

OLD WORLD CLIMBING FERN (*LYGODIUM MICROPHYLLUM*) SPORE GERMINATION IN NATURAL SUBSTRATES

ERYNN M. CALL⁽¹⁾, LAURA A. BRANDT⁽¹⁾, AND DONALD L. DEANGELIS⁽²⁾

⁽¹⁾U.S. Fish and Wildlife Service – A.R.M. Loxahatchee NWR, Boynton Beach, FL, USA

⁽²⁾U.S. Geological Survey, Department of Biology, University of Miami, Coral Gables, FL, USA

⁽³⁾Current Address - Everglades Division, South Florida Water Management District, 3301 Gun Club Road, West Palm Beach, FL, USA

ABSTRACT: *Old World Climbing Fern (Lygodium microphyllum) is a non-native invasive fern that is established within South Florida and poses a significant threat to native habitats. We examined spore germination of L. microphyllum spores in three natural substrates; soil, leaf litter, and water. Germination rates on day 28 ranged from 0 to 99% across all substrate treatments. Germination on soil ranged from 84 to 97% (median 93%; mean \pm SD 92 ± 4) and in water ranged from 52% to 99% (median of 85%, mean \pm SD of $79\% \pm 16$). Germination on leaf litter was highly variable (overall range 0 to 98%), possibly due to the predominance of wax myrtle (*Myrica cerifera*) leaves in the leaf litter and its associated allelopathic qualities. These results confirm that *L. microphyllum* spores have high germination potential on a variety of substrates. We have seen the implications of this invasive life history characteristic in the actual and predicted spread of this species. This information highlights the importance of finding and implementing control strategies that stop the plants from producing spores or limit/eliminate spore germination. Without such mechanisms encroachment of *L. microphyllum* will continue to pose a formidable challenge to restoration of the Everglades.*

Key Words: A.R.M. Loxahatchee National Wildlife Refuge, Everglades, *Lygodium microphyllum*, spore germination

OLD WORLD climbing fern (*Lygodium microphyllum*) is a Category 1 exotic species (Florida Exotic Pest Plant Council), which is defined as a species that is invading and disrupting native plant communities in Florida (FLEPPC, 2005). This native of the old world tropics (Africa, Australia, Asia, and Melanesia) was first collected from a nursery in Delray Beach, Florida in 1958 and a naturalized population was subsequently discovered in 1965 in Martin County. Today *L. microphyllum* is expanding at an alarming rate, destroying vast expanses of natural habitat (Pemberton and Ferriter, 1998).

The effect of invasive, exotic plants on the natural ecosystem can be quite severe and has been documented extensively (Soulé, 1990; Pysek, 1995; Gordon, 1998). *L. microphyllum*, in particular, negatively affects native communities in several ways. It alters fire ecology by carrying searing fires into the canopy, destroying trees that would normally survive a ground fire. The exotic fern also affects the plant community structure by engulfing and smothering native vegetation, preventing growth of such plants as the rare tropical curlygrass fern (*Actinostachys pennula*) and *Tillandsia utriculata* L. and

other rare bromeliads (Craddock Burks, 1996). Entire tree islands in the Everglades are destroyed as *L. microphyllum* fronds topple trees and blanket native vegetation. Tree islands extensively infested with *L. microphyllum* were found to have a lower percent cover of native plant species (Brandt and Black, 2001). *L. microphyllum* may also affect wildlife species by ensnaring animals in its extensive rachis mat (Darby and McKercher, 2002). Loss of tree islands also has negative implications for endangered avian species such as the wood stork and Everglades snail kite that use this habitat frequently. Destruction of tree island habitat is a serious setback to the restoration efforts because these unique areas are a critical component to the proper function of the Everglades system and provide much of the system's biocomplexity (Brandt et al., 2000). In the northern region of the Everglades landscape, tree islands and other habitats in the Arthur R. Marshall National Wildlife Refuge (Refuge) have been particularly impacted by *L. microphyllum*. As of surveys conducted in February 2005, approximately 25,200 hectares (43% of the Refuge) were infested at various intensities (Woodmansee et al., 2005).

