Austromusotima camptozonale (Lepidoptera: Crambidae) Herbivory Results in Frond and Rhizome Mortality of the Invasive Fern Lygodium microphyllum (Schizaeles: Lygodiaceae)

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AUSTROMUSOTIMA CAMPTOZONALE (LEPIDOPTERA: CRAMBIDAE) HERBIVORY RESULTS IN FROND AND RHIZOME MORTALITY OF THE INVASIVE FERN *LYGODIUM MICROPHYLLUM* (SCHIZAELES: LYGODIACEAE)

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Abstract

Old World climbing fern, *Lygodium microphyllum* (Cav.) R. Br.; Schizaeles: Lygodiaceae), is among the most problematic invasive weeds in southern Florida, USA, where it smothers and displaces native vegetation. Chemical and mechanical control methods that target aerial fronds may not provide adequate control of *L. microphyllum* as underground rhizomes produce new fronds following treatment. Alternatively, biological control involves persistent feeding damage from introduced herbivores and may be an additional control measure for the management of the exotic weed. Herein, we hypothesized that high levels of persistent herbivory will reduce foliar biomass, kill underground rhizomes, and increase the number of other plant species. This hypothesis was tested over an 18 month period by placing 6 cages over *L. microphyllum* patches growing in a natural setting and repeatedly inoculating 3 of the cages with the Australian moth *Austromusotima camptozonale* (Hampson) (Lepidoptera: Crambidae) while the remaining 3 caged patches served as untreated controls. Growth of *L. microphyllum* was markedly reduced in herbivore treated patches versus untreated controls. Rhizome density and growth also decreased in herbivorycages, where rhizomes stopped producing fronds within 12 months and destructive sampling at 18 months showed complete rhizome mortality within these patches. The number of species within patches, however, remained similar among herbivory and control treatments. These findings represent the first evidence that chronic herbivory by arthropods can kill *L. microphyllum* rhizomes.

Key Words: Old-world climbing fern, biological control, rhizome density, chronic herbivory

Resumen

El helecho trepador del Mundo Antiguo, *Lygodium microphyllum* (Cav.) R. Br.; (Schizaeles: Lygodiaceae), es una de las malezas invasoras más problemáticas en el sur de Florida, EE.UU., donde ahoga y desplaza la vegetación nativa. Los métodos químicos y de control mecánico que se dirigen a las frondas aéreas possiblemente no proveen un control adecuado de *L. microphyllum* dado que los rizomas subterráneas producen nuevas hojas después del tratamiento. Como alternativa, el control biológico implica un daño persistente por la alimentación de los herbívoros introducidos y puede ser una medida de control alternativa para el manejo de la maleza exótica. Tenemos una hipótesis de que altos niveles de herbivoría persistente reducirán la biomasa foliar, matará los rizomas subterráneos, y aumentará la diversidad de especies de plantas. Esta hipótesis se puso a prueba durante un período de 18 meses mediante la colocación de 6 jaulas sobre un grupo de plantas de *L. microphyllum* que crecieron en un entorno natural y la inoculación repetida de 3 de las jaulas con la polilla australiana, *Austromusotima camptozonale* (Hampson) (Lepidoptera: Crambidae), mientras que las otras 3 jaulas servian como controles no tratados. El crecimiento de *L. microphyllum* se redujo notablemente en las jaulas tratadas con herbívoros que en los controles no tratados. La densidad y el crecimiento de los rizomas también disminuyeron notablemente en las parcelas con herbívoros, donde los rizomas dejaron de producir frondas dentro de 12 meses y un muestreo destructivo a los 18 meses mostraron una mortalidad completa de los rizomas dentro de las jaulas tratadas. La diversidad de especies dentro de las jaulas, sin embargo, se mantuvo similar entre los tratamientos de herbivoría y control. Estos hallazgos representan la primera evidencia de que la alimentación crónica por artrópodos herbivoros puede matar las rizomas de *L. microphyllum*.

