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13 **Pre-release Assessment of Impact on Biomass Production of an Invasive Weed, *Lygodium***
14 ***microphyllum* (Lygodiaceae: Pteridophyta), by a Potential Biological Control Agent,**
15 ***Floracarus perrepae* (Acariformes: Eriophyidae).**

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1 **ABSTRACT** A pre-release assessment of impact by a potential biological control agent,
2 *Floracarus perrepae* Knihinicki & Boczek, on the invasive weed, *Lygodium microphyllum*
3 (Cav.) R. Br., was conducted in a two-year study in their native range - Australia. Thirty-two
4 pairs of test plants were planted in a field plot with two levels of shade, with one plant in each
5 pair treated bi-weekly with the miticide abamectin. The mite caused a significant reduction
6 in biomass of above ground stems and leaves and below ground roots and rhizomes. The mean
7 leaf longevity was significantly longer for the treated versus the mite infested untreated plants.
8 Populations of native predator mites were low throughout the study, however, the mite pathogen
9 *Hirsutella thompsonii* Fisher was common in the second year of the study, but neither reduced
10 the impact of *F. perrepae*. Based on its potential to cause significant damage to *L. microphyllum*
11 under field conditions in the native range and extremely narrow field host range, *F. perrepae* is
12 an excellent candidate for biological control of this invasive fern in Florida, USA.

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14 **KEY WORDS** Predictive studies, biological control weeds, Florida Everglades
15 restoration.

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1 Predicting the impact of natural enemies is an important but underdeveloped part of the science
2 of biological control (Shea and Possingham 2000, Wratten and Gurr 2000). Pre-release
3 assessment of impact caused by candidate agents is one of the tools that may be useful in the
4 prioritization and selection process in a biological control program. Prioritizing the agents that
5 show the greatest potential to control the target organism could potentially minimize the numbers
6 of species released in a program (Hoddle and Syrett 2002, Balciunas 2004). Limiting the
7 number of species released may reduce risk to non-target species and improve success (McEvoy
8 and Coombs 1999, Strong and Pemberton 2001). However, there are valid concerns that
9 effective agents could be overlooked if the prioritization process is not realistic.

10 Few biological control programs have attempted to quantitatively assess the impact on
11 plant biomass of a candidate biological control agent before release. Waloff and Richards (1977)
12 in an 11-yr insecticidal exclusion study in Britain demonstrated that broom, *Cytisus scoparius*
13 (L.) Link (= *Sarothamnus scoparius*), when protected from insect attack outgrew plants exposed
14 to the native insect fauna. Balciunas and Burrows (1993), also using chemical exclusion,
15 demonstrated reduction in biomass of the sapling paperbark trees, *Melaleuca quinquenervia*
16 (Cav.) S. T. Blake, by naturally occurring herbivores. Cage studies have also been used to
17 demonstrate impact of agents. Kleinjan et al. (2003), in greenhouse cage studies, measured the
18 direct impact on the tuber biomass of bridal creeper, *Asparagus asparagoides* (L.), related to
19 above ground feeding by the cicadellid *Zygina* sp. This study was used in the prioritization
20 process of the bridal creeper biological control program, which led to selection of this agent for
21 release. *Zygina* sp. has become established and initial reports of impact are positive (Batchelor
22 and Woodburn 2002). Briese (1996) assessed the potential impact of the weevil, *Lixus cardui*
23 Olivier, on the growth of the *Onopordum* spp. thistles using field cage studies. *Lixus cardui* was

1 found to reduce plant height and biomass by up to 50 % and the plants produced 80 % fewer
2 viable seeds. Post release studies of *L. cardui* in Australia confirm the earlier predictions of
3 impact (Swirepik and Smyth 2002). Field studies in the native range of the thistle, *Carduus*
4 *nutans* L., were conducted to assess the impact of root-feeding insects (Sheppard et al. 1995).
5 The studies showed that two weevils, *Hadroplontus trimaculatus* F. and *Trichosirocalus*
6 *horridus* Panzer, mainly altered plant architecture, whereas the syrphid fly, *Cheilosa corydon*
7 Harris, reduced seed production by 45 %. Although *C. corydon* was prioritized in the biological
8 control program, rearing difficulties in quarantine prevented further study of this potential agent.