The rapidity of this exotic's invasion may be explained by its efficient spore dispersal and reproductive strategies. Spores are released from leaves high in the tree canopy and dispersed mainly by wind. *L. microphyllum* plants produce a large number of spores. Each leaf has on average 133 sori, each sorus has approximately 215 spores, so each fertile leaf has the potential to produce 28,600 spores (Volin et al., 2004). *L. microphyllum* also has the ability to reproduce from a single spore by intragametophytic selfing (the union of the egg and sperm occurs on the same gametophyte) (Lott et al., 2003). Because of the large number of spores being released and the potential for each spore to develop into a new plant, the level of invasiveness of *L. microphyllum* is quite severe. Within this study, we investigated the rate of spore germination on three substrates; soil, leaf litter collected from tree islands, and water. Prior research has utilized agar for the growth of *L. microphyllum* (Lott, 2001; Brown, 1984); however, we wanted to determine germination rates of unsterilized spores within natural substrates (soil, leaf litter, and water) to more accurately represent conditions within the Refuge. The questions addressed in this study are: 1) What are the germination rates on natural substrates? 2) Are there differences in germination rates on different substrates? 3) What is the timing of germination? 4) At what point in time is the maximum germination rate achieved?

METHODS—Fertile *L. microphyllum* fronds were collected within the Refuge on March 17 (Tree island 1: 26°30'01.4, 80°18'03.5), and May 5 (Tree island 2: 26°28'51.8, 80°14'23.7), 2004, and a spore germination trial ensued after each collection date. Two trials of the experiment were run to increase sample size and better characterize variability. Fronds were placed into sealed buckets for transport to avoid spreading spores in transit. Fronds were stored in open bags at ambient temperature and dried for two to three weeks, allowing for the release of spores. Once the spores were released, the bottom corner of the plastic bag was cut and the spores were poured into a vial.

Spores were not sterilized with a Clorox solution or rinsed with fungicide, as has been done in prior *L. microphyllum* germination studies (Brown, 1984; Lott, 2001). It was found that this may

alter the germination rates of *L. microphyllum* (Brown, 1984) and fern spores in general (e.g. Miller, 1968; Raghavan, 1989; Camloh, 1993;1999; Sheffield, 1996).

Spores were transferred using a pair of forceps to a test tube containing 5 ml of distilled water. A vortexer maintained an equal distribution of spores in solution and 10 μ l was taken from the spore suspension using a Pipetman (West Coast Scientific, Oakland, CA) microliter pipette (Ko et al., 1973). The solution was placed on a glass slide and the spores were counted. This process was repeated 10 times to maintain consistency and determine the dilution factor that would allow all spores to be counted within a sampling day.

Soil and leaf litter were collected from two Refuge tree islands on March 31 (experimental trial 1) May 26 (experimental trial 2). Since we were unable to locate an island without any *L. microphyllum*, we collected substrates from islands that had low infestations (a few clusters of climbing vine). Soil was collected from a depth of 0 to 15 cm after surface litter was cleared. Leaf litter was collected on the same island as the soil. In trial 1, the litter was composed of dahoon holly (*Ilex cassine*), smilax (*Smilax laurifolia*), swamp bay (*Persea pulustris*), button bush (*Cephalanthus occidentalis*), swamp fern (*Blechnum serrulatum*), chain fern (*Woodwardia virginica*), and wax myrtle (*Myrica cerifera*). In the second trial the leaf litter was composed predominantly of wax myrtle.

Soil was prepared for germination by placing approximately 25 g in a Petri plate (100 mm diam.). In trial 1 and 2, 12 and 24 Petri plates were prepared for each treatment respectively. The soil was compressed to form a disk (ca. 60 mm diam.), the surface was leveled with a sterilized spoon, and a sterile polycarbonate membrane (8 μ m, 47 mm diam.; Nuclepore Co., Pleasanton, CA) was laid on top of the soil. A drop (10 μ l) of spore suspension was added to each soil disk in a Petri plate (Ko, 2003). Petri dishes containing leaf litter and distilled water were inoculated following the same protocol as for the soil, except for the disc compression. Distilled water was added to the Petri dishes containing soil and litter throughout each of the two experimental trials to maintain disk moisture.

Three sets of control treatments were performed; soil with no inoculate, leaf litter with no inoculate, and Petri dishes with a strip of tape covered in a film of grease (Burkard Manufacturing Co Ltd, 1952). The first two controls were used to determine if the soil and leaf litter contained spores before inoculation. The greased tape was used to determine if spores were suspended in the air of the terrariums.

