UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
OFFICE OF INTERNATIONAL RESEARCH PROGRAMS
**AUSTRALIAN BIOLOGICAL CONTROL LABORATORY**

# 2005 Annual Report

prepared by

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Staff January 1 – December 31, 2005

**Mr. Matthew Purcell** – Acting Director, Experimental Scientist
**Mr. Tony Wright** - Experimental Scientist
**Mr. Jeff Makinson** - Research Officer
**Mr. Ryan Zonneveld -** Research Officer
**Mr. Bradley Brown** – Research Officer
**Mr. Dalio Mira** - Plant Culture (part-time, 65%)
**Mr. Gio Fichera** – Research Officer (part-time, 10%)

**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 In order to prevent the spread of out-of-date or inaccurate information, this report should not be quoted or cited without verifying accuracy with the USDA-ARS Australian Biological Control Laboratory.

**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 |
| **CQ** | - Central Queensland Coast |
| **CSIRO** | - Commonwealth Scientific and Industrial Research Organisation |
| **IPRL** | - Invasive Plant Research laboratory |
| **nNSW** | - Northern New South Wales, north of Coffs Harbour |
| **NQ** | - North Queensland, north of the Tropic of Capricorn |
| **NSW** | - New South Wales |
| **OIRP** | - (USDA-ARS) Office of International Research Programs |
| **QLD** | - Queensland |
| **SEL** | - (USDA-ARS) Systematic Entomology Laboratory |
| **SQ** | - South Queensland, south of the Tropic of Capricorn |
| **SFWMD** | - South Florida Water Management District |
| **TAG -** | - (USDA-APHIS) Technical Advisory Group Biological Control of Weeds |
| **USDA** | - United States Department of Agriculture |

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## Report Summary

During 2005, our research continued to focus on the biological control of weeds outlined in the ABCL project plan: the broad-leaved paperbark tree, *Melaleuca quinquenervia*; Old World climbing fern, *Lygodium microphyllum*; and Australian Pine, *Casuarina* spp. Significant programs also exist for hydrilla, *Hydrilla verticillata*, and skunk vine, *Paederia foetida*.

Research on the biological control of *M. quinquenervia* focused on developing the bud/tip feeding weevil *Haplonyx multicolor* as an agent. Difficulties in rearing the gall-former, *Sphaerococcus ferrugineus*, the focus of research during 2004, led to the reprioritization of agents and the renewed focus on *H. multicolor*. Research concentrated on developing rearing methods for this weevil. Its ability to kill flower buds could have a major impact on the reproductive potential of *M. quinquenervia*. Because of concerns on the host-specificity of the foliage-feeding moth *Poliopaschia lithochlora*, host-specificity tests were conducted on another moth with a similar habit, *Eochrois leiochroa*.

The first agent for *L. microphyllum*, the defoliating moth *Austromusotima camptozonale*, was released in Florida in February. Applications for release of an additional two agents evaluated by ABCL, *Neomusotima conspurcatalis* and the mite, *Floracarus perrepae*, have been submitted. Exploration was conducted in previously unexplored areas of the native range of *L. microphyllum* along the coast of Queensland, Australia. ABCL staff member, Jeff Makinson, was posted overseas to Singapore to conduct research on a stem-boring Lepidoptera. This moth has only been found in Singapore and is the highest priority for current research on *L. microphyllum* at ABCL. Assistance was also given to Prof. John Volin from the Florida Atlantic University who was on sabbatical in Australia studying the growth rates and nutritional allocation of *L. microphyllum* in Australia for comparison with his parallel research being conducted in Florida.

We continued to contract Dr. Gary Taylor from the University of Adelaide to conduct exploration for biological control agents of *Casuarina* spp. Of major interest is the seed-feeding torymid wasp, *Bootanelleus orientalis* which will be the focus of further research. ABCL funds have been offered to support an Australian Research Council research grant application being submitted jointly by University of Adelaide and ABCL. If successful, resources available for research on these weeds will double and Gary Taylor will work full-time on the co-evolutionary aspects of *Casuarina* and its associated insect herbivores.

Surveys for herbivores of *Hydrilla verticillata* continued in Indonesia, Malaysia, Thailand and Singapore. Research continued to focus on the stem-boring aquatic *Bagous* weevils found in Thailand, and on the defoliating moth, *Paracymoriza vagalis*, from Indonesia.

*Paederia foetida* surveys continued in Thailand and Singapore and several promising agents have been located and could be targeted in the future for use as biological control agents. The current scope of the project is only for exploration and identification of *Paederia* species and agents.

**Overview**

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

Many invasive weeds in the USA such as the broad-leaved paperbark tree, *Melaleuca quinquenervia*; Old World climbing fern, *Lygodium microphyllum*; hydrilla, *Hydrilla verticillata*; 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 continue northward from Australia into tropical and subtropical Southeast Asia, including Indonesia, Malaysia, Thailand, Vietnam, Papua New Guinea, India, and southern China, and out to New Caledonia, while other targets such as *Paederia foetida* are found only in Asia. 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, 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.

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

## Administration and Support

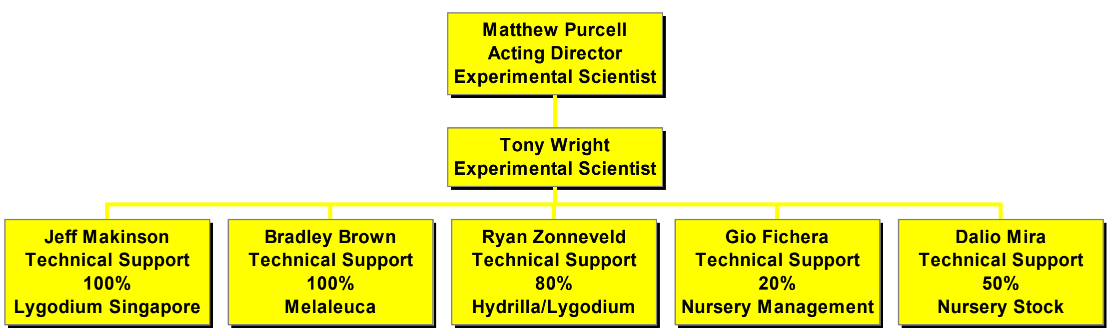
The ABCL is a research unit within the USDA-ARS, Office of International Research Programs (OIRP). The personnel and facilities of the ABCL in Australia are provided through a co-operative agreement between the USDA-ARS and CSIRO Entomology.

A coalition of federal, state and local agencies fund the overseas research on biological control of *M. quinquenervia*, *L. microphyllum* and *H. verticillata.* South Florida Water Management District and the Florida Department of Environmental Protection contributed substantially to ABCL research during 2005.

ABCL works closely with the following USDA-ARS project leaders to co-ordinate the research: Dr. Ted Center (overall program and *Melaleuca quinquenervia* ); Dr. Bob Pemberton (*Lygodium microphyllum* and *Paederia foetida*); Dr. Paul Pratt (*M. quinquenervia*); and Dr. Greg Wheeler (*Hydrilla verticillata* and *Casuarina* spp.). ARS National Program leaders Dr. Ernest Delfosse (Weeds) and Dr. Kevin Hackett (Biological Control) assist with program direction.

## Staff and Projects

ABCL staff consists of four full-time CSIRO personnel and three part-time staff members. In April 2005, Bradley Brown returned to ABCL after an 18-month break traveling overseas. A breakdown of staffing is given below.




Matthew Purcell is the Acting Director of ABCL and is the lead scientist on the *Melaleuca*, *Hydrilla* and *Casuarina* projects. Tony Wright is the lead scientist on the *Lygodium* and *Paederia* projects. Both scientists conduct most of the foreign exploration in Southeast Asia. Jeff Makinson provides technical support and is temporarily based in Singapore for one year working on the *Lygodium microphyllum* stem-borer. When in Australia, Jeff provides technical support in all projects as well as records management, curation and database management. Ryan Zonneveld is the technical support for both the *Lygodium* and *Hydrilla* projects and is the primary quarantine technician. Bradley Brown provides technical support for the *Melaleuca* project. Dalio Mira and Gio Fichera are responsible for plant propagation and maintenance for all ABCL projects.

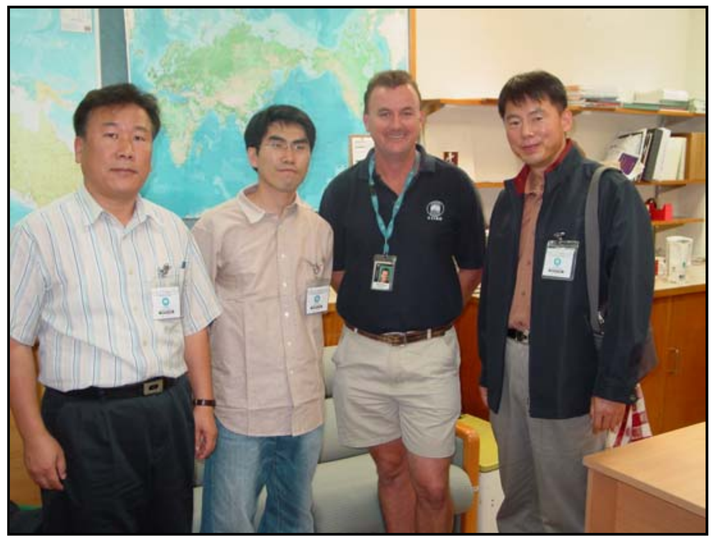
## ABCL Weed Projects and Agents

| Target/Stakeholders | Research Stage | Agent | Mode of Attack |
|---|---|---|---|
| *Melaleuca quinquenervia* (Broad-leaved Paperbark Tree)<br>**USDA ARS** | Released | *Oxyops vitiosa* | Leaf-feeding weevil |
| | | *Boreioglycaspis melaleucae* | Sap-sucking bug |
| | | *Fergusonina turneri* | Bud-galling fly |
| | Quarantine | *Lophodiplosis trifida* | Stem-galling fly |
| | Australian evaluation | *Haplonyx multicolor* | Bud-feeding weevil |
| | | *Eochrois leiochroa* | Leaf-binding moth |
| | | *Poliopaschia lithochlora* | Leaf-binding moth |
| | | *Sphaerococcus ferrugineus* | Gall-forming scale |
| | | *Cerambycidae* | Wood-boring beetles |
| *Lygodium microphyllum* (Old-World Climbing Fern)<br>**USDA ARS**<br>**Florida DEP**<br>**SFWMD** | Released | *Austromusotima camptozonale* | Defoliating moth |
| | Quarantine | *Neomusotima conspurcatalis* | Defoliating moth |
| | | *Floracarus perrepae* | Leaf-feeding mite |
| | | *Neostrombocerus albicomus* | Defoliating sawfly |
| | Singapore evaluation | *?Ambia* | Stem-boring moth |
| *Hydrilla verticillata* (Hydrilla)<br>**USDA ARS**<br>**Florida DEP** | Released | *Bagous hydrillae* | Stem-boring weevil |
| | | *Hydrellia balciunasi* | Leaf-mining fly |
| | Under evaluation in Australia/Asia | *Bagous spp* | Stem-boring weevils |
| | | *Paracymoriza vagalis* | Defoliating moth |
| *Casuarina* spp. (Australian Pine)<br>**USDA ARS**<br>**Florida DEP** | Exploration phase, no targeted insects | | |
| *Paederia foetida* (Skunk Vine)<br>**USDA ARS**<br>**Florida DEP** | Exploration phase, no targeted agents | | |

## Travel to the USA and Visitors to ABCL

Matthew Purcell made two trips to the United States in 2005. In February, Matthew participated on a TAME Melaleuca field day organized by the USDA ARS Invasive Plant Research Laboratory (IPRL) in Florida. The presentations demonstrated the integration of ecologically and economically sustainable *Melaleuca* control to public land managers. He also discussed ABCL exploratory research with project scientists, visited release sites for *Melaleuca* agents, and attended the official release of *Austromusotima camptozonale*, the first biological control agent released on *Lygodium microphyllum*. In December, Matthew revisited IPRL for further discussions on research progress then traveled to Washington to meet with the new Director of OIRP, Dr. Pai-Yei Whung, Deputy Director, Dr. Richard Greene, OIRP administrative officers and National Program Staff. He also gave a presentation on the past, present and future directions of ABCL. Dr. Whung then accompanied Matthew to the IPRL in Fort Lauderdale where they were given a tour of the new quarantine and office facilities by Dr. Ted Center and given presentations by project staff members.

Dr. Desmier DeChenon (below left), our collaborator in Indonesia, visited Brisbane in January to discuss continuing exploratory research on *Hydrilla verticillata* and *Lygodium microphyllum*. In October, Sang-Boem Lee, Jory Ho Park and Kyoung Yul Ryu from South Korea (below right) visited the laboratories to discuss biological control programs at ABCL and CSIRO. In December, Dr. Sonja J. Scheffer (bottom, with Bradley Brown and Matthew Purcell) from the USDA SEL in Washington visited ABCL to inspect survey and collection sites of the gall-forming fly, *Fergusonina turneri*. Dr. Scheffer was a major collaborator in the development of this agent, which was approved for release in the US in 2004.



## Biological Control of *Melaleuca quinquenervia*

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

*Melaleuca quinquenervia* is widespread along the eastern coast of Australia, usually occurring in swamps and other wetlands. The Australian range is roughly from Sydney to the tip of Cape York Peninsula. The most extensive stands are located in SQ and nNSW).

Since 1986, surveys have been conducted in Australia to find potential biological control agents of *M. quinquenervia*, and more than 450 insect herbivores have been collected (Balciunas *et al*. 1994). Two species have been released in Florida. A foliage-feeding weevil, *Oxyops vitiosa* Pascoe (Coleoptera: Curculionidae) (inset left), was released in 1997 (Center *et al*. 2000) and a sap-sucking psyllid, *Boreioglycaspis melaleucae* (Hemiptera: Psyllidae) (inset right), was released in 2002 (Wood and Flores 2002). Both insects have established (Pratt *et al.* 2003) and are having a serious impact on the weed (Wood and Flores 2002), though more agents are required. The petition to release a third insect, the gall fly *Fergusonina turneri* and its obligate nematode, *Fergusobia quinquenerviae*, has been approved by TAG. The insect is currently being mass reared for field releases. A second stem galling fly, *Lophodiplosis trifida*, is under evaluation in quarantine in Florida after being shipped from Australia back in 2003.

## Melaleuca Field Collections and Exploratory Surveys in 2005

A total of 48 collections were made in Australia in 2005 for the *Melaleuca* project. Most of the collections of *Melaleuca* species were made in Queensland. A summary of all collection is given in Table 1. Local collections in SQ and nNSW were made to collect priority agents for laboratory rearing and testing. Most of these collections were from *M. quinquenervia* though one collection from *Callistemon viminalis* was made to validate a field host record for the moth *Poliopaschia lithochlora*. Peregian Environmental Park, north of Brisbane, was surveyed on a regular basis to collect the bud-weevil *Haplonyx multicolor*. This is one of the few sites in SQ where this high-priority insect can be collected with a majority of specimens coming from NQ.

**Table 1**. Summary of field collections made during 2005 in north Queensland (NQ), southeast Queensland (SQ), central Queensland coast (CQ), northern NSW (nNSW) and the Northern Territory (NT) related to *Melaleuca* research.

| Plant | | Number Collections | Number Sites | Regions |
|---|---|---|---|---|
| **Family** | **Species** | | | |
| Myrtaceae – *Melaleuca leucadendra* complex | *Melaleuca argentea* | 5 | 5 | NT |
| | *Melaleuca cajuputi* | 1 | 1 | NT |
| | *Melaleuca dealbata* | 4 | 4 | CQ, NQ, NT |
| | *Melaleuca nervosa* | 3 | 3 | NQ, NT |
| | *Melaleuca leucadendra* | 3 | 3 | NQ |
| | *Melaleuca quinquenervia* | 30 | 22 | SQ, NQ, CQ |
| | *Melaleuca viridiflora* | 10 | 10 | CQ, NQ, NT |
| Other Myrtaceae | *Callistemon viminalis* | 1 | 1 | nNSW |
| Mimosaceae | *Acacia* sp. | 1 | 1 | SQ |

## Surveys remote from ABCL

Surveys were conducted in NQ mainly to collect the leaf binder *Eochrois leiochroa* and the bud-weevil *Haplonyx multicolor*. Specimens were collected from both *M. quinquenervia* and *M. viridiflora* and were used to establish laboratory colonies for preliminary biology and host-range testing. Surveys from Rockhampton to Cairns were undertaken in October-November to find new sites for collecting *Haplonyx multicolor*.

Surveys between Woodgate and Rockhampton were conducted in June to collect the psyllid, *Boreioglycaspis melaleucae* for genetic typing. Stem-boring *Rhytiphora* sp (Coleoptera: Cerambycidae) larvae were also collected from Woodgate in large numbers. Further surveys were conducted in October when 30 larvae were collected and used for colony establishment.

In September, collections of *Fergusonina turneri* galls were made to supply the Gainesville quarantine facility with insects for field releases. Galls were collected from Bribie Island east of Brisbane and a shipment of 172 galls was made to Florida.

*Fergusonina turneri* galls were difficult to collect as field numbers were generally low throughout 2005.

**Flower-Feeding Weevil - *Haplonyx multicolor***

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


In 2005, many adults were collected feeding on young *M. quinquenervia* saplings at Edmund Kennedy National Park and the Atherton Tablelands of NQ and at Peregian in SQ. These adults fed on the stem bases of young tips causing them to fracture and die. Larvae and adults were also collected on *M. viridiflora* in NQ, as they have in previous years, and these adults tended to be much bigger (inset left) than those collected from *M. quinquenervia*, though we have confirmed that they are the same species. In the past, buds and dead tips of *Melaleuca* have contained larvae, pupae and adults. We believe these weevils diapause in these buds given that adults are found 6-9 months after the buds were produced. Adults can also be found year-round on the young foliage. This mechanism for surviving between seasons makes this agent very attractive as a candidate for the biological control of *M. quinquenervia*. Its dual mode of feeding on both flowers and leaves would also assist populations persisting in the field. Its ability to kill flower buds could have a major impact on the reproductive potential of trees growing in Florida.

**Colony establishment, rearing techniques**

Field collected adults were given flowers and leaf buds from field sites within a sealed plastic container. The adults fed on buds but only several infertile eggs (inset right) were found on the outside of leaf buds. Damaged buds collected from the same field site contained weevil larvae and were reared on a combination of artificial diet and fresh buds. Larvae were inserted into artificially created cavities in the buds but none survived. Several adults were reared on artificial diet, though some adults were deformed while others died using this technique.

In August 2005, field collected weevil larvae at Peregian where reared through to adult and added to the laboratory colony (inset left). The field-collected and laboratory-reared pupae were reared to the adult stage by placing them within paper-lined sealed containers which where sprayed with water as needed. Some larvae were introduced onto a mix of finely ground, dried leaves and stem material, generated from an electric coffee grinder. The fine groundings were packed into recessed tubes in Styrofoam trays. These trays were placed into sealed containers, with and without a glycerol/water solution, to control humidity (inset right). The tray was moistened with water until damp, and larvae were placed within each recess. Larvae failed to feed on the material available and no adults were reared.

We suspected that plants with higher nutrition were required to induce oviposition by *H. multicolor*. When fertilized weekly, young shoots and buds were larger and thicker and foliage became deep green in colour. These thickened tips and buds are presented to adults weekly with immediate damage present after several days. We found feeding holes on buds and also the stem to which the bud was attached usually had been partially severed, effectively killing the bud above and causing it to hang (inset left). Damaged tips were collected and placed into ventilated or sealed containers, both in the glasshouse and laboratory. Additionally, some sapling tips damaged were not removed and left on the

plant within a gauze cage. In December, larvae were found on the tips held within sealed containers, but not within the ventilated containers. Larvae had developed in a cavity made within each tip and then fed upon the dead plant material (inset left). It was observed that some larvae, once disturbed, would exit the tip and die. Several of these larvae were transferred to artificial diet. The damaged tips on live plants were not disturbed.