9 In our study, the impact of a single species of a native herbivorous mite on its native host
10 plant was measured in a field plot study prior to its release as a biological control agent.

11 Although the study was conducted in the native range within close proximity to native stands, the
12 plot itself was not a natural stand. We chose to create the study site in order to standardize plant
13 size, provide optimal growing conditions, and avoid ethical concerns related to destructive
14 sampling and chemical use in natural areas. The study was conducted for two years to evaluate
15 long-term impacts of the mite on the fern. These studies were intended to: 1) confirm field
16 observations that plant damage was caused by the mite, and 2) measure the mite's impact on
17 plant biomass production.

18 The target weed in this study is *Lygodium microphyllum* (Cav.) R. Br. (Lygodiaceae,
19 Pteridophyta), the Old World climbing fern. It is native to the Old World wet tropics and
20 subtropics of Africa, Asia, Australia, and Oceania (Pemberton 1998). It is an aggressive invasive
21 weed in moist habitats of southern Florida (Pemberton and Ferriter 1998) and is classified as a
22 Category I invasive species by the Florida Exotic Plant Pest Council (Langeland and Craddock
23 Burks 1998). Exploration for natural enemies of this weed was conducted between 1997 and

1 2002 in Australia, China, India, Indonesia, Malaysia, New Caledonia, Singapore, Taiwan,
2 Thailand, and Vietnam. Two species of mites and 20 insect species were collected (Goolsby et
3 al. 2003). The eriophyid mite, *Floracarus perrepae* Knihinicki & Boczek, was given the highest
4 priority for further evaluation as a biological control agent, including host range testing. This
5 agent was prioritized from the list of herbivores collected in the surveys because it was the most
6 widely distributed and appeared from field observations to have a significant debilitating impact
7 on the plant over time. Feeding by the adults and immatures causes formation of leaf roll galls
8 (Freeman et al. 2004), which leads to necrosis and defoliation of *L. microphyllum* pinnules, and
9 appears to limit plant growth. Although the use of eriophyid mites in biological control of weeds
10 shows great promise, several authors including Briese and Cullen (2001) have stated that there
11 are not yet any dramatic successes that can be attributed to the singular impact of an eriophyid.
12 Bearing this in mind, we sought to measure the impact of *F. perrepae* on *L. microphyllum* in an
13 experimental field setting in the native range.

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Materials and Methods

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The field study was conducted over two seasons from March 2001 to March 2003. A plot of land at CSIRO Long Pocket Laboratories in Indooroopilly, Queensland (27° 30.70'S & 152° 59.81'E) measuring 30 m by 5 m was used for the layout of the 64 *L. microphyllum* test plants. Orientation of site length was approximately east to west and received full sun. The site was leveled prior to planting and 30 liter pots were arranged in a grid with plant pot centers 1 m apart along the column and the columns 1.5 m apart. This utilized the available space and arranged the plant pots in a grid three columns by 22 rows. The plot was split into four blocks on the basis of different shading from adjacent buildings; each block had 16 pots. The plot was

1 shaded with black shade cloth on a metal-framed structure. Blocks 1-3 were covered with two
2 layers of 25% shade cloth (heavy shade), overlaid to represent field light levels in *Melaleuca*
3 *quinquenervia* swamp forests (Goolsby unpublished data). Block 4 had only one layer of shade
4 cloth (light shade). A trellis was constructed over each pot to allow for growth of the climbing
5 fern. The trellis was constructed with 1.8 m high tomato stakes and galvanized chicken wire.
6 Each trellis was oriented north by south to allow for maximum sunlight capture. Each pot was
7 drip irrigated and controlled by an electronic timer. The amount of water was adjusted by season
8 to account for plant growth. A 100mm thick layer of pine bark mulch was used to top dress the
9 entire site to keep weeds to a minimum.