Palabras Claves: helecho trepador del Mundo Antiguo, control biológico, densidad de rizoma, herbivoría crónica

Old World climbing fern, *Lygodium microphyllum* (Cav.) R. Br. (Schizaeles: Lygodiaceae) is native to the Old World tropics of Africa, Asia, Australia and the Pacific Islands. Since the first report of its naturalization in the southeastern coast of Florida (Beckner 1968), this exotic fern has rapidly spread across the south Florida landscape, where it damages plant communities by blanketing vegetation in wetland habitats (Langeland & Burks 1998; Pemberton et al. 2002; Volin et al. 2004). Recent reports indicate that Florida's *L. microphyllum* population originated from north Queensland, Australia via England through the ornamental plant trade (Goolsby et al. 2006). During 1960-70s, *L. microphyllum* was restricted to parts of Palm Beach and Martin Counties on the east coast of Florida but has since spread to > 25 counties in the central and southern portions of the state (Ferriter & Pernas 2006); more recently, it has been reported from Jacksonville area of Duval County, Florida (Smith et al. 2014). This high rate of spread may be attributed, in part, to the high rate of suspended spores in the air (800 spores/m^3/h) available for dispersion (Pemberton & Ferriter 1998) via prevailing winds. Populations of the fern are predicted to expand into northern Florida, Texas, Mexico, Caribbean islands, and portions of Central and South America (Goolsby 2004). Pemberton et al. (2002) has noted naturalization of *L. microphyllum* in parts of Jamaica and Guyana.

Lygodium microphyllum is difficult to manage using controlled burns, mechanical removal, or herbicide applications due to the plant's ability to regenerate from underground rhizomes (Stocker et al. 1997; Goolsby et al. 2003, Hutchinson et al. 2006). Despite satisfactory kill of aerial vines from herbicides, 3-5% of the *L. microphyllum* individuals regrow and require repeated treatments to achieve mortality of the perennial underground rhizomes (Ferriter 2006; Lockhart 2007; Boughton & Pemberton 2008). Until recently, however, chemical and mechanical control measures were the only options for suppressing the invasion of the exotic fern. A biological control program was initiated in 1998 with the expectation that introduced herbivores would provide a long-term, environmentally benign solution to the management of *L. microphyllum* in Florida (Pemberton 1998). Following extensive host range testing, the Australian moth *Austromusotima camptozonale* (Hampson) (Lepidoptera: Crambidae) was released into *L. microphyllum* dominated sites of Florida in 2005. Small field releases were made at 17 *L. microphyllum* patches in Palm Beach County in an effort to establish a viable population in Florida (Boughton & Pemberton 2008). However, *A. camptozonale* populations did not persist beyond the 2nd generation due, in part, to predation and high temperatures (Boughton & Pemberton 2008). A laboratory colony of *A. camptozonale* was maintained to continue establishment efforts.

Following the failure of *A. camptozonale* to establish, focus was redirected to other permitted *Lygodium* biological control agents. The leaf galling mite, *Floracarus perrepae* Knihinicki & Bockzeck (Acari: Eriophyidae), was released in 2008 and established a small population at the release site in Palm Beach County (Boughton & Pemberton 2011). The foliage feeding moth *Neomusotima conspurcatalis* Warren (Lepidoptera: Crambidae), introduced in 2008 from northern Australia also did establish at the same Palm Beach County study site (Boughton & Pemberton 2009). Dispersal and feeding damage from both established biological control agents were initially limited, being restricted to the immediate vicinity of the release foci (Boughton & Pemberton 2009). Considering the apparent ease of establishing *N. conspurcatalis*, renewed efforts were returned to the release and evaluation of *A. camptozonale*.

Herbivory from any successful *L. microphyllum* biological control agent must deplete rhizome carbohydrate reserves to effectively suppress regrowth and survival of the invasive fern. Can persistent feeding by a folivore reduce or eliminate vine production from *L. microphyllum* rhizomes? The objectives of this study were to: 1) establish a population of *A. camptozonale* in a more southern region of the target weed's geographic distribution in Florida and 2) quantify the influence of chronic feeding of the aerial fronds by *A. camptozonale* on rhizome regrowth and survival.

Materials and Methods

Research Site

A research site was selected near Homestead, Florida (N 25° 28' 22.4" W 80° 21' 16.4") based on the presence of discrete *L. microphyllum* patches that served as replicated study cages. All *L. microphyllum* patches were carefully inspected to ensure that they were *F. perrepae* and *N. conspurcatalis* free. The site was characterized as a brackish wetland dominated by a *Laguncularia racemosa* L. (white mangrove) and *Conocarpus erecta* L. (button-wood) forest with the exotic *Casuarina* sp. intermixed. The soil type was predominantly organic and remained saturated with water for about 9 months during the experimental period.