We will continue to develop methods to generate a viable colony in 2006. The use of heavily fertilized plants will be developed further and oviposition and rearing of immatures on live plants will be further evaluated. Artificial diets will also be pursued given recent success. The crucial factor for rearing immatures seems to be maintaining the food source (leaf tip or diet) at the correct moisture level .

**Leaf Binder - *Eochrois leiochroa***

The larvae of *Eochrois leiochroa* (Lepidoptera: Oecophoridae) web the leaves of *M. quinquenervia* together, binding them to the stems on saplings/suckers (inset below). Unlike *P. lithochlora*, the leaves are plastered together face against face with the immatures between them. The larvae form small colonies and their feeding skeletonizes leaf tissue. A check of 14 adult specimens at the ANIC revealed that these moths have only been collected from broad-leaved *Melaleuca* spp. hosts.

**Rearing and colony establishment**

The larvae have been collected from two sites, Edmund Kennedy National Park and Lake Mitchell in NQ, and from two hosts, *M. quinquenervia* and *M. viridiflora*. The current culture of moths was established from field material collected from Lake Mitchell in May 2005. Initially larvae where reared to the adult stage in plastic containers using cut foliage.

Leaves were bunched together with string or metal ties and field foliage with larvae placed in the middle. Though larvae readily fed on cut foliage and adults were reared, it was a time-consuming process needing maintenance at least twice per week. Transferring larvae to live plants was a much simpler and successful technique. Foliage with immatures was attached to a laboratory sapling. Once a week, plants were checked to determine development of *E. leiochroa* immatures. Pupae were placed into sealed plastic containers, transferred to a controlled temperature cabinet, and monitored daily for adult emergence. Adults (inset left) were induced to oviposit when larger plants were pressed against the gauze sides of cages. Egg masses were predominantly found on older stem material and leaf nodes against the gauze. In some cases eggs had pushed through the gauze mesh and rested on the outside of the cage.

Larvae hatch after 6 days and were found mostly at leaf nodes, creating a webbed tunnel by tying the leaf to the stem. Larvae skeletonize leaves by feeding on the epidermis. Young larvae occasionally feed on the stems of *Melaleuca* but this wasn't observed for older larvae. Multiple larvae form small colonies consisting of continuous shelters made of frass, webbing and damaged leaves between nodes along branch stems. The larval stage is approximately 45 days and the pupal duration is 7 days. Pupae are found within the webbing. Females can emerge and oviposit within 24 hours and lay up to 70 eggs in a lifetime (mean 23 eggs).

**Host range testing**

No-choice tests were conducted using gauze cages (80x25x25 cm). *Melaleuca quinquenervia* and one-three test plants were placed individually in test cages so that foliage was pressed against the gauze. Two newly emerged pairs of adults were released into each cage and a 10% honey solution was supplied as a food source (replaced daily). A dampened cloth was placed on the top of the cage to provide moisture for adults to utilize. All adult deaths were recorded and eggs were counted before hatching. Trials are preliminary and ongoing and eight plant species have been tested; *Melaleuca alternifolia*, *M. linariifolia*, *M. styphelioides*, *Callistemon citrinus*, *C. rigidus*, *C. viminalis*, *Eucalyptus citriodora* and *E. camaldulensis*. *Eochrois leiochroa* oviposited and developed on all species except *M. styphelioides* (oviposited only) and *E. citriodora*. With a broad host range, it is unlikely that *E. leiochroa* would be specific enough to be used as a control agent for *M. quinquenervia*. Limited testing is planned for this insect.

**The Puff-Ball Gall-Former – "*Sphaerococcus*" *ferrugineus***

Galls, commonly referred to as "puffball galls", are abundant on the broad-leaved paperbark tree, *Melaleuca quinquenervia*, throughout its range along the east coast of Australia. They are found on the ends of branches or along the sides of branches in a leaf axil. Formation of the gall will often cause severe deformity of branch ends, restricting the growth of the tree. The galls are formed by a scale insect, "*Sphaerococcus" ferrugineus*, which resides within the centre of the tightly packed plant tissue. Although the puffball galls are usually found in moderate numbers at most field sites, some trees can be severely infested. The nutrient drain, branch deformation and growth restrictions on the tree make it a possible candidate for the biological control of *M. quinquenervia*.

**Taxonomy**

**Order:** Hemiptera
**Suborder:** Sternorrhyncha
**Superfamily:** Coccoidea
**Family:** Eriococcidae
**Genus:** "*Sphaerococcus"* (likely to be incorrect)
**Species:** *ferrugineus*

*"Sphaerococcus" ferrugineus* was originally described by Froggatt (1898) and placed in the gall-forming Brachyselinae, using specimens collected from Queensland and New South Wales. Froggatt (1921) described the adult female from *Melaleuca* as "reddish-brown, marked with dull yellow on the dorsal surface, and is embedded in the central woody cavity. General form oval, dorsal surface conical". Individuals of *"Sphaerococcus" ferrugineus* are imbedded into the gall tissue with their pointed posterior section of the body exposed (Hendricks and Kosztarab 1999). Froggatt (1921) also described the galls formed by this species as "reddish brown to dull green rounded fluffy excrescenses, composed of many fine filaments or bracts radiating from the basal gall, on the side or tip of the branches of several species of *Melaleuca*" (inset above). Our observations have found the galls to be green to brown in colour, roughly spherical (up to 3 cm diameter) with proliferations of tightly clustered, dwarfed shoot tissue usually situated in a leaf axil or branch tip.

The genus *Sphaerococcus* is part of the family Pseudococcidae (mealybugs) and it should really contain only two species, *S. casuarinae* and *S. durus*. "*Sphaerococcus" ferrugineus* is not a mealybug. Since the genus was described by Maskell (1892), it was unfortunately a "dumping ground" for rotund scale insects that did not fit elsewhere (Miller *et al.* 1998). There were 38 species or varieties that were placed in *Sphaerococcus*, and the correct family placement for each was unknown (Beardsley 1984). Miller *et al.* (1998) summarised the placement of all 38 taxa so that they could be properly catalogued in "ScaleNet", a database of the scale insects of the world (see http://www.sel.barc.usda.gov/scalenet/scalenet.htm). It should be noted that currently ScaleNet incorrectly places "*Sphaerococcus" ferrugineus* in the genus *Beesonia* within the family Beesoniidae (P. Gullan, UC Davis, pers comm.). Using syntype specimens from Froggatt's collection at the CSIRO Australian National Insect Collection in Canberra, Australia, Miller *et al.* (1998) suggested that

*"Sphaerococcus" ferrugineus* could be moved to the family Beesoniidae. They observed that "mature females have shallowly invaginated quinquelocular pores (like Beesoniidae and some Eriococcidae) and large bases to the clypeolabral shield (like Beesoniidae)". Miller *et al.* (1998) suggested that young adult females and first-instar nymphs were needed before the family placement could be confirmed. Subsequent genetic analysis suggests that it is related to other eriococcids on *Melaleuca* (P. Gullan, UC Davis, pers comm.). Hendricks and Kosztarab (1999) list both the family and genus as "uncertain".

***<u>'Sphaerococcus' ferrugineus</u>* <u>galls on</u> *<u>Melaleuca quinquenervia</u>***

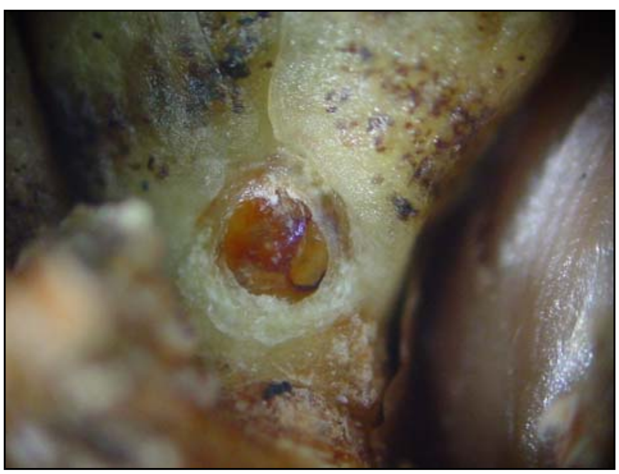
*'Sphaerococcus' ferrugineus* forms galls on the leaf and flower buds of at least six species of broad-leaved Melaleuca in Australia. There seems to be a tendency towards sporadic distribution; occasionally a single tree will be heavily infested with galls yet no other trees in the vicinity will be affected.

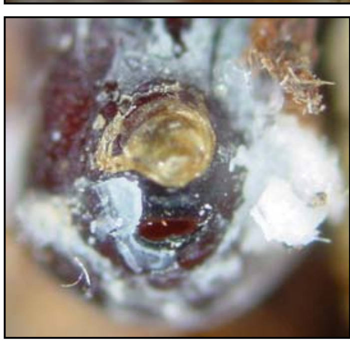
A gall can develop from the feeding of a single mature female, while dispersal is achieved through the highly mobile crawler stage. Research on other Eriococcids shows that the crawler can be very good at dispersal; in *'Sphaerococcus'* this may also be the case but it is also common to see very complex gall clusters where a number of immature

female crawlers have settled at the base of the growing "budlets" of the original gall. The picture (inset left) shows a mature female in the bottom left corner. Circled in red is a recently settled crawler, which is being enveloped by the growing tissue of the "budlet". If it survives, this immature female will mature and a fresh gall will develop out of the original one. This process can be repeated many times, creating a gall complex that may act as a nutrient sink in the host plant. In the picture, below, the gall was dissected by pulling "budlets" individually from the woody base of the gall. The photo at the top shows old brown tissue on either side of the stem and fresh green and red tissue growth where new galls have developed alongside the old. Over 1000 budlets were removed from this gall complex, to reveal a woody skeleton in which over 60 mature females were present.

Hundreds of galls, from six species in the *M. leucadendra* complex, were dissected in order to find all life-stages of this Eriococcid. Representatives of the specimens recovered were sent for classical taxonomy and genetic analysis. The results of genetic analysis suggest that specimens from NSW/SQ, NQ, and NT form 3 distinct groups – the level of difference was not specified. Within the NQ group, each specimen from a different host revealed a unique sequence, which may point to host differences.

The adult male was found enclosed in a white sac, nestled in a bract of the leaf bud growth.



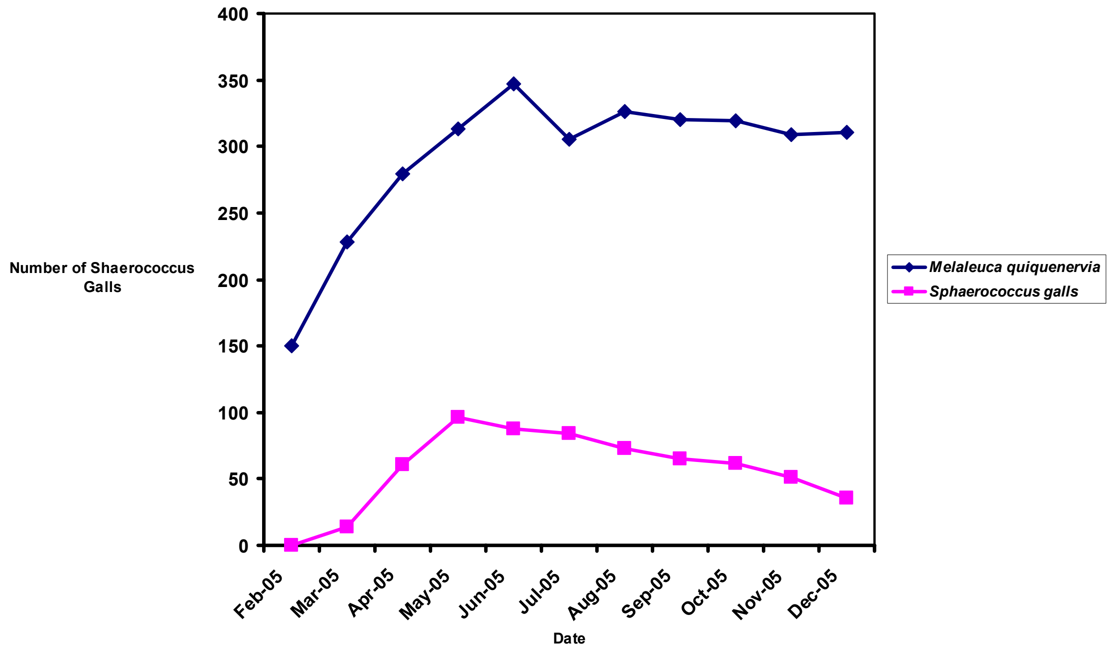


The mature female starts by feeding on the external surface of a budlet. The plant tissue gradually grows around it until only the dark sclerotized end is visible. This is usually covered by a waxy exudate.

Young suckers of *M. quinquenervia* infested with galls have been dug from the ground and potted and some of the galls have survived, but they are yet to self-transfer to clean plants. Attempts to establish a laboratory colony of galls, from scratch, are yet to be successful. Many immatures have been found during dissections, but most of them have already attached to the plant tissue and started feeding. Whilst the elongated stylet can be pulled out of the tissue, it does not seem that the juvenile can then recommence feeding on the new plant. A few of the true crawlers (inset right) that have been transferred seem to have formed some minor deformation of the plant tissue, but none have gone on to establish a proper gall.

A site at Ewan Maddock Dam, near Landsborough on the Sunshine Coast, is being used to determine the dispersal ability of *'Sphaerococcus'*. A 50-metre stretch of ground between two power poles running through the site was cleared in November 2004 (inset left). By the end of 2005 over 300 *M. quinquenervia* suckers had regrown in this area and *'Sphaerococcus'* was well established (See Fig.1 over page). Close to 40% of the mature trees lining this site have evidence of *'Sphaerococcus'* infestation. Data indicates that this gall-former is quite mobile and could readily establish quickly if released as a biological control agent in Florida.

**Figure 1**. The recolonisation of *Melaleuca quinquenervia* and *Sphaerococcus ferrugineus* in a cleared paddock at Ewan Maddock Dam during 2005.




## Results of Genetic Analysis

Genetic analysis undertaken by CSIRO Entomology in Canberra has revealed some promising information about the host-specificity of 'Sphaerococcus'. Firstly, the analyses confirmed that we have isolated the mature male and immature female stages of this scale insect; the sequences match with the only previously known stage of this species – the mature female. Representatives of what is believed to be the immature male failed to sequence. Further analysis, combined with the pending results of classical taxonomy, will be required to confirm this belief.

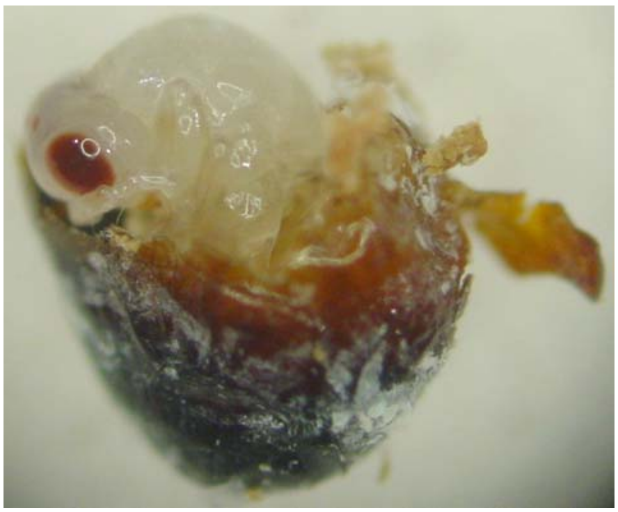
Four mature females and an adult male dissected from galls collected from 3 sites between Brisbane and the southern limits of *M. quinquenervia* in NSW yielded identical sequences for the mitochondrial gene that was amplified. These specimens form a 'southern' group. Four mature females from NQ, one each from *M. quinquenervia*, *M. cajuputi*, *M. leucadendra*, and *M. viridiflora* were found to be similar to those from SQ/NSW, but still form quite a separate group. Further, each of these NQ sequences was unique, suggesting some level of host differentiation.

Two specimens from *M. argentea* in NT, one mature and one immature female, were shown to be identical to each other but represented a third distinct group. It is not known whether this is a geographical difference or just a host difference, as no other specimens from NT were able to be analyzed. Considering the results from specimens collected in eastern Australia, it is likely that both geographical and host barriers will play a role in these genetic differences.

Early indications are that *'Sphaerococcus' ferrugineus* will prove to be host specific.

**Parasitism**

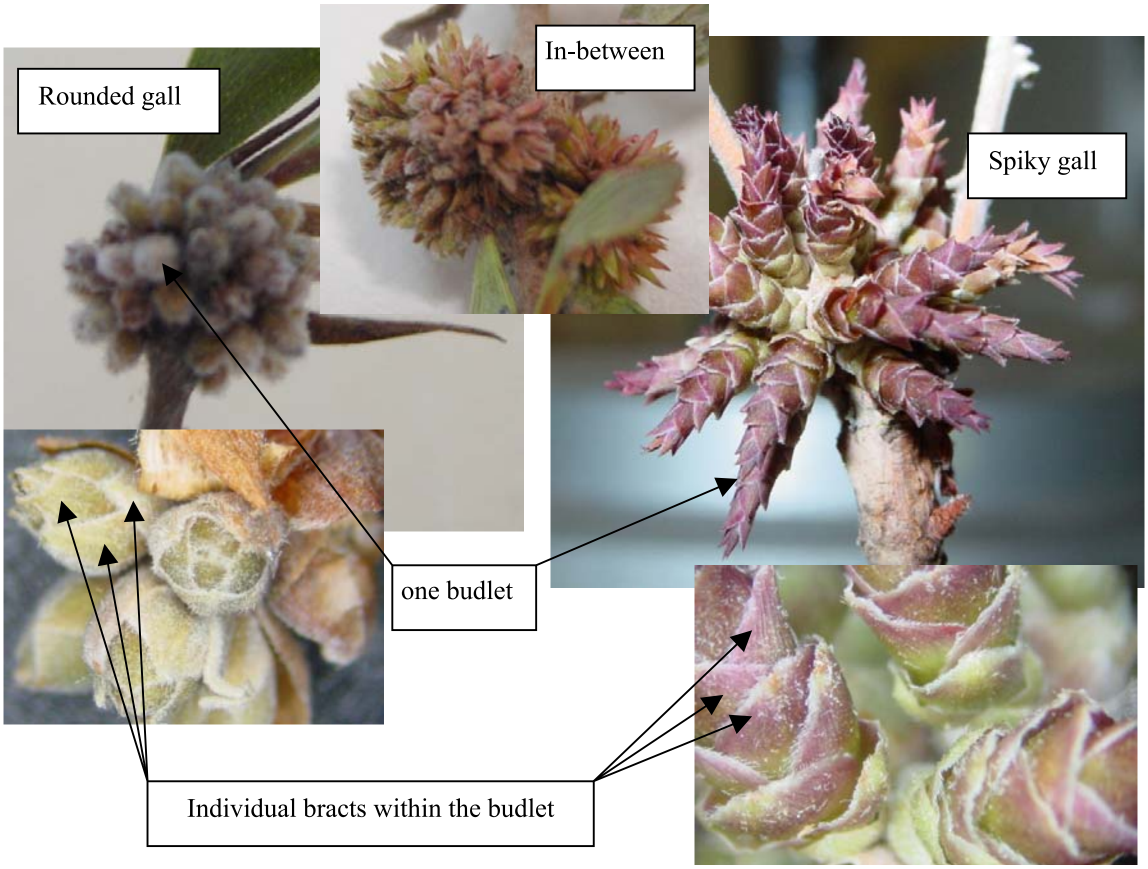
Hymenopteran endo-parasitoids within the mature females have occasionally been found. A couple of these pupae have been mature enough to rear through on their own to adults, but the species involved are yet to be identified. One wasp was also found within the white sac of the adult male *'Sphaerococcus'* on *M. argentea*. The pictures below show a pupa and larva within the, otherwise emptied, mature female.



