10 Old World Climbing Fern

R. W. Pemberton,[1] J. A. Goolsby,[2] and T. Wright[3]

[1]Invasive Plant Research Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Fort Lauderdale, Florida, USA
[2]Australian Biological Control Laboratory, Brisbane, Australia
[3]Commonwealth Science and Industrial Research Organization, Brisbane, Australia.

PEST STATUS OF WEED

Old World climbing fern, *Lygodium microphyllum* (Cav.) R. Br. (Lygodiaceae) (Fig. 1), is an aggressive invasive weed of moist habitats in southern Florida (Pemberton and Ferriter, 1998). This rapidly spreading weed colonizes new areas without the need of habitat disturbance and frequently completely dominates native vegetation. Herbicidal and mechanical controls are expensive, temporary solutions, and are damaging to non-target plants. The fern, first found to be naturalized in 1965, has become one of the most dangerous weeds in southern Florida.

Figure 1. *Lygodium microphyllum* showing sterile leaflets with entire margins and fertile leaflets with sporangia-bearing teeth. (Photo by R. W. Pemberton.)

Nature of Damage

Economic damage. Although primarily a weed of natural areas, *L. microphyllum* infests residential landscapes, horticultural nurseries, and other managed lands near infested natural vegetation. Current control costs for chemical control of *L. microphyllum* range from $325 to $1,250/ha (D. Thayer, pers. comm.). But for areas that are hard to reach, such as the *L. microphyllum* infestations on the Loxahatchee National Wildlife Refuge in Florida, the cost for a single application was $3,750/ha in 2000, and since the fern has regrown the infestations were retreated in 2001 (M. Bailey and W. Thomas, pers. comm.).

Ecological damage. *Lygodium microphyllum*'s ability to grow up and over trees and shrubs and to run horizontally allows it to smother whole communities of plants (Figs. 2 and 3) reducing native plant diversity. It is difficult for other plants to grow through the thick fern mats (up to 1 m thick). *Lygodium microphyllum* is common in bald cypress (*Taxodium distichum* [L.] Richard) stands, but also infests pine flatwoods, wet prairies, saw grass (*Cladium jamaicense* Crantz) marshes, mangrove communities, Everglades tree islands, and disturbed areas. Some Everglades tree islands are so completely blanketed by the fern that it is not possible to see trees and other vegetation beneath the fern mat (Fig. 3). *Lygodium microphyllum* also threatens rare plants. In Loxahatchee Slough in Palm Beach County, Florida, the fern is an "imminent danger" to *Tillandsia utriculata* L. and other rare bromeliads (Craddock Burks, 1996). Infestations of this fern alter the impact of fire, which is a naturally occurring element and a management tool in many Florida communities (Roberts, 1998). Thick skirts of old fronds enclose trees and serve as ladders that carry fire into tree canopies. Trees that can withstand ground fires are killed when fire is brought into the canopy. Fires that usually terminate at the margins of cypress sloughs during the wet season can burn into and through cypress sloughs infested with *L. microphyllum*. Portions of burning fern also can break free and spread fire to new areas. *Lygodium*

Figure 2. *Lygodium microphyllum* blanketing an Everglades tree island in Loxahatchee National Wildlife Refuge, Florida. (Photo by Amy Ferriter, South Florida Water Management District, West Palm Beach.)

Figure 3. Adult of *Cataclysta camptozonale*, an Australian pyralid moth currently under study as potential biological control agent of *Lygodium microphyllum*. (Photo by Christine Bennett, Florida Biocontrol Lab, USDA, ARS, Florida Division of Plant Industry, Gainesville.)

microphyllum appears to be long-lived and persistent, and can occupy a large proportion of suitable habitats in a relatively short time. The fern is classified as a Category I invasive species (the most invasive species) by the Florida Exotic Plant Pest Council (Langeland and Craddock Burks, 1998).