10 *Lygodium microphyllum* plants with rhizomes 3-5 cm in length infested with *F. perrepae*
11 were harvested from a native stand along Running Dog Creek, near Logan, Queensland (27°
12 07.30'S & 152° 58.50'E). Voucher specimens *L. microphyllum* and *F. perrepae* are lodged at
13 the Queensland Herbarium, Brisbane, Australia and Agricultural Scientific Collections Unit,
14 Orange, New South Wales respectively. The plants were placed in pots and allowed to grow for
15 a period of two months. From this collection, a subset of plants was identified for uniformity.
16 Sixty-four *L. microphyllum* with uniform infestations of *F. perrepae* were ranked by size and
17 plants of approximately the same size were selected as pairs. Eight pairs of plants were allocated
18 to each of four plots.

19 The *L. microphyllum* plants were transplanted into the 30 liter pots using a commercially
20 available organic potting mix, with a ratio of 85 parts (1 - 10 mm composted pine bark) organic
21 material: 15 parts medium grade washed river sand. Osmocote® Plus Exact (15N-4P-7.5K +
22 micronutrients) 5 - 6 months slow release fertilizer was added at the time of planting. Five
23 months after the start of the experiment leaf samples were analyzed for nutrient levels and were

1 compared to samples from field sites. Additional fertilizer was added to test plants on two
2 occasions to maintain similar nutrient levels to natural stands. On 21 September 2001, each pot
3 received a soluble fertilizer Aquasol[®] (23N-4P-8K + micronutrients), and 5 - 6 months slow
4 release Osmocote[®] Plus Exact, at label rates. In the second year of the study on 16 August 2002,
5 10 gms of IBDU (Isobutylidene diurea -31% N) was added to each pot. Weeds were removed
6 regularly from the pot surface to reduce competition for nutrients and water.

7 One plant from each pair was randomly selected to receive the treatment, a bi-weekly
8 application of a miticide for the duration of the experiment. Treated plants were sprayed with
9 the miticide abamectin, Vertimec[®], at a rate of 4 ml per liter. A plastic curtain was used to
10 prevent over-spray onto nearby plants. All plants also received a weekly application of *Bacillus*
11 *thuringiensis*, Dipel[®], to control Lepidoptera larvae, especially *Spodoptera* spp.

12 A pair of plants from each of blocks 1-3 was harvested every three months, with two pairs of
13 plants harvested from block 4 at six-month intervals over the two seasons of growth. Above
14 ground leaves and stems were separated from below ground roots in separate drying bags. Dry
15 weights were recorded after two weeks in a drying oven at a temperature of 50 °C. Below
16 ground biomass was further processed using sieves and hand sorting to remove extraneous
17 material clinging to root fibers. After completion of this process the remaining biomass was
18 dried again and weights recorded after a period of approximately two weeks.

19 To determine mite population levels on the plants, one newly expanded mature sterile
20 pinnule (leaflet) was randomly selected from each plant and the numbers of curled (with mites)
21 and uncurled subpinnae (leaves) was recorded. Pinnules were marked with a dot of paint to
22 prevent recounting in subsequent months. To determine the density of mites within curls, a
23 single leaf curl was harvested in an unbiased manner from each plant and the numbers and life

1 stages of *F. perrepae* were counted. We also identified and counted the predator mites within
2 each curl and assessed the presence or absence of the mite pathogen, *Hirsutella thompsonii*
3 Fisher. Observations and counts of mites and pathogens within the curl of the infested
4 subpinnule were undertaken using a dissecting microscope at approximately 100X.
5 Representative adult *F. perrepae* and predator species were removed and placed in 70% ethanol
6 for identification. To measure longevity of the pinnules on plants in both treatments, one newly
7 formed pinnule from each plant was tagged each month and its development followed until
8 senescence and abscission.