Some tentative estimate of parasitism could be given, but the data is considered too preliminary for any form of publication.

**Host Variation: *Melaleuca argentea***

The form of the galls ranged from rounded 'budlets' to spiky, including galls with both forms. Many of the spiky 'budlets' had multiple crawlers and other early-instar *'Sphaerococcus'* at the base of their bracts.




One, often 2 or 3, and sometimes 4 or 5 crawlers/recently settled juveniles were found at the base of a single bract of one of the budlets. Generally, they were not found in the basal third or the very tip of the budlet, but they would be in almost every other bract.

**Impact and potential as a biological control agent**

*"Sphaerococcus" ferrugineus* appears to attack both the lateral and terminal buds of *M. quinquenervia*. This scale insect forms large galls consisting of tightly packed stunted shoots with a woody center. The resultant disruption to normal plant function results in termination of growth at the bud or deformation of the branch structure beyond the gall. Additionally, it causes extra branching, changing the structural growth of the tree (inset left).

*"Sphaerococcus" ferrugineus* will attack saplings as well as older trees. There is massive germination of *M. quinquenervia* seedlings in Florida and plants can grow in very high densities as the lack of attack by natural enemies allows the growth of single stemmed, unbranched individuals. Infestation by *"Sphaerococcus" ferrugineus* scales could induce branching at an early stage of growth, radically altering the stand structure. Additionally, formation of galls can also act as nutrient sinks, draining plant resources that would usually be allocated for foliage growth or reproduction, ultimately reducing the vigor of the tree. The degree to which puffball galls act as nutrient sinks is yet to be determined. Gall tissue will be analyzed to identify the quantities of compounds such as lignin that require the allocation of significant plant resources to be produced.

Since the crawler stages of coccids are very mobile, distribution of this insect should occur relatively rapidly if released in Florida. These nymphs are likely to be dispersed by wind, and given the occurrence of the hurricane season and regular strong winds in that region, natural distribution should occur readily.

**<u>Cecidomyiid Stem Galler - *Lophodiplosis trifida*</u>**

Small stem galls caused by the fly *Lophodiplosis trifida* (Diptera: Cecidomyiidae) are frequently seen in very low numbers on young tips of *M. quinquenervia* at field sites in Australia (inset right). In the laboratory, these gall-formers readily colonize saplings, and once free from parasites, produce large numbers of galls. The plants are severely damaged; some die while others suffer severe dieback. If galls are large enough, it appears that they can sever vascular connections.

The adults are short lived, surviving for one to two days, though females can lay hundreds of eggs during that period. The red eggs are oviposited on both the leaves and stems of young foliage. Eggs hatch within 3-5 days. The neonate larvae emerge and burrow into the stem. Development from egg to adult is approximately 25-35 days.

In 2005, laboratory saplings with galls were shipped to the Gainesville quarantine facility to boost cultures for host-range testing. Preliminary testing in Australia indicted that these insects are specific to broad-leaved *Melaleuca* species.

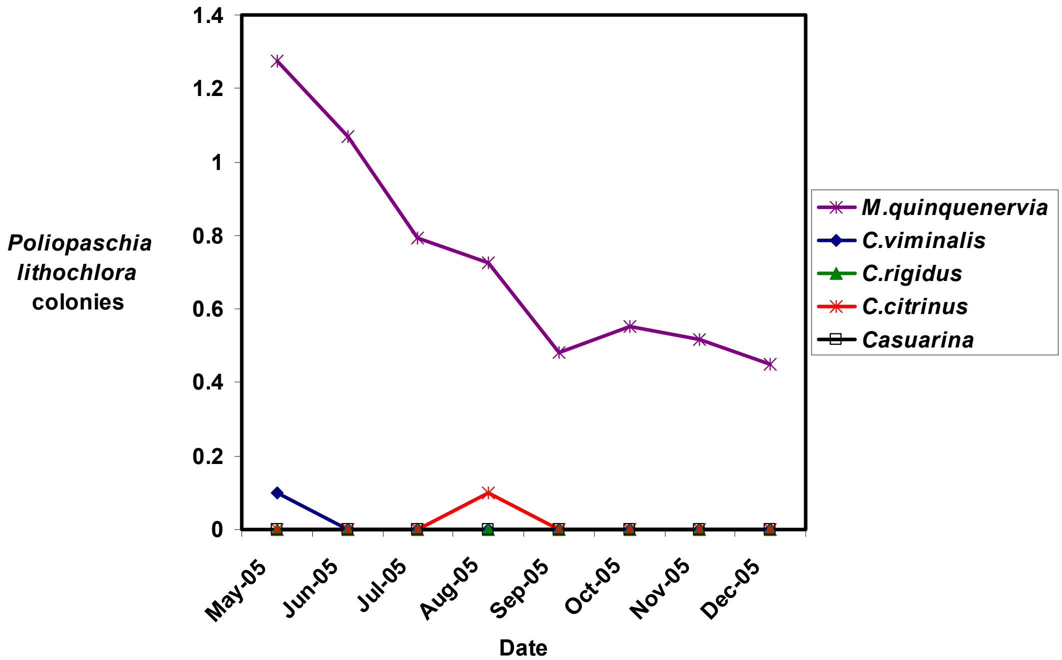
**<u>Tube-Dwelling Moth – *Poliopaschia lithochlora*</u>**

*Poliopaschia lithochlora* (Lepidoptera: Pyralidae) have been under evaluation at ABCL since 1996. Because this moth severely damages saplings and suckers of *M. quinquenervia*, prefers low-lying humid sites, and can be successfully mass-reared, it is rated highly for its potential as a biological control agent. The larvae are voracious leaf feeders, concealed in tubes that are usually found in small colonies attached to leaves and stems (inset left). Larvae move from these tubes to feed on surrounding leaves; saplings and suckers are frequently defoliated. Prepupae form sealed bulbs in the larval tubes in which they pupate. Adult females are mainly active and oviposit at night. Development from egg to adult occurs in approximately 80 days.

*Poliopaschia lithochlora* can be found at most field sites in all regions where broad-leaved *Melaleuca* trees grow in Australia. They have a strong preference for low lying sites (usually seasonally inundated) where there is a grass under-storey and the humidity is high. The grass under-storey may provide higher humidity with dew formation during the early morning periods. Larvae live in tubes and feed on both young and old leaves. Although found on older trees, saplings and suckers are preferred. For these reasons it may be an excellent agent for the wetland areas in Florida, especially where extensive stands of seedlings predominate.

The biology of this insect has been published (Galway and Purcell, 2005). The seasonality and field host specificity of this insect has also been studied intensively, and laboratory host-specificity tests have been completed. Concerns over its laboratory specificity, especially feeding on *Callistemon* and *Eucalyptus* species, have lead to this moth being given a lower priority for introduction into quarantine facilities in the United States for final testing. Interestingly, the moth was never collected from non-Melaleuca hosts in field surveys.

**Figure 2.** Results of surveys of *Poliopaschia lithochlora* colonies on *Melaleuca quinquenervia*, *Callistemon citrinus*, *C. rigidus* and *C. viminalis* in a field plot at Tyagarah in northern New South Wales.




While ABCL reprioritized research to focus on "*Sphaerococcus*" *ferrugineus*, it was decided to attempt to validate the non-target results observed in laboratory host range tests with field studies. A small field study was initiated at Tyagarah in NSW in 2003 to determine the preference of this moth under "more natural" conditions. The field site is a grazing paddock with ornamental plantings of *M. quinquenervia* along its perimeter fence. A very large population of *P. lithochlora* has always existed at Tyagarah and it's been the predominant location for collecting this moth for laboratory studies. Three *Callistemon* species (10 trees each of *C. rigidus*, *C. citrinus* and C. *viminalis*), where planted between the existing saplings of *M. quinquenervia*. Some *Casuarina* trees that incidentally fell within the plot and were also surveyed. A white tag with row number, species code and identification number was attached to the stem of each plant. Monthly surveys initiated in May 2005 recorded the height and the number of *P. lithochlora* colonies for each plant. Results are given in Figure 2 above. *Poliopaschia lithochlora* larvae have been found on *M. quinquenervia* and all of the *Callistemon* species (inset above on *C. viminalis*) though numbers are far higher on *M. quinquenervia*

and it appears to be the preferred host. This data indicates that if it were released in Florida as a control agent, some damage could be expected on ornamental *Callistemon* trees. No *P. lithochlora* larvae were recorded on *Casuarina* trees.

**Stem-boring Cerambycidae**

A species of cerambycid beetle from the sub-family Strongylurini, which is quite common throughout the native range of *M. quinquenervia*, was frequently observed at field sites throughout 2005. It has previously been collected from *M. nervosa* (NQ), *M. quinquenervia* (SQ), *M. viridiflora* (NQ), and *Callistemon* sp. (SQ). These larvae feed within the stems of *M. quinquenervia*. They cause extensive damage and even large branches are killed. Larvae tunnel the stems in a spiraling motion toward live tissue. They mostly consume tissue of the phloem, effectively ring-barking the tree. These beetle larvae are long-lived and we suspect that there may only be one generation per year. Larvae could only be reared to adult when left on damaged branches collected from the field and will not transfer to live saplings or cut branches. Due to the slow development time of these insects, questionable specificity, and rearing difficulty, no further research is planned.

In June 2005, a large population of a second cerambycid species, *Rhytiphora* sp. (inset above), was discovered on *M. quinquenervia* at Woodgate on the central coast of Queensland. A damaging population still existed in October during a return visit to the site. Between these surveys, several adults were collected and more than 30 larvae and pupae were observed or extracted from the field-collected damaged branches. Adults have been reared from immatures developing within the original field-collected branches while others were reared successfully on artificial diet (inset above right). Emerging adults were set up on caged saplings, but no oviposition was observed. Other larvae from the field were transferred to laboratory saplings and are continuing to develop. *Rhytiphora* sp. has a similar feeding habit to the Strongylurini species described above, but differs in that larvae can be transferred to live saplings. Further evaluation of this cerambycid beetle is planned in 2006.

**Melaleuca Sawfly – *Lophyrotoma zonalis***

Larvae of *Lophyrotoma zonalis* (Hymenoptera: Pergidae) defoliate broad-leaved *Melaleuca* spp. trees mostly in tropical regions of Australia. Larvae pupate in the papery bark and this insect can complete its whole life cycle on the tree. Adults (inset left) are very mobile, especially males who swarm around trees preparing to mate with emerging females. For these reasons, *L. zonalis* could have been an effective biocontrol agent, especially in the remote wetland areas of southern Florida. However, research conducted in Australia through the ABCL liasing with the National Research Centre for Environmental Toxicology (NRCET) in Brisbane, determined that *L. zonalis* larvae contained two toxins, lophyrotomin and pergidin. These toxins may pose a significant risk to animals in the United States, particularly starving migratory birds. Therefore, no releases are planned for this insect in the US even though all testing in Australia and in quarantine in Florida has been completed, and the insect is known to be highly specific to *Melaleuca* spp. and severely damages trees through complete defoliation.

In 2005, we observed further larval outbreaks (inset right) of *L. zonalis* in SQ. In 2003, larvae were first observed in SQ at sites near and on Bribie Island, north of Brisbane. Previously the insect was not known from south of Mackay in central Queensland (Burrows and Balciunas 1997). It is possible that nursery plants contaminated by *L. zonalis* have been transported from NQ for ornamental plantings. Defoliation has now been observed at several sites on the mainland approximately 15-20 km west of the sites on Bribie Island. As before, it has predominantly been on ornamental plantings of *M. leucadendra*. The complete defoliation of *M. leucadendra* and the existence of hundreds of thousands of *L. zonalis* with limited resources for feeding and oviposition should have pushed *L. zonalis* onto *M. quinquenervia* if it was an equally acceptable host. However, Bribie Island is predominantly covered by *M. quinquenervia*, and no stands appear to have been damaged by this sawfly. This indicates that *M. quinquenervia* may not be a primary host of *L. zonalis*. Before incursions into SQ, our observations indicated that this sawfly did not extend further south than the known native range of *M. leucadendra*. Given its potential toxicity to native wildlife, and concerns over whether *M. quinquenervia* is a primary host, it is unlikely this insect will be released in Florida.

**<u>Melaleuca Psyllid - *Boreioglycaspis melaleucae*</u>**

The sap-feeding psyllid, *B. melaleucae* (Hemiptera: Psyllidae) damages *M. quinquenervia* by promoting leaf drop. Both adults and nymphs feed on the leaves, but the nymphs cause the most damage. *Boreioglycaspis melaleucae* are easily detected on *M. quinquenervia* plants by the presence of white flocculant threads secreted by the nymphs. It was the second biological control agent released for *M. quinquenervia* in Florida. In 2005, ABCL staff collected and preserved *B. melaleucae* from QLD and NSW and shipped the specimens to Dr. Paul Pratt at the USDA ARS IPRL in Florida. The specimens are to be sequenced to compare the genetic variability within its native range with that found in the introduced range in Florida.

**<u>Demographic Studies</u>**

Since 2003, Dr. Steve Franks (University of California) and Dr. Paul Pratt (USDA IPRL) have been conducting a demographic study of *M. quinquenervia* at two sites in Australia, Bribie Island north of Brisbane in SQ, and at Port Macquarie in NSW. Approximately 1000 trees and saplings have been tagged at Bribie Island and over 600 at Port Macquarie. Surveys have been carried out each year with ABCL providing staff and logistical assistance. In 2005, scientists from the U.S. were unable to travel to Australia, and ABCL staff completed the surveys at both locations in September. The stem/trunk diameter of all trees was measured (inset above right) and the presence/absence of fruits/flowers was recorded. The survival of seedlings was also monitored (inset above left). A forest fire had passed through the Bribie Island site since the 2004 survey, and some of the tags were

damaged by forest fires (inset below).  However, the tree numbers could be verified by cross-referencing data with measurements obtained in 2004.

**Melaleuca Future Plans**

In 2006, *M. quinquenervia* research will focus on the flower-feeding weevil, *Haplonyx multicolor*.  ABCL staff will perfect the rearing technique for this insect and begin biology and host-specificity studies.  Since this weevil is from NQ, several trips are planned to collect large numbers of these weevils from field sites.  Shipments to the Gainesville quarantine facility are also planned.  The field host range study for *Poliopaschia lithochlora* will continue and further surveys are planned to determine the habitat preferences for this moth.  Field colonization surveys of the puff gall-former, "*Sphaerococcus*" *ferrugineus*, will also continue at Ewan Maddock Dam north of Brisbane.  Opportunistic studies on stem-boring Cerambycid and weevil species will also be conducted.

# Biological Control of *Lygodium microphyllum*

## Introduction

The native range of *Lygodium microphyllum* (inset right) is the Old World from Africa to India, Southeast Asia, Australia, and some Pacific Islands (Pemberton 1998). *Lygodium microphyllum* is found in a variety of habitats across its native range. In the states of Queensland and New South Wales in eastern Australia and in New Caledonia, *L. microphyllum* is common in the freshwater creeks and depressions of the coastal wetlands, often growing with *Melaleuca quinquenervia* (Cav.) S. T. Blake (Myrtaceae). In tropical north Queensland, *L. microphyllum* is sympatric with *Lygodium reticulatum,* but the latter prefers the upland rainforest habitats. In the monsoonal climate of northern Australia (Western Australia and Northern Territory), *L. microphyllum* is sympatric with *Lygodium flexuosum* and grows in forest patches and along perennial creeks with abundant, slow-moving fresh water. In the Kimberley range of Western Australia, *L. microphyllum* was found growing in a canyon near permanent springs. In Southeast Asia, India and Papua New Guinea, *L. microphyllum* is common along the edges of lowland rainforests in peat soils, in coastal wetlands, and in wet, lateritic clay soils. Near the northern limit of its range in southern China, *L. microphyllum* is sympatric with *L. japonicum* and *L. flexuosum*. *Lygodium japonicum* appears to be more competitive in the drier rocky soils, often found growing in a low prostrate form over whole hillsides.

## Weed Status

Old World climbing fern, *Lygodium microphyllum* (Cav.) R. Br., has rapidly invaded southern Florida. It climbs over other plants, including tall trees, forming massive walls of vegetation (Wood 2000). It also forms thick mats on the ground that smother native plants. Fires can be carried quickly to the tops of trees by the burning fern and then spread by the floating pieces (Wood 2000). In 1973, Old World climbing fern was limited to small portions of Martin and Palm Beach counties along the eastern coast and to some sites in the inland Highlands county (Pemberton and Ferriter 1998). By 1993, it had expanded greatly in Martin and Palm Beach counties and was also found in Glades County for an estimated infestation of 11,213 hectares. In 1997, an estimated 15,892 hectares were infested in 15 counties from coast to coast along the southern half of the Florida peninsula. By January 2002, the infestation was estimated at more than 40,470 hectares and still expanding (Wood and Garcia 2002). By early autumn 2002, weed managers were reporting in meetings that small, colonizing plants that could not be distinguished during aerial surveys were widespread throughout the Florida Everglades region, suggesting that the surveys underestimate the levels of infestation. The range of *L. microphyllum* is predicted to expand into central Florida and large parts of the Neotropics (Pemberton and Ferriter 1998, Goolsby 2004).

**<u>Potential spread</u>**

Pemberton and Ferriter (1998) predicted the fern would spread northward in Florida along the coasts within the boundaries of USDA hardiness Zone 9A. The confirmation by Pemberton (2003) of *L. microphyllum* in Orange Co., FL (Zone 9A) supports this prediction. John Volin (Florida Atlantic University) predicted that *L. microphyllum* would spread to most of south Florida by 2014 (Volin *et al.*, 2004). Goolsby (2004) used the climate-matching program CLIMEX (Sutherst *et al.* 1999) to predict the potential distribution of *L. microphyllum* in North and South America (Fig. 3 over page). The CLIMEX model was fitted from climate data from the known distribution of the fern in the Old World. The model was based on intensive surveys for the plant near its ecoclimatic limits in China and Australia. The model predicts that the climate is suitable for further expansion of *L. microphyllum* northwards into central Florida and southward into large parts of the Caribbean, Central and South America.

