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Evaluation of novel triclopyr formulations for control of Old World climbing fern (*Lygodium microphyllum*)

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Abstract

Old World climbing fern [*Lygodium microphyllum* (Cav.) R. Br] is a smothering vine that has invaded thousands of hectares of wetlands in southern and central Florida, including the Everglades. For more than two decades, the standard management approach in natural areas has been to cut the vines at waist height, leaving climbing rachis to desiccate in the tree canopy (poodle cutting) and subsequently treat all rooted ground cover with a foliar application of a 3% v/v solution of glyphosate. While this is generally effective, there is increasing interest in providing additional control options and more selective treatments. Along with glyphosate, triclopyr is widely used in invasive plant management and may also provide increased selectivity when treating the ground cover. However, it has not been well tested on *L. microphyllum*, especially the more recently developed acid and choline formulations. In a series of field trials, we compared the acid, amine, and choline formulations of triclopyr against glyphosate as a positive reference and nontreated plots as a negative reference based on control of *L. microphyllum* at three wetland sites in southern Florida over the period of 2016 to 2020. Significant reductions in *L. microphyllum* cover were measured at 1 mo after treatment (MAT) and continued to the termination of the studies at 12 and 28 MAT. We found all three triclopyr formulations applied with a single-nozzle backpack sprayer at 5.4 g ae L^{-1} provided comparable activity to glyphosate applied at 14.4 g ae L^{-1}. There were few differences in *L. microphyllum* efficacy among the three triclopyr formulations at each site. These results indicate that triclopyr is a suitable alternative to glyphosate for *L. microphyllum* control in wetland ecosystems. Future research should evaluate triclopyr efficacy on *L. microphyllum* in varied hydrologic conditions to better refine treatment prescriptions for wetlands.

Introduction

Old World climbing fern [*Lygodium microphyllum* (Cav.) R. Br] is an invasive, climbing vine that is steadily expanding its range in southern and central Florida (EDDMapS 2022; Hutchinson et al. 2006). *Lygodium microphyllum* is native to tropical and subtropical regions of Africa, Australia, and southeast Asia (Pemberton and Ferriter 1998). It was first verified in the United States as an ornamental plant in the nursery industry of southeast Florida in the late 1950s (Pemberton and Ferriter 1998). Less than a decade later, *L. microphyllum* escaped cultivation and was reported to be naturalized in areas of Martin County, FL (Beckner 1968). By the late 1990s, populations spread north of Lake Okeechobee and to counties along the west coast of Florida (Ferriter and Pernas 2006). Aerial surveys conducted by the South Florida Water Management District (SFWMD) in 2005 estimated an expansion in coverage of *L. microphyllum* to more than 70,000 ha throughout southern and central Florida (Ferriter and Pernas 2006). The current distribution of the fern spans nearly two-thirds of the Florida peninsula, ranging from dense infestations in the Florida Everglades in the south to isolated populations in Duval County, the northernmost county on Florida's east coast (EDDMapS 2022).

Lygodium microphyllum has quickly become one of the most destructive invasive plants in Florida, transforming natural ecosystems by smothering native plant communities under dense rachis mats that can be up to 1-m deep and climbing indeterminately into forest canopies (Hutchinson et al. 2006; Pemberton and Ferriter 1998). Its rapid spread and successful establishment is attributed to its prolific production of wind-dispersed spores, with each fertile leaflet containing up to 28,000 spores (Volin et al. 2004). In addition, *L. microphyllum* is capable of a mixed mating sexual reproduction system that includes intragametophytic selfing, allowing a single spore to produce a viable sporophyte at great distances from its source population (Lott et al. 2003). Intragametophytic selfing in homosporous ferns is rare, but *L. microphyllum* uses this reproductive tactic to colonize new habitats and can exhibit selfing rates of >75%

Management Implications

Lygodium microphyllum (Old World climbing fern) is one of the worst invasive plants in Florida's wetlands. Management is frequently herbicide driven, and glyphosate is the primary product that is used. However, glyphosate's lack of selectivity has prompted interest in treatment alternatives. We tested three aquatic-labeled triclopyr formulations (acid, amine, and choline) against the management standard glyphosate for control of *L. microphyllum* in southern Florida wetlands. We found that when applied at 5.4 g L^{-1}, all three triclopyr formulations provided control that was comparable to glyphosate applied at 14.4 g L^{-1} (3% v/v) up to 28 mo after treatment. These concentrations correspond to recommendations stated in the labels as spot treatments at 1.6%, 1.5%, and 1.1% v/v for the acid, amine, and choline formulations, respectively. Given the need for glyphosate alternatives in certain areas in Florida, these results indicate triclopyr can be a suitable alternative to glyphosate, providing an opportunity to diversify management strategies, increase potential selectivity in mixed plant communities, and result in herbicide use reductions of greater than 60%.