Six incipient *L. microphyllum* patches (ranging from 81-105 cm diam and 106-168 cm height) were selected within the research site and each patch was enclosed in a screen-covered cage (1.83 m^3) fitted with vertical zipper. Cages were randomly assigned to either herbivory (from *A. camptozonale*) or control (no herbivory) treatments. All discernible plants but *L. microphyllum* within cages were counted. Growth of *L.*

microphyllum in each cage was also quantified as the maximum height, average patch width [(broadest + narrowest points)/2] and *L. microphyllum* coverage (proportion of the cage blanketed by the fern as visually estimated by 2 or more persons and averaged). Cage assessments were conducted at the onset of the experiment (4th week of Jul 2011) and again during the 3rd week of Jul 2012.

Austromusotima camptozonale Colony, Releases, and Assessment

A colony of *A. camptozonale* was maintained at the USDA/ARS Invasive Plant Research Laboratory following the procedure described in Boughton et al. (2008). Herbivory treatment cages were inoculated in Jul 2011 with *A. camptozonale* larvae (Fig. 1). Because the cages varied in patch size, the total number of larvae released in each herbivory cage varied (17,225, 19,100, and 19,825 moth individuals in cages 1, 2 and 3, respectively). Subsequent larval releases were made at ca. one month intervals to exploit vegetation regrowing from rhizomes; no releases were made in Sep 2011 and Mar 2012 as no regrowth was apparent. The last larval release was made on May 5 2012. The proportion of dead frond (brown foliage with no living (green pinnules) coverage in relation to total *L. microphyllum* coverage was estimated for each cage through visual estimation as described above. Following each larval release, about 50 gm of granular insect bait Maxforce Complete® [(Hydramethylnon: tetrahydro-5, 5-dimethyl-2(1H)-pyrimidinone, (3-(4-trifluoromethyl) phenyl)-1-92-(4-(trifluoromethyl)phenyl) etyhynyl)-2-proponylidine)hydrazone); Bayer Environmental Science, 2 T. W. Alexander Drive, Research Triangle Park, North Carolina 27709] was sprinkled on the ground within caged area to avoid possible ant predation of released moth larvae. Assessments of all cages were done and data were gathered at the onset of the experiment, the 3rd week of Jul 2012, and the 4th week of Dec 2012. During these assessments, the percentage (of the total *L. microphyllum* cover in caged area) of dead fronds was estimated for both control and moth cages. Additional monthly assessments of dead frond coverage were also made for herbivory cages only to monitor the rate of frond mortality.

In addition to the releases in the caged patches, *A. camptozonale* larvae were also liberated in open field *L. microphyllum* patches adjacent (within 100 m) to the study cages at the same intervals as those released into the herbivory cages. These open field releases also ranged in size, from 150–19,000 larvae per release, and a total of 43,985 larvae were inoculated over the 9 release events.