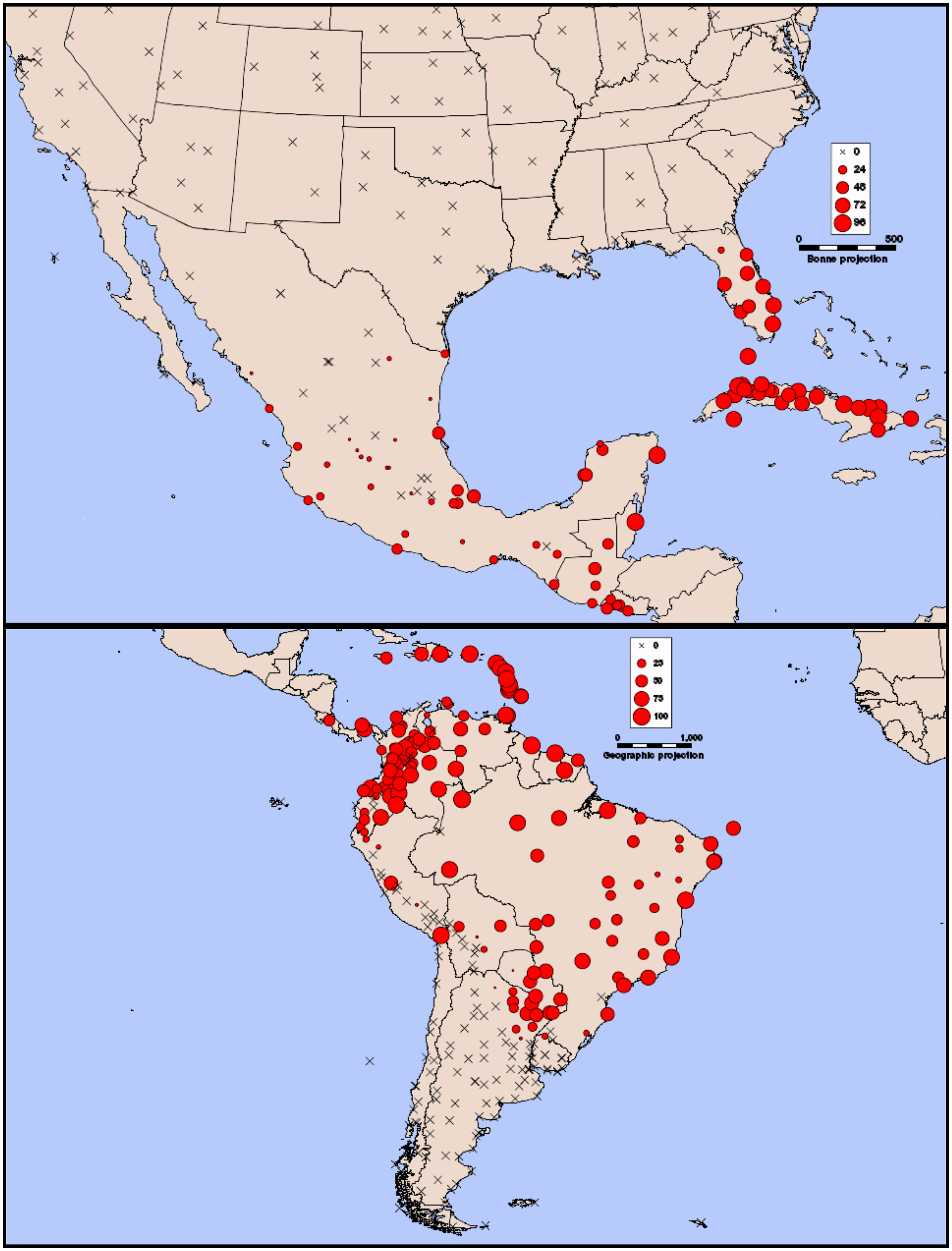
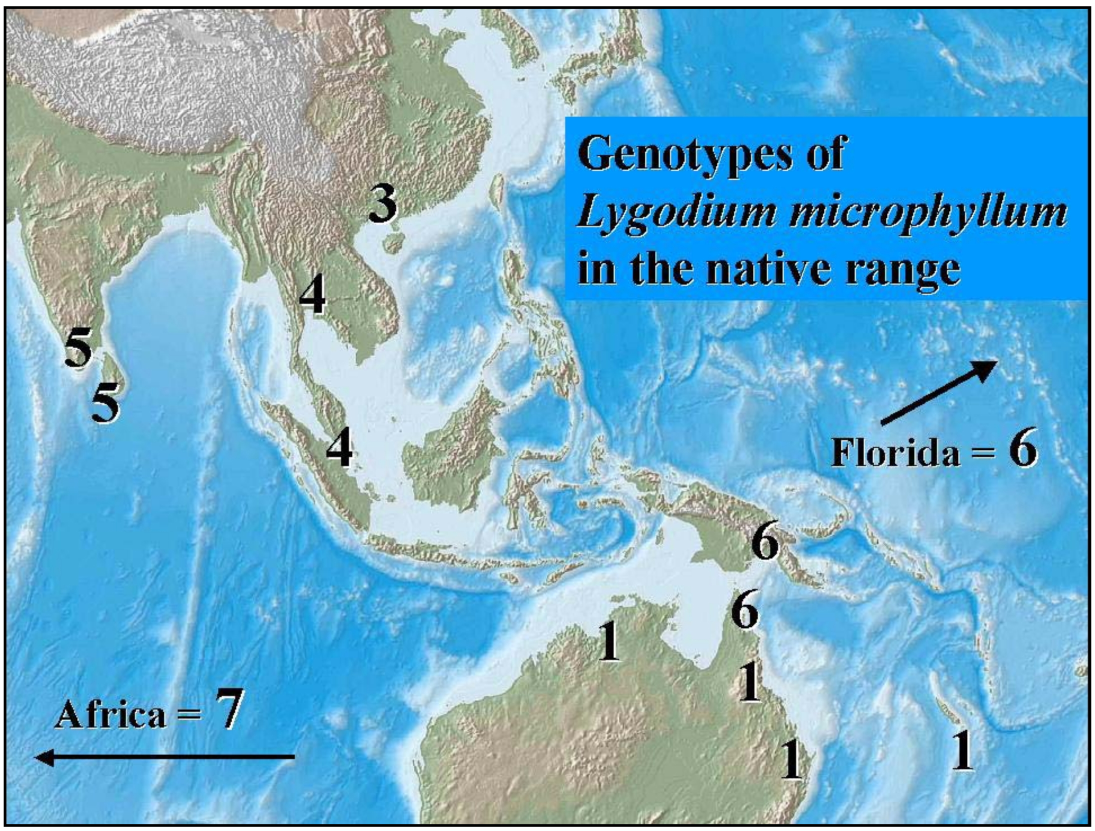



**Figure 3**. Predicted distribution of *Lygodium microphyllum* in North and South America based on CLIMEX. Size of dots indicates suitability of area for growth of fern. The locations with 'x' indicate the climate is not suitable for the fern.

**Genetic variability**

Populations of *L. microphyllum* from Africa, Asia, Australia and Oceania were characterized using molecular analysis with the intent of finding the origin of the invasive Florida population (Goolsby *et al.* 2004). Two genes from the chloroplast DNA were selected because they showed the most variation between populations. Several distinct genotypes were discovered (Figure 4 below). The population from the tip of Cape York in far north Queensland was found to be an exact match with Florida for the two genes (Goolsby *et al.* 2003). *Floracarus perrepae* collected from the Cape York *L. microphyllum* genotype were found to be the best adapted to the invasive Florida *L. microphyllum*, so both genetic and biological indicators point to Cape York as the source of the original material.

**Figure 4.** Distribution of *Lygodium microphyllum* genotypes in the native range. Genotypes are based on unique differences in the chloroplast DNA intron sequences, RPS-4 and Trn-L. Different numbers indicate unique genotypes.




There have been no reports on genetic variability of *L. microphyllum* in Florida. The range expansion pattern from a small area in Martin County in 1965 to the current range suggests that there has not been multiple introductions. Individual plants from several populations in south Florida were analyzed using molecular diagnostics. The plants analyzed from Florida all had the same identical gene sequences (matching the Cape York, Australia population). This evidence supports the assertion that there has been only one source population introduced into Florida.

## Life History

*Lygodium microphyllum* reproduces sexually during the sporophyte and gametophyte stages. Fertile pinnae of the sporophytes produce spores within sporangia (inset above right). When released from the sporangia, the spores will, given needed moist environmental conditions, germinate into tiny, thin-tissued plants called prothallia, the gametophyte generation (where eggs and sperm are formed). The gametophytes have both male and female organs producing sperm and ovules. Fertilization gives rise to the familiar plants (the sporophytes). Spores of the *Lygodium* genus have very thick walls, giving these propagules long environmental viability (Tryon, 1999, pers. comm.). In sub-tropical climates, *L. microphyllum* is evergreen and may actively grow throughout the year as both sporophytes and gametophytes. *Lygodium microphyllum* is a homosporous fern and may engage in three types of sexual reproduction: 1) intragametophytic selfing, involving the union of egg and sperm from the same gametophyte; 2) intergametophytic selfing, the cross-fertilization of gametophytes produced by spores from the same sporophytes; and 3) intergametophytic crossing, the cross fertilization of gametophytes arising from different sporophytes (Lott *et al.*, 2003). Recent research (Lott *et al.* 2003) found that *L. microphyllum* can self intragametophytically. The gametophytes first grow archegonia - the female organs - and remain in the female form for several months to encourage outcrossing. If there are younger presexual gameotophytes in or near to the female-stage gametophyte, she will induce them hormonally to grow male organs (antheridia), which produce swimming sperm that move to her archegonia and ovules effecting fertilization. If a female gametophyte is not fertilized, she grows male organs and self fertilizes. The fern is thus an ideal weed. A single spore can blow to a new area and start a new population. An established population or a recently colonizing population optimizes outcrossing.

In its natural range, *L. microphyllum* is found in a variety of habitats including open forests, rain forest, swampy areas and high altitude. *L. microphyllum* is now well established in central and southern peninsular Florida (inset right), where it grows in a number of wetland habitats including hammocks, cypress swamps, flatwoods, and disturbed sites (Nauman 1993, Pemberton and Ferriter 1998). It has been observed in scrub sites in Martin County (Thayer and Ferriter, pers. observations). Unlike some invasive exotic plant species, *L. microphyllum* does not appear to require human disturbance in order to spread and become established. *L. microphyllum* has been found in very isolated areas including remote locations in Everglades National Park and the Fakahatchee Strand State Preserve. Water and wind of storm events may help disperse millions of tiny spores over long distances, although little is known about *Lygodium* dispersal mechanisms." (Ferriter 2001).

It is suspected that the principal factors that contribute to the explosive weediness of this plant are the spores, the climbing habit, the peninsular location, and the south Florida climate. Spores are small, like dust, and can be spread easily by wind and water. Apparently one spore can give rise to new plants, unlike many ferns. The climbing fronds, often in the tops of large trees, maximize the effect of spore dispersal by winds and also allow the young plant to escape competition by overgrowing its competitors. The peninsula of Florida has daily winds coming inland from the ocean, which would speed up range expansion. The south Florida subtropical climate, except during occasional periods of drought, has abundant rain, which leaves soils moist. This would promote spore germination and gamete success.

**Review of Foreign Exploration and Agent Development**

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. Research in Australia and Southeast Asia commenced under the leadership of Dr Charles Turner in 1996.

*L. 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 2005, exploration for biological control agents for *L. microphyllum* occurred in Australia and Southeast Asia (Table 3 over page). A list of collections is given below. Most collections were from the primary target, though other *Lygodium* and associated ferns and vine species were surveyed to verify host specificity of agents as well as sources of insects for laboratory studies.

**Table 3**. Summary of field collections made during 2005 in Australia and Southeast Asia related to exploration for biological control agents of *Lygodium microphyllum*.

| Plant | | Number Collections/Number Sites | | | |
|---|---|---|---|---|---|
| **Family** | **Species** | **Australia** | **Malaysia** | **Singapore** | **Thailand** |
| Schizaeceae | *Lygodium circinnatum* | - | - | 5/3 | - |
| | *Lygodium flexuosum* | - | - | 6/3 | 2/2 |
| | *Lygodium microphyllum* | 21/20 | 5/5 | 28/12 | 4/4 |
| | *Lygodium reticulatum* | 1/1 | - | - | - |
| | *Dicranopteris sp.* | - | - | - | 2/2 |
| | *Aspleniun longissimum* | - | - | - | 1/1 |
| Unknown | Unknown vine sp. | - | - | 1/1 | - |

A list of the herbivores of *Lygodium* spp. collected in Asia and Australia since 1996 is given in Table 4.

## Activities in Australia

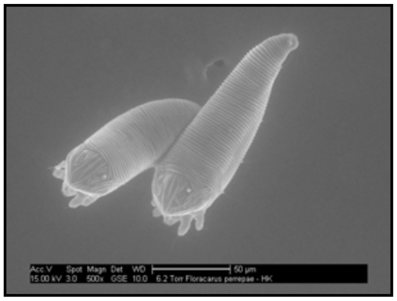
Research in Australia includes field surveys to locate and collect potential biological control agents, insect biology host-range studies, maintenance of plant stocks (Lygodium and test-plants), and establishment and maintenance of colonies of potential agents from Australia and (in quarantine) from Southeast Asia.

Besides the regular surveys in southeast Queensland, several surveys were undertaken at *L. microphyllum* sites for commencement of plant growth and insect population studies. Further north, two surveys were conducted to determine the distribution of *L. microphyllum* in previously unexplored areas along the Queensland coast. Ryan Zonneveld and Bradley Brown surveyed the central Queensland coast from Maryborough to Townsville. In the first survey new *L. microphyllum* sites were located at Bundaberg, Deep Water Creek and Agnes Waters. The moth released in Florida, *Austromusotima camptozonale* was found at Deep Water Creek north of Bundaberg. In the second survey, new sites were found in the Mackay area and another new record of *A. camptozonale* at Keeleys Road, Mackay (inset right). In August 2005, a shipment of 72 larvae of *A. camptozonale* from ABCL was shipped to the Gainesville quarantine facility to boost numbers for field releases.

**Table 4.** Herbivores of *Lygodium* spp. from Asia and Australia (1996-2005).

| | | |
|---|---|---|
| *Floracarus perrepae* Knihinicki and Boczek<br>Acarina: Eriophyidae | Australia, China, India, Indonesia, Malaysia, New Caledonia, Papua New Guinea, Singapore, Sri Lanka, Thailand | *L. microphyllum* (Cav.) R. Br.<br>*L. reticulatum* Schkuhr |
| *Brevipalpis* sp.<br>Acarina: Tenuipalpidae | China, Singapore, New Caledonia | *L. microphyllum* (Cav.) R. Br.<br>*L. japonicum* (Thunb.) Sw. |
| *Neomusotima conspurcatalis* Warren<br>Lepidoptera: Pyralidae | Australia (Qld, NT, WA), China, India, Indonesia, Malaysia, Papua New Guinea, Singapore, Thailand | *L. microphyllum* (Cav.) R. Br. |
| *Austromusotima camptozonale* (Hampson)<br>Lepidoptera: Pyralidae | Australia (Queensland) | *L. microphyllum* (Cav.) R. Br. |
| *Austromusotima metastictalis* (Hampson)<br>Lepidoptera: Pyralidae | Australia (Queensland) | *L. microphyllum* (Cav.) R. Br.<br>*L. reticulatum* Schkuhr |
| *Lygomusotima stria*<br>Lepidoptera: Pyralidae | Malaysia, Singapore, Thailand | *L. microphyllum* (Cav.) R. Br. |
| *Neomusotima fuscolinealis* Yoshiyasu<br>Lepidoptera: Pyralidae | Japan | *L. japonicum* (Thunb.) Sw. |
| Pyraustinae sp.<br>Lepidoptera: Pyralidae | New Caledonia | *L. microphyllum* (Cav.) R. Br. |
| *?Ambia* sp. (Stem-borer)<br>Lepidoptera: Pyralidae | Singapore, | *L. microphyllum* (Cav.) R. Br.<br>*(? also L. flexuosum* (L.) Sw.) |
| *Siamusotima aranea* (Stem-borer)<br>Lepidoptera: Pyralidae | Thailand | *L. flexuosum* (L.) Sw |
| *Callopistria* sp. A<br>Lepidoptera: Noctuidae | Australia (Queensland), China, India, Thailand | *L. microphyllum* (Cav.) R. Br. |
| *Callopistria* sp. B<br>Lepidoptera: Noctuidae | Australia (Northern Territory) | *L. microphyllum* (Cav.) R. Br. |
| *Callopistria* sp. C.<br>Lepidoptera: Noctuidae | Thailand | *L. microphyllum* (Cav.) R. Br. |
| *Spodoptera litura* (F.)<br>Lepidoptera: Noctuidae | Australia | *L. microphyllum* (Cav.) R. Br. |
| *Archips machlopis* Meyrick<br>Lepidoptera: Tortricidae | Malaysia, Singapore, Thailand | *L. microphyllum* (Cav.) R. Br. |
| *Neostromboceros albicomus* (Konow)<br>Hymenoptera: Tenthridinidae | Malaysia, Singapore, Thailand, Vietnam | *L. microphyllum* (Cav.) R. Br.<br>*L. flexuosum* (L.) Sw. |
| *Metriona* sp.<br>Coleoptera: Chrysomelidae | Australia (Northern Territory, Western Australia) | *L. microphyllum* (Cav.) R. Br. |
| *Endelus bakerianus* Obenberger<br>Coleoptera: Buprestidae | Singapore, Thailand | *L. microphyllum* (Cav.) R. Br. |
| *Manobia* sp.<br>Coleoptera: Chrysomelidae | Thailand | *L. flexuosum* (L.) Sw. |
| *Lophothetes* sp.<br>Coleoptera: Apionidae | Palau (Arakabesang Is.) | *L. microphyllum* (Cav.) R. Br. |
| *Acanthuchus trispinifer* (Fairmaire)<br>Homoptera: Membracidae | Australia (Queensland, Northern Territory) | *L. microphyllum* (Cav.) R. Br. |
| *Pseudococcus longispinus* (Targioni-Tozzetti)<br>Homoptera: Pseudococcidae | Australia (Queensland) | *L. microphyllum* (Cav.) R. Br. |
| *Octothrips lygodii* Mound<br>Thrips: Thysanoptera | China, Singapore, Thailand | *L. microphyllum* (Cav.) R. Br.<br>*L. flexuosum* (L.) Sw.<br>*L. japonicum* (Thunb.) Sw. |

**Insects in culture**



Colonies of the *Lygodium* mite (inset left), *Floracarus perrepae* continue to be maintained at the ABCL in Brisbane. This mite deforms the growth of *L. microphyllum* (inset above) and causes leaf necrosis. The culture will be held in Brisbane until *F. perrepae* has been approved for release in the US. These mites were originally collected from the Iron Range in north Queensland. The fern genotype from that region is believed to be an exact match with the fern genotype in Florida and the mite from the Iron Range readily feeds on Florida *L. microphyllum*. We continue to focus on building the laboratory colony to facilitate future shipments to the Gainesville quarantine facility pending approval for release.

Colonies of the two pyralid moth species, *Austromusotima camptozonale* and *Neomusotima conspurcatalis*, are in culture at ABCL . The colony of *A. camptozonale* originated from Peregian, north of Brisbane, and is being maintained as a back-up for the culture in Fort Lauderdale being used for field releases. Further shipments of *A. camptozonale* have been requested for 2006. Since the colonies in Brisbane have been maintained for some time, moths will be collected from local field sites near Brisbane to start new colonies for improving genetic variability and vigor.

There are two colonies of *N. conspurcatalis* that originated from Wangi Falls in the Northern Territory, and from the Iron Range in north Queensland. *Neomusotima conspurcatalis* is currently undergoing evaluation in Gainesville. Both colonies are being used as a back-up for these quarantine cultures and for the culture in Fort Lauderdale used for field releases.

*Callopistria* sp. A larvae (inset left) collected from Cairns in February were used to establish a laboratory culture in Brisbane. This moth has been in culture for 8 months and host testing is progressing. We will opportunistically collect all of the *Callopistria* species encountered in Australia and Southeast Asia and conduct limited testing on each to give an indication of their host specificity. Any species with potential as agents could be subjected to more rigorous testing.