(Lott et al. 2003; Soltis and Soltis 1992). The presence of *L. microphyllum* is significantly correlated with wet and seasonally inundated conditions commonly found throughout much of Florida's mesic upland and wetland ecosystems including the Everglades, flatwoods, swamps, and hardwood hammocks (Hutchinson et al. 2006; Volin et al. 2004).

The tree islands of the Arthur R. Marshall Loxahatchee National Wildlife Refuge (LNWR) represent one of the most impacted habitats. The refuge is a 58,755-ha peat wetland remnant of the northern Everglades located in western Palm Beach County, FL. The overall landscape is a mosaic of sloughs, sawgrass [*Cladium mariscus* (L.) Pohl ssp. *jamaicense* (Crantz) Kük.] ridges, wet prairies, and forested tree islands (Brandt and Black 2001). Tree islands are slightly elevated areas surrounded by open marsh that are composed of Loxahatchee Muck (euic, isohyperthermic Typic Haplosaprists; Soil Survey Staff 2022) and support the growth of woody and herbaceous vegetation (Figure 1). Severe infestations have been observed to completely smother entire tree islands, collapsing the forest canopy and creating monotypic rachis mats (Pemberton and Ferriter 1998). Aerial surveys of the Everglades watershed in 2012 found that 74% of all *L. microphyllum* infestations in the entire watershed were concentrated on tree islands in the LNWR (Rodgers et al. 2014).

Herbicide applications are currently the primary management technique in the effort to control *L. microphyllum* (Hutchinson et al. 2006). Stocker et al. (1997) originally determined that treatments of glyphosate, triclopyr amine, and 2,4-D resulted in complete short-term control of aboveground biomass, but all treatments were susceptible to resprouting from the rhizomes and resulted in considerable damage to desirable native plants. Studies by Thomas and Brandt (2003) and Barrett et al. (2006) determined that a cut-and-spray method, referred to as poodle cutting, whereby climbing rachis is separated from the belowground rhizomes and then successive treatments of glyphosate are applied is very effective at controlling *L. microphyllum*. This technique is also used to reduce the possibility of non-target damage to desirable plants below the *L. microphyllum* rachis, reduce the volume of herbicide necessary for an effective treatment, and facilitate the targeting of living portions of the plant (Hutchinson et al. 2006). This continues to be the most common method used to control *L. microphyllum* (Hutchinson et al. 2006; Hutchinson and Langeland 2006).

Concerns over damage to non-target species using broad-spectrum herbicides such as glyphosate have resulted in the demand for alternative chemistries to treat *L. microphyllum*. Greenhouse and field trials examining the effectiveness of triclopyr amine resulted in 100% necrosis for 10 wk posttreatment (Hutchinson and Langeland 2007). Triclopyr amine treatments, even at high rates of 6.7 kg ae ha^{-1} (6 lb ae $acre^{-1}$) resulted in excellent control of *L. microphyllum* with a concomitant increase in native vegetation and species richness (Hutchinson and Langeland 2007). In addition, triclopyr is absorbed more thoroughly by *L. microphyllum* at 60% when compared with the standard treatment of glyphosate at 31%, although most of the chemical remains in the leaves, showing minimal basipetal translocation regardless of the treatment (Hutchinson et al. 2010).

Recently, two new formulations of triclopyr, a pyridinyloxyacetic acid (triclopyr acid) and a choline salt (triclopyr choline) have been registered for use in non-crop and aquatic sites (Anonymous 2016, 2017), but they have yet to be evaluated for effectiveness in controlling *L. microphyllum*. Irrespective of formulation, triclopyr is a synthetic auxin herbicide with a mechanism of action that mimics the plant growth hormone indole-3-acetic acid, causing uncontrolled growth by interfering with protein synthesis (Shaner 2014). The demonstrated success of triclopyr amine at controlling *L. microphyllum* and the observation of regrowth of native vegetation warrants the evaluation of these new formulations.