9 A split plot analysis of variance was done on the dry weights. Shade and time were
10 assessed between pairs and treatment differences and their interaction with shade and time
11 assessed within pairs. Blocks were excluded from the analysis since it was determined that they
12 had no effect on the plants in high shade. Total dry weight, dry weight of leaves, and the dry
13 weight of roots were analyzed. All the weights were log transformed for the analysis to make the
14 variance independent of the mean. Estimates of treatment differences of dry weights between
15 pairs are shown on the log scale. Back transformation of these differences gives an unbiased
16 estimate of the proportional change in dry weight between treated and untreated plants. Means
17 are reported as \pm standard error throughout, unless otherwise noted.

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Results

20 The miticide was effective in controlling *F. perrepae* on the treated plants (Table 1),
21 although a minor amount of plant damage from the mite was experienced. It is likely that *F.*
22 *perrepae* dispersed by air from the untreated to the treated plants. Once settled on the leaves of
23 the treated plants, mites were able to occasionally survive and induce curls, but successful

1 development was uncommon. Two species of predator mites, *Tarsonemus* sp. and a tydaeid sp.,
2 were found in the curls, but their numbers remained low throughout the study. The predator
3 mites in the test plot were the same species that are common at field sites in southeast
4 Queensland (J.A.G., unpublished data). The mite pathogen, *H. thompsonii*, was present in the
5 field plot in the second year of the study.

6 *Floracarus perrepae* caused a significant reduction in biomass of *L. microphyllum*
7 throughout the two-year study. Figure 1, shows the mean total weights of the treated and
8 untreated plants for each sample date. The three dry weight analyses were very similar. In all
9 analyses shade and its interaction with other factors were not significant. Time was a significant
10 effect ($F_{1,23} = 128.87$, $P < 0.0001$ in total dry weight) as expected, due to growth of the plants
11 during the study. There was a small but significant interaction between time and treatment ($F_{1,23}$
12 $= 2.98$, $P < 0.02$). The treatment effect was strongest ($F_{1,23} = 131.01$, $P < 0.0001$) for total dry
13 weight. Ignoring the interaction between time and treatment, estimated treatment differences of
14 dry weights on the log scale were: total -0.26 ± 0.03 , leaves -0.29 ± 0.04 , and roots -0.19 ± 0.04 .
15 Back transformation of the logs show that overall the mite caused a 49% (95% CI 42-56)
16 reduction in above ground stems and leaves with a 35% (95% CI 23-45) reduction of roots and
17 rhizomes. The relative biomass of the fern above and below ground is seen in the mean weight of
18 the 64 plants at harvest. Average above ground dry weight in grams was greater, at 297 ± 31.9 ,
19 compared to below ground, at 123 ± 13.0 .

20 Leaf longevity was significantly different between miticide-treated and untreated plants.
21 Leaves on untreated plants lived on average 162.4 ± 7.4 ($n = 37$) and 220.8 ± 13.2 ($n = 37$) days
22 on treated plants. Leaves with mite-induced curls developed the characteristic necrotic lesions
23 seen in natural stands of *L. microphyllum* infested with *F. perrepae*. Dr. Roger Shivas, Plant

1 Pathologist, Queensland Dept. of Primary Industries, analyzed the diseased leaves. The
2 pathogens isolated were typical saprophytic fungi. Further analysis for viruses and viroids was
3 negative.

4 After six months of the study, nutrient levels in leaf tissue fell below background levels
5 assayed from native stands of *L. microphyllum* (Table 2). Fertilizer applications brought them up
6 to slightly higher levels as compared with natural stands in Queensland, but similar to levels in
7 Florida where the plant is a vigorously growing invasive weed.