**Florida Atlantic University Research**

In July, Dr. John Volin from the Florida Atlantic University arrived in Australia to initiate studies in Australia to complement his ongoing research into the spread of *L. microphyllum* across the Florida landscape. John is collaborating with scientists from the University of Queensland while ABCL has provided logistical support. Space has been made available at ABCL for John to conduct experimentation on resource allocation. Our staff also grew most of the ferns (over 200 sporelings) that were used in the study and provided vehicles for soil collections from the field and moving potted plants for experimentation both in the laboratory and field. ABCL also provided John with locations of *L. microphyllum* field sites for selection of his experimental field plot. The field study was designed to compare the ecophysiology and soil characteristics of field-grown *L. microphyllum* in its native range vs. invaded Florida habitats over 12-month period. The survival, growth and ecophysiology of *L. microphyllum* is being compared across a natural gradient of light availability using young sporophytes planted in the soil. John's principal objective is to compare sporophyte growth and survival, normalized for variation in light environment, within *L. microphyllum* exotic vs. endemic ranges.

John returned to the US in December but his research continues in Australia. John's assistants, Mike Tobin and Terri Shirshac (inset right), are maintaining the current field experiment at Serpentine Creek and are planning further growth experiments at Stradbroke and Bribie Islands near Brisbane, and similar surveys in North Queensland. Mike is also assisting ABCL with local surveys by locating new monitoring sites and providing input into how to measure growth rates of *L. microphyllum* which will link with his planned research.

**Lygodium Exploration Activities in Southeast Asia 2005**

- January 14, 2005. 100 sawfly eggs shipped directly from Thailand to Gainesville quarantine laboratory.
- February 14-24. Singapore: Bukit Timah Nature Reserve, Lower Pierce Reservoir and Sungei Buloh. Sites very dry, but *Endelus bakerianus* adults and feeding, *Callopistria* feeding damage and stem-borer damage were found. There were numerous observations of dead and dying young shoots, which is also seen in Australia. There was only evidence of the stem-borer at Bukit Timah. A *Callopistria* larva was collected at Lower Pierce. *Paederia foetida* at Bukit Timah had leafbinder leps and mite galls. Other ferns near *L. microphyllum* were checked and a stem-borer (not related to the *Lygodium* species) was found on Resam (*Dichronopteris* sp.) Met with Indonesian collaborator Roch Desmier de Chenon and visited the stem-borer site

to view typical stem-borer damage. In Thailand, *Endelus* and thrips were the only *L. microphyllum* herbivores active in the provinces of Nakhon Si Thammarat and Songkhla. *P. foetida* was flowering but not yet fruiting. Eriophyid galls were present at one site.

- May 2-13. *L. flexuosum* plants were collected at the Ban Pong site near Chiang Mai for shipment to Bangkok then on to Chris Bennett at Gainesville. There was little insect activity on *Lygodium* apart from stem-borers. *P. foetida* still showed little fruit, and collections of the fruit available did not produce Cecidomyidae adults.
- May 10, 2005. 400 sawfly eggs shipped to Gainesville quarantine facility.
- July 13-25. In Singapore, no stem-borers or new stem-borer damage was seen. *Callopistria* and *Endelus* damage was present, and just two *Lygomusotima stria* larvae at Bukit Timah. Several alternative accommodations were visited and reviewed in preparation for locating a staff member for a twelve month period. Visited the National Parks headquarters at Central Catchment to introduce Matthew Purcell. In Thailand, further collections of *L. flexuosum* were undertaken. Collections of the mite on *P. foetida* were hand carried to Kasetsart University for a meeting with Prof. Angsumarn, taxonomist who agreed at the meeting to undertake checking of the species. An attempt to collect more weevils on *Rhodomyrtus tomentosa* was not successful (these were for further checking by taxonomists at SEL, Washington).
- August 30 – September 17. With Jeff Makinson in Singapore to show the local monitoring sites for the coming year. In Thailand, used a hire car for exploration of new sites south from Bangkok to the provinces of Petchaburi, Prachuap Kiri Kan and Surat Thani. Contacted local researchers at Pranburi Research Station, and located new infestations including possible *Paederia cruddasiana* near Huay Tapaed reserviour (also a source of hydrilla). Also explored near Nakhon Nayok, but found only *Paederia* sp.
- November 6-18. With Jeff Makinson, explored the southern peninsula of Malaysia as a potential extra source of the *Lygodium* stem-borer. This country has been extensively altered and developed and little *L. microphyllum* and no *P. foetida* was found. Sampling included *Hydrilla* and *Casuarina*. In Singapore, stem-borers were collected near Mandai and pressed plant specimens of *L. circinnatum* were taken.

***Siamusotima aranea* (Thai Lygodium stem-borer) (Lepidoptera: Pyralidae)**

In northern Thailand, *S. aranea* (inset right) is reasonably common at selected field sites of *L. flexuosum* and were collected and maintained in artificial diet at the Royal Project laboratories at Chiang Mai. These were later transported to the Department of Agriculture laboratories in Bangkok, then to Gainesville quarantine laboratories. The field population of *S. aranea* suffered early in the year when widespread fires swept through the area and destroyed our sampling sites. The drought intensified throughout the region during 2004, and the stem-borer populations suffered badly. Recovery in 2005 was slow.

***? Ambia sp.* (Singapore Lygodium stem-borer) (Lepidoptera: Pyralidae)**

In Singapore, larvae of the second stem-borer species were scarce (also because of the regional drought) but an occasional specimen continued to be found by checking for dying stem tips and fresh frass at holes along the stem (inset right). However no evidence of fresh stem-borer activity was found after November 2004. The return of rain in 2005 brought a slow recovery of the insect population.

During 2005 an ABCL staff member was transferred to Singapore for a period of twelve months to find, rear and learn the biology of the insect. Progress on this project is included in the Singapore section.

**Lygodium Sawfly - *Neostromboceros albicomus***

Further biological studies by our Thai collaborators improved rearing of *N. albicomus* (inset left), allowing development of large populations for shipment to the US quarantine laboratories in Gainesville.

For mating, several males are released into the laboratory shadehouse (inset right, with scientific assistant

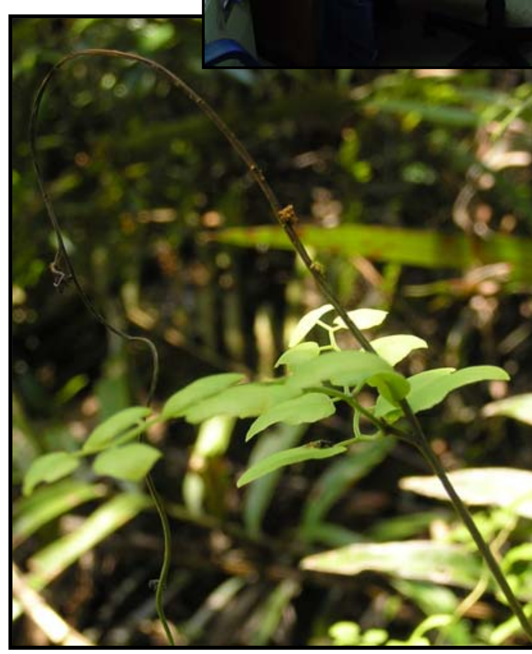
Khun Tawat) during the afternoon. The males are allowed to fly for around one hour, before a female is taken into the shadehouse and placed on a leaf. The female usually begins fanning her wings soon after. In most cases, a male is attracted immediately and mating takes place. Sexes of pupae and adults can be separated by the terminalia.

Two shipments of eggs were dispatched from Thailand in mid January and early May 2005. A quarantine colony was subsequently established in Gainesville.

## **Singapore *Lygodium* Stem-borer**

The major focus of *Lygodium* research is on a stem-boring lepidopteran from Singapore, ?*Ambia* sp. This moth has only been found in Singapore and as yet an adult has not been collected or reared for identification. Larval numbers are always very low at field sites and field-collected immatures have all died once the damaged stem is removed from the plant. Transfers to live plants have not yet been successful.

Singapore is usually visited in transit to other locations in Asia, or on returning to Australia. It was believed that locating somebody in Singapore on a permanent basis would increase the chance of developing this agent further for biological control. At the request of the Project Leader, ABCL staff member Jeff Makinson (inset right) was relocated to Singapore in August for a period of 6-12 months to concentrate solely on collecting, identifying and culturing the stem-borer. Detailed preparations began in March. Collection permits were arranged with National Parks authorities in Singapore and equipment, personal and accommodation requirements were also being addressed. Jeff is based in an apartment and all field trips are being conducted using public transport. Jeff will also be called upon to conduct exploration for *Lygodium* agents with our co-operators in Thailand, Malaysia and Indonesia.



The current survey of stem-borer activity (inset right) in Singapore commenced in August 2005, with the first larva found at the end of September. Stem-borers have since been found on three of the four *Lygodium* species occurring naturally on the island: *Lygodium microphyllum*, *L. flexuosum* and *L. circinnatum*.

Evidence of the stem-borer has been found at only 9 of the 77 Lygodium sites that have been surveyed, and so far only four of these sites have yielded active larvae. However, over the last month the number of larvae in the field appears to be increasing and this may also translate into an increased range of stem-borer activity.

As of early December, 14 larvae: from *L. microphyllum* (11), *L. flexuosum* (2) and *L. circinnatum* (1) have been collected from the field. A further 11 larvae (damaged stems with fresh frass at the exit holes, suggesting that a larva is inside) have been left in the field to monitor their progress in natural conditions. Some evidence of parasitism has also been observed.

Stem-boring activity in four other plants (*Paederia foetida*, *Dicranopteris* sp., *Asplenium ?longissimum* and an undetermined vine) has also been recorded. The *A. ?longissimum* borer is a scolytid beetle, the *Dicranopteris* sp. borer is a moth that pupates within the stem (an adult has been reared) and only a moulted head capsule was found in the stems of *P. foetida* and the vine.


The Thai stem-borer, *S. aranea*

Little is known about the Singapore stem-borer's life-history. Genetic and morphological evidence shows that it is different from *Siamusotima aranea* (inset left), the *L. flexuosum* stem-borer found in Thailand, the similarities between the two suggests that a knowledge of the life-history of *S. aranea*, and of the work done to establish a colony of it, will be beneficial in the study of the Singapore stem-borer.

The adult of *S. aranea* appears to be a spider-mimic and lives for 5-8 days under lab conditions. Adults appear twice a year, around May/June after the rains of April/May and again in October.

Little is known about oviposition behavior, though it appears eggs are laid on new leaves and, under lab conditions, they hatch after 6-7 days and move to young shoots to commence feeding.

Most mature larvae are found in May and September; though a range of larval sizes can be found throughout the year, suggesting that populations are not tightly synchronized. Larval duration under natural conditions appears to be about 4-5 months. This indicates that there are two generations per year.

Pupation is most common around April and then again in October; the pupal period appears to be between 6-11 days. Pupation occurs externally on the *L. flexuosum* stem towards the base of the plant, using material available nearby to cover the pupal case (inset below).

Success with laboratory colonies of *S. aranea* is an important consideration for the study of the Singapore stem-borer. Adults will mate in cage conditions and oviposition is thought to be enhanced with the addition of honey and water.

The use of an artificial diet for rearing larvae has been an important part of the success of the *S. aranea* colony. A diet based on *Lygodium* leaves and stems is pumped into paper straws and left to set in a fridge. While this diet does not appear suitable for the youngest larvae, more mature larvae will pupate within 2-3 months when fed on it. They will readily pupate on the side of the straw and even on tissue paper left in the container holding the straws.

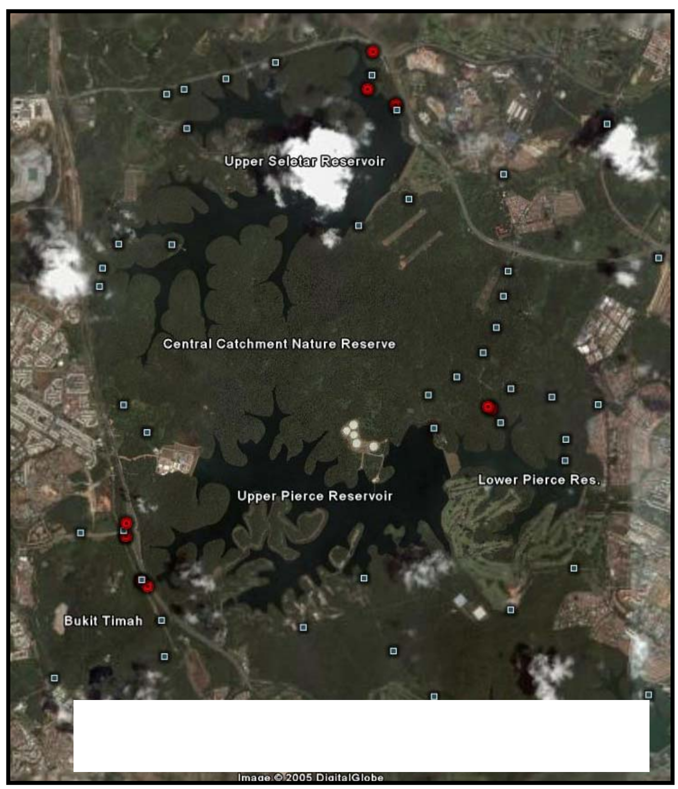
**History of Singapore stem-borer research**

Surveys of *L. microphyllum* in Singapore commenced in 1999; and up to July 2005 there have been 117 collections made during 20 trips to the country.

The first stem-borer was found in March 2000. Subsequent genetic analysis of this larva indicated that it was different from *S. aranea*. Since then, only four further larvae have been collected: in February 2003 and in May, June and August 2004, though other collections have recorded stem-borer damage. All observations of stem-borer activity were made from three sites along the Belukar track in Bukit Timah Nature Reserve and from a single site at Lower Pierce Reservoir.

**Current field searches**

The image above (Google Earth) shows all *Lygodium* spp. sites that have been surveyed between 1999 and December 2005. The red spots mark sites with stem-borer activity. Since August 2005, 66 of these 77 sites marked above have been visited – extended stretches of Lygodium are represented by only a couple of dots on the map.




The main goals have been to expand searches around known stem-borer sites in order to find the extent of activity and to search new areas in the hope of chancing upon further activity. Both methods have yielded results – stem-borer sites are marked in red on the picture, right.

By searching around the Lower Pierce site, stem-borer activity was found on *L. circinnatum*. Searches of the Belukar track at Bukit Timah are yet to yield active larvae but have extended, northwards, the known range of the stem-borer in this region.

Exploratory trips around Upper Seletar located four new sites where no evidence of stem-borers had previously been found.  Three of these sites, along Mandai Rd, have produced the majority of larvae found; the 4th site shows damage but no current activity.

The current field searches have also expanded upon the known host associations of the stem-borer.  Previously only known from *L. microphyllum*, stem-borer larvae have also been found on *L. flexuosum* and *L. circinnatum*.  Results of DNA analysis will indicate whether there is a single species feeding across the range of *Lygodium* in Singapore or if there are genotypic or species-level differences amongst them.

Top: *L. circinnatum* damage at Lower Pierce.

Middle: larva is dissected from its stem and began to feed in a *L. flexuosum* stem – it did not survive.

Bottom: damage to *L. circinnatum*.

Right: *L. flexuosum* at Mandai Rd. site.  Note the typical structure of the leaf (large circle) and the extent of damage (small circle, frass on the stem surface).  On the right, dead leaves indicate the hollow section of stem.  More, dried, frass can be seen at the far right (red arrow).  This larva was preserved for genetic comparison and a sample of leaf tissue was also collected to confirm the identity of the plant.

A major focus of the field searches has been the search for pupae. This has, so far, been unsuccessful. Pupal cases are likely to be well camouflaged against the stems (as is the case with *Siamusotima aranea* in Thailand) and plant growth at these sites tends towards thickets of Lygodium and surrounding plants with abundant leaf-litter and other ground debris, making the search for pupae time-consuming. Also, the stem-borer may not, of necessity, pupate on a Lygodium stem. It is most likely that a larva exiting a stem will pupate on that stem, but it is possible that it may pupate on the nearby stem of another plant or even on surrounding leaf-litter – stems have been found where feeding extends to the rhizome and pupation may also occur at this level. Time is spent on each field trip searching these areas for pupae.

A further aspect of field work has been the field-monitoring of stems with fresh damage. When larval numbers began to increase around November, some stems likely to contain active larvae were tagged in the field and left to monitor their progress. It is too early to make any conclusions about the success of this endeavour, in terms of gaining understanding of their biology or improving the success of pupal searches.

**Life-history of the Singapore stem-borer**

Not a lot is known about the life-history of this stem-borer. Some information, presented below, has been gathered from dissections of damaged stems and from observations made during attempts to establish a colony.

All three areas where stem-borers have been found are near water: the Lower Pierce site is right on the water's edge; all 4 sites at Upper Seletar/Mandai Rd are within 50m of the water and Bukit Timah (though separated by an expressway) is still within a couple of hundred meters of the reservoir. Also, like the Thai stem-borer, it seems likely that there is an activity spike following periods of extended rainfall.

**Larval instars**

Dissections of damaged stems have given some indication of larval activity. One old *L. microphyllum* stem, taken from the northern end of Belukar track, revealed a neat series of 5 anal shields of increasing size (inset right) from the distal to the basal end. These plates measured 0.3, 0.4, 0.5, 0.6 and 0.7mm between the roughly parallel sides of the shield. This suggests that there are at least five instars.
Other dissections have included anal shields ranging from 0.3 - 0.8mm. Observations of larvae reveal that the size of the anal shield may not be a valid indication of the relative maturity of a larva. One larva, 2.5cm

long, had a thinner anal shield than another, 1.5cm long. Either the length of the larva is a more reliable indication of larval maturity (with thickness merely being a by-product of the stem in which it feeds) or both of these dimensions play a role, such that a longer thinner larva can be on the same moult as a shorter thicker one.

One larva (inset left), dissected from an *L. microphyllum* stem, did not have an anal shield at all. Either this is the earliest instar of a stem-borer and has yet to develop a sclerotised shield, or it is a second species boring within the *L. microphyllum* stems.

**Minimum stem diameter**

Some basic, and roughly accurate, measurements have been taken of stem diameters and the size of holes formed in these stems. This is important in regard to determining the minimum requirements for plant stems grown for any future stem-borer colony. In general, stem-borer holes range from 0.2 – 0.9mm diameter and have been found in stems from 1.1 – 1.7mm wide. It is estimated that a stem diameter around 1.5mm or greater may be required for fully-matured larvae.

**Representation of typical larval feeding**

A section of *L. microphyllum* stem (inset right) was collected from Lower Pierce. It had a withered tip, a series of holes and then green pinnae below that. A new shoot of ~15cm (bottom of pic) had grown from the leaf base – presumably in response to the death of the old one.

The pattern of hole formation on this plant was relatively typical of the stem-borer, though of course each example is different in some ways. The 1st (distal) hole was found in the withered end of the stem, about 20.5 cm down from the crosier.

The bare leaves were located between the 9th and 10th holes and there was approx.10cm between the larva and the node of the green leaves.

The pattern of feeding damage in this stem is also typical of the stem-borer. When the larva is young it feeds only through about half of the protostele, but as it matures it will eat the whole protostele leaving a clean hollow stem.

**An atypical scenario – 3 larvae in a single *L. microphyllum* stem**

In November, a single damaged *L. microphyllum* stem was collected from Mandai Rd. There was about 30cm of feeding (a hollow stem with holes) towards the distal end of the stem then about 23cm of solid stem. This section of solid stem included a leaf junction with brown dried leaves, suggesting further damage below. A single hole was found

below this. The stem was split open here to reveal 3 larvae in a row (above).

Dissecting the main section of damage (the 30cm of hollow stem) revealed 6 moulted head capsules of 3 distinct sizes and 8 anal plates of 3 distinct sizes (3x 4.5mm, 3x 3mm, 2x 2mm). A couple of these 14 moulted pieces were found near each other, but generally they were scattered along the length of the hollow.