Extent of losses. It is hard to quantify the extent of the losses due to Old World climbing fern. The explosive growth and rapid spread of the fern are relatively recent. It was first collected from Jonathan Dickinson State Park in Palm Beach County in 1966 (Beckner, 1968), and by 1993, it was present on 493 ha (11%) of the park and the Loxahatchee National Wild and Scenic River (Roberts, 1998). In 1995, the weed was present on 6,800 ha (12%) of the Loxahatchee National Wildlife Refuge (Palm Beach Co.), where it was undetected in 1990 (S. Jewel, pers. comm.). The land area infested by this fern is estimated (from aerial surveys) to have increased 150% between 1997 and 1999 (A. Ferriter, pers. comm.). Present losses due to this weed are bad, yet modest compared to potential losses unless effective controls can be found and implemented soon.

Geographical Distribution

In the United States. *Lygodium micophyllum* is a subtropical and tropical plant that requires shallow aquatic habitats or moist soils. At present, *L. microphyllum* is limited to the southern third of the Florida peninsula from Brevard and Highlands County south. The area with the lowest winter temperatures currently infested is Polk and Highlands Counties (ca. 28°N) in south-central Florida. These areas are in the USDA Plant Hardiness Zone 9B (minimum of 3.9 to 6.6°C) (Cathey, 1990). Because Zone 9B extends up the eastern coast to the Georgia border (ca. 30°N), the weed may well be able to colonize this area. Zone 9B extends to just above Tampa on the west coast of Florida. If spores of the fern are carried across the Gulf of Mexico to the southern coast of Texas and Mexico, the weed could establish in those areas because of suitable climates and habitats. If it establishes in Mexico, it could spread south to much of wet tropical America. *Lygodium microphyllum* also is naturalized to a limited extent in Jamaica and Guyana (Pemberton and Ferriter, 1998), so the opportunity for it to spread within the Caribbean and other tropical portions of the Americas already exists.

Native range. *Lygodium microphyllum* has an exceptionally large native range, occurring in much of the moist Old World tropics and subtropics (Pemberton, 1998). The fern's temperate limits are between 28°S and 29°S in Australia and South Africa, and 25° N and 27°N in Assam (northeastern India) and the Ryuku Islands (the southwestern most part of Japan). It spans more than half of the world's circumference from 18°E in Senegal to 150°W in Tahiti. In Africa, *L. microphyllum* ranges from Senegal south and east through most of West Africa to Zaire,

then south to Angola, east to East Africa, and then south to South Africa. In Asia, the fern is distributed from India and Nepal, east through much of Southeast Asia, and north through the warmer provinces of southern China to Taiwan and Okinawa. It also occurs throughout many of the Southeast Asian islands to Australia and, in the Pacific, east to Tahiti.

BACKGROUND INFORMATION ON PEST PLANT

Taxonomy

The correct name of Old World climbing fern is *L. microphyllum* (Alston and Holttum, 1959; Hanks, 1998), but the species is occasionally referred to as *Lygodium scandens* (L.) Sw., particularly in older works. As many as 40 species have been placed in the genus *Lygodium* (Mabberley, 1997), but a recent revision has reduced this number to 26 (Hanks, 1998). All but two species are from areas with tropical or subtropical climates. The genus *Lygodium* has usually been placed in the Schizaeaceae, a small primitive family that also includes the genera *Actinostachys, Schizaea, Anemia*, and *Mohria* (Prantl, 1881; Holttum, 1973; Hanks, 1998). Since *Lygodium* is unique morphologically, it is sometimes interpreted to comprise its own monogeneric family, the Lygodiaceae (Bierhorst, 1971; Wagner and Smith, 1993). The relationship of the Schizaeaceae (Lygodiaceace *s.s.*) to other groups of ferns is unclear (Smith, 1995).