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Discussion

10 *Floracarus perrepae* had a significant impact on *L. microphyllum* in our study. The
11 damage and impact on the plant was consistent over the two-year period. Although there was a
12 significant interaction between the treatment effect and time, this was only observed in the last
13 quarter of the experiment. In the last quarter, the difference in the size of the treated and
14 untreated plants began to narrow. This may be due to the untreated plants becoming slightly root
15 bound and lacking additional trellis to climb upwards. The impact of *F. perrepae* was not
16 different between the two shade levels. Although the plants appeared smaller in the low-shade
17 block, the proportional differences between treated and untreated were the same as in the high
18 shade blocks. We set up the low-shade block to replicate the higher light levels that *F. perrepae*
19 might encounter in Florida. In Florida, *L. microphyllum* is often found growing in nearly full sun
20 and across the canopies of tree islands. It does not appear that these conditions will negatively
21 affect the mite. The impact of *F. perrepae* was greater on the above ground biomass (leaves and
22 stems) than roots. The mite did not visibly alter plant architecture. However, we may have been
23 able to measure a difference in height of the climbing vines if we would have used taller trellises.

1 During our exploration in Australia and Asia for potential biological control agents, *F. perrepae*
2 appeared to cause considerable debilitation and defoliation of *L. microphyllum* (Goolsby et al.
3 2003). We could not be certain if this effect was due to the action of the mite. The effects of *F.*
4 *perrepae* in the biomass plot confirm the plant damage we observed in the field across its native
5 range. The differences observed in longevity of the leaves between treated and untreated plants
6 illustrate this impact. Leaves infested with active colonies of the mite undergo rapid necrosis
7 and senescence, but may not abscise immediately. In retrospect we should have measured the
8 date at which infested leaves became necrotic instead of the date of abscission. This measure
9 may have been a better indicator of leaf damage and impact of the mite. However, it seems
10 logical that this persistent damage to the leaves by the mite reduces the photosynthetic ability of
11 the plant, which in turn limits the growth of the plant. The damage caused by *F. perrepae* is not
12 readily apparent, but when measured over the long term its significance is clearly evident.
13 Population levels of *F. perrepae* in the biomass plot were higher than found in natural stands
14 (J.A.G., unpublished data). This may be due to the low levels of predation experienced in the
15 biomass plot (Table 1). Although we identified the same species of predators that occur in
16 nearby natural stands of *L. microphyllum*, other factors in the biomass plot environment did not
17 allow them to reach high population levels. This is fortuitous because this low level of predation
18 may be similar to what *F. perrepae* will encounter in Florida in the absence of its indigenous
19 predator species.

20 The mite pathogen, *H. thompsonii*, was common in the second year of the study.
21 Although it caused considerable mortality to *F. perrepae* populations, the level of plant damage
22 was relatively unchanged (Table 1). *Hirsutella thompsonii* occurs in Florida where it is a natural
23 control for citrus rust mite, *Phyllocoptruta oleivora* (Ashmead), (Muma 1955, McCoy and

1 Couch 1982). Therefore, we should expect this pathogen to infect *F. perrepae* if it is released in
2 Florida. Based on our experience in Australia with a locally occurring strain of *H. thompsonii*, it
3 does not appear that this pathogen will greatly minimize the impact of *F. perrepae* on *L.*
4 *microphyllum* in Florida.

5 In summary, the assessment of *F. perrepae* shows that it can have significant impact on
6 the target weed *L. microphyllum* in the native range. This attribute plus its apparently narrow
7 host range makes *F. perrepae* an ideal candidate for biological control of *L. microphyllum*.

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1 **Table 1. Percentages of leaves curled and numbers of mites in leaf curls by sample date* on untreated and**
 2 **plants treated with miticide**