These larvae went through three moults in a single stem, then exited, to re-enter together through a single hole approx. 20cm further down the stem.

**Evidence of parasitism**

Two different types of empty parasitoid pupal cases have been found in stem-borer damaged *Lygodium* stems. Concrete evidence of parasitism is still yet to be recorded.

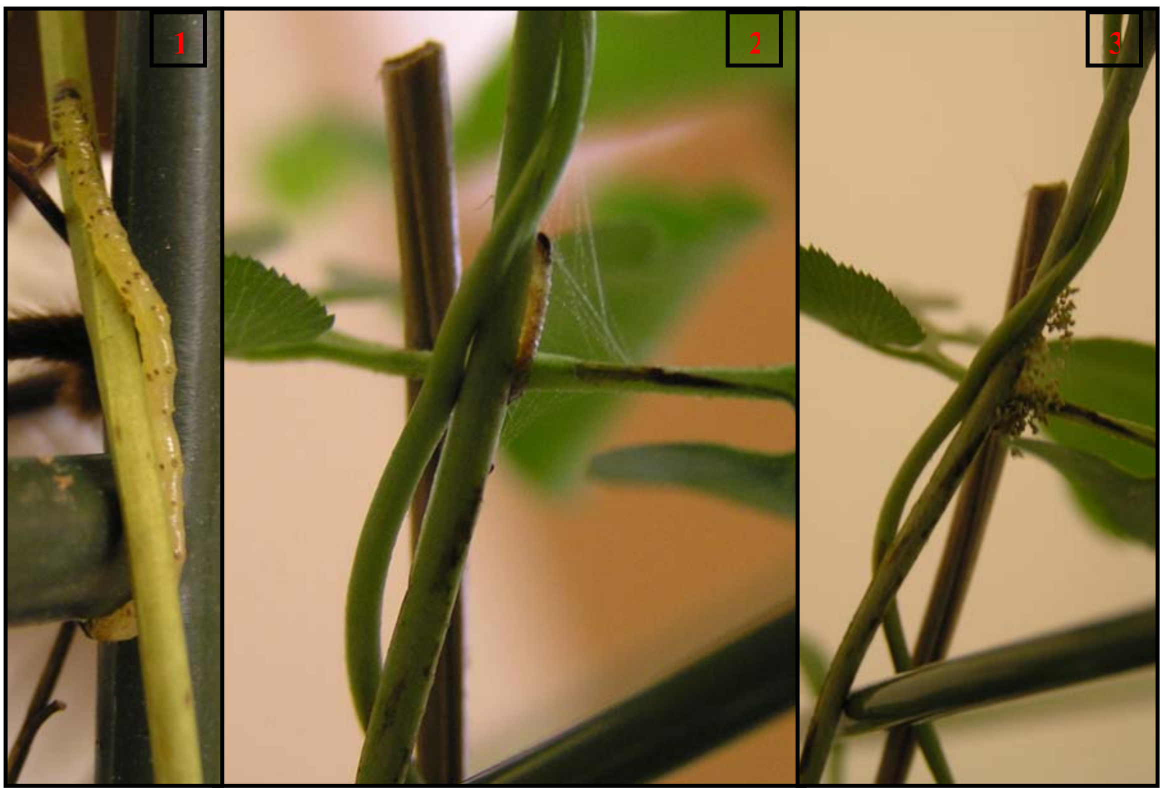
Five clear/white pupal cases were dissected from the end of a hollow section of a *L. circinnatum* stem from the Lower Pierce site.

A cluster of four empty tan pupal cases were then found at the end of a hollow section of stem, with a moulted anal shield nearby.

**Colony / Lab rearing of larvae**

Attempts have been made to rear field-collected larvae. Difficulties associated with this include an incomplete knowledge of the stem-borer's life-history and cues for pupation, an inability to consistently grow the thick stems necessary for larval development, and an inconsistent response from larvae transfering from cut material to test plants.

Some useful observations have been made in attempting to rear the stem-borer. A general outline of how a larva enters a stem is offered in the picture, below, with accompanying text:




1. A larva may spend 10-20 mins moving along the stem, presumably looking for an appropriate entry point. It may fall off the plant a number of times (this may be an artefact of the un-natural process of removal from the host-stem and manipulation on to the new plant).
2. Once a suitable site is found, the larva will produce a silken enclosure often around the 'prop' of a leaf junction, a twig around which the Lygodium has twined, or even an artificial plant support. This enclosure may provide protection or offer support during entry. All things going well, 60-90 mins after being placed on the stem the larva will be partially inside the stem, as shown.
3. Within 3-4 hours the larva is fully within the stem, exiting its frass as it feeds. By the next morning the stem had wilted over and the larva had created a second hole to exit

accumulated frass.  A *L. microphyllum* larva will enter the stem of a *L. flexuosum* test plant, and vice versa.  As observed in the field, larvae will exit and re-enter a stem under lab conditions.

Whether there is a period of diapause or larvae are awaiting some cue for pupation, there is a period of inactivity where no new holes are made and no fresh frass deposited.  I have, mistakenly, assumed that the larva has exited the stem and been lost, or died within it, but on opening the stem have found the larva alive but inactive.  It is unlikely to enter a new stem after this has occurred.

A range of conditions has been provided in the attempt to induce pupation.  Plants have been set-up in gauze cages, within plastic containers and left exposed.  For ease of observation, the plants are generally kept on a covered patio, but one was also set-up outside, shaded but exposed to wind and rain.

**Other stem-borers**

***Lygodium circinnatum*** An empty Lepidopteran pupal case (inset below) was dissected from a hollow stem of *L. circinnatum* collected from the Lower Pierce site in September. No moulted anal shields, typical of the stem-borer, were found in this stem, though they may have been ejected with the frass through one of the holes. This may suggest that there are two types of stem-borer in *L. circinnatum*, or that the stem-borer found within this Lygodium is a different species from the Singapore stem-borer from *L. microphyllum*.

***Paederia foetida*** A dead shoot of *P. foetida*, riddled with holes (inset below) was found near one of the stem-borer sites on the Belukar track. Upon dissection a single moulted

Lepidopteran-looking head capsule was found, but no anal shields were seen. Two holes were found towards the tip of the stem, below that was 21mm of hollow stem without any holes and then there were 14 holes, and the head capsule, in the next 28mm of stem. This pattern of hole formation is different from what occurs in *Lygodium* stems; the *Lygodium* stem-borer produces a cluster of holes at the distal end of the stem when the larva is youngest, the distance between holes increases and less holes are made as the larva matures and moves basally down the stem. Subsequent searches of *P. foetida* have failed to locate more of this damage.

**Undetermined Vine**

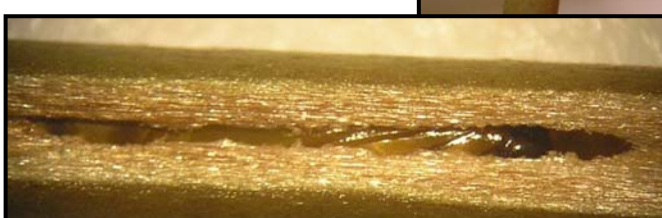
A number of dead stems of an undetermined vine species (inset left) were found at Sungei Buloh Wetland Reserve, no stem-borers have been found on Lygodium in this region. Only a couple of these dead stems had holes in them (inset left). These were

dissected and a single Lepidopteran-looking head capsule was found in one of them. Again, no anal shields were seen in these stems.

***Dicranopteris* sp.**

On Old Upper Thomson Rd, within 1km of the Lower Pierce stem-borer site, three dead and dying fronds of resam, *Dicranopteris* sp., were collected. Undamaged *L. microphyllum* was also growing at this site. The resam crosier (inset right) bears a resemblance to that of Lygodium, though more robust, and the new shoot is a seemingly ideal habitat for a stem-borer.

Two of the fronds had hollow stems with holes and frass within the stem. No anal shields or other sclerotised material was found in the stem or amongst the frass.
The other frond had a 1mm hole in the 2.3mm stem with green leaves below and brown leaves above the hole. The first 3.5 cm of hollow stem had lots of frass (couldn't find any larval remnants amongst it). The next 5.3cm was clean and a pupa was found above that. The adult emerged 10 days later.



The larva appeared to feed up the stem; unlike the Lygodium stem-borer. Also, there was only one hole in the whole stem and the pupal case was different from that found in the *L. circinnatum* stem. It is likely to be a unique species.

***Asplenium ?longissimum***
Stem-boring damage was found in this fern growing at one of the *Lygodium* stem-borer sites on Mandai Rd. The stem was dissected to reveal scolytid beetle adults (inset right), larvae and eggs.

**Immediate goals**

The major goal for the continuing work of this project is to establish the identity of this stem-borer. Pending results of DNA analysis will indicate whether there is a single species feeding across the range of *Lygodium* hosts or if there is a suite of species, or genotypes, each unique to its host.

Confirmation of the genetic analysis will be attempted through the continued search for adults. This will involve further searches for pupal cases on *Lygodium* stems, the stems of other plants growing with *Lygodium* and in the leaf-litter and other debris.

To complement this search, a series of black-lighting trips, in conjunction with National Parks, Singapore, have been proposed; night collection of *S. aranea* adults has been successful in Thailand.  All stem-borer sites are located within Nature Reserves, so the consent and participation of National Parks representatives is essential.  It would be ideal to rear an adult prior to any black-lighting, so that the search would not be 'blind' as such, but if this is not possible then the option would be to collect anything that resembles *S. aranea* and then attempt to initiate a cage colony of stem-borers from these adults.

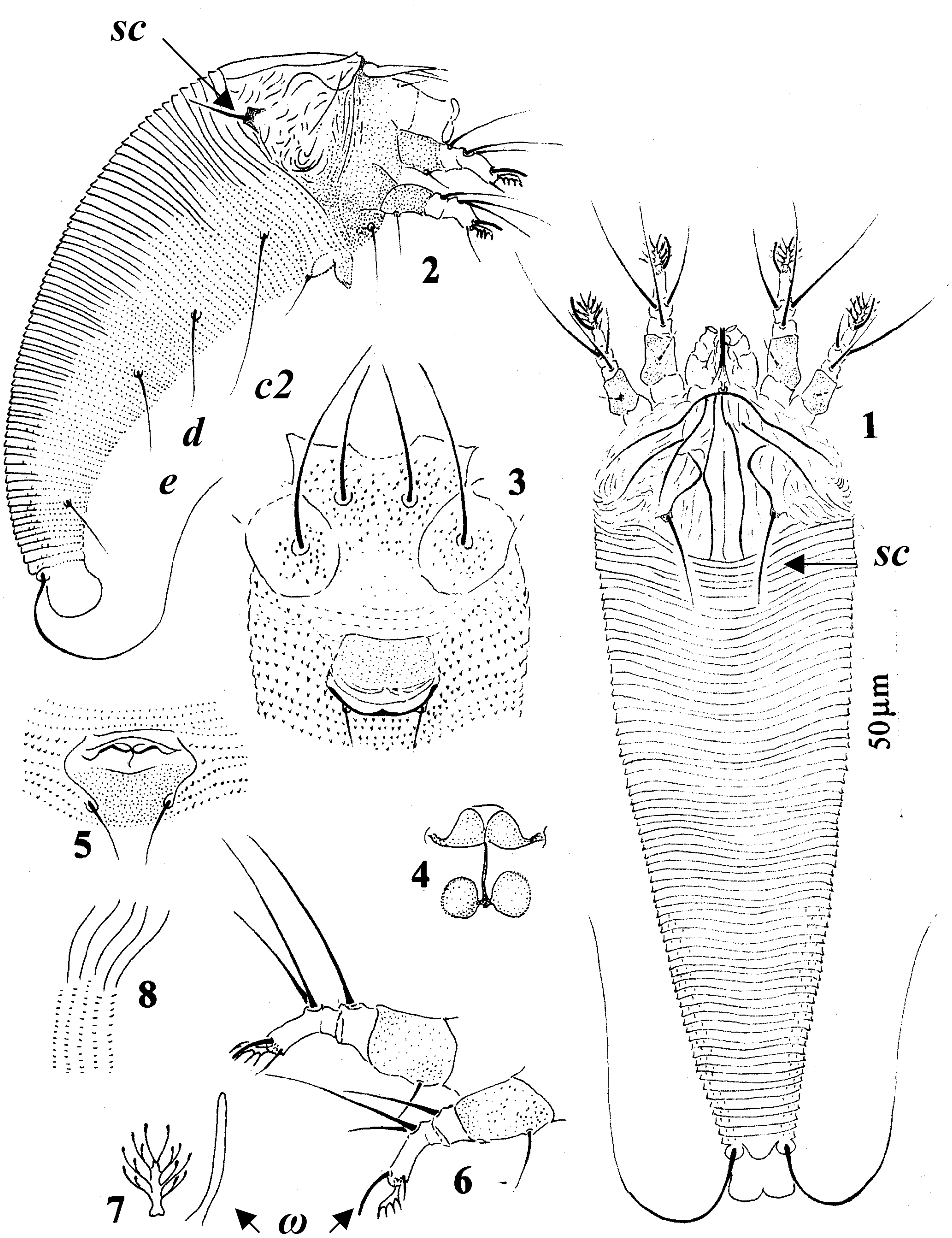
Beyond this, there is a need to find new sites with current or prior stem-borer activity and to expand upon the area that borers can be found at the current sites.  This will enhance the collection options when/if the borer is considered for the Ft. Lauderdale quarantine. To this end, new sites around Singapore are visited weekly and a trip to the south-east of Sumatra is being finalised for late January 2006.  In conjunction with this, monitoring of larvae in the field will be continued.

A batch of Lygodium diet was prepared in Brisbane for delivery to Singapore.  Some larvae are being transferred to this diet, though others will be retained in live plants.  It is hoped that this will improve the chances of rearing adults and will provide a useful tool for work both in Singapore and Florida.

## Leaf Curling Mite – *Floracarus perrepae*

Eriophyoid mites (also known as gall, blister, rust, bud or erinose mites) are a large group of highly specialised plant-feeders. They are minute in size (about 0.1 to 0.3 mm in length), slow moving, worm-like in shape, with only two pairs of legs. The morphology of *Floracarus perrepae* from Knihinicki and Boczek (2002) is given below

242 Knihinicki & Boczek 2002




Figs. 1-8. *Floracarus perrepae* n. sp. (all female except Fig. 5) - 1. Dorsal view; 2. Lateral view; 3. Genitalia and coxae; 4. Apodeme of genitalia; 5. Male genitalia; 6. Lateral view of Legs I and II; 7. Tarsal empodium and solenidion; 8. Lateral view of dorsal and ventral annuli region.

Dr. Danuta Knihinicki of New South Wales Agriculture, Orange, NSW Australia first identified the mite as a member of the genus *Floracarus*. Knihinicki and Boczek (2002) described the mite, which included specimens from Australia, China, and New Caledonia in the original description of the species.

**DNA Analysis**

*Floracarus perrepae* populations from throughout its native range were collected and analyzed using sequence data from the nuclear rRNA D2 and mitochondrial CO1 genes using the methods of DeBarro *et al*. (2000). This technique identified six distinct genotypes: southeast Queensland, New South Wales, Western Australia, and the Northern Territory of Australia (A); New Caledonia (B); China (C); Thailand, Indonesia, Malaysia (D); India/Sri Lanka (E); and Cape York, Queensland Australia, and Papua New Guinea (F)(Figure 5).

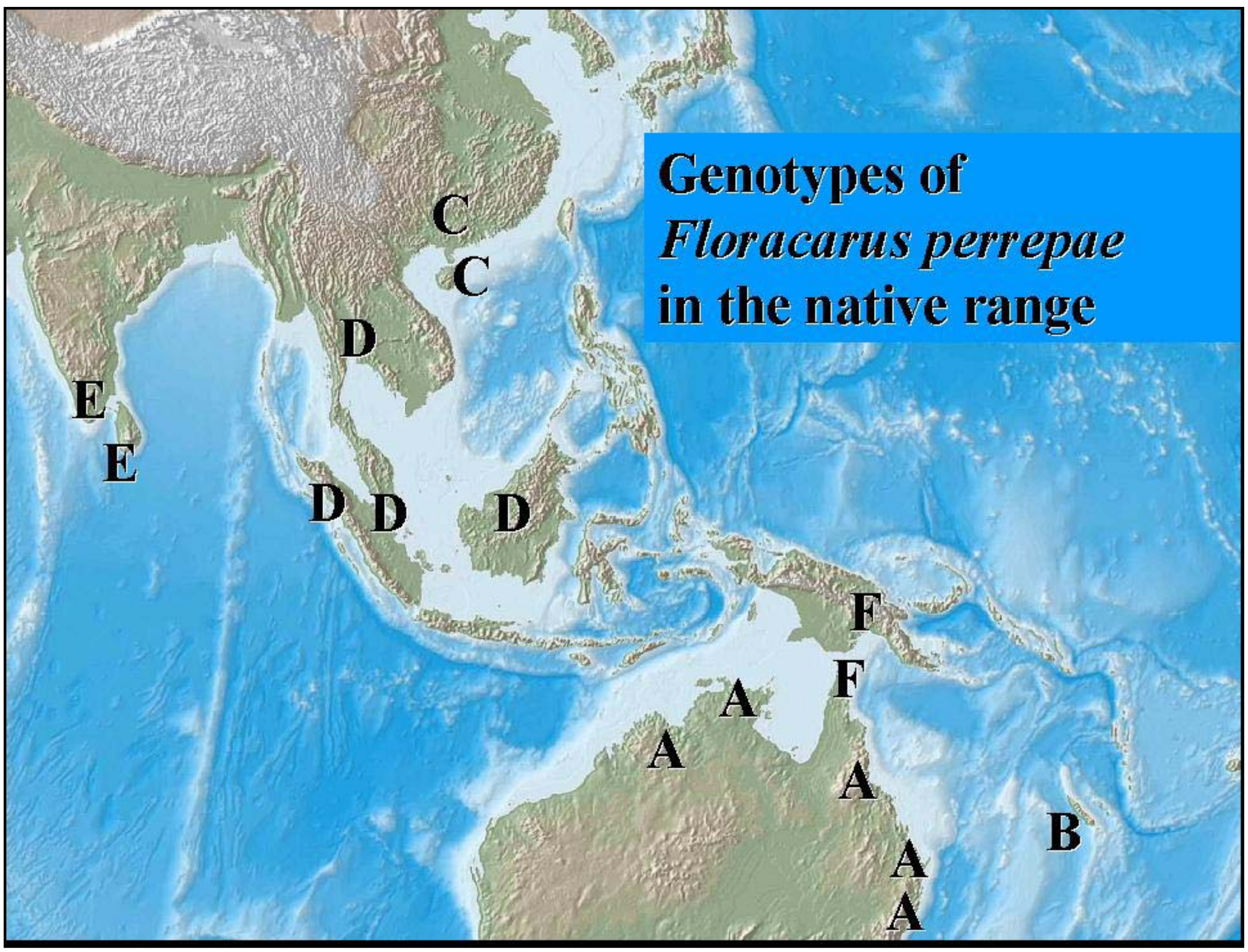



**Figure 5.** Distribution of *Floracarus perrepae* genotypes in the native range of *L. microphyllum*. Different letters represent unique populations based on DNA sequences from the nuclear D2 and mitochondrial CO1 genes.

The holotypes have been deposited in the Australian National Insect Collection, Canberra, ACT Australia. Paratypes and vouchers have been sent to: Queensland Museum, Brisbane, QLD, Australia; USDA-ARS, Systematic Entomology Laboratory, Beltsville, MD, USA; and the Florida Insect Collection, Gainesville, FL, USA.

