies fund the overseas research on biological control of *M. quinquenervia, L. microphyllum, P. gossypiella* (Pink Bollworm) and *M. hirsutus* (Pink Hibiscus Mealybug).

South Florida Water Management District, Jacksonville District of United States Army Corps of Engineers, Florida Department of Environmental Protection, ARS-Western Cotton Research Laboratory, and USDA-APHIS National Biological Control Institute contributed substantially to the research during 2000.

ABCL works closely with the following project leaders to coordinate the research: Dr. Ted Center (Melaleuca & Hydrilla), Dr. Bob Pemberton (Lygodium), Dr. Alan Kirk (Pink Hibiscus Mealybug), and Dr. Steve Naranjo (Pink Bollworm). ARS National Program leaders are Dr. Ernest Delfosse (Weeds) and Dr. Kevin Hackett (Biological Control).

Staff and Facilities

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

Greenhouse facilities at ABCL were upgraded to allow for efficient rearing of tropical insects during the dry winter months. Our main greenhouse was retrofitted to allow for evaporative cooling in the summer and below-bench hot water pipe heating in the winter. Insect colonies are flourishing in the new facility. Additional shadehouses were added to allow for increased production of *M. quinquenervia* and additional ferns on the *L. microphyllum* host-test list.

Travel and Visitors

In May, John Goolsby visited the Office of International Research Programs (Beltsville, MD) and the Invasive Plant Research Laboratory (Ft. Lauderdale, FL). Dr. Goolsby presented a paper to the Annual Meeting of the Florida Exotic Plant Pest Council, entitled: "Contributions of the ABCL towards biological control of Florida weeds."

Dr. Robert Vlasik on assignment with USDA-APHIS (Weslaco, TX) to oversee fumigation of cottonseed infested with *Pectinophora* spp. visited the lab in June 2000. Dr. Alan Kirk, EBCL visited in July 2000 to travel with Dr. Goolsby to the Northern Territory and Western Australia.

Kelly Cutchin and Laurie Hewitt spent three months (Sept-Nov) 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 field collection of *Fergusonina* galls. 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, *M. quinquenervia*, was introduced into Florida as an ornamental at the beginning of this century. There has been a rapid expansion of this pest's range in southern Florida over the past 30 to 40 years . It now infests half a million acres, resulting in extensive environmental and economic damage.

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 (Fig 1). 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). In Australia, *M. quinquenervia* is also sold as an ornamental by commercial nurseries.

<u>Melaleuca Field Collections and Exploratory Surveys in 2001</u>

10-13 April: - Surveys between Townsville and Daintree National Park in north Queensland (NQ) to collect larvae of *Careades plana* (Lepidoptera: Noctuidae).

29 May – 1 June: - Surveys in Darwin and Litchfield National Park in the Northern Territory (NT) to collect *Lophyrotoma zonalis* (Hymenoptera: Pergidae) for toxicity tests.

11-15 September: - Surveys between Townsville and Daintree National Park to collect *L. zonalis* larvae for toxicity tests and *Fergusonina* spp. (Diptera: Fergusoninidae) galls.

30 October- 2 November 2000: - Surveys for temperate agents in central NSW (cNSW). Surveyed the Myall Lakes, Forster and Port Macquarie.

16-21 December 2000: - Surveys between Townsville and Daintree National Park, NQ, to collect *L. zonalis* larvae for toxicity tests.

During 2000, most of field collections (170) were of *M. quinquenervia* in SQ to obtain *Fergusonina* bud galls for shipment to quarantine in Florida, and to collect and identify moths that severely damage flowers. Some of these collections were from cNSW near Forster and Port Macquarie, regions that were not explored in previous years. We also made 36 field search collections from eight other broad-leaved *Melaleuca* species, mainly to collect bud galls to determine the species complex of *Fergusonina* flies, and to collect *L. zonalis* larvae for toxicity tests. A breakdown of all collections is given in Table 2 while a complete list of collection records is supplied in Appendix 1.

Table 2. Summary of field search collections made during 2000 in north Queensland (NQ), south-east Queensland (SQ), northern NSW (nNSW), central NSW (cNSW), southern NSW (sNSW) and the Northern Territory (NT) relating to *Melaleuca* research.

Plant		Number Collections	Number Sites	Regions
Family	**Species**			
Myrtaceae – *M.leucadendra* complex	*M. argentea*	3	2	NQ
	M. cajuputi	8	4	NQ/NT
	M. dealbata	3	3	NQ
	M. fluviatilis	2	2	NQ
	M. leucadendra	1	1	NQ
	M. nervosa	7	5	NQ
	M. quinquenervia	170	61	NQ/SQ/nNSW /cNSW/sNSW
	M. stenostachya	2	1	NQ
	M. viridiflora	6	6	NQ/SQ
Other Myrtaceae	*Callistemon* sp.	1	1	SQ
	Callistemon viminalis	2	2	SQ
	Eucalyptus seeana	1	1	SQ

New South Wales Surveys. In November we conducted exploratory surveys of *M. quinquenervia* in cNSW, near Forster, Port Macquarie and the Myall Lakes. Some of these areas have never been searched for herbivores. We wanted to determine if there was a different complex of insect herbivores that attack *M. quinquenervia* in more temperate regions. Many of the insects that we have collected in the sub-tropical and tropical areas further north were observed, including *Pterygophorus insignis* (Hymenoptera: Symphyta), *Poliopaschia lithochlora* (Lepidoptera: Pyralidae), *Lophodiplosis indentata* (Diptera: Cecidomyiidae) galls, and *Fergusonina* sp. galls.

We collected flea beetles (Coleoptera: Chrysomelidae: Galerucinae) feeding on small saplings of *M. quinquenervia* at the Myall Lakes. The adults scarred the leaves though no larvae could be found in the field, or in the laboratory when set up on potted plants. Larvae of flea beetles can feed on foliage, but many feed below ground on roots. *Rhyparida* (Chrysomelidae) adults were also collected from many sites feeding on young stems, but like the flea beetles, larvae could not be found in the field or laboratory. Extensive damage was observed to tips of *M. quinquenervia*, especially in the Myall Lakes region, where moth larvae had burrowed through the young stems. This damage has been recorded before, though not to the extent observed in cNSW. Unfortunately, searches of the damaged material yielded few specimens, and most of the agents had left their host plants. Some immature specimens were reared and these adults have been curated for identification.

Similar surveys are planned for 2001, corresponding with the flowering season and periods of new foliage growth. Following the damage observed to young tips in the November 2000 survey of the Myall Lakes, field excursions will be planned for September/October in an attempt to recover larger numbers of the Lepidoptera larvae boring within young stems.

Melaleuca Sawfly – *Lophyrotoma zonalis* (Hymenoptera: Pergidae)

Larvae of the sawfly *Lophyrotoma zonalis* defoliate broad-leaved *Melaleuca* spp. trees in the northern regions of Australia. Larvae pupate in the papery bark of broad-leaved *Melaleuca* spp., and this insect completes its whole life cycle on the tree . Adults are very mobile, especially males who 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. After intensive studies in Australia, *L. zonalis* was shipped to quarantine in Florida in 1992. All host-specificity screening has been completed, though studies on the toxicity of larvae are to be undertaken before release.

In 1999, Dr. Peter Oelrichs, from the National Research Center for Environmental Toxicology (NRCET) in Brisbane, determined that *L. zonalis* larvae contained 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). Dr. Oelrichs was the first to identify toxins in sawflies, isolating lophyrotomin from *L. interrupta*, a sawfly that feeds on *Eucalyptus melanophloia*, and poisoned cattle in Queensland (Oelrichs 1982, McKenzie *et al*. 1984).

Because *L. zonalis* is a high priority candidate for biological control of *M. quinquenervia*, we will undertake toxicity studies of large animals to determine the potential of this sawfly to poison animals if releases are to be made in Florida. Although both toxins have been isolated from the larvae of *L. zonalis*, the levels vary between sawfly species. *Lophyrotoma zonalis* has high concentrations of pergidin and slightly lower levels of lophyrotomin, the latter being more toxic (Oelrichs pers com.). This is the reverse of data recorded for the larvae of *L. interrupta* where lophyrotomin is in higher concentrations. Therefore, toxicity tests conducted for *L. interrupta* can be no guide to the toxicity of the *L. zonalis*. Behavioural factors must also be considered. There are differences between the larval habits of *L. zonalis* and that of other sawflies that have been implicated in animal poisonings around the world. Unlike these other sawflies, *L. zonalis* larvae pupate individually in the papery bark of *Melaleuca* trees, inaccessible to many animals. This is also one of its perceived benefits as a biological control agent, being able to complete its life cycle in the vast wetland areas of southern Florida. Many sawflies, including those involved in cattle poisoning, form large gregarious masses that move to the soil to pupate. 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, they may be unpalatable to many animals.

Both NRCET and Queensland Department of Primary Industries (QDPI) have facilities to conduct toxicology studies, and both organizations have been contacted regarding possibilities for testing. Negotiations to conduct the tests during 2001 are underway. The tests will follow the same experimental protocol used by the QDPI for *L. interrupta*. Over 30,000 larvae will be required.

Initially we were going to rear larvae in the laboratory for toxicity tests, as *L. zonalis* is difficult to collect in large numbers from *M. quinquenervia* field sites. They are frequently found on closely related broad-leaved *Melaleuca* species, though we were concerned that the levels of toxins may vary depending on the species of *Melaleuca* consumed. *Lophyrotoma zonalis* are not found on *Melaleuca* spp. in southern Queensland, and must be collected from tropical regions of northern Australia.

Over 2000 larvae were collected from *M. cajuputi* trees in Darwin, NT during May, and half of these larvae were placed onto foam boarding for pupation. The remaining larvae were freeze-dried and sent to Dr. Oelrichs for toxin analysis. Adults emerging from the foam were released into cages containing *M. quinquenervia* saplings or into sleeves surrounding branches. Emerging larvae remained on the saplings until all leaf material was consumed after which they were reared on cut material in plastic containers. In September, John Goolsby and Matthew Purcell visited NQ where they collected small numbers of *L. zonalis* larvae from *M. dealbata* to boost laboratory numbers.

Unfortunately, most immatures held within the sealed containers became diseased and over 90% died. Moisture within the containers facilitated the rapid infection of larvae by non-motile bacteria. Dr. Bob

Teakle, an insect pathologist from QDPI, confirmed the existence of these bacteria following dissections of infected larvae. Rearing within containers was abandoned, and larvae were then reared on cut *M. quinquenervia* branches, made into bouquets, then placed in water within gauze cages. Although this technique was successful in reducing bacterial infection, it was highly labour intensive with plant material needing to be replaced every two days. To rear sufficient larvae for testing using this technique would have taken considerable time.

The results of toxin analysis of *L. zonalis* larvae collected from *M. cajuputi* in Darwin determined that the levels of lophyrotomin and pergidin toxins were no different to that found on *M. quinquenervia*. Therefore, we decided to collect larvae from any *Melaleuca* species, and have sub samples tested for toxins. In November, Matthew Purcell travelled from Townsville to Cairns, NQ. In this region, only three *M. viridiflora* trees at one site near Gordonvale, south of Cairns, were infested with *L. zonalis* larvae. However, over 5,000 larvae were collected from this site over three days. Unlike 1999, when extensive defoliation by this sawfly was observed over extensive areas, only small pockets of defoliation were observed this year, possibly due to an increase in parasitization or variations in seasonal climatic conditions. Regional sawfly abundance during a second trip to the region by Matthew Purcell and Ryan Zonneveld in December was similar, with large numbers of larvae found on three *M. leucadendra* trees from a site in southern Cairns . Over 25,000 larvae were collected from these trees over four days.

During the collection of *L. zonalis* larvae from the sites in NQ, observations were made of the daily habits of larvae, not recorded in previous studies . Larvae were found on the base of the trunks of trees throughout the day, mostly mature larvae searching for pupation sites in the papery bark. However, during the November/December collections, massive numbers of larvae moved down from the upper branches between 9.00am and 11.00am on consecutive days. Again, many of these larvae were mature, searching for pupation sites. However, there were also large numbers of immature larvae, possibly moving down the tree from defoliated branches, and then returning upwards to limbs with leaves. It is unclear why these habits occur during the stated hours, or whether they occur throughout the season. Most larvae for the planned toxicity tests were collected during this morning period. Collecting for the remaining hours of the day was uneconomical.

All larvae reared in the laboratory or field collected have been frozen for further testing. There should be sufficient stocks of larvae for toxicity testing, however should more be required, *Melaleuca* sites in North Queensland are being monitored for *L. zonalis* by collaborators based in Cairns and Townsville.

Tip-Binding/Flower-Feeding Moth – *Holocola* sp. (Lepidoptera: Tortricidae)

During 1999, Dr. Thai Van from the USDA Invasive Plants Research Laboratory in Fort Lauderdale, and Dr. Min Rayachhetry from the University of Florida, visited Brisbane to research the ecology of *M. quinquenervia*. They had collected ecological data on *M. quinquenervia* stands growing in Florida, including standing biomass, seed production and litter fall, and wanted similar data from Australia for comparison (Rayachhetry *et al* 1998). After analyzing litter fall samples collected by a collaborator, Dr. Margaret Greenway from Griffith University in Brisbane, they immediately noted the high rate of abortion of young flower buds in native stands of *M. quinquenervia*. From our extensive Melaleuca samples collected since 1986, we were aware that many Lepidoptera larvae attacked flowers, though we hadn’t isolated species that fed on buds and immature inflorescences. Flower-feeding insects had not been targeted as biological control agents, mainly due to difficulties in mass rearing and testing. Additionally, insects specific to *M. quinquenervia* that solely feed on flowers may not be successful as biological control agents in Florida. These specific flower-feeding herbivores are likely to die out during the nonflowering season, having no alternate hosts to sustain their populations between seasons. We were aware that some moths possibly have a dual mode of feeding, both on inflorescences and young foliage (tips), allowing them to survive year round. Research was then directed to verifying which species exhibited these habits.

In March we began regular collections of flower buds, immature inflorescences and leaf tips of *M. quinquenervia* at two sites in SQ north of Brisbane, Landsborough State Forest and Peregian Environmental Park. These sites were visited weekly and 30-50 buds or immature inflorescences (with obvious damage if possible) were collected and held in the laboratory for emergence of moths. Flowering of *M. quinquenervia* is followed by a flush of young foliage. Once flowering material could not be collected, 30-50 young foliage tips (again with damage/leaf binding if possible) were collected and held for emergence of moths. All of the adults were preserved and labeled. Surveys continued until October.

Curation of the reared moths is continuing. However, it appears that the larvae of at least one species of *Holocola* damages both the flowers and leaves of *M. quinquenervia*. Larvae tunnel through flower buds or through the stems of the immature inflorescences, preventing the formation of flowers and the subsequent production of seeds. These larvae also bind young leaves together, feeding on the leaf material within. Notes obtained with previous identifications of *Holocola* by Dr Marianne Horak, a taxonomist at the CSIRO Australian National Insect Collection (ANIC), indicated that members of this genus are specific to feeding on Myrtaceae. Additionally, each species is likely to be host specific. Unfortunately, the genus *Holocola* requires a major revision and there are many unnamed species. The adults are also sexually dimorphic, further impeding our attempts to separate species. Representatives of our specimens collected during 2000 will be sent to Dr. Horak during 2001 for identification. We are also likely to employ DNA techniques to verify species differences before commencing extensive biology and host-range studies. During 2001, we will conduct wide ranging collections of flowering material of *M. quinquenervia* to isolate and mass rear moths identified as having the dual flower/leaf mode of feeding. If successful, host-range testing will be initiated.

The importance of this initiative cannot be underestimated. Impeding the vast reproductive potential of *M. quinquenervia* is imperative if adequate control of this invasive weed is to be achieved.

Gall Fly/Nematode Complex – *Fergusonina* sp.(Diptera: Fergusoninidae) / *Fergusobia* sp. (Nematoda: Tylenchida: Sphaerulariidae)

The gall-making fly *Fergusonina* sp. Malloch and its obligate nematode, *Fergusobia* sp. Currie, form galls in the leaf and flower bud tissue of *M. quinquenervia* . It is likely that the nematode initiates gall formation (Giblin-Davis unpublished data). Galls of the leaf buds inhibit terminal and lateral branch growth, while galls of flower buds prevent or inhibit flower formation and seed set. This insect has been a high priority research target at the ABCL for some time. Collaborators from the ARS Invasive Plant Research Laboratory, ARS Systematic Entomology Laboratory, University of Florida, University of Missouri and taxonomists from the University of Adelaide in Australia, are currently determining the complex of *Fergusonina* spp. on broad-leaved *Melaleuca* species and other Myrtaceae. Dr. Robin Giblin-Davis and colleagues from the University of Florida are also conducting research on the biology of the fly/nematode association through sectioning galls.

In August, the first shipments of *Fergusonina*/*Fergusobia* galls collected from *M. quinquenervia* were sent to the Gainesville quarantine facility in Florida. Between August and November, 13 shipments of galls were made, the result of 42 field collections from 27 sites in SQ and nNSW. Almost 10,000 galls were shipped. Most were collected by the two Berea University students, Kelly Cutchin and Laurie Hewitt (see inset picture below). Each shipment was carefully packed with ice bricks to avoid temperature extremes on route to Florida. A miniature temperature/humidity recorder was also included and returned to ABCL by Gainesville quarantine staff. Adjustments in cooling procedures were then made in further shipments so that ideal conditions could be maintained within shipment packages.

Because the level of parasitism increased throughout the season, the number of *Fergusonina* flies within galls decreased accordingly. Thus, large numbers of galls were shipped to ensure colony establishment and host-testing procedures could proceed in Florida. Toward the end of the season, most of the field-collected galls had emergence holes from most chambers. Galls of suitable quality for shipment (majority of chambers intact) became difficult to collect in sufficient numbers, hence the high number of collection sites visited by staff. Sub-samples of each shipment were retained at the ABCL to compare emergence rates with the shipped samples so that any adverse effects of shipping could be identified. Quarantine testing in Florida is proceeding on schedule.

Collections were also made on other broad-leaved *Melaleuca* species during the year to supplement research on the species complex of *Fergusonina*/*Fergusobia* initiated during 1999. Adult flies were reared from galls collected on *M. argentea* for the first time. Flies were also collected from *M. fluviatilis, M. nervosa*, *M. quinquenervia, M. stenostachya* and *M. viridiflora* to verify existing data.