Greenhouse studies by Dias et al. (2017) found that the new formulations of triclopyr exhibit similar activity on common crop species. However, field studies using the new formulations revealed that there may be some variability depending on the target species, as the acid formulation was very effective at controlling hen's eyes (*Ardisia crenata* Sims), but the choline formulation did not result in significant control of this species (Cristan et al. 2019). Mechanisms for this have not been well studied, especially for the acid and choline formulations. Applicators in the field have reported that the triclopyr acid formulation is effective at controlling *L. microphyllum* at lower rates than triclopyr amine and glyphosate, which could reduce the total volume of herbicide necessary for effective control (EM Donlan, personal communication); however, this concept has not been rigorously tested.

The objective of this study was to evaluate the efficacy of the new triclopyr formulations, triclopyr acid and triclopyr choline, using ground treatments and to compare the results with the efficacy of triclopyr amine and the current commercial standard *L. microphyllum* glyphosate treatment. A better understanding of these triclopyr formulations could provide land managers with additional tools for *L. microphyllum* management.

Materials and Methods

A field study was established at three sites in southern and central Florida in 2016, 2017, and 2018. The first site was in Strazzulla Marsh near Wellington, FL (26.57°N, 80.24°W) and the second was adjacent to Nubbin Slough near Okeechobee, FL (27.25°N, 80.69°W). The third site was located on tree islands in the LNWR (26.50°N, 80.33°W) in western Palm Beach County.

Figure 1. Aerial image of a tree island in the Arthur R. Marshall Loxahatchee National Wildlife Refuge (LNWR) in January 2018. *Lygodium microphyllum* is blanketing the native vegetation on the island and climbing into the canopy. A contractor crew is spread out across the island carrying out a cut-and-spray (poodle cutting) treatment technique.

Strazzulla Marsh and Nubbin Slough

In December of 2016, the first site was established in Strazzulla Marsh near Wellington, FL. Strazzulla Marsh is 1,035 ha and contains a portion of the largest remaining cypress-dominated wetland in Palm Beach County, FL (USFWS 2019). At this location, *L. microphyllum* has formed dense monocultures and mixed stands in areas that were previously occupied by invasive melaleuca trees [*Melaleuca quinquenervia* (Cav.) S.F. Blake]. The melaleuca trees had been treated several years earlier, opening the understory to sunlight and leaving snags that can be used as vertical structure for *L. microphyllum* to climb. The *L. microphyllum* rachis mat can be up to 1-m thick, preventing regrowth of native vegetation in areas that were previously occupied by bald cypress [*Taxodium distichum* (L.) Rich.], buttonbush (*Cephalanthus occidentalis* L.), pickerelweed (*Pontederia cordata* L.), giant leather fern (*Acrostichum danaeifolium* Langsd. & Fisch.), swamp fern (*Blechnum serrulatum* Rich.), chain fern [*Woodwardia virginica* (L.) Sm.], hottentot fern [*Thelypteris interrupta* (Willd.) K. Iwats.], and other native ferns, herbaceous species, and bromeliads. Strazzulla Marsh is a rainfall-driven wetland with mesic soils primarily composed of Riviera fine sand (loamy, siliceous, active, hyperthermic Arenic Glossaqualfs; Soil Survey Staff 2022; USFWS 2019). Water depth typically fluctuates from saturated soils in the dry season (December to May) to frequent ponding with depths ranging from 0.3 to 0.8 m in the wet season (May to October). Upon initiation of the study, no standing water was present. By 6 mo after treatment (MAT) in June 2017, steady rainfall at the onset of the wet season had increased water levels to an average depth of 0.3 m throughout the site, and they did not recede through the termination of the study at 12 MAT.

The second site was at Nubbin Slough near Okeechobee, FL. Nubbin Slough is a forested drainage way that runs through thousands of hectares of cattle pasture and discharges water from the Nubbin Slough Basin into Lake Okeechobee. The site consisted of an open canopy with the dominant tree species being slash pine [*Pinus elliottii* Engelm.]. Most trees were impacted by vertical *L. microphyllum* growth that ascended to the canopy. *Lygodium microphyllum* has formed a monotypic stand and replaced much of the understory plant community, which was historically composed of saw palmetto [*Serenoa repens* (W. Bartram) Small], native ferns such as bracken fern [*Pteridium aquilinum* (L.) Kuhn], *B. serrulatum*, *W. virginica*, and other woody and herbaceous species. The slough contains areas of Basinger fine sand (siliceous, hyperthermic Spodic Psammaquents) and Placid fine sand (sandy, siliceous, hyperthermic Typic Humaquepts) that exhibit ephemeral ponding during periods of frequent rainfall (Soil Survey Staff 2022). Throughout the duration of the study, occasional inundation of up to 15 cm occurred from torrential rain events but drained rapidly.