**Geographical Range**

*Floracarus perrepae* was collected from subtropical and/or tropical locations in Australia, China, India, Indonesia, Malaysia, New Caledonia, Papua New Guinea, Singapore, Sri Lanka and Thailand. It is not known if *F. perrepae* occurs in Africa where *L. microphyllum* is also native. The northern limit is likely to be in mainland Asia where *L. microphyllum* is found near 24 ºN at Wushi in Guangdong, China (Goolsby *et al.* 2003b). The southern limit is likely to be near Yamba in NSW, Australia, where *L. microphyllum* is found at near 30ºS. *Floracarus perrepae* is adapted to all the climates in which *L. microphyllum* grows. The climatic tolerance of the mite allows it to be widely distributed with its host plant across Asia, Australia and Oceania.

**Host Range.**

Field research at ABCL has established that the primary host of this mite is *L. microphyllum.* Other *Lygodium* species were surveyed in their native habitat for the presence of *Floracarus*, including: *L. japonicum*, *L. flexuosum*, *L. circinnatum*, *L. dimorphum* and *L. versteegii*, and were not found to be hosts of this mite. *Floracarus perrepae* has been collected from the Australian native *L. reticulatum,* which occurs in north Queensland, but they did not develop on *L. microphyllum* under laboratory conditions. It is possible that the population of *F. perrepae* on *L. reticulatum* is a host specific biotype. This seems likely given the degree of specialization of *F. perrepae* to *L. microphyllum* genotypes, which will be discussed shortly.

**Life History**

*Floracarus perrepae* has a simple life cycle, consisting of an egg, larva, nymph, imago chrysalis, and adult (Ozman and Goolsby 2005). Eggs are very small, about 0.041mm diameter, spherical and translucent. The larvae are transparent and lack segmentation as compared to the nymphs. Nymphs are similar in appearance to adults (0.173-0.226 mm long), but smaller in size. A quiescent or resting stage occurs between the nymph and adult, called the imago chrysalis. Males are similar to females, but slightly smaller. Newly emerged adults are cream in color, changing to light brown or a creamy-yellow color 6-7 days later.

Adults prefer to feed on young, quickly expanding subpinnae, inducing the formation of swollen, rolled leaf margins. Immature stages feed inside the leaf curl. At 70% RH eggs hatch within 5.0 days at 21 °C and 3.9 days at 26 °C. A quiescent or resting stage occurs between the larva and nymph, and again between the nymph and adult (imago). Immatures feed and develop inside the protective leaf curls. Development time from first instar to adult takes 4.0 days at 21 °C and 3.1 days at 26 °C. The mean fecundity is 59.0 and 35.4 progeny at 21 and 26 °C, respectively. Adult longevity is 35.3 and 19.6 days at 21 and 26 °C, respectively. Fertilization occurs in the leaf roll, and we have observed the male spermatophores. The sex ratio of the mite colony is female biased (10.5 : 1). Eggs are laid in the leaf roll where the adult females feed.

Mite feeding stimulates plant epidermal modifications resulting in rolling of the subpinnae and eventual necrosis. Mature female mites prefer new sterile subpinnae for feeding and oviposition. To feed, the mite attaches itself to the subpinna surface by means of an anal sucker and then forces its stylets into the epidermal layer of the leaf tissue. Mouthparts of eriophyoid mites are complex. The gnathosoma consists of a subcapitulum (rostrum), which encloses the cheliceral stylets, and oral stylet. Stylets are generally visible only during feeding. Newly formed subpinna rolls were found to contain from one to five adult mites, although a single mite may cause the subpinna to roll. Subpinnae curl over, downward and inward, rolling over themselves two to three times. Several hundred eggs can be laid inside the roll. The immatures and adults feed on the specialized leaf tissue in the leaf rolls. The subpinna eventually dries and abscises as a result of the continued feeding, by which time the adult mites move to another young subpinna and repeat the cycle. In response to mite feeding the normal epidermal cells become significantly enlarged, causing the subpinna to roll. Mature epidermal cells become meristematic, greatly increasing their cytoplasmic content and metabolic activity to form the nutritive tissue of the roll.

## Evaluation of *Floracarus perrepae* Genotypes

In preliminary host-range testing we found that the QLD 'A' genotype of the mite was not able to induce leaf rolls and complete development on the Florida '6' genotype of *L. microphyllum.* Additional collecting and genetic characterization revealed several distinct genotypes. It is expected that one or more of the mite genotypes would be suited to the invasive Florida genotype of *L. microphyllum.*

## Damage to Target Weed

*Floracarus perrepae* cause damage directly to *L. microphyllum* by the feeding action of adults and immatures, and indirectly through the formation of leaf rolls, which leads to necrosis and early senescence of leaves. Infested plants become debilitated and produce very little new growth. Leaf necrosis is caused by wounding of the leaf tissue by the mite and subsequent infection by saprophytic fungi and bacteria. These pathogens are not vectored by the mite and pose no risk to non-target or economic plants.

## Geographical source of the agent

The populations studied in the host-range tests came from *Lygodium microphyllum* at the Iron Range National Park, along Scrub Hen Creek in Cape York, Queensland, Australia at latitude 14ºS (Figure 6 overleaf)). The Iron Range is a rainforest area near the northern tip of Cape York. The genotype of the *L. microphyllum* at this location matches the invasive Florida genotype of the fern, for two separate chloroplast DNA sequences. Thus, we collected the best-adapted known genotype for use as a biocontrol agent.

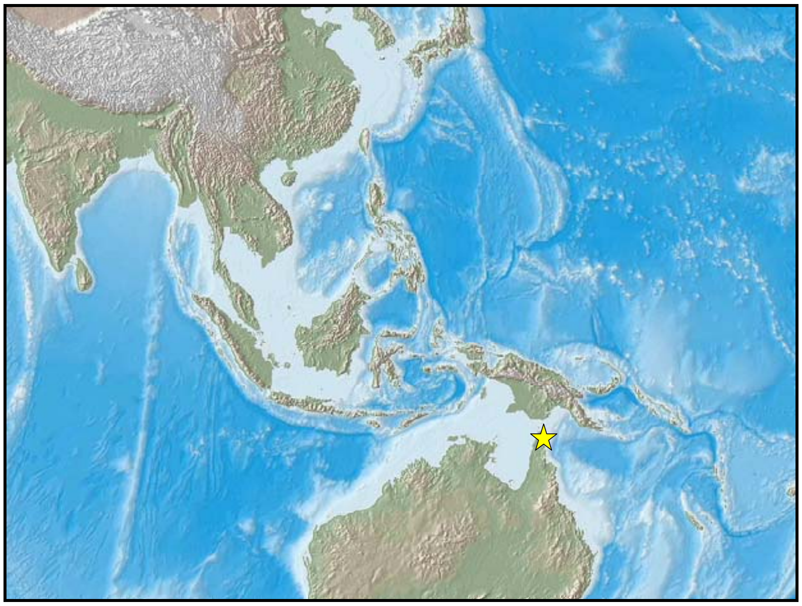


**Figure 6.** Star symbol represents collection location for *Floracarus perrepae*, Iron Range National Park, Cape York, Queensland, Australia.

**Plans for 2006**

In Australia, field visits to *Lygodium* sites will continue in conjunction with the *Melaleuca* program. John Volin returned to the US in December 2005 but his research will be continued in Australia by Mike Tobin and Terri Shirshac until March 2006. ABCL staff will continue collaboration during 2006 by monitoring growth studies of *L. microphyllum*. The colony of *Floracarus perrepae* will be maintained pending approval to ship a starter colony to Fort Lauderdale. We expect to continue rearing *Austromusotima camptozonale*, although new collections to revitalise the old colony are planned.

The stem-borer research in Singapore should continue until July 2006, at which time a decision regarding continued stationing of a staff member in Singapore to work on this project is expected. If rearing problems can be overcome, a shipment of stem-borer larvae direct to Fort Lauderdale is advocated.

Elsewhere in Southeast Asia, we aim to make further collections of insects, particularly *Callopistria* sp. and *Lygomusotima stria*, to establish colonies in the Brisbane quarantine for host-specificity testing. We can also consider recommencing work on *Siamusotima stria* and undertaking further shipments to Florida.

# Biological Control of *Casuarina* spp.

Funding from DEP was specifically used to support survey activities to discover and evaluate potential biological control agents of *Casuarina* spp. The project is a joint initiative between USDA-ARS collaborators in Australia and Florida. In particular, this project is focused on herbivores that feed on seed cones and flowers thus limiting the reproductive potential of *Casuarina* where it is invasive.

During the first year of this contract the main objectives were addressed: 1) A review of the literature to determine what is currently known about the taxonomy and distribution of *Casuarina cunninghamiana* Miq. (River She-Oak), *C. equisetifolia* L. (Australian-pine), and *C. glauca* Seiber (Grey She-Oak) in Australia; 2) A review and collation of ABCL records of *Casuarina* herbivores; 3) planning to prioritize areas in eastern Australia that should be targeted for initial surveys; 4) preliminary surveys of herbivores in key parts of the native range.

Plans are underway to proceed with biology studies and host-range testing of herbivores with potential as biological control agents.

**<u>Casuarina in Florida</u>**

Australian pine, *Casuarina* spp., has become a serious invasive weed of coastal areas in the United States especially in southern Florida, including the Everglades National Park and neighboring areas. With its rapid growth, dense coverage, and thick litter accumulation, Australian pine dramatically alters the habitat of infested areas inhibiting the growth of native plants and their associated herbivores. A salt tolerant species, Australian pine may be found on coastal dunes where it increases beach erosion and interferes in the nesting by endangered sea turtles and the American crocodile. As this species spreads primarily by seed production into natural areas, a reduction in reproductive output would decrease its invasiveness. Reproduction may also occur by root suckers and thus herbivores attacking young plants would reduce establishment of saplings. Australian pine is also highly valued as an ornamental tree providing shade throughout its range. Control efforts that target the reproductive structures and saplings would potentially reduce its spread into natural areas while not affecting its horticultural value. Preliminary surveys of the insect herbivore fauna associated with the Casuarinaceae commenced in Australia in mid 2004.

**<u>Field Exploration</u>**

Surveys in 2005 continued to concentrate mainly on *Casuarina equisetifolia* (inset left), *C. glauca* and *C. cunninghamiana*, the target species for potential biological control agents for possible introduction in the U.S. (see Table 5 over page) Surveys have concentrated in the Brisbane area and from

Brisbane to Rockhampton in eastern coastal to sub-coastal Australia where these species commonly occur, though occasional surveys have been made north and south of these areas. Survey methodology includes recording site locality, including GPS coordinates, visual inspection of plant for gall material and large insect specimens and sweep netting of foliage. Small insects are aspirated into collection tubes and placed in ethanol. These are subsequently sorted and where possible point-mounted on card, or with large numbers, transferred to gelatin capsules. Cones are collected, returned to the laboratory and placed on absorbent paper in ventilated rearing containers for subsequent emergence of cone-feeders or granivores. Where appropriate, botanical samples are taken to confirm host identifications.

**Table 5**. Summary of field collections made during 2005 in Australia and Southeast Asia related to exploration for biological control agents of *Casuarina* spp.

| Plant | | Number Collections | Number Sites | Regions |
|---|---|---|---|---|
| **Family** | **Species** | | | |
| Casuarinaceae | *Allocasuarina distyla* | 7 | 7 | NSW |
| | *Allocasuarina inophloia* | 1 | 1 | NQ |
| | *Allocasuarina littoralis* | 16 | 16 | NSW, NQ, CQ, SQ |
| | *Allocasuarina torulosa* | 11 | 11 | NSW, NQ, CQ, SQ |
| | *Allocasuarina verticillata* | 10 | 10 | SA |
| | *Casuarina cristata* | 2 | 2 | NSW, VIC |
| | *Casuarina cunninghamiana* | 26 | 26 | NSW, NQ, CQ, SQ, ACT, NT |
| | *Casuarina equisetifolia* | 46 | 46 | NSW, NQ, CQ, SQ, NT, WA, TH |
| | *Casuarina glauca* | 23 | 23 | NSW, NQ, CQ, SQ, SA |
| | *Casuarina leuhmannii* | 3 | 3 | NQ,SQ, VIC |
| | *Casuarina pauper* | 1 | 1 | SA |

From 9th to 23rd January 2005, a survey of Brisbane and environs (11 sites), and Brisbane to Rockhampton and north to Byfield (20 sites) was undertaken. Included in this survey period was a trip to northern New South Wales (10 sites). Coastal sites were sampled from Coolangatta to Ballina, then inland via Lismore, Casino and Grevillea.

From 25th April to 8th May 2005, field surveys were undertaken in the vicinity of Brisbane (7 sites), and from Brisbane to Rockhampton and Yeppoon (24 sites). Additional surveys were conducted from Townsville to Daintree (coastal sites), and in the Atherton Tablelands (inland sites, inset right). In addition to all five species of casuarinas recorded for the Brisbane region, also sampled was the disjunct northern population (near Mareeba) of *C. luehmannii*, and a newly sampled species, the disjunct northern population (near Herberton) of *A. inophloia* (Stringybark She-Oak). In these surveys a total of 56 sites were sampled.

Other surveys have been conducted in South Australia and the Northern Territory. In South Australia, *Allocasuarina verticillata* (Drooping She-Oak) has been sampled from 20 localities: in the Adelaide Hills (10 sites), Yorke Peninsula (13 sites), mid-north of South Australia (3 sites) and Kangaroo Island (1 site); *A. muelleriana* (Slaty She-Oak) from the Adelaide Hills (1 site) and Kangaroo Island (2 sites); *A. striata* (Small Bull Oak) from Kangaroo Island (1 site); *A. pauper* from near Blanchetown (4 sites) and Yorke Peninsula (1 site); and from ornamental *C. cristata*, *C. cunninghamiana, C. glauca* and *C. obesa* from various localities including the Waite Arboretum in Adelaide.

In the Northern Territory, *C. equisetifolia* has been sampled from two localities: Dudley Point (near East Point) and Lee Point in Casuarina Coastal Reserve. Other incidental collecting by collaborators included *C. equisetifolia* from New Caledonia, and *A. distyla* (Scrub She-Oak) (7 sites) from New South Wales.

Two surveys were conducted in the Northern Territory in 2005. Surveys in Darwin, and environs indicated significant populations of an undetermined micro-lepidopteran from the cones of *C. equisetifolia*. At one locality, several fruiting cones of some trees were observed with the webbed frass of a lepidopteran larva. On dissection, these cones were found to contain webbed tunnels within, and the empty exuvium of a small moth pupa. This site was subsequently revisited and adult micro-lepidoptera were reared. The second survey in August continued with a road trip from Darwin in the Northern Territory through northern Western Australia to Broome, in the northwest of Western Australia. The trip yielded few field sites, as *Casuarina* has a limited distribution in this area and occurred only as ornamentals in some localities. One of these was a small stand of *C. equisetifolia* in Katherine, another a single specimen of *C. glauca* in Whyndam, and *C. equisetifolia* in Derby. Even so, we managed to sample naturally occurring *C. cunninghamiana* at the very western limit of its range, with several new insect associations collected. At Broome, *Casuarina equisetifolia* occurs extensively as an ornamental in excess of 1000 km from its natural range. Cone material (3 sites) is currently in culture to determine what herbivores, if any, occur in this disjunct, western-most population of this plant species. With the apparent paucity of insect herbivores at this site, its growth and dispersal may provide some interesting comparisons, as *C. equisetifolia* appears to be invading the local coastal sand dune system.

Following the trip to the Northern Territory/Western Australia, additional sites were sampled in the greater Brisbane area. These included sampling *C. equisetifolia* (7 sites), *C. glauca* (4 sites), *C. cunninghamiana* (1 site), and *A. littoralis* (1 site). As for previous surveys, cone material is currently in culture for emergence of insect herbivores.

In December, a road trip from Adelaide to Canberra was undertaken. Casuarina species sampled were *A. cristata* (1 site), *A. pauper* (1 site) and *A. leuhmannii* (1 site) in western Victoria, *C. cunninghamiana* (1 site in central New South Wales, 2 sites in Canberra, ACT). This trip included visits to the Australian National Insect Collection in Canberra to meet with various taxonomic specialists, and identification of insect specimens, particularly the micro-Lepidoptera and Coleoptera.

Further collections in Adelaide, South Australia include *Allocasuarina verticillata*, naturally occurring and exceedingly abundant in the Mt Lofty Ranges/Fluerieu Peninsula and the common ornamentals *C. cunninghamiana* and *C. glauca*.

*Casuarina equisetifolia* grows extensively along the coastlines of Thailand and Malaysia. In April 2005, cones were collected from trees at Cha Am on the east coast of Thailand (inset left), south-west of Bangkok. These cones were handed to local collaborators at the Royal Irrigation Department who will collect the emerging herbivores. Further collections of cones from southeast Malaysia were made in November.

**Insect Fauna**

Preliminary observations have indicated that each species of *Allocasuarina*/*Casuarina* sampled has its own suite of associated insect fauna, i.e. the insects. Particularly, sap-sucking Hemiptera appear to exhibit a high degree of host specificity. Furthermore, even though the survey is very much in its initial stages, it appears that where a particular host has been sampled across a number of localities, some useful distributional data is being collected that show that some species may be widespread, but also indicate the extralimital boundaries of other species. For example, on *A. verticillata* in South Australia, some common triozids that occur on the mainland do not appear to be present on Kangaroo Island, and the distribution of a brown planthopper that occurs commonly throughout the Fleurieu Peninsula of South Australia does not appear to extend to both adjacent Kangaroo Island or Yorke Peninsula.

These surveys have indicated the presence of a complex of new, undescribed species of the psylloid family Triozidae, and a suite of species from various families of planthoppers. Notable, particularly on *C. glauca* is the spittlebug, *Philagra parva*, and on *C. equisetifolia*, a large undetermined mirid bug, a minute green mirid, *Campylomma austrina*, and a lygaeid bug, *Germalus humeralis*. Many casuarinas also support phytophagous pentatomid bugs including *Avicennia virescens* (particularly on *C. equisetifolia*), and *Poecilometis* sp. Some of these larger bugs aggregate on cones and may be granivorous, but further observations are required to elucidate this. There is also a number of curculionids (weevils), including several species of brown weevil (including *Haplonyx* sp. that appears to feed in cones) and a suite of smaller species including an iridescent green species (*Misophrice* sp.). For these, biological associations with the host plant are not yet known, although it has been observed that there is considerable inter-tree variation in the composition of the curculionid fauna each tree may support. Further to the micro-lepidopteran reared from *C. equisetifolia* from northern Australia, there are also a suite of micro-lepidopterans that appear to feed indiscriminately in the cones of *C. glauca*, *A. littoralis* and *A. torulosa* in eastern Australia, and in *A. verticillata* in Southern Australia. Identifications and host associations for these have yet to be determined.

Of particular note is the emergence of the torymid wasp, *Bootanelleus orientalis* from incubated cones of *C. equisetifolia*. Examination of the associated *Casuarina* seed, that are shed from the cones as the fruiting body dries, show that many contain the exit hole of the emergent wasp. Each parasitised seed is hollow and non-viable. This species has been widely collected from Ballina in northern New South Wales to Yeppoon in Queensland. Few other wasps are reared from cones containing *B. orientalis* indicating that hyperparasitism is negligible. Another cohort of *Bootanelleus* wasps was reared from *C. cunninghamiana* from Forbes in inland New South Wales. It is not yet known if it is conspecific with *B. orientalis*. These wasps are less that half the size as those reared from *C. equisetifolia*, but this may be a function of resource size. The seeds of *C. cunninghamiana* (inset below left) are about half the size of those of *C. equisetifolia* (inset below right).