Parasitism of *Fergusonina* in Australia. In order to understand the impact of parasitism, we first needed to establish which species of wasps are primary parasitoids of the *Fergusonina* sp. and which are hyperparasitoids or inquilines. A large complement of primary parasitoid species may indicate that parasitism plays a significant role in regulating *Fergusonina* sp. populations in Australia. Understanding the regulatory effect of natural enemies on a potential biological control agent in its native range is a useful predictor of its success in its adventitious range.

The biologies of eleven species of Hymenoptera associated with the gall-making fly, *Fergusonina* sp. were investigated. More than 2000 wasps were reared from 1100 galls collected in New South Wales and Queensland, Australia over a two-year period from 1997 to 1999 from *M. quinquenervia* (Table 3). Additional galls from each site were dissected for observation and description of the immature stages. A molecular technique, which involved sequencing D2 expansion domain of the 28S rRNA gene, was used to match the identity of the immature wasps with their adult forms.

Table 3. Numbers of *Fergusonina* and parasitoids reared from galls during two-year field study at three locations: Peregian (P) and Morayfield (M) in QLD and Woodburn (W) in NSW.

Site	P	M	W	All Sites
# Galls	366	493	263	1122
Fergusonina sp. (Fergusoninidae)	372	483	420	1275
Eurytoma (Eurytomidae)	300	473	13	786
Coelocyba (Pteromalidae)	276	33	122	431
Neanastatus (Eupelmidae)	65	144	35	244
Cirrospilus (Eulophidae)	113	19	0	132
Bracon (Braconidae)	28	42	47	117
Eupelmus (Macroneura) (Eupelmidae)	4	99	0	103
Chromeurytoma (Pteromalidae)	15	50	5	93
Megastigmus (Torymidae)	17	35	28	80

Eupelmus (Eupelmus) (Eupelmidae)	3	5	2	10
Euderus (Eulophidae)	3	4	2	9
Poecilocryptus (Ichneumonidae)	0	1	4	5
Total Hymenoptera	824	905	258	2010
% Parasitism	68.9%	65.2%	38.1%	61.2%

At Morayfield and Peregian the mean level of parasitism was approximately 67% (493 galls at 68.9% and 366 galls at 65.2% respectively) but dropped to 38.1% (from 263 galls) at Woodburn possibly due to the absence of *Eurytoma*, the most common parasitoid of the twelve wasp species. *Eurytoma* was determined to be a primary ecto- and endo-parasitoid of *Fergusonina* larvae and pupae. The next most common wasps, *Coelocyba* and *Neanastatus*, (and *Megastigmus* appearing in smaller numbers at all sites), have also been confirmed as primary parasitoids, but unlike *Eurytoma* they seem to develop upon a single *Fergusonina* larva. Like *Eurytoma*, *Bracon* is a voracious feeder, parasitizing multiple *Fergusonina* larvae and pupae, but it also appears capable of acting as a hyperparasite. *Eupelmus* (Macroneura) and *Chromeurytoma* appear to be exclusively hyperparasitic, from the small numbers of specimens we have examined.

Of the eleven species of Hymenoptera associated with the *Fergusonina* sp. gall, two were classified as primary endoparasitoids, three as solitary primary ectoparasitoids, three as predator ectoparasitoids feeding on multiple hosts, and three as hyperparasitoids. The predator ectoparasitoid species have specialized biologies, which enable them to chew through plant tissues to access gall inhabitants. We do not know which of the eleven species are gall specific, but did find it interesting that none of these species were reared from other galls on *M. quinquenervia* formed by Cecidomyiidae or Homoptera (unpublished data). None of the Hymenoptera in this study could be described as inquilines. The term inquiline is defined by Torre-Bueno (1989) as a commensal that lives in a very close spatial relationship with its host, in its shelter, not feeding on it, but frequently destroying it. We did occasionally observe species of Lepidoptera acting as inquilines, including one species, *Holocola* sp., which is known to feed on plant tips of *M. quinquenervia* (unpublished data).

We found the D2 molecular method to be robust for characterizing all life stages including eggs and small larvae. Further, the D2 gene sequence was consistent for each species and between adults and immatures. The molecular technology provides many advantages in the study of cryptic immature insects. The amount of time and effort required to identify immatures is greatly reduced because rearing to the adult stage is not needed. The biology of the immatures can be observed in vivo and matched with adults without speculation or comparison to known biologies of related species. A greater number of gall-inhabiting insects are likely to be discovered using this technique as compared to other techniques. Removal of the gall from the plant and holding it for insect emergence subjects the inhabitants to changes in plant turgor, humidity and temperature. All of these factors could be critical to the survival of the immatures. Sleeving the gall for collection of emerging insects may be biased against late arrivals such as hyperparasitoids.

In biological control programs directed against weeds, agents must reach high population levels in order to control their host. Development of high population levels in the region of introduction is promoted initially by an almost unlimited food supply and by release from the agent's natural enemies (Harley & Forno 1992). *Fergusonina* sp. is likely to be introduced to Florida, USA, where it will find an abundance of suitable *M. quinquenervia* plant buds which it needs to form galls (Goolsby *et al.* 2000a, Van *et al.* 2000). In its region of origin *Fergusonina* sp. is heavily attacked by natural enemies, including eight primary parasitoids. One would predict that fewer parasitoid species would attack *Fergusonina* sp. in

Florida, and that they would be less co-adapted than those in Australia. Fergusoninidae are not represented in the New World, so the association with this family of gall-making flies would be novel for the indigenous parasitoids. In the absence of its co-evolved natural enemies, *Fergusonina* sp. could reach much higher populations levels, potentially having an impact on *M. quinquenervia*. We hope that our study provides the basis for future comparisons of natural enemies of *Fergusonina* sp. in both its native and adventitious range. This research would further our ability to predict the impact of indigenous parasitoids on introduced biological control agents.

***Careades plana* (Lepidoptera: Noctuidae)**

The larvae of the noctuid moth, *Careades plana,* feed mainly on the older leaves of *M. quinquenervia* . As reported in 1999, in previous surveys of Melaleuca spp. in Australia, this moth was only found in very low numbers, almost entirely on *M. quinquenervia*, with one collection from *M. cajuputi*. This moth has been collected at 13 field sites in tropical NQ, though the larvae have never been collected south of Townsville. In 1998 and 1999, specimens were also collected from *M. cajuputi* in southern Thailand, the same as collected in Australia, verified by analysis of the D2 gene.

In April Matthew Purcell conducted field surveys for *C. plana* in NQ. Two larvae were collected from *M. viridiflora* for the first time at Pandanus Road, a site north of the Daintree River. Another 28 larvae and one pupa were collected from a stand of *M. quinquenervia* nearby. Additionally, four larvae and one pupa were collected from Edmund Kennedy National Park, near Cardwell, north of Townsville. These are the first collections since surveys of *Melaleuca* began where significant numbers of larvae have been collected.

Careades plana collected from the field in NQ were hand carried back to the Brisbane laboratory. Most of the larvae reached the pupal stage and adults were released onto saplings of *M. quinquenervia* held in gauze cages. No mating or oviposition occurred. Further attempts at mating were conducted, including using a variety of cage sizes (including a walk in), gauze colors, lighting and temperature regimes (indoor and outdoor). All attempts were not successful. After consulting with Dr. Don Sands, an expert in rearing noctuids, he indicted that rearing moths in this sub-family could be very difficult, requiring highly specific conditions to induce mating. Conditions in SQ during May are much colder than in tropical NQ. Therefore, providing the moths with warm conditions under natural lighting was impossible. We now have a new controlled environment glasshouse that could provide the conditions necessary to induce mating. If possible, further attempts will be made to collect and colonize *C. plana* during 2001.

***Paropsisterna tigrina*? (Coleoptera: Chrysomelidae)– Leaf Beetle**

Chrysomelid larvae were collected feeding on *M. cajuputi* in Darwin in NT in May . Adults reared from these larvae appear to be similar to specimens previously collected from NT on *M. cajuputi*, and identified as possibly *Paropsisterna tigrina*. We have also collected *P. tigrina?* from *M. leucadendra*, *M. nervosa*, *M. quinquenervia* and *M. alternifolia*. This beetle is a serious pest of *M. alternifolia* plantations in nNSW where this tree is grown commercially for “tea tree oil”, an essential oil extracted from the leaves. Adults and larvae of *P. tigrina*? feed on the young leaves. Our specimens from *M. cajuputi* appear to be lighter in color and much larger than specimens collected from *M. alternifolia*. We intend to verify if more than one species exists through DNA analysis.

A small colony of *P. tigrina*? has been established in Brisbane. Some very preliminary host-range testing was completed in September/October using *Callistemon viminalis* and *M. viridiflora*. Tests of each species were replicated three times using *M. quinquenervia* as a control. Five mated pairs of *P. tigrina*? were placed onto a sapling of a test species inside a gauze cage. The same number of pairs was set up on *M. quinquenervia* in a separate cage. After 7 days, the adults were removed and the eggs were counted. Once eclosion occurred, larvae were transferred to cut plant material in plastic containers for rearing to the adult stage. Results of these tests are given in Table 4.

Table 4. Results of no-choice oviposition/survival tests of *Paropsisterna tigrina*? on two test plants. Each test was replicated three times with *Melaleuca quinquenervia* used as a control.

Plant Species	Mean oviposition (eggs/test)	Mean oviposition (control) (eggs/test)	Mean % survival after hatch	Mean % survival after hatch (control)	Mean % survival to adult	Mean % survival to adult (control)
Callistemon viminalis	96.3	203.7	1.9	62.4	0.0	4.1
Melaleuca viridiflora	174.3	172.7	81.4	71.8	7.1	3.8

It was not surprising that *M. viridiflora* was an acceptable host of *P. tigrina?*, considering that this insect has been field collected from other *Melaleuca* spp. in Australia. *Callistemon* is a closely related to *Melaleuca* and is not native to the USA, but *C. viminalis* and other members of this genus are highly valued ornamentals in Florida. Although adults laid large numbers of eggs on this species, it was less than half that recorded on *M. quinquenervia* and there was no survival of immatures. Further comprehensive tests will be required to assess the potential of this insect as a biological control agent for *M. quinquenervia*. Mass rearing is underway in Brisbane to facilitate the completion of host testing.

<u>Other Insects</u>

Pea galls were very rare in the field during 2000, thwarting attempts to conduct further studies on the causative agent, *Lophodiplosis indentata* (Diptera: Cecidomyiidae). Therefore attempts to colonize and conduct impact, biology and host-range studies of this insect have been postponed until this insect can be recollected from field sites, possibly during 2001.

Cecidomyiid galling of the stems of young *M. quinquenervia* saplings and seedlings was observed at several field sites, especially at Roy's Road north of Brisbane. The stem deformation caused by these flies may severely impede the growth of these young trees. The reared adults will be sent to Dr. Raymond Gagné at the Systematic Entomology Laboratory for identification. If sufficient specimens can be collected, attempts will be made to colonize this gall fly.

The tube-dwelling moth, *Poliopaschia lithochlora*, was observed in very low numbers during the year. Our main collection site at Tyagarah in nNSW was destroyed due to construction of a new highway. Laboratory cage tests conducted during 1999 indicated that *Callistemon* spp. might be acceptable hosts of *P. lithochlora*. This year we had planned to collect and rear this moth so that adults could be released into the Garden Plot at ABCL. We could than monitor the host preferences of *P. lithochlora* under more natural conditions. Unfortunately, the extremely low numbers at field sites prevented this research from proceeding.

Ecology of *Melaleuca* in Australia

Scientists from USDA-ARS, Ft. Lauderdale visited in 1999 to study the ecology of *M. quinquenervia* in Australia. Investigations were performed to study the regeneration potential of *M. quinquenervia*: including biomass allocation, seed production, seed set and seed viability, and rate of seed rain. Litter samples were collected by ABCL during 2000 for sorting by our collaborator Dr. Margaret Greenway (Griffith University – Brisbane). Data will be forwarded to Dr. Thai Van (ARS-Ft. Lauderdale) for analysis.

Survey for Pathogens of Melaleuca

Surveys. Dr. Min Rayachhetry, Alex Racelis, and ABCL Staff conducted fungal pathogen surveys during November and December 1999. Collections were made from many of the species in the *Melaleuca leucadendra* complex. Additionally, most of the sampling was conducted in the Townsville – Cairns area in NQ, where *Melaleuca* diversity is highest and climatic conditions are most similar to south Florida. As of January 2000, 15 different pathogens have been isolated and identified. Dr. Roger Shivas (Plant Pathologist, QDPI, Indooroopilly) was contracted to identify and voucher the fungi. One of the pathogens, *Phyllosticta* sp. was found to have potential as a biological control agent. Efficacy testing of this pathogen is described below.

Pathogen Testing. Preliminary pathogen studies were conducted at ABCL by Min Rayachhetry and Alex Racelis. The fungal pathogen, *Phyllosticta* sp. was evaluated in greenhouse efficacy tests (Fig 2). *Melaleuca quinquenervia* plants were inoculated with live fungal isolates. Tests were completed in April 2000. Dr. Roger Shivas (QDPI) scored the pathogen for pathogenicity. It does not appear that this pathogen is sufficiently virulent to be considered as a biological control agent. ABCL will continue to survey for additional *M. quinquenervia* pathogens.

Melaleuca Research Plans for 2001

The main priority for 2001 will be the initiation and completion of toxicity tests of large animals using *L. zonalis* sawfly larvae. Host testing has been completed on this sawfly at the Gainesville quarantine facility in Florida, and the results of toxicity tests will determine whether or not this insect is released. *Lophyrotoma zonalis* severely damages *Melaleuca* through the complete defoliation of trees. As it completes its life cycle entirely on the tree, it could be an exceptional biocontrol agent in the wetland areas of Florida. Therefore this research has been given the highest priority. Negotiations are underway between ABCL and researchers from the NRCET and QDPI regarding conducting these tests in Brisbane early in 2001.

We will continue to make regular collections of flowers in SQ to collect moth larvae that have a dual mode of attack, feeding on both immature flowers and buds as well as young foliage of *M. quinquenervia*. Initially, specimens curated from 2000 will need to be sorted and checked by taxonomists. Once species with potential have been identified, we will attempt to establish colonies, following which host testing and biology studies will proceed.

Now that a colony of *P. tigrina*? has been established, preliminary host testing of this insect will be completed in the coming year. Its species identification will be confirmed through DNA analysis by comparing specimens from NT with *P. tigrina?* collected from *M. alternifolia* plantations in NSW. Tests will also be conducted to determine whether *P. tigrina?* can complete its development on the tree.

Specimens in the laboratory have pupated on soil as well as in cage sealing. The papery bark of *Melaleuca* trees may be an acceptable pupation site.

If the pea gall former, *Lophodiplosis indentata*, is collected from field sites, then colonization, impact studies, biology studies and host testing will be initiated. Searches will also be conducted to locate large populations of *Poliopaschia lithochlora* so that adults can be reared and released into the garden plot to validate laboratory host testing of this insect. If possible, *Careades plana* larvae will be collected from NQ in a second attempt to colonize this moth. The updated, controlled environment glasshouse should provide much better conditions to induce mating and oviposition by *C. plana*.

Surveys are planned for temperate regions of NSW in an attempt to locate new insect herbivores of *M. quinquenervia* adapted to cooler climates. This has recently become a high priority due to the discovery of *M. quinquenervia* infestations in the Okefenokee Swamp in Georgia, much further north (colder) than the current infestations in Florida. Not only do we hope to discover new insects, but also to determine the geographic range over which the existing agents attack *M. quinquenervia*.

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 (Fig 3). 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 hydroperiod 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.

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. *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.

During 2000, exploration was conducted in Australia, Indonesia, Malaysia, Singapore, Thailand, and China. Many herbivores of *Lygodium microphyllum* were collected (Table 5). Preliminary host-range testing has been completed for three leaf-feeding pyralids, *Cataclysta camptozonale*, *Neomusotima conspurcatalis*, and *Musotima* sp. Two new species of noctuid moths in the genus *Callopistria* and an eriophyid mite, *Floracarus* sp. are beginning preliminary host testing. Field studies are underway to measure the impact of the mite on disease incidence and biomass production of *L. microphyllum*.

Table 5. *Lygodium* herbivores collected from Australia and Southeast Asia.

Name	Collection Locations	Host Plant
Cataclysta camptozonale Lepidoptera: Pyralidae	Australia (Queensland)	*L. microphyllum* *L. reticulatum*
Neomusotima conspurcatalis Lepidoptera: Pyralidae	Australia (Queensland, Northern Territory, Western Australia), Indonesia, Malaysia, Singapore, Thailand, China	*L. microphyllum* *L. flexuosum*
Musotima sp. Lepidoptera: Pyralidae	Malaysia, Singapore, Thailand	*L. microphyllum*
Callopistria sp. A Lepidoptera: Noctuidae	Australia (Queensland), Indonesia, Thailand	*L. microphyllum*
Callopistria sp. B Lepidoptera: Noctuidae	Australia (Western Australia & Northern Territory)	*L. microphyllum*
Endelus bakerianus Coleoptera: Buprestidae	Singapore	*L. microphyllum*
Lepidoptera: Limacodidae	Thailand	*L. microphyllum*
Lepidoptera: Tortricidae (possible 3 spp.)	Thailand, Malaysia, Singapore	*L. microphyllum*
Isotenes miserana Lepidoptera: Tortricidae	Australia (Queensland)	*L. microphyllum* *M. quinquenervia*
Stem-borer Lepidoptera: Pyralidae	Singapore	*L. microphyllum*
Neostrombocerus albicomus Hymenoptera: Tenthridinidae	Malaysia, Singapore, Thailand, Vietnam	*L. flexuosum*
Tortoise beetle Coleoptera: Chrysomelidae	Australia (Northern Territory)	*L. microphyllum*
Manobia sp. (Flea beetle) Coleoptera: Chrysomelidae	Thailand	*L. flexuosum*
Metriona sp. Coleoptera: Chrysomelidae	Australia (Northern Territory)	*L. microphyllum*
Lophothetes sp. Coleoptera: Curculionidae	Palau	*L. microphyllum*
Leaf-miner Diptera: Agromyzidae	Malaysia	*L. microphyllum*
Hemiptera: Miridae	Australia (Northern Territory)	*L. microphyllum*
Acanthuchus trispinifer Homoptera: Membracidae	Australia (Queensland)	*L. microphyllum*
Thrips	Malaysia, Thailand, Singapore,	*L. microphyllum*

Thysanoptera	China	*L. japonicum*
Floracarus sp. Acarina: Eriophyidae	Australia, Indonesia, Malaysia, Singapore, Thailand	*L. microphyllum*

***Lygodium* Research in Australia**

Exploration for natural enemies of *L. microphyllum* continued in 2000 with trips to Queensland (7), New South Wales (2), the Northern Territory (2), and Western Australia (2). *Lygodium microphyllum* would not be considered a weed in any of these habitats and is associated with a complex of herbivorous insectsand mites (Fig 4). From Australia, five species of Lepidoptera and an eriophyid mite from the genus *Floracarus* have been collected and established in culture.