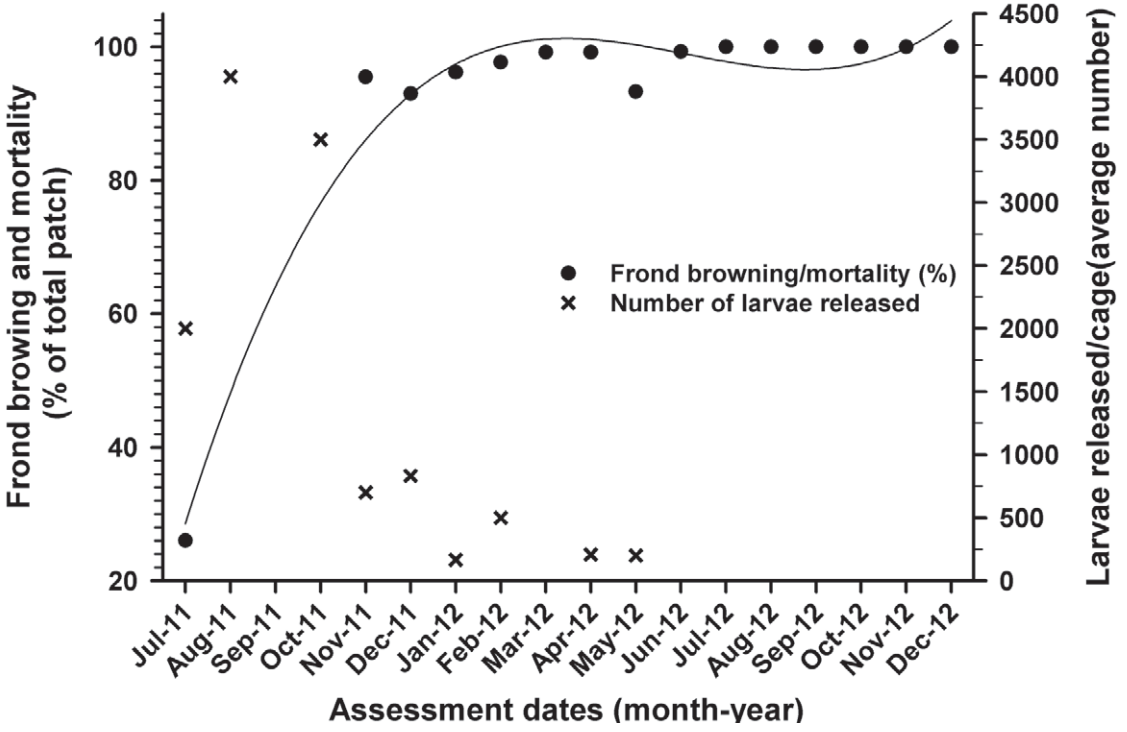

Fig. 1. Mean densities of released *Austromusotima camptozonale* larvae and the resulting percentage of dead *Lygodium microphyllum* foliage in caged patches.

Also, herbivory cages and the open field release areas were monitored for biocontrol moth establishment by searching *L. microphyllum* vegetation for *A. camptozonale* adults, larvae or eggs.

Lygodium microphyllum Rhizome Incidence, Growth and Mortality Assessment

A permanent central point within each cage was marked using a flag. Twelve transects were delineated within each cage, originating at the central point and radiating to the edge of the *L. microphyllum* patch. Transects were uniformly distributed around the central point at 30° angles, like spokes on a wheel. The presence or absence of a *L. microphyllum* rhizome was quantified at 3 randomly selected points along each transect. To avoid confusion with other species, some rhizomes were partially exposed by carefully removing the soil cover to confirm identifications. Assessments were conducted during the 4th week of Jul 2011 and the 3rd week of Jul 2012. At the conclusion of the study (Dec 2012), transects in each cage were re-examined for the presence of live fronds arising from underground rhizomes. In addition, all rhizomes occurring below 6 of the 12 transects in each cage were harvested, washed and examined under a dissecting microscope (40 ×). Rhizomes with new growth, green distal tips, or white central tissues were considered healthy while dried rhizomes with brown distal ends and discolored (light-brown to dark-brown) central tissues were considered dead.

Data Analysis

Statistical analyses were performed using the General Linear Models procedure (PROC GLM) in SAS (1999). Proportional data (dead: live fronds, percent coverage) were arcsine transformed before analysis; non-transformed means and percentages are presented in the tables. Initial measures of within treatment patch height, patch width, changes in percent cover, species diversity, rhizome length and incidence were compared with those gathered at the end of the experiment (Jul 12, 2012). Mean separations were performed using Duncan's Multiple Range Test procedure.

Results

An average of 18,717 larvae were released within each herbivore cage during the 12-month period (Fig. 1). Larval feeding resulted in browning of the *L. microphyllum* patches, which were initially replaced by new vines from the pinnule-axils. The proportion of dead to live fronds was greater in the herbivory versus control treatments ($P < 0.0001$, $F = 1767.5$, df = 1), where the ratio of dead fronds steadily increased during the first 4 month evaluation period and fluctuated slightly during the remaining 7 months. However, mortality (100% browning) of all fronds was achieved in patches by Jul 2012 and patches did not produce new growth through the end of Dec 2012 (Fig. 1); at least 6 additional casual observations were made as of Dec 2013 but no new growth was present.

The proportion of the dead fronds in *L. microphyllum* patches at the onset of the experiment was not significantly different between herbivory and control treatments (Table 1). However, the dead fronds in *L. microphyllum* patches were greater in herbivory cages than in control cages for both 12 month and 18 month assessments (Table 1).