**<u>Plans for 2006</u>**

Surveys will continue in current collection locations to monitor season variations in herbivore activity. Additionally, cones in Southeast Asia, particularly from Thailand, will be collected and seed feeders reared for identification. Preserved insect herbivores of *Casuarina* cones will be forwarded to taxonomists for identification. Host records will then be sought from the literature to assess their potential as biological control agents.

Thus far surveys have concentrated on seed feeding insects though this may be expanded to include other foliage feeding herbivores. Those insects with the most potential as biological control agents will be subjected to biology, host range and impact studies. *Casuarina* stocks are being increased at ABCL greenhouse facilities for cage testing of insects. Also, seedlings have been planted in the ground at ABCL. These trees may take some time to mature, though in future cones could be collected for laboratory testing.

ABCL funds have been offered to support an Australian Research Council research grant application being submitted jointly by the University of Adelaide and ABCL. If successful, resources available for research on these weeds will double and Gary Taylor (inset below) will work full-time on Casuarina, mostly based in Adelaide but with a significant component of field exploration near Brisbane and around Australia. The project would be researching the systematics and coevolution of insect herbivores on casuarinas, and exploring congruence between potential biocontrol agents and their plant hosts.

## Surveys for herbivores of *Paederia foetida* in Southeast Asia

*Paederia* species occur from northern Thailand down the Malay peninsula to Singapore. Consumption of *P. foetida* as a vegetable is common in Thailand, much less so in Malaysia and rarely in Singapore, so plants are more commonly seen in Thailand than elsewhere. During exploration as part of the Lygodium project, *Paederia* was observed in several localities: in Thailand in the provinces of Songkhla, Nakhon Si Thammarat, Surat Thani, Chiang Mai and Phetchaburi; and in Malaysia in Pahang.

Eight species of *Paederia* are listed for Thailand.
*P. calycina* Kurz (Southwestern)
*P. foetida* Linn. (Mae Hong Son to Central and down the Peninsula)
*P. hirsuta* Craib (Central)
*P. kerrii* Craib (Chiang Mai)
*P. linearis* Hook. (Northern, Central and Peninsula)
*P. pilifera* Hook. (Northern)
*P. pilosa* Roxb. (Southeastern)
*P. tomentosa* Bl. var. *glabra* Kurz (Northern, Southwestern and Peninsula)

Records of insects attacking *P. foetida* in Thailand are rare, with only two Sphingid moths recorded in the 1965 book "Host list of insects of Thailand": *Gurelca hylas hylas* Walk. and *Macroglossum sitiene* Walk. (Sphingidae), both leaf eating caterpillars. *M. sitiene* is also known to feed on other plants in the Rubiaceae including *Morinda citrifolia* (Robinson *et al*. 2001).

Project activities to date include the following:

- Searching herbarium records and distribution with Thai botanists to map and report distribution of *P. foetida*
- Growing multiple specimens of *P. foetida* in the DOA laboratory plant nursery.
- Collecting living mature and immature insects for rearing on nursery plants at the Bangkok laboratory.
- Recording field information including GPS locations, habitat, plant observations and insect damage.
- Insect herbivores are pinned/preserved and sent to local taxonomists and SEL via ABCL for determination.

### Origin and geographic distribution

*Paederia foetida* occurs from Japan south to Timor and west to Nepal. In Southeast Asia it grows wild and is cultivated for food. In Thailand *P. foetida* occurs mostly in the southern peninsula and the east coastal belt to Klong Yai District. It is also common in Malaysia and Singapore, and listed as very common in Hong Kong.

*P. foetida* was introduced by the U.S. Department of Agriculture before 1897 as a potential fiber plant, but it soon showed its potential as a weed. It is now listed as a Category 1 plant on the Florida Exotic Pest Plant Committee database.

During 2005, five exploratory trips in association with the *Lygodium* field work in Southeast Asia were undertaken in February, May, July, September and November. Identification of the various *Paederia* species is a continuing problem, because flowers and fruit are required. A collection of specimens is being assembled in Bangkok for examination by Dr Christian Puff, University of Vienna, Austria. Dr Puff has offered to examine the specimens when next visiting Thailand, expected sometime during 2006. Colleagues at CSIRO Canberra are experimenting with separation of *Paederia* species using leaf DNA. Although this appears to be mostly successful, identification of actual species is not possible because Genbank does not yet hold species data for most *Paederia* species.

## Insects found associated with *Paederia foetida* in Southeast Asia

### *Zipangia* sp. poss. *cheni* Scherer (Coleoptera: Chrysomelidae)

This flea beetle was collected on *P. foetida* in the south of Thailand near Nakhon Si Thammarat in August 2004. It was identified by Dr A. Konstantinov at SEL. Adults feed on leaves causing damage shown in the photo. Larvae are possibly root feeders but have not yet been found, so they may be associated with other plant species.

### *Dulinius conchatus* Distant (Hemiptera: Tingidae)

Pemberton and Pratt previously collected the lace-bug *D. conchatus* in Japan. In Thailand, it is probably the most common insect on *P. foetida*, and consistently damaging. It is not being collected further because of insufficient host-specificity.

***Thrips morindae* (Thysanoptera: Thripidae)**
Two thrips species were collected: the common pest species *Thrips hawaiiensis* Morgan (Thripidae); and *Thrips morindae*, a species given as a synonym of *T. javanicus* by Palmer (1992). *T. morindae* is unusual in that adults are brown in both sexes, whereas most brown species have pale males. Dr Laurence Mound, Australian National Insect Collection (ANIC), CSIRO Entomology, Canberra made all identifications. Because of uncertainty regarding Palmer's report, Mound examined the original specimens of *T. javanicus* from Tjibodas, Java, and of *T. morindae* from "Batavia", on loan from Frankfurt, and concluded that *morindae* is a valid species needing to be distinguished from *javanicus*.

**Flower and Fruit borers**
Observations to date indicate flowers and fruit are only available during the period February – March. Further collections of the insects shown below were therefore not possible during the second half of the year. The only fruit encountered on *Paederia* was at one of the Don Sak sites near Surat Thani, however this fruit was very old and there was no insect activity. Additional collections of these insects are planned for February/March 2006 in southern Thailand when the insects should again be active.



**(a) Flower borers**
An unknown Lepidoptera species is responsible for damage observed on young flowers of *P. foetida*. Although heavy damage has so far been seen at just one site near Surat Thani in southern Thailand, lower level damage is common. Flower buds affected do not develop.

**(b) Fruit borers: unidentified Cecidomyiidae**
In southern Thailand, this gall midge was found infesting more than 60% of fruit of *P. foetida* in early 2004. Fruit production in early 2005 was low, possibly due to drought and collections were limited. Cecidomyiid taxonomist Prof. Junichi Yukawa of Kyushu University has agreed to study the taxonomy of the insect. His initial impression is that the midge belongs to the Asphondyliini, probably genus *Asphondylia*. Two midges in the Asphondyliini occur on *P. foetida* in Japan. We are now attempting to collect larvae and pupae as well as material for DNA analysis to help with his studies.



**Leaf binder Lepidoptera**

Several Lepidoptera larvae have been found associated with leaves of *P. foetida*. Leaf binders are consistently present. Collections are continuing. Specimens have been forwarded to SEL for identification.



**Stem galls**



Stem galls in FPA solution were sent to Dr Min Rayamajhi. He reported that the galls were caused by a pathogen, *Endophyllum paederiae*, with the entire gall surface “covered with rust pustules while the gall-tissues are made of hypertrophied parenchymatous cells loaded with starch granules. Some galls appear to develop discoloration probably due to colonization by secondary pathogens”. Further study is planned. Soil pH at some sites isvery high (>8.0). We will plan for a collection and survey trip when quarantine arrangements in Fort Lauderdale are complete.

**Leaf miner**



Leaf miner damage (inset above) to *P. foetida* is relatively common and occasionally severe. The Singapore leaf miner (no photo available) is a different species. Similar damage has also been seen on other Paederia species, for example at Phrae.

**Staphylinid**

This insect has been found at widely separated locations in the north-eastern, central and south-western of Thailand. Leaf damage is slight but leads to bending of the lamina.

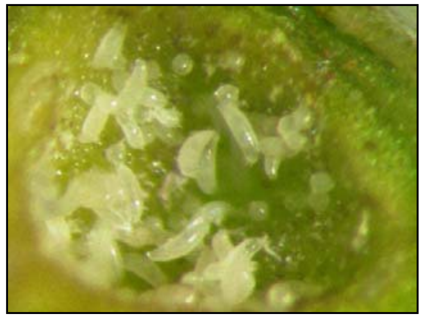
**Eriophyid mites**

Mite damage to *P. foetida* was first observed at one site in southern Thailand, south of Nakhon Si Thammarat in February 2004. However, the damage was relatively unimpressive, being single galls near the mid-vein of 10% of leaves. Since then more damage, often very intense damage causing leaf deformation, has been observed in southern Thailand. In February 2005, very heavy galling was present during a dry period. A large number of mites were seen in young galls. This damage appeared more like a further development of the leaf vein gall. However during a repeat visit in May 2005 after rain, little evidence of mite damage was present at the site. Further collections of mites in southern Thailand were possible in July. No predatory mites have yet been found.

Two genera of eriophyid mites were isolated from the heavily galled leaves, and were tentatively identified by Prof. Angsumarn Chandrapatya (Kasetsart University, Bangkok) as *Aceria* sp. and *Cecidophyopsis* sp. Further taxonomy is being undertaken. Prof. Chantrapatra and colleague Prof. James Amrine (West Virginia University) agree the *Aceria* sp. mite is a new species and they would like to describe it, however pressure of work has delayed completion of this process. Mites have not yet been isolated from the narrow twisted leaves shown above. Galling is also present in Singapore but at a much lower level, perhaps due to the more even rainfall throughout the year.

Single gall on mid-vein

Different gall type on curled, stunted leaf

Heavy damage to *Paederia foetida* by the eriophyid mite

**Research Plans for 2006**

Our research plans for 2006 include the identification of pressed specimens of Paederia species from Thailand by a local or visiting specialist. We will also confirm by DNA the identity of *P. foetida* from Singapore. Further collections of the cecidomyid midge from fruit are required for identification by Prof. Yukawa. Additional exploration to identify sites with the gall pathogen, *Endophyllum paederiae*, will allow a study and collection visit to Thailand by pathologist Dr Min Rayamajhi during 2006. If funds are available, we plan to support the study and description of the leaf galling eriophyid mite in Thailand.

## Biological Control of *Hydrilla verticillata*

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

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

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

Additional foreign exploration is needed to find new agents that are adapted to the range of environments where hydrilla is invasive, in particular, constant level rivers and lakes.

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

**Foreign exploration**

In 2005, ABCL staff and international cooperators made 44 collections of *Hydrilla* in Australia and Southeast Asia. Most of these collections (37) were made in Thailand which has been the main focus of exploratory research.

In April 2005, surveys were conducted in Thailand to primarily search for aquatic moth species in the river and irrigation systems east and west of Bangkok. The surveys were performed in conjunction with Dr. Manop Siriworakul from the Royal Irrigation Department of Thailand (RID) (inset left). The only aquatic moth species located was *Parapoynx diminutalis*, the pyralid moth already present in the United States. Aquatic *Bagous* (Coleoptera: Curculionidae) weevils were collected from several sites and were brought back to ABCL quarantine. One species that was collected from the Huay Yang Chun River (inset right) in the Kaeng Krachan district is possibly new and we are currently trying to establish a culture in Brisbane.

In April, surveys were also conducted near Indonesian Oil Palm Research Institute in North Sumatra, particularly in Sungei Maningkring site. Dr. Desmier de Chenon and staff are cooperators with ABCL and assisted Matthew Purcell to collect larvae of the aquatic moth

*Paracymoriza vagalis*. Over 300 of the larvae were collected (inset left) and hand-carried back to quarantine at ABCL in Australia. These moth larvae prefer hydrilla growing in flowing streams rather than ponds or lakes.

In July, surveys were conducted in Singapore. Hydrilla samples were taken from the Sungei Buloh Nature reserve but few insects were collected. Due to an outbreak of Dengue Fever on the island, all wetland areas were being regularly fogged with insecticides to control mosquitoes. This may have had a negative impact on hydrilla herbivores.

The first surveys by ABCL staff for hydrilla agents in Western Australia were conducted in September. Hydrilla was collected from a natural lake at Kununurra and Lake Argyle (inset right) near the Northern Territory border. The only insect of interest collected were *Hydrellia* leaf-mining flies. Records of hydrilla also exist for the Fitzroy River closer to the Western Australian coastline. Unfortunately the river was not flowing due to dry conditions and no hydrilla could be found in the remaining isolated ponds.

In October, Singapore was revisited on route to Thailand and once again hydrilla sites at Sungei Buloh (inset left) were surveyed. Few insects were collected with the main herbivore being *Parapoynx diminutalis*, the foliage-feeding moth already present in the US. We attempted to find new sites near local reservoirs but only the aquatic plant *Egeria densa* was located. ABCL now has a staff member temporarily based in Singapore who has been trained in collection and processing of hydrilla samples. When possible, Jeff Makinson will make collections of hydrilla and locate new hydrilla sites.

A survey of hydrilla throughout northern and central Thailand was also conducted with the Royal Irrigation Department in October. Collections of hydrilla were made at Bung Boraphet and an irrigation canal at Mae Gua north of Bangkok. No insects of interest were collected from samples taken from these sites. Hydrilla was scarce in the north and only a single collection was made at Phayao Lake south of Chiang Rai. The main insect of interest collected was a Chironomidae fly found at Phayao Lake (inset below) which was hand-carried to quarantine in Brisbane. We are currently attempting to colonize this insect so as to further evaluate its impact and host specificity. Usually these flies attack the growing tips of hydrilla.

Collections were also made by the staff of RID in our absence to the north-east and south-west of Bangkok. All insect herbivores were preserved and are yet to be formerly identified. There are specimens of weevils, leaf-mining flies and defoliating moth larvae in 12 collections.

A survey was conducted in peninsular Malaysia during November 2005 to recollect mites feeding on hydrilla at irrigation canal northwest of Jahor Buru. On revisiting the site no hydrilla could be found. Because of the monsoon, these canals are frequently flushed of aquatic plant material.

**<u>Potential agents</u>**

***Paracymoriza vagalis***

The aquatic moth, *Paracymoriza vagalis* (inset right) was imported into quarantine in January 2005. Our cooperator, Dr. Desmier DeChenon, collected over 100 larvae from the rivers surrounding the Indonesian Oil Palm Research Institute (IOPRI) in Marihat, North Sumatra, Indonesia. This is the most damaging moth found in surveys throughout Southeast Asia. In high populations, the larvae completely defoliate hydrilla in these flowing rivers. The populations fluctuate dramatically depending on rainfall, which frequently flushes the hydrilla from the system. However, the moth population recovers quickly once the hydrilla regenerates.

The distribution of *P. vagalis* is widespread throughout Asia. Little is known of the host plants of this moth, though the genus has been recorded from Podostemaceae. There are eight described species in this genus from the oriental region, six of which are from Southeast Asia. The moths were identified by Dr. Marianne Horak at the Australian National Insect Collection using a colour plate from a publication on moths of Southeast Asia (Robinson et al. 1994). Therefore, the species determination is only tentative. Preliminary host-specificity tests were conducted in Indonesia in 2004 and in starvation tests of 15 test plants, the caterpillars of *P. vagalis* (inset above left) fed on several plant species but could only pupate and complete their development on hydrilla.

During 2005 we imported into Brisbane quarantine two consignments of the foliage-feeding moth *Paracymoriza vagalis* but all attempts at mating and colony establishment failed. Currently our cooperators in Sumatra are making field observations of the moth to determine what are its requirements for mating and oviposition. It is possible lighting may be a critical factor and rearing techniques are being developed to explore this possibility. Further shipments of the larvae to quarantine in Brisbane from Indonesia are planned for February/March 2006.

***Bagous* weevils**

Three colonies of *Bagous* weevils are being maintained in quarantine in Brisbane. Several colonies were destroyed as species were found to be non host-specific. The weevils in the remaining colonies appear to be similar to those already tested, though their habits appear to be slightly different. All species will be genetically sequenced early in 2006 to confirm whether cryptic species may be present. Further host-range testing will be commenced if unique and untested species are identified.

**<u>Future plans</u>**

Dr. Roch Desmier DeChenon, our cooperator in Sumatra, Indonesia, will be visiting our laboratory in early January 2006. We will be discussing arrangements for shipping further consignments of the defoliating moth, *Paracymoriza vagalis*, and planning surveys in Sumatra for 2006. Final surveys of Thailand are planned for February/March when water levels begin to recede and hydrilla has had a significant period of growth following the monsoon. Ideally, this is when insect populations on hydrilla should be at a maximum.

Host-range testing on *Bagous* weevil species will be completed by ABCL staff in quarantine in Brisbane. A series of *Bagous* species will be sent to CSIRO in Canberra for genetic sequencing.

Foreign surveys will be conducted during 2006 in: 1) peninsular Malaysia; 2) Singapore; 3) Thailand and 4) Indonesia. Sites will be selected based on previous collections and insect species will be targeted that have potential as biological control agents. Approaches will also be made to the representatives of the Mekong River Commission to determine whether surveys in Vietnam and Cambodia are feasible over the coming year, while Western Australia will also be revisited.

Surveys in southeast Queensland will also be increased to locate aquatic mites for further evaluation, and to recollect *Bagous hydrillae* for field and laboratory evaluations. *Bagous hydrillae* was introduced into Florida but establishment was never confirmed. Further study of the field biology of this insect may determine factors that would improve chances of establishment in Florida.

## Acknowledgements

We would like to acknowledge the financial support of the South Florida Water Management District, Florida Department of Environmental Protection, ARS National Program Staff, and the ARS Invasive Plant Research Laboratory.

We would like to thank our many co-operators in Australia including: Environmental staff of Caboolture, Pine Rivers and Redlands Shires; Queensland National Parks and Wildlife staff of Bribie Island; Chris Burwell (Queensland Museum); Eric Claussen and Amanda Smith (NSW, NPWS); Sarah Russell, Bill Crowe (AQIS); Marjorie Gunasekara (CSIRO Entomology - Indooroopilly); Lawrence Mound, Diana Hartley (CSIRO Entomology – Canberra).

We thank our international collaborators in Indonesia, Singapore, Thailand and the UK. In Indonesia: Roch Desmier de Chenon and staff (based at the Indonesian Oil Palm Research Station, Marihat); Singapore: Benjamin Lee, Ng Sock Ling, James Gan, Chew Ping Ting (National Parks), Tay Boon Liang; Thailand: Amporn Winotai and staff (DOA), Manop Siriworakul (Royal Irrigation Department), Usanee Chattrakul, Suriont Rimbut and Amara Thagong (Royal Project Foundation); and the United Kingdom: Shen-Horn Yen (British Museum of Natural History).

In the USA, we would like to thank: Pai-Yei Whung, Rich Greene, Louise Waugh, Heather Phelps (OIRP); Judy St. John, Ernest Delfosse and Kevin Hackett (NPS); Ted Center, Bob Pemberton, Gary Buckingham, Chris Bennett, Alan Dray, Luke Kasarjian, Paul Madeira, Paul Pratt, Min Rayamajhi, Phil Tipping, Thai Van, Greg Wheeler and Susan Wineriter (ARS Invasive Plant Research Lab, Ft. Lauderdale, FL); Robin Giblin-Davis (University of Florida); Alma Solis, Pete Touhey, David Smith, and Ethan Kane (ARS-SEL).

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