Lygodium microphyllum is a distinctive fern in North America. This vine has pinnately compound fronds (pinnules), except the sterile fronds (pinnae), which have entire margins. *Lygodium japonicum* (Thunberg ex. Murray) Swartz, another invasive species in the southeastern United States, has twice pinnately compound fronds with sterile fronds that have toothed margins. *Lygodium palmatum* (Bernh.) Sw., a temperate native member of the genus living from Appalachia north to New England, has palmately compound fronds. *Lygodium microphyllum* has been placed, based on morphology, in the subgenus Volubilia; the native *L. palmatum* in the subgenus Palmata; and *L. japonicum* in the subgenus Flexuosa (Prantl, 1881; Hanks, 1998).

Biology

The biology of *L. microphyllum* is not well studied. The fern is a long-lived perennial vine. The aerial vines are actually very long leaves with a stem-like rachis and leaflet-like pinnae and pinnules comprising the photosynthetic tissue. The plant bears both fertile leaflets with sporangia bearing teeth along the edge of the blade, and sterile leaflets with entire margins. The true stems are underground rhizomes. These vines can be 30 m in length. Growth and sporulation appear to occur all year. The fern produces large numbers of spores; more than 800 spores/m^3/hour were trapped in one Florida infestation (Pemberton and Ferriter, 1998). Spores can germinate in six to seven days (Brown, 1984). Dried spores taken from the plants have germinated after two years (Lott and Pemberton, unpub.). The life cycle of *L. microphyllum* is the same as with other ferns. The spores require moist conditions to germinate and grow into small, liverwort-like gametophytes. Male and female organs are produced on the same gametophytes and fertilization occurs when the swimming spermatozoid swims from the male organ to a female organ to penetrate the ovule. Fertilization gives rise to the familiar large leafy fern, which is the sporophyte stage. The fern spreads locally by vegetative growth and over long distances by wind-borne spores. The plant can grow in standing water and wet soils, and either in full sun or shade.

Analysis of Related Native Plants in the Eastern United States

In addition to *L. palmatum* (discussed above), there are West Indian *Lygodium* species that are being considered in the biological control program because of the proximity of this region to southern Florida. *Lygodium volubile* Sw., which occurs in Cuba and other areas of the West Indies, belongs to the same subgenus Volubilia as *L. microphyllum*. *Lygodium cubense* Kunth. (a Cuban endemic), *Lygodium venustum* Sw. (found in the West Indies), and *Lygodium oligostachyum* (Willd.) Desv. (endemic to the Dominican Republic and Haiti) (Prantl, 1881; Hanks, 1998) all belong to the subgenus Flexuosa.

Except for *L. palmatum*, the only native North American plants that have been considered related to *L. microphyllum* are three species of *Anemia*, one species of *Actinostachys*, and one species of *Schizaea* (Wagner and Smith, 1993; Nauman, 1993; Mickel, 1993). *Anemia mexicana* Klotzsch occurs in southwest Texas and northern Mexico. *Anemia adiantifolia* (L.) Swartz is locally common in Florida and the West Indies. *Anemia wrightii* Baker in Hooker and Baker is a tiny rare fern limited to lightly shaded solution holes and limestone sinks in southern Florida and the West Indies. Both Florida *Anemia* species are broadly sympatric with *L. microphyllum*. *Schizaea pusilla* Pursh is found in the northeastern part of North America. *Actinostachys pennula* (Swartz) Hooker is a widely distributed species in tropical America whose only known North American population is on Everglades tree islands that are being overgrown by *L. microphyllum*.

The relationships among the genera of the Schizaeaceae s.l. are unclear (Smith, 1995). Recent molecular research (*rbc*L) on the phylogeny of ferns found that *Lygodium*, *Actinostachys*, and *Anemia* have more intrageneric distance than occurs between most fern families (Hasebe *et al.*, 1995). The research also showed the family to be very isolated, with more intrafamilial distance between it and other fern families than occurs between most fern families (Hasebe *et al.*, 1995). The molecular data and the antiquity of the Schizaeaceae (*Anemia* spores are known from the Cretaceous [Smith, 1995]), suggest that the family arose earlier than other fern groups.