Unexplored areas within the native range of *L. microphyllum* in WA were surveyed in 2000. Remote, isolated patches of *L. microphyllum* were located near Kununurra in the Kimberley Region. The fern is found deep within steep, shaded canyons where conditions are just wet enough for it to survive. In this environment, *L. microphyllum* grows from seeps and fissures in the canyon walls. The *Floracarus* sp. mite was collected from *L. microphyllum* in several locations. We compared the mites collected from WA with those from QLD, NSW and NT using DNA sequencing. The *Floracarus* from WA has identical D2 sequences to all the other Australian populations, therefore we can assume it is the same species widely distributed across the range of *L. microphyllum*. *Neomusotima conspurcatalis* and *Callopistria* sp. B were also collected. It appears from remnant damage on the plants that populations of these defoliating species build during the wet season and cause considerable impact on the fern. In 2001 we will endeavor to access these flooded environments to assess this effect and determine if other herbivore species are present. Considerable planning and coordination with locals is a must due to impassable roads, and the threat of crocodiles and poisonous snakes, which are more active during the wet season.

Surveys in NT recorded the same complex of Lepidopteran herbivores and *Floracarus* sp. that were found in the previous year. Two species of Hemiptera (true bugs) were found in very high numbers on *L. microphyllum* during the dry season. Dr. Geoff Monteiff (Queensland Museum) indicates that this a common survival tactic for these species. Later in the year, the bugs disperse from the shaded fern wetlands to reproduce on their favored host plants.

Regular surveys of field sites in SQ continued through 2000. Data is being gathered on the seasonal phenology of *L. microphyllum* and two herbivores, *Cataclysta camptozonale* and *Floracarus* sp. A large outbreak of *C. camptozonale* occurred at Carbrook Creek near Logan, QLD. Larvae of this species defoliated the fern along a 60-meter strip along the creek.

Australian Exploratory Trips

- SQ - Monthly visits to field sites to determine phenology of *C. camptozonale* and *Floracarus* sp.
- NQ - (Dec 6 – 10, 1999) Traveled to *Melaleuca* and *Lygodium* sites with collaborators from Ft. Lauderdale, Florida.
- SQ - (Dec. 10 – 18, 1999) Set-up *Melaleuca* field ecology experiments.
- NT & WA - (July 19 – 28) Surveyed previously unexplored areas in the distribution of *L. microphyllum*.
- NQ (Oct. 11-15) Collect field host-range data for *Lygodium* agents.
- WA & NT (Nov. 7-13) Revisited Kununurra area to collect herbivores.

Lygodium Exploration in Southeast Asia

Over much of Southeast Asia, very hot, dry conditions were followed by record rains late in the year, which brought severe flooding to many areas. As a consequence, insect populations on *L. microphyllum* were generally low during the year and we relied heavily on sites that have previously indicated a diverse insect fauna. However, as well as revisiting previously productive sites, exploration also included exploratory visits to major areas not previously visited in China and Thailand (Fig 5). A *Lygodium* workshop in Kuala Lumpur involving local collaborators from Sumatra, Malaysia and Thailand clearly demonstrated the value of collaboration, and this year new and valuable contacts were established with workers in Singapore, Hong Kong, Hainan and southern mainland China. As in previous years, we endeavored to increase efficiency by visiting more than one country per trip and by gathering information on other weedy species of US interest.

Exploration in Thailand included visits to sites in northern areas as well as to the less inhabited areas of Mae Hong Son in the west and adjacent to the Cambodian border in the east. Collaboration with the Department of Agriculture, in particular Khun Pimpolporn Nanta and Dr Amporn Winotai, has been invaluable and this year saw further strengthening of ties between ABCL and DOA. The project now uses DOA greenhouse space in Bangkok and staff of the Biological Control Research Group have assisted with collecting, rearing and liaising with other groups in the country.

The area around Mae Hong Son (an isolated basin surrounded by mountains and adjacent to the Burmese border) has many interesting historical records of isolated plant communities including *L. microphyllum* and *P. foetida*. However the drought conditions during the year also affected this area and *L. salicifolium* was the only *Lygodium* species found during the visit in February. At the Pangtong Royal Project Development Center where the Department of Agriculture monitors leaf-miner flies, contact was made with staff for future reference. To date, Thailand's great geographic diversity has resulted in the most productive collection areas found so far. Many species have so far been collected for identification or further study. One of these, a chrysomelid *Manobia* sp. from northern Thailand, could not be reared on *L. microphyllum* in Brisbane quarantine and it appears to be a *japonicum/flexuosum* specialist which could be useful in future projects.

A collection of the moth *Neomusotima conspurcatalis* in cooler areas of northern Thailand confirms its place as the most widespread herbivore of *L. microphyllum*, but in most places populations of this species were badly affected by the drought. However during the dry period it was surprising to find a very damaging population of *N. conspurcatalis* at Ongkharak near Bangkok, where a study of fluctuations in standing crop *of L. microphyllum* is commencing. (The study involves developing monitoring methods to compare actual and expected dry weights of the plant, the difference reflecting herbivore intensity.) The fact that heavy damage was observed in early August when maximum populations of the moth are not expected before late November, indicates that the species maintained its population at an unexpectedly high level during an unfavorable period.

Insect activity was also low in Singapore, however a further collection of *Musotima sp*. (currently being described) was made in early March for rearing, host testing and temperature-tolerance testing in Brisbane quarantine. This moth has only been found from southern Thailand, Malaysia and Singapore and appears much more restricted to the humid tropics than *N. conspurcatalis*. Singapore is also home to a leaf mining buprestid collected previously and identified as *Endelus bakerianus* Obenberger by Dr. Svatopluk Bílý (Department of Entomology, National Museum, Prague). Because the Brisbane quarantine colony of *E. bakerianus* did not survive, this species was targeted for collection this year, however during the year only two adults were seen in the field. The beetle has also been collected at the insect-rich Wat Node site in southern Thailand, so both areas will be intensively searched during 2001.

There were also difficulties rearing the stem-boring moth in quarantine and because of its extreme rarity in the field in Singapore, more widespread searching on thick-stemmed *Lygodium* in Malaysia and southern Thailand may be necessary. Similar stem-boring damage has been observed on *L. flexuosum* so the possibility exists that the insect may also utilize the closely related *L. japonicum*, although in southeast Asia stems of *L. japonicum* are not as thick as those of mature *L. microphyllum* and *L. flexuosum*. It may be possible to conduct long term field studies in Singapore in order to further study this insect.

Contact was made with Mrs Yang Chang Man of the University of Singapore/Zoological Reference Collection (Raffles Museum) and representative specimens of *N. conspurcatalis* were lodged with the Collection. It is hoped that opportunities for future collaboration with Museum/University staff may be developed in the coming year, especially with respect to further study on the stem-boring lep.

In Hong Kong, contact was made with the Director (Mr Richard Yip) and staff of the Department of Agriculture, Fisheries and Conservation. Valuable assistance with specimens, historical records and field visits was provided by Mr Patrick Lai and staff associated with the DAFC Hong Kong Herbarium. Many sites including areas of Lantau, Lamma and Hong Kong Islands and both the eastern and western New Territories (with Dept. staff) were visited in August and November. Insects collected on *L. microphyllum* included a *Callopistria* sp. and undetermined tortricids. Specimens of both of these moths were taken to the Brisbane quarantine facilities where a colony of the *Callopistria* was established (the tortricid was regarded as an unlikely prospective agent so further study was postponed). There was little insect activity on *L. japonicum*, a very common fern of the area, but this may be a result of unusual climatic conditions. Of particular interest was the abundance of *Triadica sebifera* (= *Sapium sebiferum*) (Chinese tallow) in Hong Kong, including places where it and *L. microphyllum* grow in close proximity. Historical records for *Lygodium* and *Triadica* were checked at the Hong Kong Herbarium, Kowloon.

In November, a brief visit to Macau revealed *L. japonicum*, *L. flexuosum*, *P. foetida* and *T. sebifera* but no *L. microphyllum*. Later on mainland China, travel with Hong Yin Chen from the USDA-ARS Sino-American Biological Control laboratory, Beijing, was undertaken through various parts of southern China. Unfortunately, relatively little *L. microphyllum* was encountered during much of the travel in Guangdong Province, with just mites, thrips and old damage typical of *N. conspurcatalis* being seen. Conditions were quite dry until the final day when a typhoon crossed the coast bringing good rains to southern China. Hainan Island, which has much more *L. microphyllum* than the mainland, benefited from the rain and collecting was also much improved. Of most interest were collections of *Callopistria* sp. from Hainan, which were added to collections from Hong Kong for culture in ABCL quarantine in Brisbane.

South-East Asian Exploratory Trips:

- Singapore (Feb 20-22). The aim was to visit two sites with Indonesian collaborator Dr. R. Desmier de Chenon (CIRAD-Sumatra) to see typical damage of *Lygodium* herbivores for comparison with other Indonesian sites.
- Thailand (Feb 22-26) To explore part of the northern region and the isolated area of Mae Hong Son, following records indicating the presence of *L. microphyllum* and *P. foetida*.
- Malaysia (Feb 26-28) To visit wetland sites and the Bagan Lalang site to recollect *Musotima* sp. and check for *Endelus* sp.
- Singapore (Feb 28-Mar 2) For collection of *Musotima* sp. and *Endelus* sp. at both Sungai Buloh and Bukit Timah. Also collected insects seen on earlier visit, including *Callopistria* sp., an undetermined tortricid and stem-borer moth larva.
- Singapore (Jul 30-Aug 2) To inspect the insect collection at the Raffles Museum and in particular their holdings of the buprestids, *Endelus* spp., and lodge specimens *of N. conspurcatalis*. Also to check field sites for *Endelus*.
- Malaysia (Aug 2-3) To check the mines site for insect activity and pick up pinned specimens from Malaysian collaborator.
- Thailand (Aug 3-7) To check eastern provinces of Trat, Rayong and Prachinburi and along the Thai side of the Cambodian border to Hat Lek.
- Hong Kong (Aug 7-11) To review *Lygodium* specimens at the Hong Kong Herbarium, contact staff of the Department of Agriculture Fisheries and Conservation, visit sites in the New Territories and Lantau Island. Check distribution records for *L. japonicum* and *Triadica sebifera*.
- Hong Kong (Nov 1-4) To introduce John Goolsby to local collaborators and extend collection to other areas on Lantau Is., Lamma Is., Hong Kong Is. and the New Territories.
- Macau (Nov 4) To survey for *L. microphyllum* and other US weeds.
- Guangdong (Nov 5-9) To establish local contacts and explore for prospective agents on *L. microphyllum*.
- Hainan (Nov 9-12) To establish local contacts and explore for prospective agents on *L. microphyllum*.
- Hong Kong (Nov 12-16) To revisit sites from previous visit where insects were seen and collect for transfer to ABCL quarantine.

<u>Leaf Feeding Moth - *Cataclysta camptozonale* (Hampson) (Lepidoptera: Pyralidae)</u>

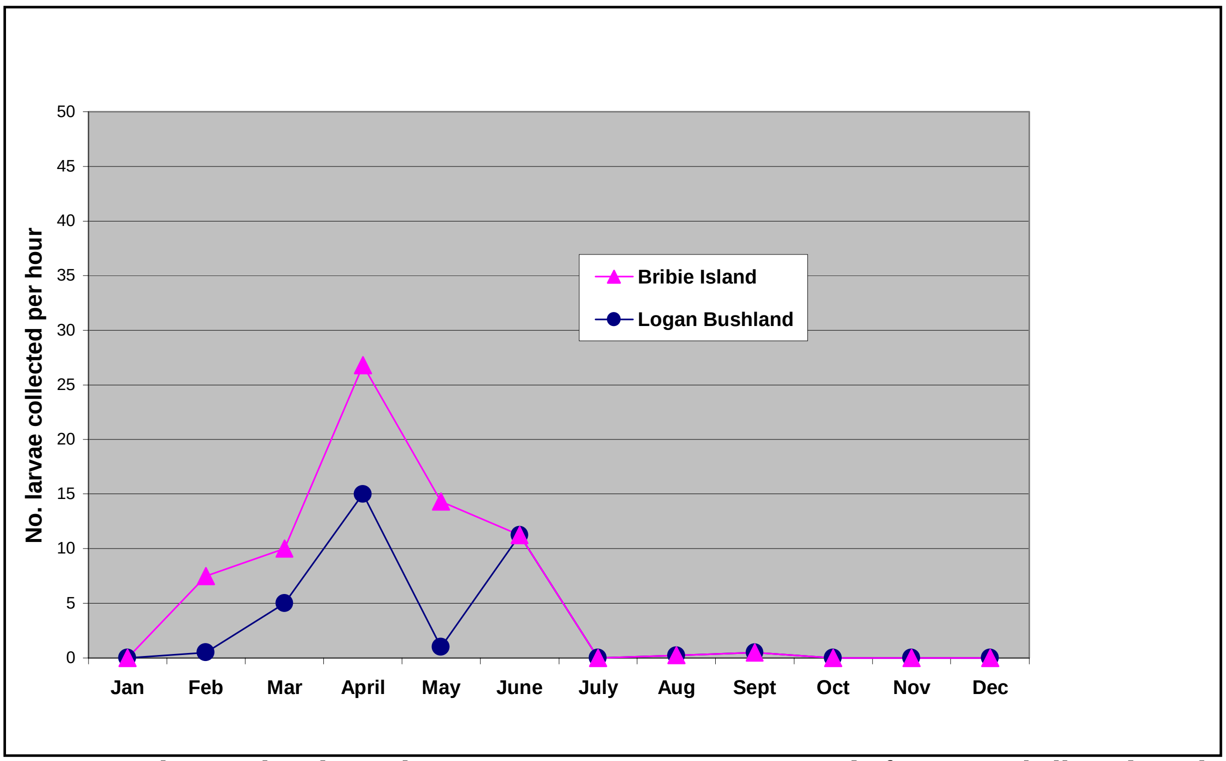
Distribution. *Cataclysta camptozonale* is distributed from subtropical SQ and nNSW to tropical NQ.

Biology. Eggs are yellow and laid singly or in clusters mostly on the new growth. Larvae skeletonize *L. microphyllum* leaves, with fourth instar larvae consuming entire leaves. The developmental period from egg to adult at 27 C^{o} is 21 days.

Seasonal Phenology. Populations of *C. camptozonale* were monitored monthly at two locations near Brisbane. The numbers of larvae collected per hour was recorded as a measure of density (Fig 6). Populations peaked during April (Fall) and remained at moderate levels through the Winter months at both locations. Populations were below detectable levels during the Spring and Summer.

Fig 6. Seasonal population levels of *Cataclysta camptozonale* at two locations in SQ.

Carbrook Creek Outbreak. During April and May of 2000, we observed an outbreak population of *C.*



camptozonale at Carbrook Creek near Logan, QLD. An entire stand of *L. microphyllum* along the creek was defoliated covering an area of 1800 square meters. At the peak of the outbreak, density of the larvae reached 65 per hour. We attempted to measure biomass destruction using paired replicates of covered and uncovered potted ferns. Although we observed extensive damage to the uncovered plants the difference was not significant. Following the defoliation event at the site, two fern species, *Hypolepis muelleri* and *Blechnum indicum* increased in dominance. Nine months after defoliation, the *L. microphyllum* stand had still not regrown to its former density. The empirical data gathered from this outbreak shows the tremendous potential of *C. camptozonale* as a biological control agent.

Host-range. In order to expedite the host-range testing of *C. camptozonale* the test plant list was split between ABCL and quarantine facilities in Gainesville. ABCL screened species of ferns that were more readily available in Australia. Twenty-nine fern species were tested in no-choice tests (Table 6). Two pairs of newly emerged moths were placed inside a moistened plastic bag that enclosed a single fern leaf, or small group of pinnae. Adults were left in the bags until death or five days, if eggs were visible.

Table 6. List of species to be tested in Australia

Family	Plant Species	Comments
Schizaeceae	*Lygodium microphyllum*	Australian form
	L. microphyllum	Florida form
	L. japonicum	Related species in Australia/SEA
	L. palmatum	Closest relative in N. America
	L. reticulatum	Related species from Australia
	Anemia adiantifolia	Related species in West Indies
	Schizaea bifida	Related species in West Indies
Aspleniaceae	*Asplenium nidus*	Rare in Florida, in same habitat
	Phyllitis scolopendrium	Ornamental
Cyathaceae	*Cyathea cooperi*	
Dennstaediaceae	*Hypolepis muelleri*	Ornamental
	Pteridium aquilinum	
Dryopteridaceae	*Nephrolepis biserrata*	
	Polystichum acrostichoides	Ornamental
	Rumohra adiantifolis	Ornamental
Gleicheniaceae	*Sticherus flabellatus*	In same habitat in Australia
Lycopodiaceae	*Lycopodiella cernuum*	Ornamental
Ophioglossum	*Ophioglossum petiolatum*	Ornamental
Osmundaceae	*Osmunda regalis*	Common in Australia
Parkeriaceace	*Ceratopteris thalictroides*	In same habitat in Australia
Polypodiaceae	*Phlebodium aureum*	Common in Australia
	Platycerium hillii	
Psilotaceae	*Psilotum nudum*	Ornamental
Pteridaceae	*Acrostichum speciosum*	Related species in West Indies
	Adiantum capillus-veneris	Related species from Hispanola
Salviniaceae	*Salvinia molesta*	Ornamental
Selaginellaceae	*Selaginella emmelliana*	
Thelypteridaceae	*Thelypteris patens*	Ornamental

Host-range data indicates that *C. camptozonale* is a genus-level specialist (Table 7). *Lygodium microphyllum* (Florida & Australia) and *L. palmatum* had the highest mean number of eggs deposited. It appeared that these two species were preferred over *L. japonicum* and *L. reticulatum*. More replicates are needed to determine if this is a true indication of host preference. Other neotropical *Lygodium* spp. should be tested in 2001 to better understand host suitability and preferences of *C. camptozonale* within the *Lygodium* genus.