At these two sites, 32 plots, each 25 m^2 in size (5 by 5 m), served as experimental units and were established in dense, horizontally growing patches of *L. microphyllum*. Plots were marked at the center point with a single permanent polyvinyl chloride (PVC) pipe 2.5 m in height and established in a grid pattern with a buffer of at least 1 m between each plot to reduce the likelihood of overspray into neighboring plots. Conditions within each site were homogenous. The experiment was a completely randomized design (CRD). Eight treatments were applied, including three triclopyr formulations (triclopyr acid, Trycera™ herbicide, 344 g L^{-1}, Helena Agri-Enterprises, Collierville, TN, USA; triclopyr

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amine, Garlon® 3A, 360 g L^{-1}, Corteva Agriscience, Indianapolis, IN, USA; triclopyr choline, Vastlan™ herbicide, 480 g L^{-1}, Corteva Agriscience) at two concentrations each (5.4 and 10.8 g L^{-1}); glyphosate (Roundup® Custom, 480 g L^{-1}, Bayer CropScience, Monheim am Rhein, Germany) at the commercial standard concentration of 14.4 g L^{-1} (which is equivalent to 3% v/v; Hutchinson et al. 2006); and a nontreated reference. Triclopyr concentrations were chosen based upon previous research that indicated triclopyr amine applied at 7.2 g ae L^{-1} resulted in >90% control at 6 MAT (Hutchinson and Langeland 2007). Each treatment was replicated four times. A nonionic surfactant (Induce® NIS, Helena Agri-Enterprises) was added to all herbicide treatments at 0.5% v/v.

Treatments were applied using a carbon dioxide (CO_2) pressurized backpack sprayer with a single adjustable cone nozzle at a pressure of 276 kPa and an application volume of 374 L ha^{-1}. The sprayer was calibrated before use to ensure a consistent volume of solution was applied to each plot, and the applicator made multiple practice attempts on simulated plots to minimize potential error. At Strazzulla Marsh, herbicide treatments were applied over a 2-d period on December 10 and 11, 2016. Treatments at the Nubbin Slough were initiated and completed on May 26, 2017.

Data collection at these two sites consisted of a visual estimate of percent vegetation cover using a 1-m^2 quadrat placed in four locations within each 25-m^2 plot. The values from these four subplots were then averaged together to provide the estimate for the entire 25-m^2 plot. Baseline data were collected just before herbicide application. Posttreatment cover evaluations were collected at 1, 2, 3, 6, and 12 MAT.

LNWR

The third site was established in January 2018 in LNWR and was set up differently due to conditions in the refuge. Twenty islands ranging in size from 0.08 ha to 0.45 ha (average 0.13 ha) were randomly selected and served as the experimental units. Common native woody species identified on the tree islands were wax myrtle [*Morella cerifera* (L.) Small], dahoon holly (*Ilex cassine* L.), swamp bay [*Persea palustris* (Raf.) Sarg.], and *C. occidentalis*. The understory was composed of an abundance of native ferns, including cinnamon fern (*Osmunda cinnamomea* L.), sword fern [*Nephrolepis exaltata* (L.) Schott], *B. serrulatum*, *T. interrupta*, *W. virginica*, and other herbaceous species. Each island had a moderate or dense infestation of *L. microphyllum*, characterized by smothering horizontal growth in the understory and ascension into the canopy of most trees. Islands were randomly assigned to one of the five treatments, with four replicate islands per treatment. Three 25-m^2 (5 by 5 m) subplots were established within infestations on each island, totaling 60 subplots across the 20 experimental units. Subplots were marked at the center point with a 2.5-m PVC pipe.