Within treatment analysis showed no significant changes in *L. microphyllum* patch height, width and coverage between Jul of 2011 and 2012 sampling dates for control cages (Table 2). In herbivore treatment cages, patch width and overall coverage were reduced from Jul 2011 to 2012; however, height difference was not statistically different due to higher within treatment variability (Table 2).

A comparison of underground rhizome length in control patches showed a slight ($P = 0.4986$; $F = 0.46$; df = 1) increase from Jul 2011 to 2012 (Fig. 2). In the herbivory treatment, in contrast, live rhizome length significantly decreased ($P < 0.0001$; $F = 188.06$, df = 1)due to rhizome mortality during the same sampling period (Fig. 2).

The incidence (= relative density) of the underground rhizomes of *L. microphyllum* in control patches did not change ($P = 0.1210$; $F = 2.12$; df = 1) over time. In moth herbivore treated cages, however, the rhizome incidence decreased significantly ($P < 0.0001$; $F = 85.58$; df = 1) from Jul 2011 to Jul 2012 (Fig. 3). Overall, total plant densities other than *L. microphyllum* did not change in either the control ($P = 0.7857$; $F = 0.14$; df = 1) or herbivore ($P = 0.3641$; $F = 0.84$; df = 1) treatments over time (Fig. 4).

Surveys of the general release site outside cages revealed F_1 (first generation) larvae and larval feeding damage up to 120 m from the release foci on 2 occasions (Dec 2011 and Feb 2012).

Table 1. Percentage of dead fronds in replicated *Lygodium microphyllum* patches in moth treated and control cages.

Date of assessment	Control	Herbivory
July 2011	17.3 (± 4.3) a	26.0 (± 7.0) a
July 2012	21.3 (± 3.0) b	99.3 (± 0.6) a
December 2012	24.0 (± 3.1) b	100.0 (± 0.0) a

Numbers in the parentheses represent standard error of the mean. Means associated with different letters are significantly different from each other ($P = 0.05$, $n = 3$) as per Duncan's Multiple Range Test.

TABLE 2. *LYGODIUM MICROPHYLLUM* PATCH HEIGHT (CM), DIAMETER (CM), AND COVER PERCENTAGE OF TOTAL CAGE AREA DURING THE 18-MONTH EXPERIMENTAL PERIOD.

Treatment plots (patch)	Assessment dates	
	July 11, 2011	July 12, 2012
Control		
Height	132.3 (± 4.6) a	173.7 (± 5.5) a
Diameter	101.7 (± 26.0) a	120.3 (± 24.9) a
Cover	32.7 (± 6.1) a	49.3 (± 48.6) a
Moth-treated		
Height	114.0 (± 4.6) a	41.7 (± 41.7) a
Diameter	82.3 (± 23.87) a	3.7 (± 3.7) b
Cover	26.7 (± 3.2) a	0.3 b (± 0.3) b

Numbers in the parentheses represent standard error of the mean. Means associated with different letters are significantly different from each other ($P = 0.05$, n = 3) as per Duncan's Multiple Range Test.

No life stages of *A. camptozonale* or larval feeding damage were observed during subsequent assessments. Often, predators, including ants and exotic lizards, were observed capturing and feeding on the larvae shortly after release even though the insect bait was added at the time of previous month's releases.

DISCUSSION

Underground rhizomes of perennial plants are known to influence aboveground stem densities, growth, and reproduction (Ashmun & Pitelka 1985; Cain 1990). Below ground, rhizome expansion results in increased lateral spread within a patch as well as increases interspecific competition. One explanation for the superior competitive ability of *L. microphyllum* is the rapid expansion of perennial rhizomes that, in conjunction with fast growing rachises, displace native species in most natural systems in southern Florida (Pemberton & Ferriter 1998). The data presented herein indicate that chronic feeding damage from *A. camptozonale* on *L. microphyllum* pinnulets limits the plant's ability to replace damaged rachises. This finding demonstrates that *L. microphyllum* is unable to compensate for high levels of herbivory on above ground foliage. Additionally,