Females were induced to lay eggs on nine fern species outside the *Lygodium* genus (Table 7). In all cases the naïve first instar larvae died shortly after hatching. In most instances we observed that the larvae attempted to feed on the test plant but did not find it suitable. Unfortunately we were not able to move naïve 1st instars onto all the plant species in the test list due to difficulties in removing eggs from leaves. Tests to determine the physiological host-range of experienced third instar larvae will be conducted in 2001.

In 2001, additional neotropical *Lygodium* species from the West Indies will be tested because of the proximity of this region to southern Florida. *Lygodium volubile,* which occurs in Cuba and other areas of the West Indies, belongs to the same subgenus Volubilia as *L. microphyllum*. *Lygodium cubense* a Cuban endemic, *Lygodium venustum* in the West Indies, and *Lygodium oligostachyum* endemic to Hispaniola (the Dominican Republic and Haiti), belong to a separate subgenus Flexuosa. With the addition of the neotropical species, we will be testing eight Lygodium species. This should give us a better understanding of the specificity of *C. camptozonale* and the other candidate Lepidoptera species on plants at the subgenus level.

Table 7. Host-range Testing Results for *Cataclysta camptozonale.*

Family	Plant Species	No. Reps	Total Eggs	Mean No. Eggs ± SE	Mean No. Adults Reared ± SE
Schizaeceae	*Lygodium microphyllum*- (AU)	13	385	29.6 ± 8.6	20.3 ± 5.9
	L. microphyllum – (FL)	9	183	20.3 ± 7.6	12.8 ± 7.0
	L. japonicum	9	25	2.8 ± 2.1	1.9 ± 1.6
	L. palmatum	4	117	29.3 ± 1.4	13.8 ± 4.8
	L. reticulatum	1	2	2.0	0.0
	Anemia adiantifolia	12	90	7.5 ± 6.9	0.0 ± 0.0
	Schizaea bifida	7	0	0.0 ± 0.0	-
Aspleniaceae	*Asplenium nidus*	11	5	0.5 ± 0.3	0.0 ± 0.0
	Phyllitis scolopendrium	4	0	0.0 ± 0.0	-
Cyathaceae	*Cyathea cooperi*	10	3	0.3 ± 0.3	0.0 ± 0.0
Dennstaediaceae	*Hypolepis muelleri*	7	0	0.0 ± 0.0	-
	Pteridium aquilinum	10	0	0.0 ± 0.0	-
Dryopteridaceae	*Nephrolepis biserrata*	9	5	0.6 ± 0.5	0.0 ± 0.0
	Polystichum acrostichoides	7	0	0.0 ± 0.0	-
	Rumohra adiantifolis	11	15	1.4 ± 1.1	0.0 ± 0.0
Gleicheniaceae	*Sticherus flabellatus*	4	5	1.3 ± 0.7	0.0 ± 0.0
Lycopodiaceae	*Lycopodiella cernuum*	4	0	0.0 ± 0.0	-
Ophioglossum	*Ophioglossum petiolatum*	0	0	0.0 ± 0.0	-
Osmundaceae	*Osmunda regalis*	8	15	1.9 ± 1.1	0.0 ± 0.0
Parkeriaceace	*Ceratopteris thalictroides*	4	0	0.0 ± 0.0	-
Polypodiaceae	*Phlebodium aureum*	10	21	2.1 ± 1.8	0.0 ± 0.0
	Platycerium hillii	4	0	0.0 ± 0.0	-
Psilotaceae	*Psilotum nudum*	4	0	0.0 ± 0.0	-
Pteridaceae	*Acrostichum speciosum*	7	0	0.0 ± 0.0	-
	Adiantum capillus-veneris	11	13	1.2 ± 1.1	0.0 ± 0.0
Salviniaceae	*Salvinia molesta*	7	0	0.0 ± 0.0	-
Selaginellaceae	*Selaginella emmelliana*	7	0	0.0 ± 0.0	-
Thelypteridaceae	*Thelypteris patens*	7	0	0.0 ± 0.0	-

<u>Leaf Feeding Moth – *Neomusotima conspurcatalis* (Lepidoptera: Pyralidae)</u>

Distribution. *Neomusotima conspurcatalis* has been collected from Australia and many parts of Southeast Asia. This year we extended the known range of *N. conspurcatalis* to include southern China. Collections were made in Hong Kong, Guandong and Hainan. Both *L. japonicum* and *L. circinatum* were found in the habitats we surveyed. We did not see any damage or collect any larvae from either species. This is good evidence that the field host-range of this insect may be more narrowly specific at the subgenus level.

Molecular Data. In order to determine if we were dealing with a complex of species across this wide distribution, we used a molecular genetic tool to analyze the DNA of the different populations. Molecular sequencing of the nuclear rRNA D2 gene showed an exact match between the specimens from Australia, China, and other parts of Southeast Asia. This indicates that we are dealing with one species throughout its distribution.

Biology. Eggs are yellow and laid singly or in clusters mostly on the new growth. Larvae skeletonize *L. microphyllum* leaves, with fourth instar larvae consuming entire leaves. The approximate developmental period from egg to adult at 25 C^o is 25 days.

Host-range Testing. Thirteen plant species were evaluated in preliminary host-range testing of *N. conspurcatalis* (Table 8). Like the other pyralid species under evaluation, *N. conspurcatalis* appears to be a genus-level specialist, as larvae were only able to complete development on *Lygodium* species (Table 8). *Lygodium japonicum* appears to be a more suitable host for *N. conspurcatalis* than for *C. camptozonale*. This would be expected since *L. microphyllum* and *L. japonicum* are sympatric throughout most of the range of *N. conspurcatalis.*

Preliminary tests to determine the physiological host-range of naïve immatures were conducted. Females were induced to lay eggs on eight fern species outside the *Lygodium* genus (Table 8). Over 288 eggs were laid on these eight species, yet none of the larvae lived past the first instar.

Table 8. Host-range testing results for *Neomusotima conspurcatalis.*

Family	Plant Species	No. Reps	Total Eggs	Mean No. Eggs ± SE	Mean No. Adults Reared ± SE
Schizaeceae	*Lygodium microphyllum* – (AU)	3	101	33.7 ± 12.0	29.7 ± 17.8
	L. microphyllum – (FL)	4	89	22.3 ± 14.3	16.5 ± 11.1
	L. japonicum	4	35	8.8 ± 5.9	6.8 ± 6.1
	L. palmatum	2	56	18.7 ± 3.3	3.0 ± 1.4
	Anemia adiantifolia	2	107	53.5 ± 13.1	0.0 ± 0.0
Aspleniaceae	*Asplenium nidus*	3	6	2.0 ± 1.7	0.0 ± 0.0
Cyathaceae	*Cyathea cooperi*	3	38	12.7 ± 9.3	0.0 ± 0.0
Dennstaediaceae	*Pteridium aquilinum*	3	0	0.0 ± 0.0	-
Dryopteridaceae	*Nephrolepis biserrata*	3	17	5.7 ± 2.5	0.0 ± 0.0
	Rumohra adiantifolis	3	6	2.0 ± 1.7	0.0 ± 0.0
Osmundaceae	*Osmunda regalis*	3	57	19.0 ± 5.2	0.0 ± 0.0
Polypodiaceae	*Phlebodium aureum*	4	10	2.5 ± 2.5	0.0 ± 0.0
Pteridaceae	*Adiantum capillus-veneris*	3	47	15.7 ± 6.9	0.0 ± 0.0
Salviniaceae	*Salvinia molesta*	3	0	0.0 ± 0.0	-

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

Distribution & Taxonomy. *Musotima* sp. has been collected in the lowland tropical parts of Malaysia and Thailand. Specimens were identified by Alma Solis (ARS-SEL). Dr. Solis forwarded them on to Dr. Shen Horn Yen of the Chinese Academy of Sciences, Beijing. Dr. Yen is currently at the British Museum of Natural History revising the genus and intends to describe this new species.

Molecular Data. Specimens from Thailand and Malaysia had the same D2 sequence, which was distinct from the other two pyralids in culture.

Biology. Eggs are yellow and laid singly or in clusters mostly on the new growth. Larvae skeletonize *L. microphyllum* leaves, with fourth instar larvae consuming entire leaves (see inset pictures). The approximate developmental period from egg to adult at 25 C^o is 25 days.

Host-Range Testing. Thirteen plant species were evaluated in preliminary host-range testing of *Musotima* sp. (Table 9). Like the other pyralid species under evaluation *Musotima* sp appears to be a genus-level specialist, as larvae were only able to complete development on *Lygodium* species (Table 9). *Lygodium japonicum* appears to be a more suitable host for *Musotima* sp than *C. camptozonale*. This would be expected since *L. microphyllum* and *L. japonicum* are sympatric throughout most of the range of *Musotima* sp. Females were induced to lay eggs on four fern species outside the *Lygodium* genus (Table 9). Over 139 eggs were laid on these four species, yet none of the naïve larvae lived past the first instar.

Table 9. Host-range testing results for *Musotima* sp.

Family	Plant Species	No. Reps	Total Eggs	Mean No. Eggs ± SE	Mean No. Adults Reared ± SE
Schizaeceae	*Lygodium microphyllum* – (AU)	3	8	2.7 ± 1.9	3.0 ± 2.6
	L. microphyllum – (FL)	3	87	29.0 ± 20.7	23.7 ± 12.6
	L. japonicum	3	151	50.3 ± 36.0	30.7 ± 15.8
	L. palmatum	3	32	10.7 ± 7.6	4.0 ± 1.8
	Anemia adiantifolia	3	75	25.0 ± 17.9	0.0 ± 0.0
Aspleniaceae	*Asplenium nidus*	2	0	0.0 ± 0.0	-
Cyathaceae	*Cyathea cooperi*	3	0	0.0 ± 0.0	-
Dennstaediaceae	*Pteridium aquilinum*	2	34	17.0 ± 17.0	0.0 ± 0.0
Dryopteridaceae	*Nephrolepis biserrata*	3	27	9.0 ± 6.4	0.0 ± 0.0
	Rumohra adiantifolis	3	3	1.0 ± 0.7	0.0 ± 0.0
Osmundaceae	*Osmunda regalis*	3	0	0.0 ± 0.0	-
Polypodiaceae	*Phlebodium aureum*	3	0	0.0 ± 0.0	-
Pteridaceae	*Adiantum capillus-veneris*	3	0	0.0 ± 0.0	-
Salviniaceae	*Salvinia molesta*	2	0	0.0 ± 0.0	-

Leaf Feeding Moth - *Callopistria* spp. (Lepidoptera: Noctuidae)

Distribution and Molecular Data. *Callopistria* specimens have now been collected from *Lygodium microphyllum* in WA, NT, NQ, Thailand, and now China. D2 sequences were analyzed from specimens collected at each location. The molecular data indicates that we are dealing with three separate species. The three putative species being, 'A' type from QLD and China; 'B' type from NT and WA, and 'C' type from Thailand.

<u>Leaf Feeding Moth - *Callopistria* sp. A (QLD & China)</u>

Callopistria sp. A was collected from *L. microphyllum* near Cairns in tropical NQ . As the larvae mature they consume whole leaves. Clusters of leaves with missing pinnae are characteristic of the larval feeding damage. This character can be used to locate 'hot spots' of *Callopistria*. In China, *Callopistria* sp. A was collected from several locations, including Hong Kong and Hainan. It was most common on Lantau Island in Hong Kong.

Colonies of *Callopistria* sp. A are in culture from QLD and China. We are conducting preliminary host-range testing and cold temperature tolerances for both populations. Pupae from the Chinese population are tolerant of freezing temperatures. Mortality at – 3 ^{o}C was less than13% (Table 10).

<u>Leaf Feeding Moth - *Callopistria* sp. B (NT & WA)</u>

Callopistria sp. B has been collected from the Kimberly Region near Kununurra in WA and Litchfield National Park in NT. The population from WA is now in culture at ABCL. Previously we were only able to collect single individuals in the NT. The colonies of *Callopistria* sp. B are very robust, with larvae completely defoliating the plants.

We are assaying the cold temperature tolerance of this species as well as conducting preliminary host-range testing. Results of the cold temperature tests are reported in the next section. The host-range of this insect may be narrower than other known members of the genus. The population for WA was tested in a single, choice (*L. microphyllum* plus other non-target test species in one cage) and choice-minus experiment (same non-target test species caged with *L. microphyllum*), using *L. microphyllum, Anemia adiantifolia, Cyathea cooperi, Thelypteris patens,* and *Adiantum capillus-veneris.* In the choice-minus cage, eggs were laid on *Cyathea cooperi*, but no development was observed. In the choice test, large numbers of eggs were laid on *L. microphyllum*. Larvae in this test completely consumed the *L. microphyllum* plant, but no damage was observed on the other species. Further host-range testing is needed to evaluate this species, specifically its ability to oviposit and develop on other *Lygodium* spp.

<u>Cold Temperature Tests of Lepidoptera spp.</u>

Most of the insects we collect from Australia and Southeast Asia are of tropical or subtropical origin. Cold temperature tolerances are not known for these species. It is important to know thermal limits of species so that we can predict their eventual range in the Southeastern US if released. One of the critical non-target species, *L. palmatum* occurs in cool temperate parts of the Appalachian Mountains of the eastern US (see inset pictures). Establishing the minimum thermal limits of the candidate agents will help us better assess the potential risk these agents may pose to this non-target species of *Lygodium*.

Minimum temperatures and hours below freezing for a typical cold outbreak in Florida were used to develop the testing protocols. Temperatures as low as 7 ^{c}C were experienced in the Florida Panhandle for 15 hours (Fig 7). In the area north of Lake Okechobee temperatures were slightly below freezing at - 1 ^{c}C for four hours. Our tests are designed to measure the effects of a short duration mild freeze, down the scale to progressively colder temperatures.

The temperature tests were conducted using a refrigerated water bath with an attached heater. The water bath was filled with clean water for temperatures above zero and an anti-freeze solution for temperatures below zero. Cultures of live insects were maintained on *L. microphyllum* plants in insect proof cages or on cut *L. microphyllum* material in plastic containers. When pupation was observed, pupae were placed into 6mL glass tubes, sealed with plastic screw caps. The glass tubes were held firmly in a plastic rack so that they could be totally submerged in the water bath for two hours at a constant temperature. The tubes were removed and the pupae were held loosely in containers for adult emergence. The number of newly emerged adults was recorded daily.

Preliminary results of the experiment are shown in Table 10. *Musotima* sp. experienced considerable mortality below 0 ^{c}C. This species is only distributed through the tropics of Malaysia and Thailand so may not be adapted to freezing conditions. In contrast the larger *Callopistria* sp. B from China shows considerable tolerance to freezing conditions around -5 ^{c}C. Additional testing is needed to determine the lethal minimum temperatures for all five species. The results of this testing will be discussed in subsequent quarterly reports.

Table 10. Percent emergence of adults exposed to selected temperature for two hours.

^{o}C	*Musotima* sp.		*Neomusotima conspurcatalis*		*Callopistria* A	
Control	30	83.3	10	100	29	96.7
13	10	70	10	100	30	100
10	10	40	10	100	30	100
7	30	83.3	10	100	30	100
5	30	93	10	100	27	90
3	30	70	10	100	27	90
0	30	30			26	86.7
-3	30	33.3			30	83.3
-5	30	0			30	26.7
-7	30	0			15	0

Thrips –Thysanoptera

Thrips are an extremely common herbivore in Southeast Asia, sometimes occurring in large numbers and very damaging to *L. microphyllum*. There are four genera known from ferns, *Monilothrips, Indusiothrips* and two undescribed genera. Dr. Laurence Mound, ANIC, has advised us that our *Lygodium* thrips is a member of one of the undescribed genera of which specimens have been collected from Singapore, western Malaysia, Bali and the Ryukus. Dr. Mound forwarded our specimens to Dr. Shuji Okajima, Tokyo University of Agriculture, who plans to describe the insect.

Leaf Curling Mite- *Floracarus* sp. (Acari: Eriophyidae)

Taxonomy and Distribution. *Floracarus* sp. specimens were forwarded to Dr. Danuta Knihinicki of NSW Agriculture. Dr. Knihinicki is an eriophyid specialist and has described several new species from Australia. She intends to describe this mite as a new species. The name should be published in 2001.

Floracarus sp. is the most widespread arthropod associated with *L. microphyllum*. It has been collected from locations in Australia, Indonesia, Malaysia, Thailand, Singapore, and most recently, China. *Floracarus* sp. appears to be tolerant of the wide range of climates found in the tropics and subtropics of the region.

Molecular Studies. Populations of the mite from five locations in Australia were compared by sequencing the nuclear rRNA D2 gene. We found a consistent gene sequence in all the Australian specimens. *Floracarus* sp. from China showed ten base pair changes. This level of difference is not indicative of separate species, but does show considerable temporal isolation from the Australian population.

Biological Studies. *Floracarus* sp. has been studied in the laboratory since October 2000. We are developing specialized techniques that will allow us to investigate the biology and begin host-range testing experiments. Dr. Sebahat Ozman is collaborating with ABCL on these studies. Dr. Ozman is on sabbatical from Trakya University (Turkey), at the University of Queensland, studying with Dr. Dave Walters of the Department of Entomology and Zoology.

Biological studies are being carried out in an incubator at 22 °C, 14:10 (L:D) at ambient humidity. Biological observations on their development are being made on mites maintained on cut pinnae and tips of *L. microphyllum*. Cut pinnae are placed upside down on water-soaked sponges in petri dishes. Adults, nymphs and eggs were transferred onto the pinnae from infected plants grown in a glasshouse. Although adults fed on the pinnae for about two weeks, nymphs did not survive because the pinnae do not continue to grow and form leaf curls. Cut tips are suitable for oviposition and early development of nymphs. We are able to transfer nymphs to fresh tips to continue observations. However, from these trials we have determined that *Floracarus* sp. has specialized feeding requirements, and as a result culturing on cut plant material may not prove satisfactory. Mites create and feed within deformed tissues of the host plant, causing leaf curling. Nymphs are only able to feed on the deformed tissues inside the leaf curls. In an attempt to alleviate this problem, spores of *L. microphyllum* were planted to grow small plants. We will trial this method in 2001. If successful, much of the testing could be done on individual plants grown in petri dishes.