On January 31, 2018, a six-person crew provided by a private contractor (Aquatic Vegetation Control, Riviera Beach, FL, USA) initiated the treatment of the 20 tree islands by cutting the ascending portions of the fern with machetes at a height of 120 cm to disconnect any climbing rachis from the rhizomes in the soil, effectively killing the climbing biomass (Figure 1). Poodle cutting occurred on the 16 islands that were assigned to an herbicide treatment but did not occur on nontreated reference islands.

Herbicide treatments in LNWR were a subset of the treatments applied at the other two sites and included triclopyr amine at 5.4 g L^{-1}, triclopyr acid at 3.8 g L^{-1}, triclopyr choline at 5.4 g L^{-1}, and glyphosate at 14.4 g L^{-1}. These were applied over the period from February 7 to February 12, 2018. The solutions were mixed in a 378-L tank and distributed into 15-L backpack sprayers with single adjustable cone nozzles. A non-ionic surfactant was added to all herbicide mixtures at 0.25% v/v. An applicator error during batch mixing resulted in a lower dose of triclopyr acid at 3.8 g L^{-1} (1.1% v/v Trycera™) than at the other sites, which received 5.4 g L^{-1} (1.5% v/v Trycera™). The crew formed a parallel line spanning the width of each island and proceeded to spray *L. microphyllum* as they walked in a south to north direction until they reached the sawgrass ridge on the island fringe that demarcates the transition from tree island to open marsh. Crews attempted to separate any *L. microphyllum* vines from desirable species before spraying and avoided any direct exposure to nontarget vegetation. The average application volume across the 16 treated tree islands was 674 L ha^{-1}. While spot treatments using backpack sprayers offer a targeted approach and result in a reduction in volume of applied herbicide compared with broadcast applications, the dense growth habit of *L. microphyllum* often results in higher application volumes, which occurred at this site.

Baseline data were collected before poodle cutting and herbicide application. A visual estimate of percent cover of *L. microphyllum* was collected using a 1-m^2 quadrat that was placed in four locations within the 25-m^2 subplots for a total of 12 quadrats per experimental unit. Separate estimates were obtained for ground cover (<120 cm) and vertically climbing rachis (>120 cm). However, after poodle cutting, almost all climbing rachis were eliminated on treated islands. Posttreatment cover evaluations were collected at 1, 2, 3, 6, 12, 20, and 28 MAT.

Statistical Analyses

Each site was analyzed separately. For each location, a normal Q-Q plot was used to check for normality and residuals were not normally distributed; therefore, the arcsin square-root transformation was used for the analysis of percent cover for all sampling dates; however, untransformed means are presented for clarity.

For Strazzulla Marsh and Nubbin Slough, an ANOVA was performed on percent cover data for each sampling date at each site using the EMMEANS package in RStudio® (Lenth 2022; RStudio Team 2022). The treatments at Strazzulla Marsh and Nubbin Slough contained a low and high concentration for each of three triclopyr formulations as well as a nontreated reference and glyphosate standard; therefore, variance was partitioned using linear combinations of mean differences with the MULTCOMP package in RStudio® (RStudio Team 2022) to construct *F*-tests to test for differences with respect to triclopyr formulation, triclopyr concentration, and interaction of triclopyr concentration and formulation for this CRD. *F*-tests were also included to compare low and high concentrations of triclopyr averaged across formulations to the negative reference of nontreated plots and positive reference of standard glyphosate treatments. Treatment means were compared using Tukey's honest significant difference (HSD) test. At Nubbin Slough, one triclopyr choline–treated plot and one triclopyr amine–treated plot were removed due to a double application error. As a result, the low-concentration triclopyr amine and triclopyr choline treatments had three experimental units each ($n = 3$), resulting in an unbalanced statistical design for this site.

For the LNWR, a one-way ANOVA was performed on percent ground cover data for all plots at each sampling date. An additional one-way ANOVA was performed on the climbing cover within each treated plot and nontreated reference at each sampling date

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Table 1. *Lygodium microphyllum* ground cover response to herbicide treatment over time at Strazzulla Marsh (26.57°N, 80.24°W).[a]