HISTORY OF BIOLOGICAL CONTROL EFFORTS IN THE EASTERN UNITED STATES

Area of Origin of Weed

While the native range of *L. microphyllum* is well defined, it is very large and the area(s) of origin of the plants that have become invasive in Florida is unknown. To attempt to identify the region(s) of origin of the Florida plants, collections of Florida material and material from many different parts of the native range are being made for genetic comparison. The weed is of horticultural origin in Florida (Pemberton and Ferriter, 1998). It was recognized to be a potential problem not long after it was found to be naturalized (Nauman and Austin, 1978).

Areas Surveyed for Natural Enemies

A preliminary survey for the natural enemies of *Lygodium* spp. was made in Japan, northern Taiwan, and Hong Kong during the autumn of 1997 by R. Pemberton. *Lygodium japonicum*, the only *Lygodium* in Japan, was common in central and western Honshu, including Tokyo. *Lygodium microphyllum* was uncommon in northern Taiwan; only one population was found. In Hong Kong, *L. microphyllum*, *L. japonicum*, and *Lygodium flexuosum* (L.) Swartz were examined. *Lygodium microphyllum* occurred in small patches, a few meters wide, along streams and on hillsides in heavy soils. The discovery of various pyralid moths attacking the plants helped secure funding for the project.

Much of the exploratory effort for *Lygodium* natural enemies is being carried out by J. Goolsby and T. Wright. This effort began in 1998 and is focused on *L. microphyllum* and other *Lygodium* spp. (*L. japonicum*, *L. flexuosum*, and *Lygodium reticulatum* Schkuhr, Farnkr.) in Southeast Asia and Australia. *Lygodium microphyllum* is widely distributed throughout the wet tropics and subtropics of Australia and Southeast Asia. Within Australia areas in New South Wales, Queensland, the Northern Territory, and Western Australia were explored. In southeast Asia, areas in Indonesia, Malaysia, Singapore, and Thailand were surveyed. Several trips have been made to each of the collection locations to capture the seasonal diversity of herbivores, and more than 250 sites have been visited. In these areas, *L. microphyllum* is not weedy and is associated with a complex of insects, mites, and pathogens.

Lygodium microphyllum and *Lygodium smithianum* Pres. were surveyed in West Africa (Benin, Ghana, and Cameroon) by R. Pemberton in 1999. West Africa is at the western edge of *L. microphyllum*'s huge Old World distribution. The plant was found in both swamps and in diverse terrestrial habitats in high rainfall areas. The plant often was common, but not abundant or dominant. Two short visits were made by cooperators to a *L. microphyllum* population in Natal South Africa, near the plant's southern latitudinal limit.

Natural Enemies Found

More than 18 species of herbivores have been collected from *L. microphyllum* (Table 1). The pyralid moth *Neomusotima conspurcatalis* Warren is the most

Table 1. Herbivores Collected from *Lygodium* spp. in Asia and Australia

Name	Collection Locations	Host Plant
Cataclysta camptozonale (Hampson) Lepidoptera: Pyralidae	Australia (Queensland)	*L. microphyllum* *L. reticulatum*
Neomusotima conspurcatalis Lepidoptera: Pyralidae	Australia (Queensland and Northern Territory), Indonesia, Malaysia, Singapore, Thailand, Hong Kong	*L. microphyllum*
Neomusotima fuscolinealis Yoshiyasu Lepidoptera: Pyralidae	Japan	*L. japonicum*
Musotima sp. Lepidoptera: Pyralidae	Malaysia, Singapore, Thailand	*L. microphyllum*
Callopistria spp. Lepidoptera: Noctuidae	Australia (Northern Territory), Thailand, Indonesia, Malaysia, Singapore	*L. microphyllum*
Lepidoptera: Limacodidae	Thailand	*L. microphyllum*
Archips machlopis Meyrick Lepidoptera: Tortricidae	Thailand	*L. microphyllum*
Lepidoptera: Tortricidae	Malaysia, Singapore	*L. microphyllum*
Stem-borer Lepidoptera: Pyralidae	Singapore	*L. microphyllum* *L. flexuosum*
Neostromboceros albicomus (Konow) Hymenoptera: Tenthridinidae	Malaysia, Singapore, Thailand, Vietnam	*L. flexuosum*
Metriona sp. Coleoptera: Chrysomelidae	Australia (Northern Territory)	*L. microphyllum*
Endelus sp. Coleoptera: Buprestidae	Singapore	*L. microphyllum*
Manobia sp. Coleoptera: Chrysomelidae	Thailand	*L. flexuosum*
Lophothetes sp. Coleoptera: Apionidae	Palau (Arakabesang Is.)	*L. microphyllum*
Hemiptera: Miridae	Australia (Northern Territory)	*L. microphyllum*
Acanthuchus trispinifer (Fairmaire) Homoptera: Membracidae	Australia (Queensland, Northern Territory)	*L. microphyllum*
Thrips: Thysanoptera	Malaysia, Thailand	*L. microphyllum*
Floracarus sp. Acarina: Eriophyidae	Australia, Indonesia, Malaysia, Singapore, Thailand	*L. microphyllum*