Preliminary studies show that this mite has a simple life cycle, consisting of an egg, larva, nymph and adult. There are two nymphal stages. Eggs hatch within 8-9 days at 22 °C. A quiescent or resting stage occurs between the larva and nymph, and again between the nymph and adult. Development duration from first instar to adult lasted about 7-8 days. Mature females have survived for about one month on cut tips. Immatures are similar to adults in appearance but of a smaller size. Males are similar to females, but slightly smaller. Fertilization likely occurs in the leaf curl, although we have not observed the male spermatophores. 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.

Mature females prefer the new sterile pinnae on actively growing plant tips for oviposition. Observations of newly formed leaf curling revealed up to five adults, although a single gravid female is able to cause the development of the deformation (curled-leaves) for itself and later for all its progeny. With the start of feeding, deformation in the leaf tissue occurs. The tissues become swollen and have a watery appearance and curling of the leaf begins. Attack on the leaf margin induces rolling, and on some pinnae, the whole margin may be affected. The leaf curls over downwards and turns on itself 2-3 times. The leaf curling eventually dries and falls. When the leaf curling begins to dry, adults leave and go to another young pinnae for feeding.

Host-Range Testing. *Floracarus* sp. appears to have a very narrow host-range. Field surveys have detected the mite on one other species of *Lygodium*, *L. reticulatum* which is sympatric with *L. microphyllum* in NQ. We have not observed the mite on other species including: *L. japonicum, L. circinatum*, or *L. flexuousum*.

Preliminary host-range tests with the critical non-target species, *L. palmatum* have been conducted. Two techniques were used to evaluate the suitability of *L. palmatum*: hand transfers of mites to new growth, and intermingling with infested *L. microphyllum* pinnae. To date we have not observed the characteristic leaf curling on *L. palmatum* indicative of *Floracarus* sp.

Further host-range testing is planned for 2001. We will attempt to import whole plants and spores of additional Neotropical *Lygodium* species from the West Indies, Central and South America. These species include: *L. volubile, L. cubense, L. oligostachyum, L. venustum* and *L. heterodoxum*. Dr. Bob Pemberton is planning to collect many of these species from the Dominican Republic in February 2001. CSIRO collaborators in Veracruz, Mexico may collect some of these species.

Field Phenology. Regular sampling at four sites in SQ has begun in order to determine the season phenology of *Floracarus* sp. on *L. microphyllum*. Fifty leaflets (approximately 300 pinnae) were collected at each site. The numbers of curled sterile and fertile leaves are counted. The numbers of mites in a subsample of ten pinnae are counted in order to estimate the actual population size.

Mite populations at the four sites remained fairly constant throughout the year (Fig 8). The pooled yearly average of infested pinnae for all sites was 20%. Populations likely remained steady due to the fact that the *L. microphyllum* plants at each site produced new growth throughout most of 2000. This provided gravid females with an ample supply of young pinnae for oviposition. Predatory mites were present in the samples. Many leaf curls were empty which indicates all the *Floracarus* sp. eggs and nymphs may have been consumed by predatory mites. This study will continue through 2001.

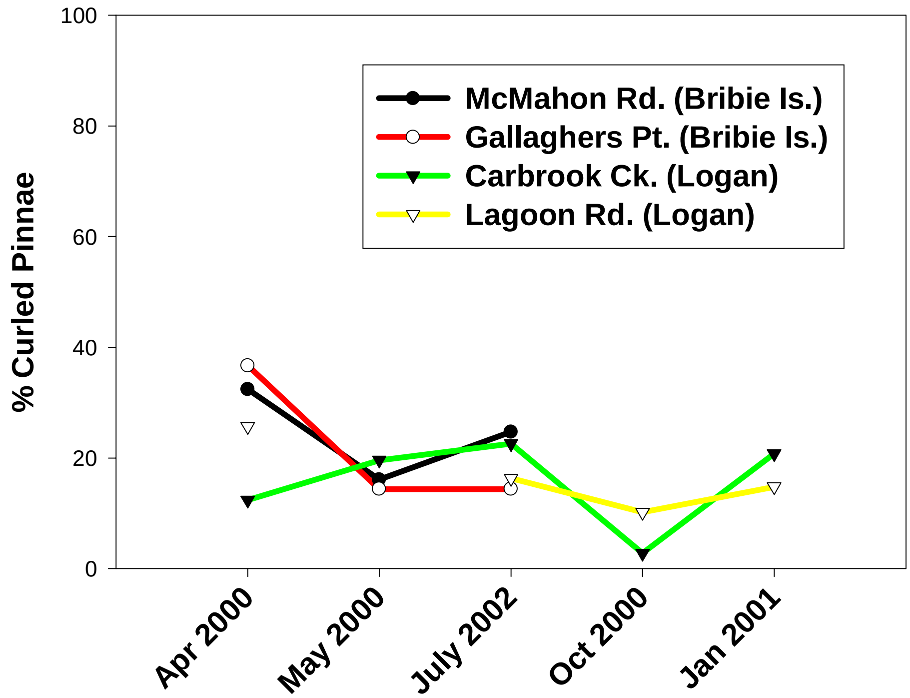


Fig 8. Percentage of sterile *L. microphyllum* pinnae infested with *Floracarus* at four locations in SQ.

<u>Research Plans for 2001</u>

Our research plans for 2001 include additional foreign exploration, biological studies of agents and host-range testing. Exploration in Southeast Asia will focus on New Guinea, New Caledonia, Palau, and Guam. We will also revisit Singapore to recover and culture the stem-boring Lepidoptera and leaf mining buprestid, *E. bakerianus*, collected in 1999. In Australia new parts of the range in WA near Shark Bay will be explored. Field studies will focus on the host-range and biology of the eriophyid mite, *Floracarus* sp. To determine the impact of the mite, a chemical exclusion test will be conducted at ABCL. We will be attempting to measure the impact on biomass production caused by *Floracarus* sp. We will continue screening of the pyralids, *C. camptozonale, N. conspurcatalis*, and *Musotima* sp. and two *Callopistria* spp. to determine if their physiological host-range includes the Neotropical species of *Lygodium*.

Biological Control of Giant Salvinia, ***Salvinia molesta***

Export of Biological Control Agent. The noxious aquatic weed *Salvinia molesta* has recently invaded Texas and Louisiana. Biological control programs conducted by CSIRO in Australia have been very successful using the weevil, *Cyrtobagous salviniae* originally imported from S. America (Fig 9). ABCL staff collected, reared and shipped over 500 *C. salviniae* weevils from nearby Wappa Dam to Phil Tipping & Ted Center (ARS-Invasive Plant Research Lab, Ft. Lauderdale, FL) and Lloyd Wendel & Daniel Flores (APHIS-Plant Protection Center, Mission, TX) .

Molecular Studies of Weevil. *Cyrtobagous salviniae* from Australia were compared with weevils believed to be *C. salviniae* from Florida using molecular sequencing (Goolsby *et al* 2000b). Sequencing of the nuclear rRNA D2 gene from both populations revealed considerable differences indicative of separate species. Based on this information weevils were exported from Australia to Florida for screening and field release. This information may also explain why *C. salvinae* from Florida failed to establish on *S. molesta* in Texas.

Prospects for biological control of Carrotwood, *Cupaniopsis anacardioides*

Cupaniopsis anacardioides has become a serious pest in localized areas in the United States. In particular, it 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 2000, nine collections were made of carrotwood in NQ and SQ. In a collection from Harvey Bay, north of Brisbane, larvae were observed feeding within the fruits. Several adults were reared, probably *Prosotas felderi* (Lepidoptera: Lycaenidae), a butterfly previously recorded feeding on *C. anacardioides* (Hawkeswood 1988). Unfortunately, this insect also feeds on *Acacia* spp, *Alectryon coriaceus*, *Macadamia integrifolia*, and *Litchi chinensis*.

Moth larvae also fed on the fruits of *C. anacardioides* at a site near Coolum on the Sunshine Coast. A stem boring larvae was collected from Stradbroke Island near Brisbane. Adults of both species have been reared and curated for identification. Galls were collected from the young foliage of a carrotwood tree at Hervey Bay. Cecidomyiidae larvae emerged from the galls soon after collection but failed to pupate. In 2001, bags will be placed on trees enclosing galls so that adults can be collected for identification.

A small number of seedlings of *C. anacardioides* are being grown at the ABCL to colonize any field-collected insects. The project remains a low priority, with opportunistic collecting continuing during 2001 alongside collections of *Melaleuca* and *Lygodium*.

Prospects for Biological Control of Skunkvine, *Paederia foetida*

Paederia foetida is an invasive weed across the southeastern U.S., especially in Florida. ARS cooperators in Ft. Lauderdale (R. Pemberton & P. Pratt) have conducted an initial biological control feasibility study for the Southwest Water Management District in Florida. It is likely that a biological control program will be developed, and ABCL is geographically well positioned to play a role in the foreign exploration.

Paederia foetida is widely distributed across the temperate, tropical and subtropical areas of east Asia. ABCL staff have located the plant in several locations in Southeast Asia. *Paederia* species occur from the cool subtropics of northern Thailand down the Malay peninsula to tropical Singapore. Consumption of *Paederia* as a vegetable is common in Thailand, much less so in Malaysia, so plants are commonly cultivated in Thailand. During exploration as part of the Lygodium project, *Paederia* was observed in several localities in Thailand, in the provinces of Songkhla, Nakhon Si Thammarat (specimen sent to B. Pemberton), Surat Thani, Chiang Mai and Phetchaburi; and in Malaysia in Pahang. Often, *Paederia* was noticed simply because it was growing with or near *L. microphyllum* .

Paederia spp. were again noted during this year's Lygodium exploratory trips. *P. foetida* was less common further north and away from wet coastal regions, apparently replaced by species appearing more hairy and less aromatic. For example, although *P. foetida* is listed as present in northern Thailand, and specifically in the Mae Hong Son area, we encountered none during a visit in February. Specimens taken from Chiang Mai and Mae Hong Son, together with ones collected by Amporn Winotai at Khon Kaen and Petchabun, were all identified as *P. linearis* by a Thai DOA botanist. Also, no *P. foetida* was seen during a visit to the strip along the Thai-Cambodian border in August although searching was limited because of bad weather, so this area can not yet be discounted. *Paederia foetida* with damage was seen in Singapore and it appears reasonably common around Hong Kong but with little damage. However in November on Colane Is. in Macau some very encouraging damage was present (but no insects on this occasion) caused by extensive feeding on the lower leaf surface, leaving only the upper translucent epidermis intact. Also in November in mainland China, two *P. foetida* plants were seen at the South China Botanic Gardens, Guangzhou.

Eight species are listed for Thailand. Many of the species can only be identified by their flowers and fruit, which are not present year round. We will continue to collect information on *Paederia* spp. in conjunction with *L. microphyllum* exploration.

Prospects for Biological Control of Hydrilla, *Hydrilla verticillata*

The aquatic plant, *Hydrilla verticillata* (Hydrocharitaceae), continues to be a problem weed in several regions of the United States since its introduction in the early 1950's. Hydrilla forms dense mats at the water surface, impeding water flow. It causes extensive environmental, economic and recreational problems including reductions in fish stocks, hampering flood control and irrigation, and obstructing navigation. Herbicidal and mechanical controls have been ineffective and very expensive. Following worldwide surveys for biological control agents of hydrilla in its native range, insects were introduced into the U.S. from Australia and parts of Asia. These agents, two *Bagous* (Coleoptera: Curculionidae) weevils and two *Hydrellia* (Diptera: Ephydridae) flies, have not established or have had a limited impact on the growth of hydrilla, and new agents are needed.

Intensive surveys of hydrilla conducted by ABCL staff were completed in 1992. However, while in southern Thailand in November of 1999, we visited several hydrilla sites. Collections were made at the Thale Noi Bird Sanctuary and on Highway 4151 south of Nakhon Si Thammarat. Hydrilla at both of these sites was growing in isolated areas in very small mats. A portable berlese funnel, as well as hand searching, was used to process the collected plant material. Small numbers of Nymphuline (Pyralidae), *Hydrellia* and *Donacia* (Coleoptera: Chrysomelidae) larvae were collected. Unfortunately, this material had to be processed quickly, possibly reducing the efficiency of the extraction process.

The center of origin of hydrilla is unclear though many botanists feel its origin lies in the warmer regions of Asia. Indonesia, Thailand, Malaysia and Vietnam have been lightly surveyed for hydrilla insects, and more extensive surveys could be useful in finding new agents. The sparse nature of hydrilla in southern Thailand suggests that natural enemies are controlling this plant. Additionally, insects collected from these habitats would be adapted to permanently flooded sites, such as lakes, irrigation canals and reservoirs. ABCL intends to canvas cooperators across the US to develop new support for exploration in Southeast Asia.

Prospects for Biological Control of Chinese Tallow, *Triadica sebiferum*

Chinese tallow, *Triadica sebiferum* (= *Sapium sebiferum*) (Euphorbiaceae) is a serious environmental and agricultural weed in the southeastern U.S. In Texas, pasturelands along the Gulf Coast are invaded by this weed changing the grass prairies to woodland thickets. In the eastern states it is invading riparian and coastal marshland habitats. Five-year-old trees can produce in excess of 100,000 seeds per year. The seeds are dispersed by birds further enhancing this species invasive characteristics. Researchers at Washington State University predict that its eventual distribution could include most of the eastern US.

The tree is a popular ornamental in the southern states, favored for its fall color. Biological control programs could target seed production, which could potentially reduce the invasiveness of this species. A limited program of this kind would still preserve the tree as an ornamental species.

Chinese tallow is native to southern China including Hong Kong and the adjacent provinces of Guanghzou, Guanxi, and Hainan. John Goolsby and Tony Wright were in southern China in November of 2000 collecting agents for *L. microphyllum*. Many sites with Chinese tallow were noted, it was often growing with *L. microphyllum*. Several insect species were observed feeding on the leaves, flowers and seeds. We found very little seed production on most trees, which should be investigated further. Overall, the potential for biological control of this species is promising. The ABCL and the Sino-American Biological Control Lab in Beijing could conduct foreign exploration in southern China.

Survey of Australian Parasitoids of Pink Bollworm, *Pectinophora gossypiella*

Pink Bollworm, *Pectinophora gossypiella* is a key pest of cotton in Northeastern Mexico, Arizona, & California (Fig 13). Pink Bollworm feeds on cotton bolls reducing both yield and quality of the lint. The introduction of transgenic Bt cotton varieties has resulted in more effective control of pink bollworm, while simultaneously reducing broadspectrum insectide use. As a result, classical biological control agents for control of pink bollworm are more likely to establish and be effective. Recent studies by Gordh (Univ. of Queensland) indicate that *P. gossypiella* is native to northern Australia. Preliminary field surveys of the parasitoid complex attacking *P. gossypiella* were conducted in NT and WA by the ARS Australian Biological Control Laboratory to assess their potential as biological control agents. This research was done in collaboration with Dr. Steve Naranjo (ARS-Western Cotton Research Laboratory, Phoenix, AZ).

Two preliminary surveys were conducted in July and October of 2000 by John Goolsby, Alan Kirk (EBCL), and Don Sands (CSIRO) in northern Australia to assess the potential for a biological control program against *P. gossypiella*. Several *Gossypium* species occur in this area including several native species such as *G. australe* and *G. rotundifolia* (Fig 14). Pink bollworm is believed to be native to this area and infests all the *Gossypium* species as well as many species of *Hibiscus*. Infested squares and bolls were collected and dissected to evaluate the parasitoid complex. Two species were parasitizing the young larvae as they entered the boll, *Apanteles onoene* and a second undescribed species of *Apantales* (Fig 15). Don Sands, who conducted a similar study in the late 1970's, had not encountered the second species and concurred that it would be the best candidate for further evaluation.

Dr. Steve Naranjo will be visiting Australia in July 2001. We will discuss the potential for additional exploration and evaluation of parasitoids. Candidate parasitoid species could be reared in Brisbane to develop rearing protocols. *Pectinophora scutigera,* a native congener of *P. gossypiella,* could be reared in Brisbane on artificial diet. The availability and local knowledge of the diet would allow for year-round evaluation of parasitoid species. Evaluation should focus on the ability of each parasitoid species to parasitize both early instars of *P. gossypiella* on the surface of the boll and latter instars that have tunneled inside.

Biological Control of Pink Hibiscus Mealybug

The Pink Hibiscus Mealybug (PHMB), *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae) has recently entered California and poses a serious threat to the forestry, agricultural, horticultural and tourist industries of the southern USA. It attacks 215 genera of economically useful plants worldwide. Chemical control is ineffective and Encyrtidae (Hymenoptera) parasitoids have been successfully used in biological control of PHMB elsewhere. ABCL in cooperation with the ARS European Biological control Laboratory is carrying out foreign exploration for PHMB natural enemies.

Exploration. Surveys were made this year in Western Australia and the Northern Territory. Two new species of parasitoids in the family Encrytidae and a predator were recovered from a wild host plant near Kununurra, WA(Table 11). Mealybugs from these collections were identified by John Donaldson (QDPI) as *M. hirsutus*. Parasitoid colonies from the WA collections have been initiated at ABCL.

Specimens from the Brisbane colony of *Gyranusoidea indica* were compared with the mass rearing colony in Brawley, CA. We compared the nuclear D2 sequence for several individuals and did not find any differences. We concluded from the data that the two colonies were the same species and subsequently terminated the Brisbane colony. Voucher specimens were lodged with the ARS-Systematic Entomology Laboratory, Australian National Collection, and the Queensland Museum.

Table 11. Natural enemies recovered from Pink Hibiscus Mealybug in Australia.