Petri dishes were placed in two terrariums (16.8 -Gallon, 6.7"H \times 43"W \times 19.5"D) using a complete randomized block design. Thermal hygrometers were placed in each terrarium to monitor the humidity and temperature. Humidity was maintained by placing open containers of water within the terrariums. Terrariums were placed under cool-white fluorescent illumination (100 μ mol m⁻² s⁻¹ photoperiod of 13/11 hrs light/dark respectively, (Lott, 2001)).

The proportion of spores that had germinated was counted on days 7, 14, 21, and 28 of each trial. These sampling days were selected based upon past *L. microphyllum* spore germination research (Brown, 1984). In these investigations, maximum germination was obtained within 28 days.

The normality of the data sets in each trial was tested using proc univariate normal procedure (SAS Institute, 1990). Nonparametric tests were used for pairwise and multiple comparisons because data did not meet assumptions of normality for parametric statistics. Wilcoxon two-sample test was used for pairwise comparisons and the Kruskal-Wallis was used for comparing multiple groups, and the Kolmogorov-Smirnov Goodness of Fit test was used to test if the timing of maximum germination was different among trials or among treatments.

RESULTS—Mean germination rates for each trial, treatment, and sample day are listed in Table 1. The mean germination rate over all sample days did not significantly differ between treatments in trial 1. In trial 2, the leaf litter significantly differed ($P < 0.001$) in the mean germination rate as compared to the soil and water treatments. The germination rates on day 28 are discussed in

TABLE 1. Mean *Lygodium microphyllum* spore germination rates for each experimental trial and treatment.

Trial	Treatment ^a	Sample Day	Mean	S.E.	n ^b
1	Soil	7	0.039	0.020	3
		14	0.335	0.160	3
		21	0.664	0.135	3
		28	0.886	0.043	2 ^c
	Leaf litter	7	0.082	0.032	3
		14	0.466	0.062	3
		21	0.742	0.137	3
		28	0.643	0.288	3
2	Soil	7	0.239	0.028	6
		14	0.848	0.039	6
		21	0.934	0.009	6
		28	0.936	0.010	6
	Leaf litter	7	0.006	0.003	6
		14	0.007	0.005	6
		21	0.000	0.000	6
		28	0.018	0.015	6
1&2 Combined	Water	7	0.290	0.078	9
		14	0.631	0.065	9
		21	0.673	0.131	9
		28	0.792	0.067	9

^a Inoculation with *Lygodium microphyllum* spores.

^b Number of petri dishes.

^c Removal of outlier value.

greater detail, as this measure has more meaning in a practical sense in the spread of *L. microphyllum*.

Germination rates on day 28 ranged from 0 to 99% across all treatments (Fig. 1). Germination on day 28 for soil ranged from 4% to 97%. All but one value, determined to be an outlier (> 2 standard deviations from the mean), ranged between 84 and 97%. Median germination rates were not significantly different between trial 1 and trial 2 with or without the inclusion of the outlier. Median and mean (\pm SD) for the two trials combined without the outlier were 93% and 92% \pm 4, respectively (combined, n = 8).

Germination on day 28 for leaf litter ranged from 0% to 98%. Median germination rates were significantly different between trial 1 and trial 2 (Wilcoxon rank sum, $Z = 1.9696$; $p = 0.0489$). Median and mean (\pm SD) for trial 1 were 87% and 64% \pm 50, respectively (n = 3). Median and mean for trial 2 were < 1% and 2% \pm 4%, respectively (n = 6). Because there were significant differences between the two trials the data were not pooled.

Germination on day 28 for water ranged from 52% to 99%. Median germination rates were not significantly different between trial 1 and trial 2. Median and mean for both trials combined were 85% and 79% \pm 16, respectively (n = 9). There was no germination in either the soil without inoculate or the leaf litter without inoculate. There was no significant difference between day 28 germination rates for soil and water. Median and