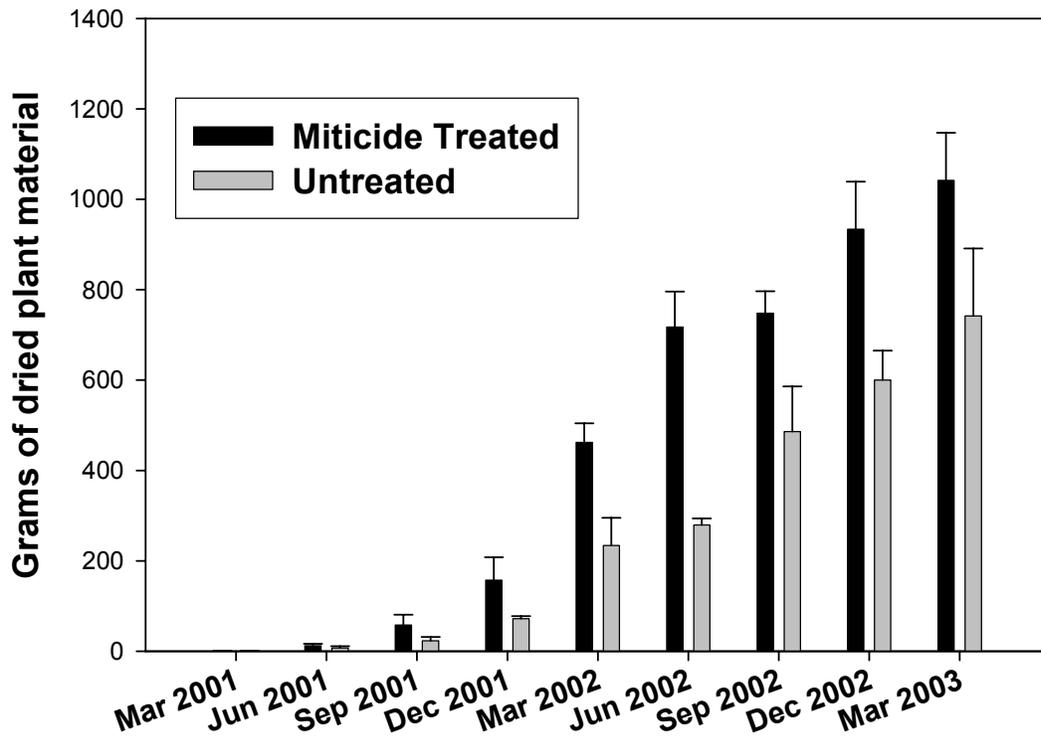
Date	Untreated			Treated				
	% leaves curled	Mean nos. of adults and nymphs in curls	Mean nos. of predator mites per curl	% of curls with mite pathogen	% leaves curled	Mean nos. of adults and nymphs in curls	Mean nos. of predator mites per curl	% of curls with mite pathogen
6/01	84	71.0	0.03	0	10	1.5	0	0
10/01	69	51.4	0	0	1	0.5	0	0
12/01	92	69.1	0.04	0	30	12.8	0.01	0
3/02	38	27.2	0.09	10	7	2.0	0	0
6/02	65	15.5	0.26	50	15	2.0	0.01	12
10/02	63	16.0	0.03	52	3	0.7	0	7
12/02	62	20.6	0.05	16	11	0.3	0	6
3/03	93	33.5	0.07	31	20	0.4	0.03	0

3 * The table shows pooled means for the three months prior to destructive sampling.
 4

1 **Table 2. Nutrient levels in *Lygodium microphyllum* leaf tissue**

Sample Location	% N \pm SD	% P \pm SD	% K \pm SD
Biomass plot untreated before fertilization	2.03 \pm 0.13	0.17 \pm 0.01	1.46 \pm 0.23
Biomass plot treated before fertilization	1.70 \pm 0.03	0.14 \pm 0.01	1.27 \pm 0.05
Biomass plot untreated after fertilization	2.68 \pm 0.11	0.30 \pm 0.13	2.03 \pm 0.18
Biomass plot treated after fertilization	2.85 \pm 0.04	0.32 \pm 0.02	1.49 \pm 0.18
Natural stand – Queensland, Australia	1.91 \pm 0.20	0.19 \pm 0.02	2.41 \pm 0.11
Weedy stand – Florida, USA	2.64	0.29	2.19

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1

2

1 **Fig. 1.** The impact of *Floracarus perrepae* on biomass production of *Lygodium microphyllum* in a two-year
2 chemical exclusion study conducted in the native range of the mite and fern. Treated plants received monthly
3 applications of Agrimec[®] miticide. Bars represent the mean total dry weights of roots, stems and leaves \pm SE.