widely distributed, followed by an eriophyid mite in the genus *Floracarus*. Most efforts to locate natural enemies have focused on the above-ground portions of the fern. Searching methods have included visual inspection, beating trays, and black-light trapping.

In South Africa, few natural enemies were found. In West Africa, the most damaging and common natural enemy of *L. microphyllum* was a *Tenuapalpis* mite (Tenuapalpidae), which caused brown, channel-like scars on the leaves. The mite also commonly fed on *Nephrolepis* ferns, which indicated a undesirably wide host range; *Nephrolepis* ferns are a modern group of ferns whereas *Lygodium* ferns are an ancient group. The paucity of natural enemies associated with *Lygodium* in Africa may relate to the time of year that the surveys were made, or to the low diversity of *Lygodium* species on the continent. Only two species of *Lygodium* occur

there, compared to about a dozen species in southeast Asia, where many natural enemies have been found.

A rust fungus, *Puccinia lygodii* (Har.) Arth., native to South America and naturalized in the United States, has recently been found infecting *L. japonicum* in northern Florida (Rayachhetry *et al*., 2001). The rust is not known to occur in the region infested by *L. microphyllum* and its capability to infect *L. microphyllum* is unknown. It is a glasshouse pest of ornamental *Lygodium* (Jones, 1987), and may have a role in *L. microphyllum* control (Pemberton, 1998).

Host Range Tests and Results

Host specificity testing schemes based on plant family affiliations, as employed for weedy flowering plant, are not suitable for use with weedy ferns because of the lack of agreement on fern families, even in recent publications. Recent molecular work (Hasebe, 1995) has helped to identifying more natural groupings of ferns. Nevertheless, fern genera are usually the reliable taxa used to orient host specificity testing. Because of the relatively modest number of genera of ferns in Florida and the southeastern United States, it is possible to test representatives of most genera. The most difficult species to evaluate are the 40 species of ferns considered threatened or endangered in Florida. Most all of these ferns are neotropical species reaching the northern limits of their ranges in southern Florida. Permits from federal, state, and local governments are needed to collect small numbers of representative species of each genus of rare ferns. Because of the taxonomic isolation of the genus *Lygodium,* we expect to find specialist natural enemies with host ranges limited to the genus.

For the moth species currently being evaluated, host range tests have been designed to measure the oviposition behavior of the adult and the suitability of the test ferns for development of the immature stages. No-choice sleeve cage tests are being used to determine oviposition responses of the adults. Oviposition in sleeves is recorded daily, along with hatching and mortality of immature stages. In many cases, adult moths do not oviposit on test plant species. For these species, naïve neonate larvae are placed on test plants to determine their ability to feed and develop. For larger larvae, which are big enough to crawl between test plants in a choice test, simultaneous presentation of cut foliage from several test plant species will be used to determine the ability of larger larvae to choose plant species best suited for completion of their development.

