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EFFORTS TO ESTABLISH A BIOLOGICAL CONTROL AGENT AGAINST INCIPIENT INFESTATIONS OF OLD WORLD CLIMBING FERN IN SOUTHWEST FLORIDA

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Old World climbing fern, *Lygodium microphyllum*, (henceforth climbing fern) has become a serious invasive weed in moist habitats across southern Florida (Volin et al. 2004). Climbing fern grows rapidly over other plants, smothering understorey species and changing local fire ecology, by carrying fires into tree canopies, killing trees that would ordinarily survive ground fires (Pemberton & Ferriter 1998). Climbing fern was likely introduced during the late 19th century as an ornamental plant (Pemberton & Ferriter 1998), subsequently escaped cultivation and was reported naturalized in coastal Martin County in southeast Florida by 1965 (Beckner 1968). Like other ferns, *L. microphyllum* reproduces by spores (Lott et al. 2003), which can be carried aerially over long distances. This has contributed to rapid spread of this weed (Ferriter & Pernas 2006). *Lygodium microphyllum* is now present in all counties in central and southern Florida below a line running from Hernando County on the Gulf Coast to Volusia County on the Atlantic coast (Anonymous 2011). Conventional management of climbing fern is not sustainable across the huge acreages and remote locations currently infested (Hutchinson et al. 2006; Stocker et al. 2008). Biological control may offer the best prospect for long-term management (Pemberton 1998). Among the 3 biological control agents that have so far been released, only the brown lygodium moth, *Neomusotima conspurcatalis* (Lepidoptera: Crambidae), readily established large field populations (Boughton & Pemberton 2009). It has successfully over-wintered for 3 years at fern-infested sites in Martin County and can reach high densities. Preliminary data indicate that when *N. conspurcatalis* populations are high, larval defoliation can suppress climbing fern at lower levels than before the agent was released (Boughton, unpublished data). The objectives of this applied study were two-fold: 1) to determine whether mass-releases could be used to establish populations of *N. conspurcatalis* against incipient (low level) infestations of climbing fern in southwest Florida, and 2) if establishment was successful, to determine whether populations of *N. conspurcatalis* could either reduce ground cover of climbing fern, or at least suppress the weed and prevent infestations from becoming worse.

Studies were conducted in Flatford Swamp, in Manatee County. Two sites where the insect was released were located 0.5-1.5 miles (0.8-2.4 km) from 2 control sites where no insects had been released. Ground cover of climbing fern was measured at the start of the project to provide a reference point for any future reductions caused by the biocontrol agent. Vegetation measurements were taken along two 20-m transects at each of the 4 sites in 2009 to collect baseline data. Transect end-points were permanently marked so that the same areas of vegetation could be re-examined during 2010 and 2011. Climbing fern was measured using linear cover methods, by visually assessing percent cover within each meter-segment underneath a graduated, rope-line transect. Percent cover was also visually assessed inside seven 0.25 m^2 quadrats located along transects at fixed distances from the origin. Two experienced researchers participated in each transect measurement, and independent assessments from both individuals were averaged to arrive at final cover estimates.

The initial mass-release of 16,400 *N. conspurcatalis* larvae was conducted during Sep 2009 from a laboratory colony maintained at the USDA Invasive Plant Research Laboratory, Fort Lauderdale, Florida. This colony was established from insects collected in Kununurra, Australia in May 2007. The insect was reared on *L. microphyllum* foliage (Boughton et al. 2009), and 4,100 larvae were distributed along each of the 2 transects at both release sites by transferring caterpillar-infested foliage from rearing boxes onto field plants. Timed-searches of climbing fern foliage (30 min search time along each transect) were conducted at release sites in Nov 2009 and in Apr 2010, but yielded no recoveries of insects or evidence of feeding damage. Following this apparent failure, methodology was changed for a second mass-release in 2010. Because establishment failures can result from reduced adaptability of insects maintained for extended periods in the laboratory (Van Driesche 1993), field-adapted *N. conspurcatalis* that had survived in the wild since being released in 2008, were used in 2010. In total, 311 larvae were field collected during Jul 2010 and returned to the laboratory for rearing. Emerging moths were allowed to mate and oviposit. A total of 7600 progeny larvae were transferred to rearing boxes, and transported to Flatford Swamp

in Aug 2010, where 1,900 were distributed along each of the 2 transects at both release sites. Using the same methodology as in 2009, timed searches of release transects conducted in Oct 2010 recovered 96 and 54 larvae, respectively, along transects at one of the release sites, while foliar damage characteristic of recent feeding by *N. conspurcatalis* larvae (Boughton et al. 2009) was present on climbing fern along both transects at the other release site. Although it is difficult to draw reliable conclusions from such a limited number of trials, the differing outcomes of releases conducted at the same sites, at similar times of the year, using fewer than half as many insects in 2010, would appear to confirm the superiority of field-collected insects for colonization efforts. Evolutionary theory would suggest that natural selection had acted upon underlying genetic variation present in field populations of *N. conspurcatalis*, giving rise to individuals that were better adapted with regard to various useful attributes, such as mate finding ability or avoidance of predation, and that these adaptive traits (Messenger et al. 1976) explain the improved ability of field-collected insects to initiate new field populations, relative to lab-reared stock.