Species	ABCL #	Location	Date	Comments
Gyranusoidea indica Hym: Encyrtidae	2000809	Brisbane, QLD	28-II-2000	D2 sequences identical to colony in Brawly, CA
Cacoxenus perspicax Diptera: Drosophilidae	2000809	Brisbane, QLD	28-II-2000	Common predator of high density PHMB
Ophelosia bifasciata Hym: Pteromalidae	2000803	Brisbane, QLD	28-II-2000	Not commonly recovered may be parasitoid of *Crytpolaemus*
Crytpolaemus montrouzierri Coleoptera: Coccinellidae	2000809	Brisbane, QLD	28-II-2000	Very common predator at medium to high densities of PHMB
Encyrtidae: Hymenoptera *Coccidoctonus* sp.?	2000892	Kununurra, WA	8-X-2000	Collected from wild Malvaceous host plant, may be a hyperparasite
Coccophagus sp. Hymenoptera: Aphelinidae	2000892	Kununurra, WA	8-X-2000	Collected from wild Malvaceous host plant
Mataeomera sp. Lepidoptera: Noctuidae	2000892	Kununurra, WA	8-X-2000	Collected from wild Malvaceous host plant

Field Studies. Little is known about the importance of natural enemies in keeping the pest status of *M. hirsutus* at an acceptable level in Australia. Field studies in the Brisbane area were undertaken to record the phenology of PHMB and its associated natural enemies. Six sites with *Hibiscus rosa-sinensis* were chosen in Sherwood, a suburb of Brisbane, for the study. Cardboard bands (6) were placed on the limbs of the hibiscus plants and collected monthly. The numbers of mealybugs and emerged parasitoids were recorded. This technique has been used by numerous mealybug researchers, and provides a standard measure of density across field sites.

Population levels of PHMB peaked in the Fall from February to April (Fig 16). Predation by *C. montrouzierri* was very high during this time period at all six sites. Infested shoots often showed the characteristic ‘bunchy-top’ growth. By Winter the density of PHMB dropped nearly below detectable levels. New growth during this period of low PHMB density did not show the bunchy-top symptoms. It appears from our study that PHMB is under excellent natural control by a combination of predators and parasitoids. We will continue the study through 2001.

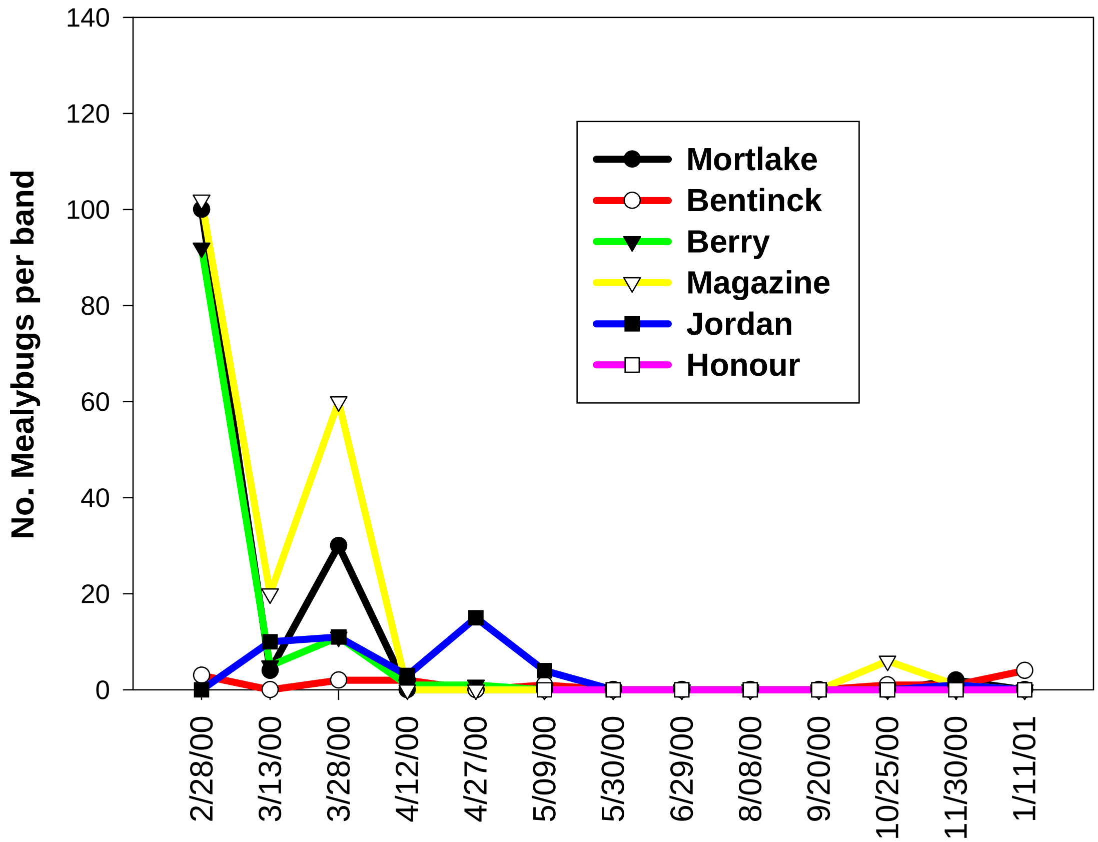


Fig 16. Seasonal population levels of PHMB at six locations in Queensland, Australia.

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ABCL Staff Publications

The following is a list of publications, authored or co-authored by ABCL staff members, which are in press or were published during 2000.

Center, T. D., T. K Van, M. Rayachhetry, G.R. Buckingham, F.A. Dray, S. A.Wineriter, M.F. Purcell, and P.D. Pratt. 2000. Field colonization of the melaleuca snout beetle (*Oxyops vitiosa*) in south Florida. Biological Control 19: 112-123.

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Goolsby, J.A., P.W. Tipping, T.D. Center, and F. Driver. 2000. Evidence of a new *Cyrtobagous* species (Coleoptera: Curculionidae) on *Salvinia minima* Baker in Florida. Southwestern Entomologist. (in press).

Goolsby, J.A., C.J. Burwell, J.R Makinson & F. Driver. Investigation of the biology of the Hymenoptera associated with *Fergusonina* sp., a gall fly of *Melaleuca quinquenervia,* integrating molecular techniques. International Journal of Hymenoptera Research (submitted).

Giblin-Davis, R.M., J. Makinson, B. J. Center, K. A. Davies, M.F. Purcell, G. S. Taylor, S. Scheffer, J. Goolsby and T. D. Center. *Fergusobia/Fergusonina*-induced shoot bud gall development on *Melaleuca quinquenervia.* Journal of Nematology (submitted).

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Appendix: 2000 ABCL Field Explorations

PLANT CODES			
ANON	No host searched	Lrt	*Lygodium reticulatum*
		Lsl	*Lygodium salicifolium*
ARdo	*Arundo donax*	Lsp	*Lygodium* sp.
Csp	*Callistemon sp.*	Mar	Melaleuca argentea
Cvm	*Callistemon viminalis*	Mcj	*Melaleuca cajuputi*
		Mdl	*Melaleuca dealbata*
CPan	*Cupaniopsis anacardioides*	Mfl	*Melaleuca fluviatilis*
		Mlb	*Melaleuca leucadendra*
Ese	*Eucalyptus seeana*	Mnv	*Melaleuca nervosa*
		Mqn	*Melaleuca quinquenervia*
Gau	*Gossypium australe*	Mst	*Melaleuca stenostachya*
Ghr	*Gossypium hirsutum*	Mvr	*Melaleuca viridiflora*
Grt	*Gossypium rotundifolia*		
		MIpg	*Mimosa pigra*
Hpn	*Hibiscus panduriformis*		
Hrs	*Hibiscus rosa-sinensis*	Pfo	*Paederia foetida*
Hsp	*Hibiscus sp.*	Psp	*Paederia* sp.
Htl	*Hibiscus tiliaceus*		
		SAds	*Sapium discolor*
Lfl	*Lygodium flexuosum*	SAsb	*Sapium sabiferum*
Lmc	*Lygodium microphyllum*		
Ljp	*Lygodium japonicum*	SLmo	*Salvinia molesta*

Appendix: 2000 ABCL Field Explorations

Collection #	Date	Host	Site	State/ Province	Country
2000001	7-Mar-00	Mqn	Brown Lake, Stradbroke Island	QLD	AUSTRALIA
2000002	7-Mar-00	Mqn	18 Mile Swamp, Stradbroke Is	QLD	AUSTRALIA
2000003	7-Mar-00	Mqn	Blue Lake Beach, Stradbroke Is	QLD	AUSTRALIA
2000004	7-Mar-00	Mqn	Flinders Beach Rd, Stradbroke Is	QLD	AUSTRALIA
2000005	7-Mar-00	CPan	Flinders Beach Rd, Stradbroke Is	QLD	AUSTRALIA
2000006	30-Mar-00	Mqn	Roy's Road	QLD	AUSTRALIA
2000007	30-Mar-00	Mqn	Landsborough State Forest	QLD	AUSTRALIA
2000008	30-Mar-00	Mqn	Peregian Beach	QLD	AUSTRALIA
2000009	30-Mar-00	Mqn	Nudgee	QLD	AUSTRALIA
2000010	6-Apr-00	Mqn	Roy's Road	QLD	AUSTRALIA
2000011	6-Apr-00	Mqn	Landsborough State Forest	QLD	AUSTRALIA
2000012	6-Apr-00	Mqn	Peregian Beach	QLD	AUSTRALIA
2000013	6-Apr-00	Mqn	Nudgee	QLD	AUSTRALIA
2000014	11-Apr-00	Mcj	Refuse Transfer Facility, Cairns	QLD	AUSTRALIA
2000015	11-Apr-00	Mcj	McDowell Billabong	QLD	AUSTRALIA
2000016	12-Apr-00	Mcj	Pandanus Road	QLD	AUSTRALIA
2000017	12-Apr-00	Mvr	Pandanus Road	QLD	AUSTRALIA
2000018	13-Apr-00	Mqn	Pacific HWY	QLD	AUSTRALIA
2000019	18-Apr-00	Mqn	Manly Golf Club	NSW	AUSTRALIA
2000020	18-Apr-00	Mqn	Wamberal Lagoon Reserve	NSW	AUSTRALIA
2000021	8-Apr-00	Mqn	Kedron Brook Bikeway	QLD	AUSTRALIA
2000022	13-Apr-00	Mqn	Roy's Road	QLD	AUSTRALIA
2000023	13-Apr-00	Mqn	Landsborough State Forest	QLD	AUSTRALIA
2000024	13-Apr-00	Mqn	Peregian Beach	QLD	AUSTRALIA
2000025	13-Apr-00	Mqn	Nudgee	QLD	AUSTRALIA
2000026	19-Apr-00	Mqn	Roy's Road	QLD	AUSTRALIA
2000027	19-Apr-00	Mqn	Landsborough State Forest	QLD	AUSTRALIA
2000028	19-Apr-00	Mqn	Peregian Beach	QLD	AUSTRALIA
2000029	19-Apr-00	Mqn	Nudgee	QLD	AUSTRALIA
2000030	6-Apr-00	Cvm	Peregian Beach	QLD	AUSTRALIA
2000031	3-May-00	Mqn	Landsborough State Forest	QLD	AUSTRALIA
2000032	5-Mar-00	Mqn	Peregian Beach	QLD	AUSTRALIA
2000033	5-Mar-00	Mqn	Roy's Road	QLD	AUSTRALIA
2000034	4-May-00	CPan	Mudjimba Esp Park	QLD	AUSTRALIA
2000035	11-May-00	Mqn	Roy's Road	QLD	AUSTRALIA
2000036	11-May-00	Mqn	Landsborough State Forest	QLD	AUSTRALIA
2000037	11-May-00	Mqn	Peregian Beach	QLD	AUSTRALIA
2000038	18-May-00	Mqn	Roy's Road	QLD	AUSTRALIA
2000039	18-May-00	Mqn	Landsborough State Forest	QLD	AUSTRALIA
2000040	18-May-00	Mqn	Peregian Beach	QLD	AUSTRALIA
2000041	18-May-00	Mqn	Cooloola Way	QLD	AUSTRALIA
2000042	26-May-00	Mqn	Roy's Road	QLD	AUSTRALIA
2000043	26-May-00	Mqn	Landsborough State Forest	QLD	AUSTRALIA
2000044	26-May-00	SLmo	Wappa Dam	QLD	AUSTRALIA
2000045	26-May-00	Mqn	Peregian Beach	QLD	AUSTRALIA
2000046	26-May-00	Csp	Peregian Beach	QLD	AUSTRALIA
2000047	29-May-00	Mvr	Amy Smith Drive	QLD	AUSTRALIA
2000048	30-May-00	Mcj	Vesteys Beach Rd	NT	AUSTRALIA

2000049	31-May-00	Mcj	Vesteys Beach Rd	NT	AUSTRALIA
2000050	31-May-00	Mcj	Vesteys Beach Rd	NT	AUSTRALIA
2000051	31-May-00	Lmc	Wangi Falls	NT	AUSTRALIA
2000052	31-May-00	Lmc	Wangi Falls	NT	AUSTRALIA
2000053	1-Jun-00	Mcj	Vesteys Beach Rd	NT	AUSTRALIA
2000054	31-May-00	Mqn	Roy's Road	QLD	AUSTRALIA
2000055	31-May-00	Mqn	Landsborough State Forest	QLD	AUSTRALIA
2000056	31-May-00	Mqn	Peregian Beach	QLD	AUSTRALIA
2000057	16-Jun-00	Mqn	Roy's Road	QLD	AUSTRALIA
2000058	16-Jun-00	Mqn	Landsborough State Forest	QLD	AUSTRALIA
2000059	16-Jun-00	Mqn	Peregian Beach	QLD	AUSTRALIA
2000060	8-Jun-00	Mqn	Roy's Road	QLD	AUSTRALIA
2000061	8-Jun-00	Mqn	Landsborough State Forest	QLD	AUSTRALIA
2000062	8-Jun-00	Mqn	Peregian Beach	QLD	AUSTRALIA
2000063	16-Jun-00	Ese	Dakabin	QLD	AUSTRALIA
2000064	23-Jun-00	Mqn	Landsborough State Forest	QLD	AUSTRALIA
2000065	23-Jun-00	Mqn	Peregian Beach	QLD	AUSTRALIA
2000066	23-Jun-00	SLmo	Wappa Dam	QLD	AUSTRALIA
2000067	29-Jun-00	Mqn	Landsborough State Forest	QLD	AUSTRALIA
2000068	29-Jun-00	Mqn	Peregian Beach	QLD	AUSTRALIA
2000069	29-Jun-00	SLmo	Wappa Dam	QLD	AUSTRALIA
2000070	6-Jul-00	Mqn	Landsborough State Forest	QLD	AUSTRALIA
2000071	6-Jul-00	Mqn	Peregian Beach	QLD	AUSTRALIA
2000072	14-Jul-00	Mqn	Landsborough State Forest	QLD	AUSTRALIA
2000073	14-Jul-00	Mqn	Peregian Beach	QLD	AUSTRALIA
2000074	20-Jul-00	Mqn	Landsborough State Forest	QLD	AUSTRALIA
2000075	20-Jul-00	Mqn	Peregian Beach	QLD	AUSTRALIA
2000076	27-Jul-00	Mqn	Landsborough State Forest	QLD	AUSTRALIA
2000077	27-Jul-00	Mqn	Peregian Beach	QLD	AUSTRALIA
2000078	3-Aug-00	Mqn	Pottsville	NSW	AUSTRALIA
2000079	3-Aug-00	Mqn	Lennox Head	NSW	AUSTRALIA
2000080	3-Aug-00	Mqn	Lake Argyle	NSW	AUSTRALIA
2000081	3-Aug-00	CPan	Lake Argyle	NSW	AUSTRALIA
2000082	3-Aug-00	Mqn	Beech St	NSW	AUSTRALIA
2000083	3-Aug-00	Mqn	Evans Head Boat Harbour	NSW	AUSTRALIA
2000084	4-Aug-00	Mqn	Landsborough State Forest	QLD	AUSTRALIA
2000085	4-Aug-00	Mqn	Peregian Beach	QLD	AUSTRALIA
2000086	7-Aug-00	Mqn	Hawthorne Park	QLD	AUSTRALIA
2000087	10-Aug-00	Mqn	Landsborough State Forest	QLD	AUSTRALIA
2000088	10-Aug-00	Mqn	Landsborough State Forest	QLD	AUSTRALIA
2000089	10-Aug-00	Mqn	Peregian Beach	QLD	AUSTRALIA
2000090	10-Aug-00	Mqn	Mountain Creek	QLD	AUSTRALIA
2000091	17-Aug-00	Mqn	Bracken Ridge	QLD	AUSTRALIA
2000092	18-Aug-00	Mqn	Roy's Road	QLD	AUSTRALIA
2000093	18-Aug-00	Mqn	Landsborough State Forest	QLD	AUSTRALIA
2000094	18-Aug-00	Mqn	Peregian Beach	QLD	AUSTRALIA
2000095	18-Aug-00	Mqn	Chelmer	QLD	AUSTRALIA
2000096	22-Aug-00	Mqn	Morayfield	QLD	AUSTRALIA
2000097	23-Aug-00	Mqn	Nudgee	QLD	AUSTRALIA
2000098	23-Aug-00	Mqn	Nudgee	QLD	AUSTRALIA
2000099	23-Aug-00	Mqn	Deagon Deviation	QLD	AUSTRALIA
2000100	24-Aug-00	Mqn	Landsborough State Forest	QLD	AUSTRALIA
2000101	24-Aug-00	Mqn	Peregian Beach	QLD	AUSTRALIA