Herbicide	Concentration	0 MAT	1 MAT	2 MAT	3 MAT	6 MAT	12 MAT
	g ae L^{-1}	% Cover[b]					
Triclopyr amine	5.4	78 a	8 bc	0 b	0 b	2 b	2 b
	10.8	87 a	2 c	0 b	0 b	1 b	5 b
Triclopyr acid	5.4	76 a	1 c	0 b	0 b	2 b	6 b
	10.8	81 a	1 c	0 b	0 b	1 b	1 b
Triclopyr choline	5.4	72 a	18 b	0 b	0 b	5 b	4 b
	10.8	78 a	3 c	0 b	0 b	2 b	2 b
Glyphosate	14.4	82 a	3 c	0 b	0 b	1 b	0 b
Nontreated	—	87 a	87 a	84 a	88 a	99 a	27 a

[a]MAT, months after treatment.
[b]Means within columns followed by the same letter are not significantly different according to Tukey's HSD (P = 0.05).

to investigate the effectiveness of poodle cutting at eliminating the climbing portion of the rachis after disconnecting it from the belowground rhizome. Treatment means were compared using Tukey's HSD test.

Results and Discussion

Strazzulla Marsh

There were no differences in baseline *L. microphyllum* percent cover before herbicide applications (72% to 87%) across experimental units (Table 1). Average low and average high triclopyr concentration differed ($P < 0.001$) from the nontreated reference for all posttreatment evaluations. By 1 MAT, there was no significant difference in percent cover between the triclopyr amine, triclopyr acid, and triclopyr choline formulations at the higher concentration of 10.8 g L^{-1} and the glyphosate (14.4 g L^{-1}) treatment. Plots treated with a lower concentration of 5.4 g L^{-1} (1.12% v/v) triclopyr choline exhibited slower symptomology with a reduction in percent cover to 18% compared with most other treatments, which had significantly lower cover. There were significant triclopyr concentration, formulation, and rate by formulation interaction ($P < 0.03$) effects at 1 MAT, but not for any other evaluation date. The nontreated reference maintained an average 87% cover of *L. microphyllum*. By 2 MAT, all treatments, including the low concentration of triclopyr, were effective and had reduced percent cover of *L. microphyllum* to less than 1%. This pattern of efficacy continued through the peak of the dry season at 3 MAT in March 2017, when no living *L. microphyllum* was observed in any treatment plots while percent cover in the nontreated reference was 88%. Average low- and high-concentration triclopyr treatments did not differ from the glyphosate standard at any sample date from 1 to 6 MAT.

Strazzulla Marsh is a rainfall-driven wetland, and with the beginning of the wet season, the site began to pond frequently. By 6 MAT in June 2017, rain events raised the water level at the site to 0.15 m. While some new sporophytes began to emerge, all herbicide treatments continued to suppress *L. microphyllum* percent cover to less than 5%, while the nontreated reference plots increased to 99%. Between June and the end of the dry season in October 2017, rain events increased the water level at the site to an average of 0.6 m, which submerged large portions of the treated and nontreated reference plots. The water did not recede by the final sampling date at 12 MAT in December 2017. The inundation of the site coincided with a reduction in percent cover of *L. microphyllum* in the nontreated reference plots from 100% at 6 MAT to 27% cover at 12 MAT. All herbicide-treated plots maintained low *L. microphyllum* cover (0% to 6%) with no differences between any of the triclopyr formulations or glyphosate (Table 1).

All three triclopyr formulations, regardless of concentration, performed as well as the commercial standard glyphosate. The rise in water levels throughout the wet season may have suppressed the emergence of new sporophytes and resprouting from the rhizomes in all experimental units. While *L. microphyllum* spores can germinate in standing water (Call et al. 2007), it is unknown whether a submerged gametophyte can produce a sporophyte. Mature plants with rachis above the waterline can survive inundation of the rhizomes, although they will demonstrate a slower growth rate and a reduction in reproductive output (Gandiaga et al. 2009).

Nubbin Slough

Baseline *L. microphyllum* cover ranged from 84% to 99% across the treatments and was not significantly different at 0 MAT (Table 2). There were no significant triclopyr concentration, formulation, or concentration by formulation interaction effects for any posttreatment evaluation. The average low- and high-concentration triclopyr treatments differed from the glyphosate standard only at 1 MAT ($P < 0.001$). Average low and high triclopyr concentration treatments differed from the nontreated reference until 12 MAT ($P < 0.001$). At 1 MAT, all three formulations of triclopyr significantly reduced live cover of *L. microphyllum* to less than 5%, which was lower than cover following glyphosate (17%). *Lygodium microphyllum* cover in the nontreated plots was 88%.

