Pre-release evaluation and host-range testing of *Floracarus perrepae* (Eriophyidae) genotypes for biological control of Old World climbing fern

J.A. Goolsby,1 J.R. Makinson,2 D.M. Hartley,3 R. Zonneveld2 and A.D. Wright2

Summary
A biological control program for *Lygodium microphyllum*, an invasive climbing fern in Florida, USA, was initiated in 1997. Surveys for natural enemies were conducted in the fern’s native range which includes Australia, Asia and Oceania. Twenty-two herbivores were documented, including an eriophyid mite, *Floracarus perrepae* Knihinicki & Boczek. Molecular diagnostics were used to match the origin of the invasive Florida population with the native range. The population from Cape York, Queensland was found to be an exact match with the invasive populations in Florida for the two chloroplast DNA sequences analyzed. Field studies of *F. perrepae* were conducted, which found that the mite was active year-round, with populations peaking during periods of ample soil moisture. Predator mites and a pathogen had significant impacts on *F. perrepae* populations, but heavy plant damage was still observed. Pre-release field impact studies revealed that *F. perrepae* caused more than 50% impact on *L. microphyllum* biomass production over a two-year period. Several genotypes of *F. perrepae* were identified from southeast Queensland, New Caledonia, China, Thailand, India/Sri Lanka, and Cape York. Each of these populations was screened for their acceptance of the invasive Florida genotype of the climbing fern. The populations from Cape York and Thailand performed best and came from fern genotypes that were most closely related to the Florida genotype.

Keywords: agent selection, matching plant origin, screening mite genotypes.

Introduction
Old World climbing fern, *Lygodium microphyllum*, is an invasive weed in southern Florida, USA, including the Everglades (Pemberton & Ferriter 1998). It is indigenous to the wet tropical and subtropical regions of the Old World (Pemberton 1998). Although the fern was introduced into Florida in the 1890s, it did not become a serious invasive weed until the 1990s. A biological control program was initiated for this weed in 1998, which is a part of the National Everglades Restoration Program.

As part of the surveys for natural enemies of *L. microphyllum*, plant samples were collected to be used for molecular analysis with the aim of matching the invasive Florida population with populations in the native range. We initially used RAPDs to distinguish populations, but then switched to gene sequencing, which proved to be more informative. Several genes were initially sequenced including: CO1, ITS1, and D2, but they failed to show significant differences. The chloroplast genes TrnF-TrnL and rps4-TrnS showed the greatest variation among populations. We used the technique developed by Thomson (2000) for the chloroplast genes and identified unique *L. microphyllum* genotypes from Ghana, Australia (Queensland), New Caledonia, China, Thailand, India/Sri

---

1 United States Dept. of Agriculture, Agricultural Research Service, Australian Biological Control Laboratory, 120 Meiers Rd, Indooroopilly, Queensland 4068, Australia.
2 CSIRO Entomology, Australian Biological Control Laboratory, Long Pocket Laboratories, 120 Meiers Rd. Indooroopilly, Queensland 4068, Australia.
3 CSIRO Entomology, Canberra, ACT 2601, Australia.
Corresponding author: J.A. Goolsby, <john.goolsby@csiro.au>.
Lanka and Australia (Cape York). The population at the
tip of Cape York at the Iron Range National Park was
found to be an exact match with the invasive populations
in Florida for the chloroplast DNA sequences.

Exploration for natural enemies of this weed was
conducted between 1997 and 2002 in Australia, China,
India, Indonesia, Japan, Malaysia, New Caledonia,
Singapore, Taiwan, Thailand, and Vietnam. Two
species of mites and 20 insect species were collected
(Goolsby et al. 2003). Over 500 collections were made
across the range of the plant over several years and
during all seasons. We did not find the plant to be domi-
nant or weedy at any location and it was always found
in a mosaic of other plant species. The eriophyid mite
Floracarus perrepae was the most widely distributed
of the herbivores and appeared from field observations
to gradually debilitate the plant over time. Feeding by
the adults and immatures causes formation of leaf roll
galls, which leads to necrosis and premature defoliation
of L. microphyllum pinnules, impacting on plant
growth. Based on its narrow field host range and
apparent impact on L. microphyllum, F. perrepae was
prioritised for further evaluation (Goolsby et al. 2003).

Mite phenology and impact

Field and laboratory studies of F. perrepae were initi-
ated in south-eastern Queensland to learn about its
phenology and quantify its impact on L. microphyllum.
Four field sites were located to the north and south of
Brisbane at Bribie Island and near Logan, respectively.
The sites are typical habitats within the native range of
both L. microphyllum and F. perrepae in subtropical,
eastern Australia. All the sites are seasonally inundated,
with standing water common during the summer
months. Monthly surveys of F. perrepae on L. micro-
phyllum were conducted at each site from November
2000 to March 2003. At each site, 30 newly expanded
sterile pinnules were selected at random and returned to
the laboratory for counting. The numbers of infested
and uninfested subpinnules were counted for each
pinnule. This count provided a measure of the propor-
tion of infested subpinnules, or mite damage, at each
location. From this sample of infested subpinnules, a
subsample of 30 was removed to count the numbers and
stages of F. perrepae within each curl (Fig. 1). We also
identified and counted the predator mites within each
subpinnule and assessed the presence or absence of the
mite pathogen Hirsutella thompsoni.