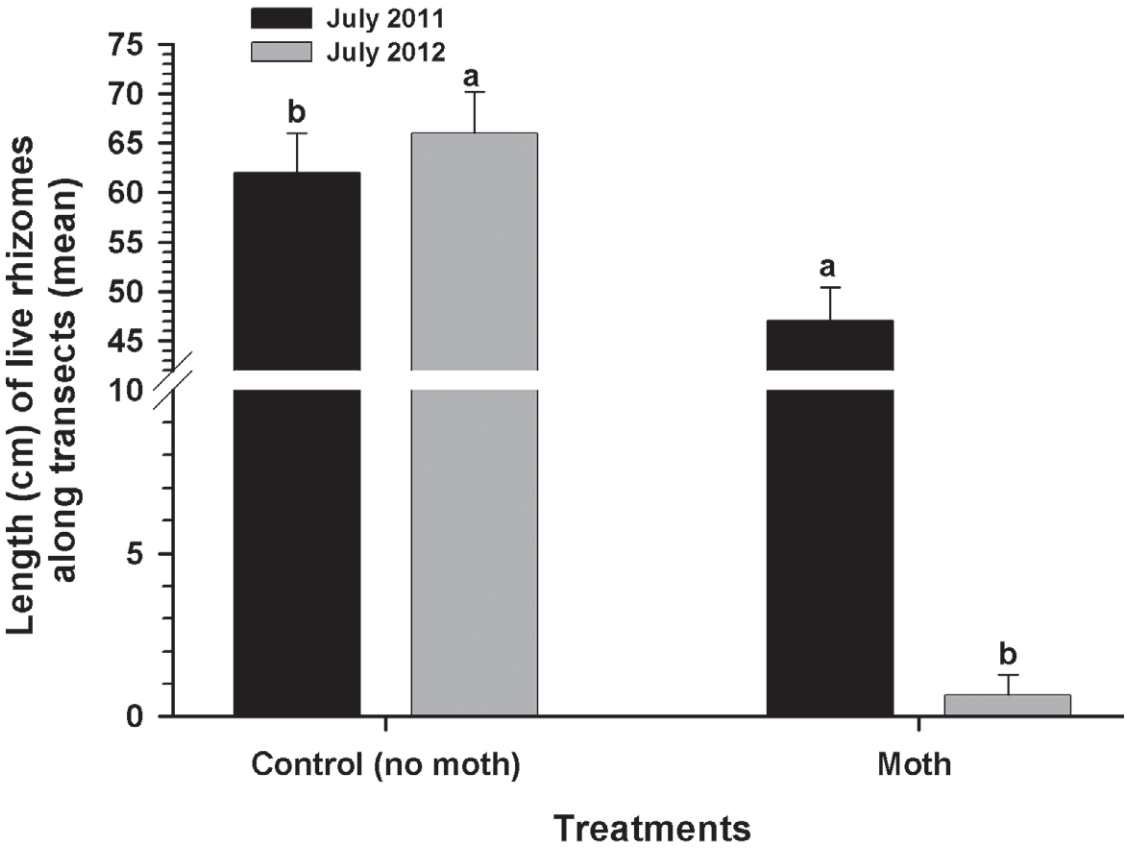

Fig. 2. Effects of *Austromusotima camptozonale* larvae on *Lygodium microphyllum* rhizome growth.