2000102	4-May-00	Mqn	Brunswick Heads Turnoff	NSW	AUSTRALIA
2000103	24-Aug-00	Mqn	Morayfield	QLD	AUSTRALIA
2000104	24-Aug-00	Mqn	Burpengary	QLD	AUSTRALIA
2000105	28-Aug-00	Mqn	Carbrook Golf Course	QLD	AUSTRALIA
2000106	28-Aug-00	Mqn	Teviot Road	QLD	AUSTRALIA
2000107	30-Aug-00	Mqn	White Patch	QLD	AUSTRALIA
2000108	29-Aug-00	Mqn	Rutter's Rd	QLD	AUSTRALIA
2000109	30-Aug-00	Mqn	McMahon Rd	QLD	AUSTRALIA
2000110	30-Aug-00	Mqn	White Patch	QLD	AUSTRALIA
2000111	29-Aug-00	Mqn	Bribie Island Rd	QLD	AUSTRALIA
2000112	31-Aug-00	Mqn	Roy's Road	QLD	AUSTRALIA
2000113	31-Aug-00	Mqn	Landsborough State Forest	QLD	AUSTRALIA
2000114	31-Aug-00	Mqn	Peregian Beach	QLD	AUSTRALIA
2000115	31-Aug-00	CPan	Yinneburra Bushland Park, Andrew St, Point Arkwright	QLD	AUSTRALIA
2000116	31-Aug-00	Mqn	Pacific Harbour, Sunderland Rd	QLD	AUSTRALIA
2000117	31-Aug-00	Mqn	Bribie Island Youth Centre	QLD	AUSTRALIA
2000118	31-Aug-00	Mqn	McMahon Rd	QLD	AUSTRALIA
2000119	4-Sep-00	Mqn	Minnippi Parklands, Stanton Rd	QLD	AUSTRALIA
2000120	5-Sep-00	Mqn	McMahon Rd	QLD	AUSTRALIA
2000121	6-Sep-00	Mqn	Kianawah Rd	QLD	AUSTRALIA
2000122	6-Sep-00	Mqn	BMX Club, Wynnum Rd	QLD	AUSTRALIA
2000123	6-Sep-00	Mqn	Minnippi Parklands, Stanton Rd	QLD	AUSTRALIA
2000124	7-Sep-00	Mqn	Bracken Ridge	QLD	AUSTRALIA
2000125	7-Sep-00	Mqn	Landsborough State Forest	QLD	AUSTRALIA
2000126	7-Sep-00	Mqn	Peregian Beach	QLD	AUSTRALIA
2000127	7-Sep-00	CPan	Yinneburra Bushland Park, Andrew St, Point Arkwright	QLD	AUSTRALIA
2000128	11-Sep-00	Mqn	McMahon Rd	QLD	AUSTRALIA
2000129	12-Sep-00	Mqn	Landsborough State Forest	QLD	AUSTRALIA
2000130	12-Sep-00	Mqn	Roy's Road	QLD	AUSTRALIA
2000131	13-Sep-00	Mqn	Landsborough State Forest	QLD	AUSTRALIA
2000132	13-Sep-00	Mqn	Peregian Beach	QLD	AUSTRALIA
2000133	18-Sep-00	Mqn	Telegraph Rd, Fitzgibbon	QLD	AUSTRALIA
2000134	19-Sep-00	Mqn	Landsborough State Forest	QLD	AUSTRALIA
2000135	21-Sep-00	Mqn	Peregian Beach	QLD	AUSTRALIA
2000136	21-Sep-00	Mqn	Landsborough State Forest	QLD	AUSTRALIA
2000137	11-Sep-00	CPan	Town Common	QLD	AUSTRALIA
2000138	11-Sep-00	Mfl	Home Hill	QLD	AUSTRALIA
2000139	11-Sep-00	Mfl	Ross River Dam	QLD	AUSTRALIA
2000140	12-Sep-00	Mqn	Pacific HWY	QLD	AUSTRALIA
2000141	13-Sep-00	Mdl	Jones St, Mossman	QLD	AUSTRALIA
2000142	13-Sep-00	Mcj	McDowell Billabong	QLD	AUSTRALIA
2000143	13-Sep-00	Mdl	Daintree Rd	QLD	AUSTRALIA
2000144	14-Sep-00	Mnv	Mt Molloy-Mt Carbine Rd	QLD	AUSTRALIA
2000145	14-Sep-00	Mst	Luster Creek	QLD	AUSTRALIA
2000146	14-Sep-00	Mar	Mary Ck, Mt Malloy-Mt Carbine Rd	QLD	AUSTRALIA
2000147	14-Sep-00	Mar	Saltbag Creek, Mt Malloy-Mt Carbine Rd	QLD	AUSTRALIA
2000148	14-Sep-00	Mnv	2km S Mt Carbine	QLD	AUSTRALIA
2000149	14-Sep-00	Mnv	Agricultural College turnoff	QLD	AUSTRALIA
2000150	14-Sep-00	Mvr	Agricultural College turnoff	QLD	AUSTRALIA
2000151	14-Sep-00	Mnv	Gilmore Rd.	QLD	AUSTRALIA

2000152	14-Sep-00	Mdl	Centenary Lakes	QLD	AUSTRALIA
2000153	26-Sep-00	Mqn	Blue Lake Beach, Stradbroke Is	QLD	AUSTRALIA
2000154	28-Sep-00	Mqn	Chelmer	QLD	AUSTRALIA
2000155	28-Sep-00	Mqn	Telegraph Rd, Fitzgibbon	QLD	AUSTRALIA
2000156	2-Oct-00	Mqn	Peregian Beach	QLD	AUSTRALIA
2000157	2-Oct-00	Mqn	Landsborough State Forest	QLD	AUSTRALIA
2000158	3-Oct-00	Mqn	Toorbul Point Rd	QLD	AUSTRALIA
2000159	3-Oct-00	Mqn	Rutter's Rd	QLD	AUSTRALIA
2000160	4-Oct-00	Mqn	Blue Lake Beach, Stradbroke Is	QLD	AUSTRALIA
2000161	9-Oct-00	Mqn	Landsborough State Forest	QLD	AUSTRALIA
2000162	9-Oct-00	Mqn	Peregian Beach	QLD	AUSTRALIA
2000163	9-Oct-00	Mqn	Sippy Downs Bike Path	QLD	AUSTRALIA
2000164	10-Oct-00	Mqn	Burpengary	QLD	AUSTRALIA
2000165	10-Oct-00	Mqn	Morayfield	QLD	AUSTRALIA
2000166	11-Oct-00	Mqn	Blue Lake Beach, Stradbroke Is	QLD	AUSTRALIA
2000167	11-Oct-00	Mqn	Myora Springs	QLD	AUSTRALIA
2000168	16-Oct-00	Mqn	Minnippi Parklands, Stanton Rd	QLD	AUSTRALIA
2000169	17-Oct-00	Mqn	Marcoola	QLD	AUSTRALIA
2000170	17-Oct-00	Mqn	Peregian Beach	QLD	AUSTRALIA
2000171	17-Oct-00	Mqn	Landsborough State Forest	QLD	AUSTRALIA
2000172	18-Oct-00	Mqn	Flinders Beach Rd, Stradbroke Is	QLD	AUSTRALIA
2000173	18-Oct-00	Mqn	Adder Rock, Stradbroke Is	QLD	AUSTRALIA
2000174	18-Oct-00	Mqn	McMahon Rd	QLD	AUSTRALIA
2000175	23-Oct-00	Mqn	Bracken Ridge	QLD	AUSTRALIA
2000176	24-Oct-00	Mqn	Pottsville	NSW	AUSTRALIA
2000177	24-Oct-00	Mqn	Brunswick Heads Turnoff	NSW	AUSTRALIA
2000178	24-Oct-00	Mqn	Beech Drive Bike Path	NSW	AUSTRALIA
2000179	25-Oct-00	Mqn	McMahon Rd	QLD	AUSTRALIA
2000180	26-Oct-00	Mqn	Landsborough State Forest	QLD	AUSTRALIA
2000181	26-Oct-00	Mqn	Peregian Beach	QLD	AUSTRALIA
2000182	3-Oct-00	Mqn	Nudgee	QLD	AUSTRALIA
2000183	30-Oct-00	Mqn	McMahon Rd	QLD	AUSTRALIA
2000184	31-Oct-00	Cvm	Chelmer	QLD	AUSTRALIA
2000185	1-Nov-00	Mqn	McMahon Rd	QLD	AUSTRALIA
2000186	30-Oct-00	Mqn	Lake Innes Nature Reserve	NSW	AUSTRALIA
2000187	31-Oct-00	Mqn	White Tree Bay	NSW	AUSTRALIA
2000188	31-Oct-00	Mqn	Sandy Point, Mungo Brush Rd	NSW	AUSTRALIA
2000189	31-Oct-00	Mqn	Seal Rocks Rd Swamp	NSW	AUSTRALIA
2000190	31-Oct-00	Mqn	Bombah Point Ferry	NSW	AUSTRALIA
2000191	1-Nov-00	Mqn	Forster High School	NSW	AUSTRALIA
2000192	1-Nov-00	Mqn	Boomerang Drive	NSW	AUSTRALIA
2000193	1-Nov-00	Mqn	Queen's Lake Nature Reserve	NSW	AUSTRALIA
2000194	6-Nov-00	Mqn	Horace Rd	QLD	AUSTRALIA
2000195	6-Nov-00	Mqn	White Patch	QLD	AUSTRALIA
2000196	6-Nov-00	Mqn	Goodwin Drive	QLD	AUSTRALIA
2000197	7-Nov-00	Mqn	Nudgee	QLD	AUSTRALIA
2000198	8-Nov-00	Mqn	Beech Drive Bike Path	NSW	AUSTRALIA
2000199	8-Nov-00	Mqn	Ballina Shire Site 1	NSW	AUSTRALIA
2000200	7-Jan-00	Lmc	Carbrook Creek	QLD	AUSTRALIA
2000201	7-Jan-00	Lmc	Carbrook Creek	QLD	AUSTRALIA
2000202	15-Feb-00	Lmc	Carbrook Creek	QLD	AUSTRALIA
2000203	15-Feb-00	Lmc	Serpentine Creek Conservation Park	QLD	AUSTRALIA

2000204	15-Feb-00	Lmc	Native Dog Creek Conservation Park	QLD	AUSTRALIA
2000205	17-Feb-00	Lmc	Bicentennial	QLD	AUSTRALIA
2000206	17-Feb-00	Lmc	McMahon Rd	QLD	AUSTRALIA
2000207	24-Feb-00	Lmc	McMahon Rd	QLD	AUSTRALIA
2000208	1-Mar-00	Lmc	Dux Creek	QLD	AUSTRALIA
2000209	1-Mar-00	Lmc	Bottoms Creek Crossing	QLD	AUSTRALIA
2000210	1-Mar-00	Lmc	McMahon Rd	QLD	AUSTRALIA
2000211	15-Mar-00	Lmc	Shara Blvd, Ocean Shores turnoff	NSW	AUSTRALIA
2000212	15-Mar-00	Lmc	Brunswick Heads Turnoff	NSW	AUSTRALIA
2000213	15-Mar-00	Lmc	Tyagarah Environmental Park	NSW	AUSTRALIA
2000214	17-Mar-00	Lmc	Carbrook Creek	QLD	AUSTRALIA
2000215	17-Mar-00	Lmc	Native Dog Creek Conservation Park	QLD	AUSTRALIA
2000216	20-Mar-00	Lmc	McMahon Rd	QLD	AUSTRALIA
2000217	20-Mar-00	Lmc	Gallaghers Point	QLD	AUSTRALIA
2000218	20-Mar-00	Lmc	Dux Creek	QLD	AUSTRALIA
2000219	22-Mar-00	Lmc	Peregian Beach	QLD	AUSTRALIA
2000220	5-Apr-00	Lmc	McMahon Rd	QLD	AUSTRALIA
2000221	6-Apr-00	Lmc	Carbrook Creek	QLD	AUSTRALIA
2000222	6-Apr-00	Lmc	Native Dog Creek Conservation Park	QLD	AUSTRALIA
2000223	7-Apr-00	Lmc	Carbrook Creek	QLD	AUSTRALIA
2000224	14-Apr-00	Lmc	Carbrook Creek	QLD	AUSTRALIA
2000225	14-Apr-00	Lmc	Martyville	QLD	AUSTRALIA
2000226	11-Apr-00	Lmc	McDowell Billabong	QLD	AUSTRALIA
2000227	14-Apr-00	Lsp	Refuse Transfer Facility, Cairns	QLD	AUSTRALIA
2000228	4-May-00	Lmc	Carbrook Creek	QLD	AUSTRALIA
2000229	11-May-00	Lmc	Native Dog Creek Conservation Park	QLD	AUSTRALIA
2000230	16-May-00	Lmc	Bottoms Creek Crossing	QLD	AUSTRALIA
2000231	16-May-00	Lmc	Gallaghers Point	QLD	AUSTRALIA
2000232	16-May-00	Lmc	Dux Creek	QLD	AUSTRALIA
2000233	16-May-00	Lmc	McMahon Rd	QLD	AUSTRALIA
2000234	18-May-00	Lmc	Peregian Beach	QLD	AUSTRALIA
2000235	18-May-00	Lmc	Yellowwood	QLD	AUSTRALIA
2000236	18-May-00	Lmc	Elanda Point	QLD	AUSTRALIA
2000237	18-May-00	Lmc	Noosa Northshore Caravan Park	QLD	AUSTRALIA
2000238	31-May-00	Lmc	Peregian Beach	QLD	AUSTRALIA
2000239	5-Jun-00	Lmc	Carbrook Creek	QLD	AUSTRALIA
2000240	5-Jun-00	Lmc	Native Dog Creek Conservation Park	QLD	AUSTRALIA
2000241	13-Jul-00	Lmc	McMahon Rd	QLD	AUSTRALIA
2000242	13-Jul-00	Lmc	Bottoms Creek Crossing	QLD	AUSTRALIA
2000243	13-Jul-00	Lmc	Gallaghers Point	QLD	AUSTRALIA
2000245	20-Jul-00	Lmc	Wangi Falls	NT	AUSTRALIA
2000246	20-Jul-00	Lmc	Green Ant Creek	NT	AUSTRALIA
2000247	21-Jul-00	Lmc	Edith Falls	NT	AUSTRALIA
2000248	22-Jul-00	ANON	Point Spring Reserve	WA	AUSTRALIA
2000249	23-Jul-00	Lmc	Backsaddle Spring	NT	AUSTRALIA
2000250	24-Jul-00	Lmc	Winama Spring	WA	AUSTRALIA
2000251	3-Aug-00	Lmc	Tyagarah Environmental Park	NSW	AUSTRALIA
2000252	3-Aug-00	Lmc	along Byron Bay - Lennox Heads road	NSW	AUSTRALIA

2000253	3-Aug-00	Lmc	Beech St	NSW	AUSTRALIA
2000254	3-Aug-00	Lmc	Wardell Turnoff, Richmond River	NSW	AUSTRALIA
2000255	9-Aug-00	Lmc	Carbrook Creek	QLD	AUSTRALIA
2000256	9-Aug-00	Lmc	Native Dog Creek Conservation Park	QLD	AUSTRALIA
2000257	30-Aug-00	Lmc	Gallaghers Point	QLD	AUSTRALIA
2000258	30-Aug-00	Lmc	Bottoms Creek Crossing	QLD	AUSTRALIA
2000260	30-Aug-00	Lmc	McMahon Rd	QLD	AUSTRALIA
2000261	28-Aug-00	Lmc	Native Dog Creek Conservation Park	QLD	AUSTRALIA
2000262	28-Aug-00	Lmc	Serpentine Creek Conservation Park	QLD	AUSTRALIA
2000263	28-Aug-00	Lmc	Carbrook Creek	QLD	AUSTRALIA
2000264	8-Sep-00	Lmc	Carbrook Creek	QLD	AUSTRALIA
2000265	12-Sep-00	Lmc	Edmund Kennedy National Park	QLD	AUSTRALIA
2000266	12-Sep-00	Lrt	Bilyand Rd, Murray Falls turnoff	QLD	AUSTRALIA
2000267	12-Sep-00	Lrt	Mitchell Park, 12 Plumb St	QLD	AUSTRALIA
2000268	12-Sep-00	Lrt	Mission Beach Rd, Tully	QLD	AUSTRALIA
2000269	12-Sep-00	Lmc	Community Centre	QLD	AUSTRALIA
2000270	12-Sep-00	Lmc	Cassowary Drive, El Arish turnoff	QLD	AUSTRALIA
2000271	13-Sep-00	Lmc	Moresby River	QLD	AUSTRALIA
2000272	13-Sep-00	Lrt	Eubenangee Swamp National Park	QLD	AUSTRALIA
2000273	13-Sep-00	Lmc	Refuse Transfer Facility, Cairns	QLD	AUSTRALIA
2000274	13-Sep-00	Lmc	McDowell Billabong	QLD	AUSTRALIA
2000275	8-Oct-00	Lmc	Ivanhoe Falls	WA	AUSTRALIA
2000276	8-Oct-00	ANON	Point Spring Reserve	WA	AUSTRALIA
2000277	8-Oct-00	ANON	Cave Spring	WA	AUSTRALIA
2000278	9-Oct-00	Lmc	Thompson Spring	WA	AUSTRALIA
2000279	11-Oct-00	Lfl	Fenton Creek	NT	AUSTRALIA
2000280	12-Oct-00	Lmc	Wangi Falls	NT	AUSTRALIA
2000281	12-Sep-00	Lmc	Bilyand Rd, Murray Falls turnoff	QLD	AUSTRALIA
2000282	12-Sep-00	Lrt	Community Centre	QLD	AUSTRALIA
2000283	13-Sep-00	Lrt	Moresby River	QLD	AUSTRALIA
2000284	19-Oct-00	Lmc	McMahon Rd	QLD	AUSTRALIA
2000285	19-Oct-00	Lmc	Gallaghers Point	QLD	AUSTRALIA
2000286	19-Oct-00	Lmc	Dux Creek	QLD	AUSTRALIA
2000287	9-Nov-00	Lmc	Carbrook Creek	QLD	AUSTRALIA
2000288	9-Nov-00	Lmc	Native Dog Creek Conservation Park	QLD	AUSTRALIA
2000289	9-Nov-00	Lmc	Refuse Transfer Facility, Cairns	QLD	AUSTRALIA
2000290	23-Nov-00	Lmc	Carbrook Creek	QLD	AUSTRALIA
2000291	23-Nov-00	Lmc	Native Dog Creek Conservation Park	QLD	AUSTRALIA
2000292	6-Dec-00	Lmc	Native Dog Creek Conservation Park	QLD	AUSTRALIA
2000293	17-Dec-00	Lmc	Martyville	QLD	AUSTRALIA
2000294	18-Dec-00	Lmc	Cairns Airport	QLD	AUSTRALIA
2000295	19-Dec-00	Lmc	Daintree Ferry	QLD	AUSTRALIA
2000296	21-Dec-00	Lmc	Refuse Transfer Facility, Cairns	QLD	AUSTRALIA
2000297	20-Sep-00	Lfl	NR Rampur		NEPAL
2000298	24-Sep-00	Ljp	Tibet Rd		NEPAL
2000299	24-Sep-00	Ljp	Thinpipali		NEPAL
2000401	24-Feb-00	Pfo	Mog Jam Pha	Mae Hong Son	THAILAND