Cold-temperature tests will be used to determine the critical lower thermal limit for survival of the target herbivores. Because the geographical range of *L. microphyllum* is from 28°N to 28°S, specialist herbivores of this fern also will be tropical or subtropical species. In cases where development of these herbivores occurs on temperate species, such as *L. palmatum* in North America, the lower thermal limit of the herbivore could reduce the risk to such nontarget species. We do not expect tropical or subtropical biological control agents to tolerate winter temperatures found in the southern part of *L. palmatum*'s range.

Releases Made

No releases of any agents have been made against Old World climbing fern in the United States as of 2000.

BIOLOGY AND ECOLOGY OF KEY NATURAL ENEMIES

Cataclysta camptozonale (Hampson) (Lepidoptera: Pyralidae)

Cataclysta camptozonale (Fig. 4) has been collected from subtropical south Queensland to tropical north Queensland. In northern Queensland, it has been collected from both *L. microphyllum* and *L. reticulatum.* Heavy damage to *L. microphyllum* has been noted despite high levels of predation and egg parasitism (Goolsby, unpub.). Larvae skeletonize *L. microphyllum* leaves, sometimes consuming much of the new growth. In laboratory cultures, larvae consume all the foliage, and scarify the stems, which kills the plants.

Developmental studies of *C. camptozonale* were conducted on two hosts, *L. microphyllum* and *L. japonicum*. Females laid an average of 25 eggs either singularly or in clusters on the upper surface of mature pinnae. Longevity of adult females was 6.2 days. Development time from egg to adult was 44 days at 24°C on both plant species. *Cataclysta camptozonale* appears to have four larval instars. Pupae can be sexed based on the presence of a slit in the middle of the second to last tergite in males only. The sex ratio of

Figure 4 Larva of *Cataclysta camptozonale* feeding on *Lygodium microphyllum*. (Photo by Christine Bennett, Florida Biocontrol Lab, USDA, ARS, Florida Division of Plant Industry, Gainesville.)

the laboratory colony used in this test was slightly female biased with a male:female ratio of 1:1.3.

Preliminary host range tests have started with *C. camptozonale* in the laboratory. Fifteen fern species have been tested in no-choice tests. Thus far it appears that *C. camptozonale* larvae develop only on *Lygodium* spp., including the North American endemic *L. palmatum*. Further testing is underway to determine the host range and critical minimum survival temperature of this moth.

Neomusotima conspurcatalis (Lepidoptera: Pyralidae)

Neomusotima conspurcatalis has been collected from many locations in northern Australia and the wet tropics of southeast Asia. Larvae defoliate leaves and skeletonize *L. microphyllum* in a manner similar to *C. camptozonale*. To determine whether *N. conspurcatalis* might be a complex of species, we used molecular genetic methods to analyze the DNA of populations from different areas. Molecular sequencing of the mitochondrial D2 gene showed an exact match between the specimens from Australia and southeast Asia. This indicates that both populations are likely to be the same species.

Fecundity, longevity, and host range of this species appear to be very similar to that of *C. camptozonale*. *Neomusotima conspurcatalis* has only been collected in tropical regions thus far. The geographical distribution of this moth seems to indicate that it is not tolerant of the cooler winters of the subtropics; however, further studies are planned to determine the exact critical low temperature.

Musotima sp. (Lepidoptera: Pyralidae)

Musotima sp. has been collected in Thailand, Malaysia, and Singapore, but only on *L. microphyllum*. It appears to be restricted to areas with tropical climates to a greater degree than either *C. camptozonale* or *N. conspurcatalis*. The moth is currently being described by Alma Solis (ARS-Systematic Entomology Laboratory, Beltsville, Maryland).

Although little is known so far, preliminary studies in quarantine indicate that adults of this species live up to 10 days, pupal development requires eight days, and the sex ratio favors females (m:f, 1:1.5). Larvae are vigorous defoliators of *L. microphyllum*, although the damage is less pronounced in the field, presumably due to parasitism and predation. The species also completes its life cycle on *L. palmatum* and *L. japonicum*.