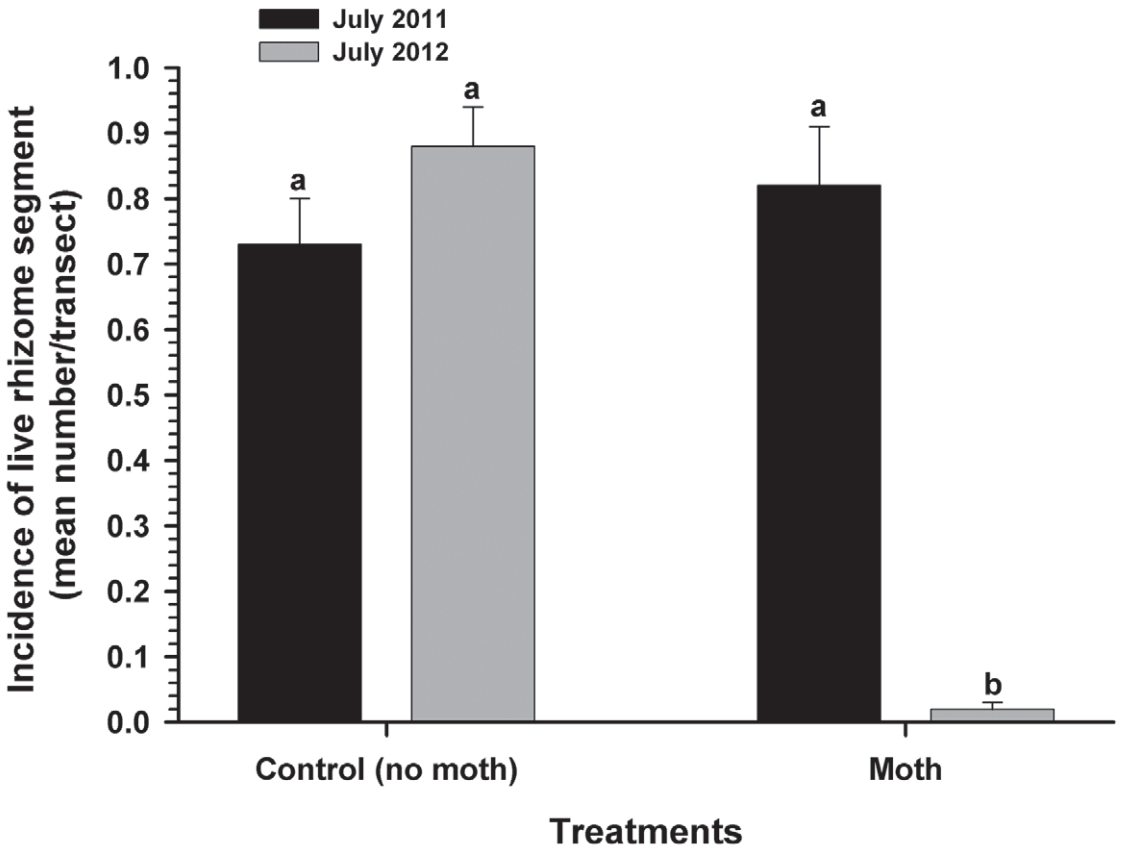

Fig. 3. Effects of *Austromusotima camptozonale* larvae on *Lygodium microphyllum* rhizome incidence.

these repeated feeding bouts also resulted in the shrinkage of *L. microphyllum* patch dimensions, reduced rhizome length, rhizome frequency, and ultimately resulted in rhizome mortality. These data represent the first evidence that herbivory can kill *L. microphyllum* plants, including their perennial rhizomes. Caution should be used when interpreting these data, however, as herbivore densities used in this study exceeded the carrying capacity and were sustained at these levels so as to elicit the greatest level of feeding damage. Despite the artificiality of the inundated *A. camptozonale* releases, it is clear that folivory can influence growth and survival of *L. microphyllum*.

These negative consequences of above ground herbivory on the performance of below ground rhizomes are not unprecedented. Harnett (1989), while studying the effects of defoliation on the different densities of the rhizomatous grasses *Andropogon gerardii* Vitman (Poaceae) and *Panicum virgatum* L. (Poaceae), concluded that defoliation caused overall reduction in biomass, tillering, rhizome densities and seed production. Similarly, Cain et al. (1991) studied the effects of the long-term chemical suppression of insect herbivores from the field populations of *Solidago altissima* L. (Asterales: Asteraceae) and concluded that the ramet height, rhizome lengths, rhizome biomass and number of daughter rhizomes were lower in herbivore defoliated cages compared with those protected from herbivores. In another study, Johnson et al. (1986) studying potato (*Solanum tuberosum* L.; Solanales: Solanaceae) plants reported the reduction of underground tuber production due to above ground herbivory.

Neither inundated releases within caged patches nor open field releases resulted in persistent populations of *A. camptozonale* at the study site. Past releases of *A. camptozonale* ranged between 1,000-2,000 larvae per site during 2004-2005 and did not result in establishment beyond the 2nd generation in Palm Beach County, Florida (Boughton & Pemberton 2008). The cumulative average number of larvae released per cage in this study was 18,717 (± 1342) but the 9 individual releases per cage over time varied from 100 to 5,000 (Fig. 1). Similarly, the cumulative number of individuals released in the open field patches of *L. microphyllum* was 43,985 larvae, with one of the releases (Feb 2012) exceeding 19,000 larvae.