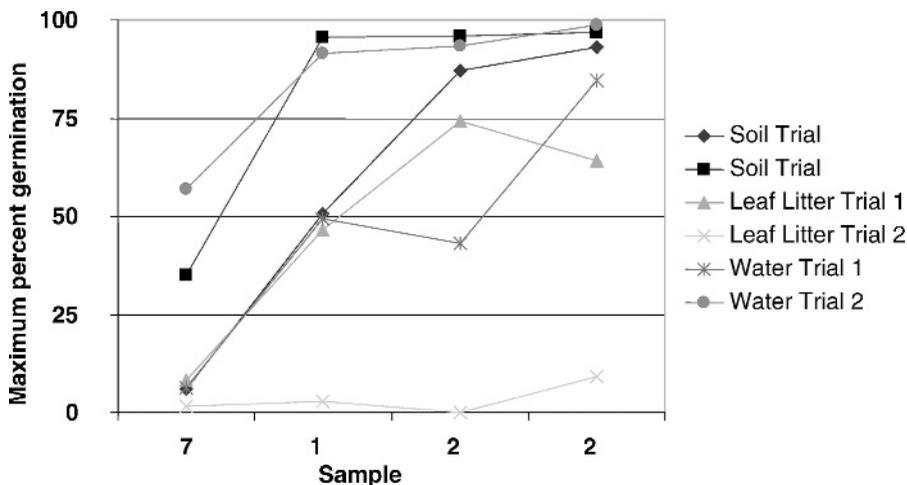


FIG. 1. Patterns of maximum *L. microphyllum* spore germination rates in soil, leaf litter, and water in two experimental trials. n = 3 for trial 1, n = 6 in trial 2.

mean (\pm SD) for the two treatments combined were 90% and 85% \pm 14, respectively (n = 17).

The timing of germination between trial 1 and trial 2 was significantly different for soil (KS = 0.5341; p = 0.016), but not for water (Fig. 1). Generally by day 14, maximum germination percent was greater than 40%. For all but the leaf litter in trial 1, overall maximum germination was observed at day 28.

DISCUSSION—In this study we have shown that *L. microphyllum* spores have a high probability of germinating on the major substrates that they may land on in the Refuge (soil, leaf litter, and water). Germination rates were consistent with what has been observed on artificial substrates (Brown, 1984). Germination patterns also were consistent with previous studies with maximum germination occurring by day 28 (Brown, 1984).

That *L. microphyllum* spores have the potential to germinate in water (median 85%) is particularly troubling from a management perspective. This means that spores landing in the marsh as well as on the tree islands may develop into mature plants. A spore landing in the water could germinate and then float to the edge of an island or other suitable spot and become established. In addition, as water levels recede during the dry season, or due to water management, spores in the water that have germinated could settle on exposed peat and become established. We do not know to what extent this is happening, but we have seen increased colonization by *L. microphyllum* in sawgrass stands and on fern tussocks. In addition, it appears that in many cases, *L. microphyllum* on Refuge tree islands establishes on the edge of the islands first.

The variability observed in the leaf litter treatments is somewhat encouraging (median of 87% and <1%, respectively for trials 1 and 2). It indicates that there may be some properties within the leaf litter on Refuge tree islands that may reduce spore germination. In the first trial, the leaf litter was composed of several common plant species found on tree islands; dahoon holly (*Ilex cassine*), smilax (*Smilax laurifolia*), swamp bay (*Persea pulustris*), button bush (*Cephalanthus occidentalis*), swamp fern (*Blechnum serrulatum*), chain fern (*Woodwardia virginica*), and wax myrtle (*Myrica cerifera*). In the second trial, leaf litter was composed predominantly of wax myrtle (*Myrica cerifera*). Low germination in trial 2 may have been the result of the influence of the allelopathic chemicals present in wax myrtle leaves (Dunevitz and Ewel, 1981). Wax myrtle has been shown to have an affect on *Schinus terebinthifolius* (Dunevitz and Ewel, 1981); thus it is not inconceivable that wax myrtle could affect spore germination of *L. microphyllum*. Future research should examine *L. microphyllum* germination in litter composed of different leaf species to determine if there are repeatable, significant affects.

Successful colonization of any species requires dispersal, germination, maturity, and reproduction. Highly invasive species are particularly effective at all of the above life cycle phases. *L. microphyllum* is no exception. We know that *L. microphyllum* is capable of producing thousands of spores at less than a year old (Lott, 2005) and that the spores are easily dispersed by wind (Nauman and Austin, 1978). It also is likely that spores are dispersed by physical contact with wildlife, people, or equipment. We have confirmed that spores have high germination potential on a variety of substrates. We have seen the implications of these highly invasive life history characteristics in the actual and predicted (Volin et al., 2004) spread of this species. This information highlights the importance of finding and implementing control strategies that stop the plants from producing spores or limit/eliminate spore germination. Without such mechanisms encroachment of *L. microphyllum* will continue to pose a formidable challenge to restoration of the Everglades.

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