The field studies found that populations of the mite
were positively correlated with minimum temperatures
and soil moisture levels. Populations of F. perrepae were
lowest during hot, dry summer conditions. The impact of
the predators and the pathogen were also significant,
though even with high levels of natural enemies, the mite
still caused obvious visual damage to the fern.

Although the use of eriophyid mites in biological
control of weeds shows great promise, several authors,
including Briese & Cullen (2001) have stated that there
are not yet any dramatic successes that can be attributed
to the singular impact of an eriophyid. Bearing this in
mind, we sought to measure the impact of F. perrepae on
L. microphyllum in an experimental field setting in the
native range. We used a field plot design with 32 pairs of
L. microphyllum plants to measure the mite’s impact on
biomass production. One plant in each pair was sprayed
monthly with Agrimec® miticide to exclude the mite
(Fig. 2). Each quarter, over a two-year period, four pairs
of the plants were harvested and the dry weights of the
roots, stems and leaves were measured. We found that
the mite caused a greater than 50% reduction in biomass
over the two-year period. The other significant aspect
of this experiment was that the local south-eastern Queens-
land population of F. perrepae did not feed and develop
on the Florida genotype of L. microphyllum. We
concluded that the locally collected F. perrepae had a
significant impact on the south-eastern Queensland
genotype of L. microphyllum, but that we needed to
search more widely for a biotype of the mite that
accepted the invasive Florida genotype of the fern.

Performance of mite genotypes

To characterize F. perrepae, populations from throughout
its native range were collected and analyzed using
sequence data from nuclear rRNA D2 and mitochondrial
CO1 genes using the methods of DeBarro et al. (2000).
This technique identified genotypes from south-eastern
Queensland, New Caledonia, China, Thailand, India/Sri
Lanka, and Cape York. Each of these unique mite geno-
types corresponded with a unique fern genotype. To
screen these genotypes of F. perrepae for acceptance of
the Florida L. microphyllum, portable screening methods
were developed to allow for in-country testing (Fig. 3).
Mites were field-collected from each location and hand-
transferred to Florida and Queensland genotype sporeling
ferns. Mites were held on the sporeling ferns for 3 to 4
weeks until completion of leaf curling, oviposition and
development of progeny. The development of leaf curls
and the numbers of progeny produced on the Florida and
Queensland ferns were recorded for each mite population
tested. F. perrepae populations from Cape York and
Thailand performed best on the Florida genotype of the
fern. The south-eastern Queensland mite genotype
performed best on its own co-evolved south-eastern
Queensland genotype of the fern, but did not develop on
the Florida fern. The mite genotype from New Caledonia
was intermediate in its performance on the Florida fern
 genotype. Genotypes from China and India/Sri Lanka
performed poorly on the Florida fern genotype. In
summary, the mites collected from the fern genotypes
that matched or were very similar to the Florida genotype
performed best. The population of the mite from Cape
York was selected for release in Florida pending the
results of full host-range testing and approval by the US
regulatory authorities.
Floracarpus for biological control of Lygodium

Figure 1. Subpinnule of *Lygodium microphyllum* showing marginal leaf curl induced by *Floracarus perrepae*.

Figure 2. Impact of *Floracarus perrepae* on *Lygodium microphyllum* growth and biomass production. Plants shown from the field plot form a paired replicate with one plant treated with Agrimec® miticide.

Figure 3. Mobile field laboratory for in-country screening of mites: top left to bottom right: dual microscope set-up used to select and transfer mites; field-collected infested leaf curls; containers of Florida and Queensland sporeling ferns used in screening tests; close-up of sporeling, *L. microphyllum* held in container to maintain high levels of humidity during transfer process.
Conclusions

We concluded that *F. perrepaec* was the best candidate agent based on its widespread distribution, its extremely narrow field host range and obvious damage caused to *L. microphyllum* across its native range in Asia, Australia and Oceania. Field studies were conducted which confirmed and quantified the impact of the mite on the fern. Field studies also elucidated the effect of climatic factors and natural enemies on population dynamics of the mite. These studies indicated that mite populations were active year-round and highest during periods of ample soil moisture and moderate temperatures. The impact of predators and pathogens was significant but did not negate the impact of *F. perrepaec* on *L. microphyllum*. Finally, the molecular diagnostics used in the biological control program were critical to discovery of the origin of the invasive fern and selection of the best adapted mite genotype. This result may have implications for other biological control programs, in that knowledge of the origin of the invasive species may lead to discovery of the most efficacious natural enemies.

Acknowledgements

The authors would like to acknowledge the following people: Bob Pemberton, Rich Greene and Ernest Delfosse (USDA–ARS) for research funding; Paul DeBarro and John Curran (CSIRO Entomology) for molecular support; Sebahat Ozman and Dave Walters (University of Queensland) for acarological instruction; and Dave Holdum (DPI) for identifying *H. thomp-soni*. The following individuals provided support and guidance in their respective countries: Hervé Jourdan and Jean Chazeau (Institute de Recherché, New Caledonia), Alex Jesudasan and Dr David (Madras Christian College, India), Amporn Winotai (Thailand Dept of Agriculture, Thailand); and Des O’Toole and Azura Tsang (City University of Hong Kong, China).

References