Floracarus sp. (Acarina: Eriophyidae)

Field collections in the Brisbane area have documented the damage caused by the mite *Floracarus* sp. (Eriophyidae) (Fig. 5) on *L. microphyllum*. Feeding by the mite on the new growth causes the pinnule (leaflet) margins to curl. It also appears that mite feeding causes disease transmission, because the feeding is associated with a black streaking and necrosis of the leaflets. Similar damage has been noted in southeast Asia as well. Fungi were isolated from the necrotic patches associated with the mite damage. The causal agent was identified as *Botryospheria* sp., which is believed to be a secondary pathogen, associated with leaf damage.

Figure 5. Leaf galls on *Lygodium microphyllum* caused by an eriophyid gall mite *Floracarus* sp., a candidate biological control agent of the fern. (Photo by John Goolsby, Australian Biological Control Laboratory, USDA-ARS, Brisbane.)

We intend to compare mite populations from Australia with those from southeast Asia, using molecular DNA tools. Analysis of the DNA (D2 gene) should indicate whether there are several species feeding on *Lygodium* in different parts of its range. Danuta Knihinicki of New South Wales Agriculture identified the mite as *Floracarus* sp. and intends to describe it as a new species. Field studies are continuing to determine the life cycle and host range of the mite. Preliminary testing indicates that this mite is highly specific to *L. microphyllum*.

RECOMMENDATIONS FOR FUTURE WORK

The biological control program against Old World climbing fern began in 1997. Although promising natural enemies have been located, none have been fully tested and none released. For this reason and because large areas of *L. microphyllum*'s native range are unexplored, surveys to locate additional natural enemies will continue in southern China, various Pacific islands, Irian Jaya, New Caledonia, and India. In addition to surveying for herbivores attacking *L. microphyllum* and other *Lygodium* spp. in the weed's native range, surveys of neotropical *Lygodium* species will be made to find natural enemies not previously associated with the weed.

Molecular phylogenetic studies are planned that will attempt to match the genotype of the *L. microphyllum* population in Florida with populations from around the world. More than 30 samples of *L. microphyllum* from Florida and various parts of its native range have been collected. Matching the invasive population with its source population should lead us to the natural enemies which with the greatest affinity for the Florida genotype. This research also will allow us to determine the degree of relatedness of species within the genus. Several subgenera have been established based on morphology. We intend to look for congruency in placement of the species in subgenera with molecular-based phylogenies. Understanding which species are most closely related to *L. microphyllum* (in the same subgroup) will allow us to develop the most meaningful host plant test list. This is especially important since many species of *Lygodium* are neotropical and could potentially be affected by introduction of biological control agents to Florida.

The climatic factors that influence distribution of potential agents also must be investigated. Because *L. palmatum* occurs in temperate areas of North America, it should not be at risk from importation of agents that have tropical or subtropical origins. Laboratory tests to establish the critical thermal limits of potential agents are planned.

More than two hundred field sites in Australia and Southeast Asia across a wide range of climates, soil types, and nutrient regimes have been surveyed for natural enemies. *Lygodium microphyllum* cannot be described as weedy in any of these locations. Yet, it is not apparent from field surveys which agent(s) most restrict population growth of this fern. Field studies are planned to determine the regulatory effects of the key agents on *L. microphyllum*. *Floracarus* sp. appears to be associated with leaf necrosis and defoliation. We intend to investigate the role of this mite in promoting infections by plant pathogens. Pathogens and insects also may be associated with the rhizome of the fern. Methods must be developed to identify rhizome natural enemies and determine if they reduce the growth of the plant.

Although current research is focused on *L. microphyllum, L. japonicum* could become a part of the program in the future. This invasive fern is naturalized in the United States from Texas to the Carolinas and southward to central Florida. Its more temperate distribution would require the use of biological control agents more tolerant of cold climates than those needed for *L. microphyllum*. Use of such cold-hardy agents might place the native species *L. palmatum* at risk. Additional information on these ferns is available in the *Lygodium* Management Plan for Florida (Ferriter, 2001).

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