2000402	24-Feb-00	Lsl	Road 1509 to MHS	Mae Hong Son	THAILAND
2000403	25-Feb-00	Psp	Roadside	Chiang Mai	THAILAND
2000404	25-Feb-00	Psp	Roadside	Chiang Mai	THAILAND
2000405	4-Aug-00	Lmc	nr. Laem Khlad village	Trat	THAILAND
2000406	5-Aug-00	Lmc	nr. Ban Phe	Rayong	THAILAND
2000407	6-Aug-00	Lmc	Road 319, nr. Nong Song Hong	Prachinburi	THAILAND
2000408	6-Aug-00	Lmc	Road 33, nr. Ban Phra	Prachinburi	THAILAND
2000409	6-Aug-00	Lmc	Klong 25, Srisa Krabua, Ongkharak	Nakhon Nayok	THAILAND
2000501	2-Jan-00	Lmc	Kuala Kubu Bahru	Selangor	MALAYSIA
2000502	2-Jan-00	Lmc	Kampung Ampang Pecah	Selangor	MALAYSIA
2000503	8-Jan-00	Lmc	3km from Kakak	Pahang	MALAYSIA
2000504	8-Jan-00	Lmc	3km from Kakak	Pahang	MALAYSIA
2000505	8-Jan-00	Lmc	Indah Mentakab Garden	Pahang	MALAYSIA
2000506	10-Jan-00	Lmc	Kampung Sungai Mai	Pahang	MALAYSIA
2000507	10-Jan-00	Lmc	Indah Mentakab Garden	Pahang	MALAYSIA
2000508	10-Jan-00	Lmc	Indah Mentakab Garden	Pahang	MALAYSIA
2000509	3-Feb-00	Lmc	Kampung Cheras Baru	Kuala Lumpur	MALAYSIA
2000510	27-Feb-00	Lmc	Taman Wetland	Selangor	MALAYSIA
2000511	27-Feb-00	Lmc	Oil Palm plantation	Selangor	MALAYSIA
2000512	28-Feb-00	Lmc	Mines Beach Resort	Selangor	MALAYSIA
2000651	9-Aug-00	Lmc	Tung Chung Valley trail	Lantau Island	HONG KONG
2000652	9-Aug-00	Ljp	Tung Chung Valley trail	Lantau Island	HONG KONG
2000653	9-Aug-00	Lmc	Tung Chung Valley trail	Lantau Island	HONG KONG
2000654	9-Aug-00	Lmc	Tung Chung Valley trail	Lantau Island	HONG KONG
2000655	10-Aug-00	Lmc	Shuen Wan	New Territories	HONG KONG
2000656	10-Aug-00	Ljp	Shuen Wan	New Territories	HONG KONG
2000657	10-Aug-00	MIpg	Shuen Wan	New Territories	HONG KONG
2000660	3-Nov-00	Lmc	Mui Wo Wetlands trail	Lantau Island	HONG KONG
2000661	3-Nov-00	Lmc	Mui Wo Wetlands trail	Lantau Island	HONG KONG
2000662	3-Nov-00	Lmc	Tung Chung Valley trail	Lantau Island	HONG KONG
2000663	3-Nov-00	SAds	Tung Chung Valley trail	Lantau Island	HONG KONG
2000664	3-Nov-00	Lmc	Tung Chung Valley trail	Lantau Island	HONG KONG
2000665	2-Nov-00	SAsb	Hong Kong Park, Botanic Gardens		HONG KONG
2000666	4-Nov-00	Lfl	Luis Camoes Grotto	Macau	CHINA
2000667	4-Nov-00	Ljp	Colane Island	Macau	CHINA
2000668	5-Nov-00	Lfl	South China Ag University	Guangdong	CHINA
2000669	5-Nov-00	Ljp	South China Botanic Gardens	Guangdong	CHINA
2000670	5-Nov-00	Ljp	South China Botanic Gardens	Guangdong	CHINA
2000671	6-Nov-00	Lmc	Guangdong Tea Institute	Guangdong	CHINA
2000672	7-Nov-00	Lmc	near Guangdong Tea Institute	Guangdong	CHINA
2000673	8-Nov-00	SAsb	Guangdong Entomological Institute	Guangdong	CHINA
2000674	9-Nov-00	Ljp	Shishan Craters	Hainan	CHINA
2000675	9-Nov-00	SAsb	Shishan Craters	Hainan	CHINA
2000676	10-Nov-00	Lmc	nr Quanghai	Hainan	CHINA
2000677	10-Nov-00	Lmc	opposit Quanghai piggery	Hainan	CHINA
2000678	13-Nov-00	Psp	Tai Tam Valley trail	Hong Kong Island	HONG KONG
2000679	13-Nov-00	Lmc	Tai Tam Tuk Reservoir		HONG KONG
2000680	13-Nov-00	Hrs	Victoria Peak	Hong Kong	HONG KONG

				Island	
2000681	14-Nov-00	Lmc	top of hill nr Sok Kwu Wan	Lamma Island	HONG KONG
2000682	14-Nov-00	ARdo	top of hill nr Sok Kwu Wan	Lamma Island	HONG KONG
2000683	14-Nov-00	Ljp	top of hill nr Sok Kwu Wan	Lamma Island	HONG KONG
2000684	15-Nov-00	SAsb	Dept. Ag, Fish and Conservation nursery	New Territories	HONG KONG
2000685	15-Nov-00	SAsb	Mai Po wetlands	New Territories	HONG KONG
2000686	16-Nov-00	Lmc	Tung Chung Valley trail	Lantau Island	HONG KONG
2000701	29-Feb-00	Lmc	Sungai Buloh Nature Park	---	SINGAPORE
2000702	29-Feb-00	Pfo	Sungai Buloh Nature Park	---	SINGAPORE
2000703	29-Feb-00	Lmc	Sungai Buloh Nature Park	---	SINGAPORE
2000704	29-Feb-00	Lmc	Sungai Buloh Nature Park	---	SINGAPORE
2000705	1-Mar-00	Lmc	Belukar Track	---	SINGAPORE
2000706	1-Mar-00	Lmc	Belukar Track	---	SINGAPORE
2000707	1-Mar-00	Lmc	Belukar Track	---	SINGAPORE
2000708	1-Mar-00	Lmc	Belukar Track	---	SINGAPORE
2000709	1-Mar-00	Lmc	Belukar Track	---	SINGAPORE
2000710	1-Aug-00	Lmc	Belukar Track	---	SINGAPORE
2000800	18-Feb-00	Hrs	Sherwood	QLD	AUSTRALIA
2000801	18-Feb-00	Hrs	Sherwood	QLD	AUSTRALIA
2000802	18-Feb-00	Hrs	Sherwood	QLD	AUSTRALIA
2000803	18-Feb-00	Hrs	Sherwood	QLD	AUSTRALIA
2000804	18-Feb-00	Hrs	Graceville	QLD	AUSTRALIA
2000805	28-Feb-00	Hrs	Graceville	QLD	AUSTRALIA
2000806	28-Feb-00	Hrs	Sherwood	QLD	AUSTRALIA
2000807	28-Feb-00	Hrs	Sherwood	QLD	AUSTRALIA
2000808	28-Feb-00	Hrs	Sherwood	QLD	AUSTRALIA
2000809	28-Feb-00	Hrs	Sherwood	QLD	AUSTRALIA
2000810	13-Mar-00	Hrs	Sherwood	QLD	AUSTRALIA
2000811	13-Mar-00	Hrs	Sherwood	QLD	AUSTRALIA
2000812	13-Mar-00	Hrs	Sherwood	QLD	AUSTRALIA
2000813	13-Mar-00	Hrs	Sherwood	QLD	AUSTRALIA
2000814	13-Mar-00	Hrs	Sherwood	QLD	AUSTRALIA
2000815	29-Mar-00	Hrs	Sherwood	QLD	AUSTRALIA
2000816	29-Mar-00	Hrs	Sherwood	QLD	AUSTRALIA
2000817	29-Mar-00	Hrs	Sherwood	QLD	AUSTRALIA
2000818	29-Mar-00	Hrs	Sherwood	QLD	AUSTRALIA
2000819	29-Mar-00	Hrs	Sherwood	QLD	AUSTRALIA
2000820	12-Apr-00	Hrs	Sherwood	QLD	AUSTRALIA
2000821	12-Apr-00	Hrs	Sherwood	QLD	AUSTRALIA
2000822	12-Apr-00	Hrs	Sherwood	QLD	AUSTRALIA
2000823	12-Apr-00	Hrs	Sherwood	QLD	AUSTRALIA
2000824	12-Apr-00	Hrs	Sherwood	QLD	AUSTRALIA
2000825	12-Apr-00	Hrs	Indooroopilly	QLD	AUSTRALIA
2000826	27-Apr-00	Hrs	Sherwood	QLD	AUSTRALIA
2000827	27-Apr-00	Hrs	Sherwood	QLD	AUSTRALIA
2000828	27-Apr-00	Hrs	Sherwood	QLD	AUSTRALIA
2000829	27-Apr-00	Hrs	Sherwood	QLD	AUSTRALIA
2000830	27-Apr-00	Hrs	Sherwood	QLD	AUSTRALIA
2000831	28-Apr-00	Hrs	Sherwood	QLD	AUSTRALIA
2000832	unknown	Hrs	Berrimah	NT	AUSTRALIA
2000833	9-May-00	Hrs	Sherwood	QLD	AUSTRALIA

2000834	9-May-00	Hrs	Sherwood	QLD	AUSTRALIA
2000835	9-May-00	Hrs	Sherwood	QLD	AUSTRALIA
2000836	9-May-00	Hrs	Sherwood	QLD	AUSTRALIA
2000837	9-May-00	Hrs	Sherwood	QLD	AUSTRALIA
2000838	9-May-00	Hrs	Sherwood	QLD	AUSTRALIA
2000839	30-May-00	Hrs	Sherwood	QLD	AUSTRALIA
2000840	30-May-00	Hrs	Sherwood	QLD	AUSTRALIA
2000841	30-May-00	Hrs	Sherwood	QLD	AUSTRALIA
2000842	30-May-00	Hrs	Sherwood	QLD	AUSTRALIA
2000843	30-May-00	Hrs	Sherwood	QLD	AUSTRALIA
2000844	30-May-00	Hrs	Sherwood	QLD	AUSTRALIA
2000845	29-Jun-00	Hrs	Sherwood	QLD	AUSTRALIA
2000846	29-Jun-00	Hrs	Sherwood	QLD	AUSTRALIA
2000847	29-Jun-00	Hrs	Sherwood	QLD	AUSTRALIA
2000848	29-Jun-00	Hrs	Sherwood	QLD	AUSTRALIA
2000849	29-Jun-00	Hrs	Sherwood	QLD	AUSTRALIA
2000850	29-Jun-00	Hrs	Sherwood	QLD	AUSTRALIA
2000852	11-Jul-00	Hrs	QLD Coast	QLD	AUSTRALIA
2000854	20-Jul-00	Hsp	Berimbah	NT	AUSTRALIA
2000855	21-Jul-00	Hsp	18 12th St. and Anzac Mem, Katherine	NT	AUSTRALIA
2000856	21-Jul-00	Hsp	Timber Creek	NT	AUSTRALIA
2000857	25-Jul-00	Hsp	60km E Fitzory Crossing	WA	AUSTRALIA
2000858	25-Jul-00	Hsp	260 Flynn St	WA	AUSTRALIA
2000859	24-Jul-00	Hsp	Hall's Creek	WA	AUSTRALIA
2000860	25-Jul-00	Hsp	Derby	WA	AUSTRALIA
2000861	25-Jul-00	Gau	Derby	WA	AUSTRALIA
2000862	26-Jul-00	Hrs	Short St	WA	AUSTRALIA
2000863	26-Jul-00	Grt	Barred Creek	WA	AUSTRALIA
2000864	26-Jul-00	Hpn	Willie Creek	WA	AUSTRALIA
2000865	27-Jul-00	Gau	Thangoo	WA	AUSTRALIA
2000866	27-Jul-00	Hpn	Cape Latouche Treville	WA	AUSTRALIA
2000867	27-Jul-00	Gau	10km inland Cape Latouche Treville	WA	AUSTRALIA
2000868	8-Aug-00	Hrs	Sherwood	QLD	AUSTRALIA
2000869	8-Aug-00	Hrs	Sherwood	QLD	AUSTRALIA
2000870	8-Aug-00	Hrs	Sherwood	QLD	AUSTRALIA
2000871	8-Aug-00	Hrs	Sherwood	QLD	AUSTRALIA
2000872	8-Aug-00	Hrs	Sherwood	QLD	AUSTRALIA
2000873	8-Aug-00	Hrs	Sherwood	QLD	AUSTRALIA
2000874	20-Aug-00	Ghr	Douglas River	NT	AUSTRALIA
2000875	20-Aug-00	Ghr	Douglas River	NT	AUSTRALIA
2000876	20-Aug-00	Ghr	Beatrice Hill	NT	AUSTRALIA
2000877	20-Aug-00	Ghr	Woolner Station	NT	AUSTRALIA
2000878	20-Aug-00	Ghr	Woolner Station	NT	AUSTRALIA
2000879	18-Aug-00	Ghr	East Arm Point	NT	AUSTRALIA
2000880	21-Aug-00	Ghr	Elsey Station	NT	AUSTRALIA
2000881	21-Aug-00	Ghr	Elsey Station	NT	AUSTRALIA
2000882	14-Aug-00	Ghr	Bowen Strait	NT	AUSTRALIA
2000883	14-Aug-00	Ghr	Smith Point	NT	AUSTRALIA
2000884	24-Aug-00	Hrs	Timber Creek	NT	AUSTRALIA
2000885	20-Sep-00	Hrs	Sherwood	QLD	AUSTRALIA
2000886	20-Sep-00	Hrs	Sherwood	QLD	AUSTRALIA
2000887	20-Sep-00	Hrs	Sherwood	QLD	AUSTRALIA

2000888	20-Sep-00	Hrs	Sherwood	QLD	AUSTRALIA
2000889	20-Sep-00	Hrs	Sherwood	QLD	AUSTRALIA
2000890	20-Sep-00	Hrs	Sherwood	QLD	AUSTRALIA
2000891	7-Oct-00	Htl	Durack, WA Ag. Station	WA	AUSTRALIA
2000892	8-Oct-00	Hsp	Ivanhoe Falls	WA	AUSTRALIA
2000893	8-Oct-00	Gau	Kununura	WA	AUSTRALIA
2000894	9-Oct-00	Hrs	Timber Creek	NT	AUSTRALIA
2000895	9-Oct-00	Gau	East of Timber Creek	NT	AUSTRALIA
2000896	11-Oct-00	Ghr	Elsey Station	NT	AUSTRALIA
2000897	11-Oct-00	Ghr	Daly River	NT	AUSTRALIA
2000898	11-Oct-00	Ghr	Woolner Station	NT	AUSTRALIA
2000899	11-Oct-00	Ghr	Lee's Point	NT	AUSTRALIA
2000900	25-Oct-00	Hrs	Sherwood	QLD	AUSTRALIA
2000901	25-Oct-00	Hrs	Sherwood	QLD	AUSTRALIA
2000902	25-Oct-00	Hrs	Sherwood	QLD	AUSTRALIA
2000903	25-Oct-00	Hrs	Sherwood	QLD	AUSTRALIA
2000904	25-Oct-00	Hrs	Sherwood	QLD	AUSTRALIA
2000905	25-Oct-00	Hrs	Sherwood	QLD	AUSTRALIA
2000906	30-Nov-00	Hrs	Sherwood	QLD	AUSTRALIA
2000907	30-Nov-00	Hrs	Sherwood	QLD	AUSTRALIA
2000908	30-Nov-00	Hrs	Sherwood	QLD	AUSTRALIA
2000909	30-Nov-00	Hrs	Sherwood	QLD	AUSTRALIA
2000910	30-Nov-00	Hrs	Sherwood	QLD	AUSTRALIA
2000911	30-Nov-00	Hrs	Sherwood	QLD	AUSTRALIA
2000950	8-Nov-00	Mqn	Lennox Head	NSW	AUSTRALIA
2000951	8-Nov-00	Mqn	Ballina	NSW	AUSTRALIA
2000952	8-Nov-00	Mqn	Woodburn	NSW	AUSTRALIA
2000953	8-Nov-00	Mqn	Pottsville Waters	NSW	AUSTRALIA
2000954	9-Nov-00	Mqn	Goodwin Drive	QLD	AUSTRALIA
2000955	9-Nov-00	Mqn	Bribie Island Rd, Ningi	QLD	AUSTRALIA
2000956	7-Nov-00	Mvr	Cardwell Swamp	QLD	AUSTRALIA
2000957	8-Nov-00	Mnv	Agricultural College turnoff	QLD	AUSTRALIA
2000958	8-Nov-00	Mvr	Leumann Rd, 5km S Gordonvale	QLD	AUSTRALIA
2000959	5-Dec-00	Mqn	nr. Yarilee State School	QLD	AUSTRALIA
2000960	5-Dec-00	CPan	nr. Yarilee State School	QLD	AUSTRALIA
2000961	5-Dec-00	Mvr	Poona National Park	QLD	AUSTRALIA
2000962	5-Dec-00	CPan	Beach front road	QLD	AUSTRALIA
2000963	17-Dec-00	CPan	Rowes Bay Golf Club	QLD	AUSTRALIA
2000964	17-Dec-00	Mqn	Pacific HWY	QLD	AUSTRALIA
2000965	19-Dec-00	Mqn	Pandanus Road	QLD	AUSTRALIA
2000966	19-Dec-00	Mar	Saltbag Creek, Mt Malloy-Mt Carbine Rd	QLD	AUSTRALIA
2000967	19-Dec-00	Mnv	Saltbag Creek, Mt Malloy-Mt Carbine Rd	QLD	AUSTRALIA
2000968	19-Dec-00	Mst	Luster Creek	QLD	AUSTRALIA
2000969	20-Dec-00	Mnv	Agricultural College turnoff	QLD	AUSTRALIA
2000970	18-Dec-00	Mlb	Supply Rd, Centenary Park	QLD	AUSTRALIA