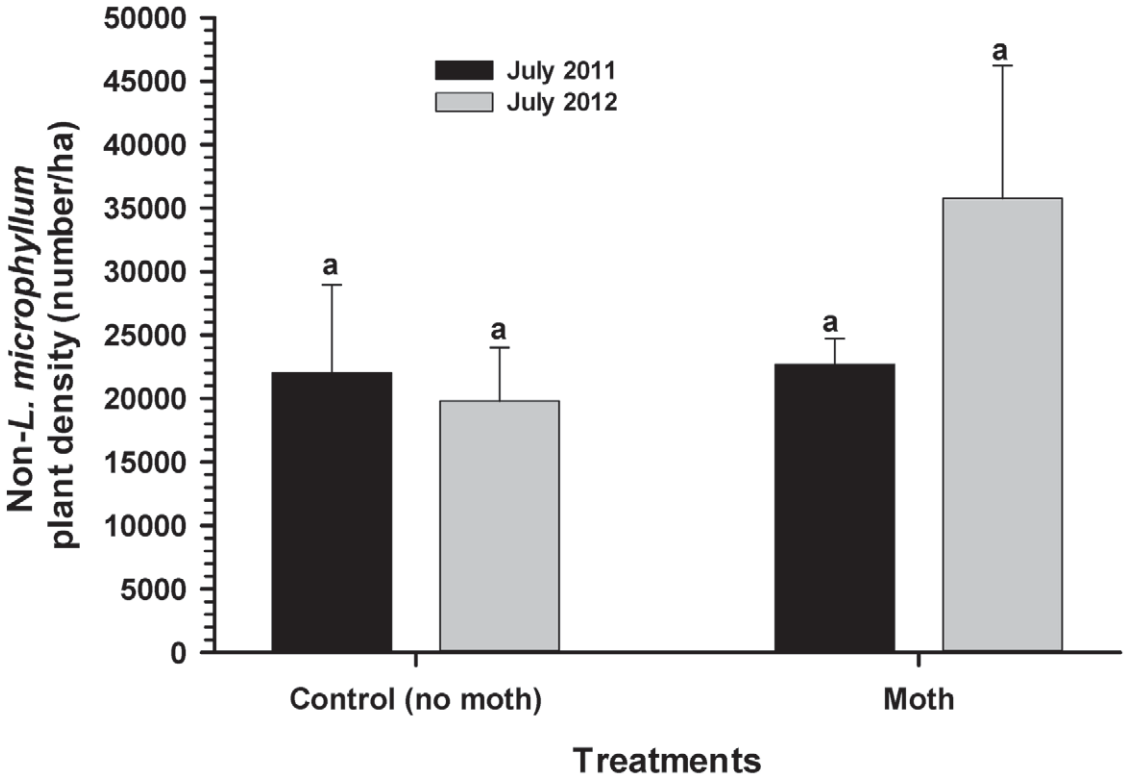

Fig. 4. Effects of *Austromusotima camptozonale* larvae on the density of plants other than the target weed*Lygodium microphyllum*.

These data corroborate the assertion by Boughton & Pemberton (2008) that establishment failure of *A. camptozonale* is not due to a small founding population size. These numbers are comparable to founding populations of other successfully established herbivores, including the gall flies *Urophora affinis* (Frauenfeld) (Tephritidae) and *Procecidochares alani* Steyskal (Tephritidae), the chrysomelid beetles *Galerucella calmariensis* (L.) and *Galerucella pusilla* (Duft.), as well as the *Melaleuca* biological control agent *Oxyops vitiosa* Pascoe (Curculionidae) (Story & Anderson 1978; Grevstad 1999; Center et al. 2000; Barton et al. 2007). In addition, these release densities were similar to the generalized minimum effective population size used as a "rule of thumb" for species conservation (Frankel & Soule 1981).

Limited observational evidence, however, indicates that predation from ants and lizards as well as other unidentified micro environmental conditions may have attributed to the herbivore's failure to persist at the research site. In previous studies, Boughton & Pemberton (2008) also reported the common occurrence of predators attacking *A. camptozonale* in Palm Beach County of Florida. However, another closely related species (*N. conspurcatalis*) did establish in the same areas of Palm Beach County and preliminary observations indicate successful establish of the moth in several *L. microphyllum* populations Miami Dade County (Smith et al. in press). Additional research is needed to investigate the disparity in establishment success between these closely related *A. camptozonale* and *N. conspurcatalis* moths in the Florida populations of *L. microphyllum*.

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