Although *N. conspurcatalis* was present at both release sites during late fall 2010, there was no evidence it was still present in Apr 2011. Typical average monthly low temperatures in this area of southwest Florida during winter are 12.3, 11.0 and 12.5 °C in Dec, Jan and Feb, respectively (Anonymous 2012). However the winter of 2010 into 2011 was unusually harsh, with average monthly low temperatures of 5.7, 9.0, and 12.3 °C during Dec, Jan and Feb, respectively, and several hard frosts caused complete dieback of climbing fern at 1 release site. *Neomusotima conspurcatalis* is a tropical species (Boughton et al. 2009) and low temperatures substantially impact populations even during the milder winters of coastal Martin County (Boughton & Pemberton 2012). This agent may not be suitable where winters are colder. Small founder populations of biological control agents, such as *N. conspurcatalis*, that are essentially monophagous and lack a diapausing life stage, may be particularly susceptible to extinction in areas of incipient weed infestations where their host plant may be rare, particularly when stochastic factors such as frost can cause localized, albeit temporary, disappearance of host plant foliage (Grevstad 1999). Future colonization efforts with *N. conspurcatalis* should be restricted to areas where climbing fern is commonly occurring, not sparse and patchy, or where the fern remains foliated year-round, so the problem of localized disappearance of the food plant is avoided.

Absent permanent establishment of *N. conspurcatalis*, transect data were combined across control and release sites and trends examined across years. Average ground cover of climbing fern assessed using linear cover methods was 19.7 ± 2.9% in Sep 2009 was little changed in Aug 2010 (21.1 ± 2.8%), but was lower (10.0 ± 1.9%) in Apr 2011 (Fig. 1A). Quadrat methods yielded similar estimates of climbing fern cover (Fig. 1B).

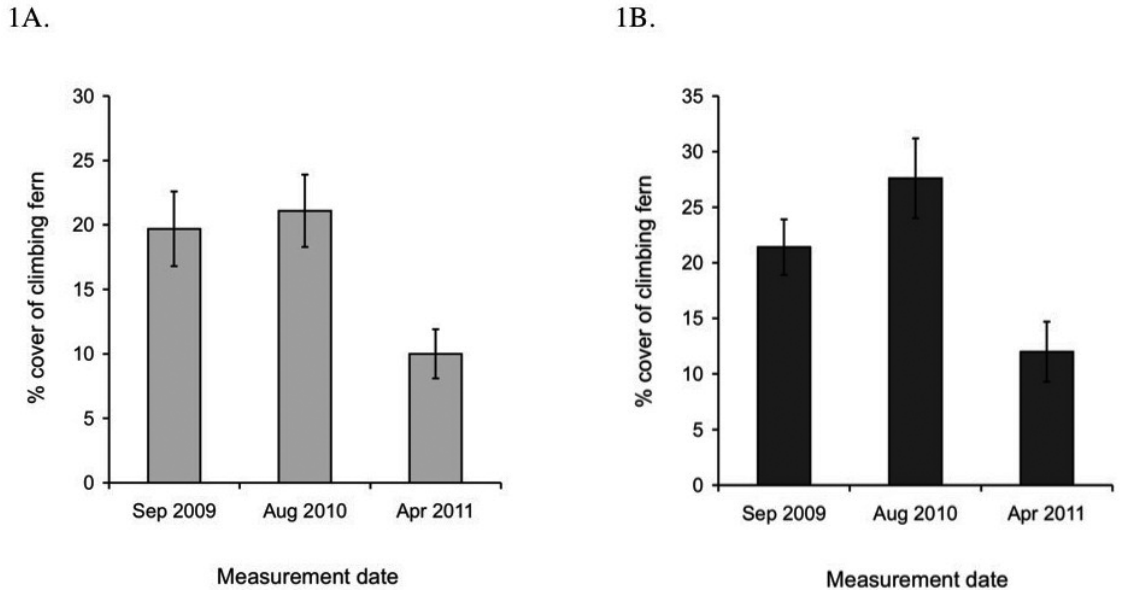

Fig. 1. Average percent ground cover of the Old World climbing fern, *Lygodium microphyllum*, measured at sites in Flatford swamp in 2009, 2010 and 2011 using linear cover (A) and quadrat cover (B) methods. Means based on data from 8 transects. Bars show standard errors.

Reductions in cover observed in 2011 were likely seasonal declines stemming from frost-induced dieback of foliage and lower water availability during the dry season.

Summary

When available, field-adapted insects should be selected for colonization and redistribution, because they appear to offer better prospects for establishment than laboratory-reared insects. Small founder populations of monophagous biocontrol agents that depend on a patchy, rare host plant are susceptible to extinction, especially when stochastic weather factors cause temporary disappearance of the host plant. Populations of *N. conspurcatalis* did not survive the winter of 2010/2011 in Manatee County, likely due to a combination of low-temperature mortality and frost events.

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