At 2 MAT, all herbicide treatments reduced *L. microphyllum* cover to 5% or less, significantly lower than results for the nontreated reference (86%). Similar to 1 MAT, there was no difference in *L. microphyllum* cover among any triclopyr formulation treatments. Cover in glyphosate-treated plots was reduced to <6% and was not different from cover in any triclopyr treatment. At 3 MAT, there were no significant differences in cover among any of the herbicide treatments, but cover was lower for all three compared with the nontreated reference. A similar pattern was observed at 6 MAT. At this sample date, almost all plots began to show some resprouting from surviving rhizomes. Regardless, all herbicide-treated plots still contained less than 19% regrowth compared with the nontreated reference at 61%. Some reduction in cover in the treated and nontreated reference plots may be attributed to the brown *Lygodium* moth [*Neomusotima conspurcatalis* Warren (Lepidoptera: Crambidae)], which became established at the site. The moth has been documented to cause temporary, localized defoliation of the plant (Boughton and Pemberton 2009).

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Table 2. *Lygodium microphyllum* ground cover response to herbicide treatment over time at Nubbin Slough (27.25°N, 80.69°W).[a]

Herbicide	Concentration	0 MAT	1 MAT	2 MAT	3 MAT	6 MAT	12 MAT
	g ae L^{-1}	% Cover[b]					
Triclopyr amine	5.4	94 a	2 c	0 b	1 b	9 b	32 a
	10.8	84 a	0 c	0 b	0 b	2 b	3 a
Triclopyr acid	5.4	92 a	1 c	0 b	1 b	7 b	22 a
	10.8	90 a	1 c	0 b	0 b	4 b	23 a
Triclopyr choline	5.4	80 a	0 c	0 b	1 b	4 b	16 a
	10.8	99 a	4 c	7 b	15 b	19 b	37 a
Glyphosate	14.4	95 a	17 b	5 b	2 b	2 b	5 a
Nontreated	—	85 a	88 a	86 a	86 a	61 a	43 a

[a]MAT, months after treatment.
[b]Means within columns followed by the same letter are not significantly different according to Tukey's HSD (P = 0.05).

Table 3. *Lygodium microphyllum* ground cover response to operational herbicide treatment over time on tree islands in Arthur R. Marshall Loxahatchee National Wildlife Refuge (LNWR; 26.50°N, 80.33°W).[a]

Herbicide	Concentration	0 MAT	1 MAT	2 MAT	3 MAT	6 MAT	12 MAT	20 MAT	28 MAT
	g ae L^{-1}	% Cover[b]							
Triclopyr amine	5.4	66 a	2 b	1 b	0 b	3 b	4 b	8 b	19 b
Triclopyr acid	3.8	77 a	2 b	1 b	0 b	3 b	9 ab	18 b	22 b
Triclopyr choline	5.4	65 a	1 b	0 b	0 b	2 b	4 b	5 b	14 b
Glyphosate	14.4	82 a	4 b	0 b	1 b	4 b	7 b	14 b	26 b
Nontreated	—	55 a	57 a	52 a	52 a	56 a	35 a	73 a	54 a

[a]MAT, months after treatment.
[b]Means within columns followed by the same letter are not significantly different according to Tukey's HSD (P = 0.05).

By 12 MAT (May 2018), there were no significant differences between any of the herbicide treatments or the nontreated reference using Tukey's HSD. *Lygodium microphyllum* percent cover in all herbicide-treated plots ranged from 3% (triclopyr amine) to 37% (triclopyr choline), while cover in the nontreated reference plots was 43%. In January 2018, the region experienced a cold front during which nighttime temperatures dropped below 0 C for 3 consecutive days, causing the aboveground biomass throughout the site to brown out. Sustained cold temperatures that produce frost can effectively kill the exposed aboveground biomass and reduce the survivability of gametophytes and sporophytes but may not harm the lower layers of the rachis or the rhizomes (Hutchinson and Langeland 2014; Volin et al. 2004). Within 1 mo, aboveground biomass resprouted from the rachis and rhizomes in most plots. However, percent cover in the nontreated reference plots did not reach pretreatment levels by the final sampling date.

LNWR

At 0 MAT in February 2018, there were no significant differences between any treatment islands or the nontreated reference. Percent ground cover values ranged from 55% to 82% (Table 3). By 1 MAT in March, all triclopyr formulations reduced *L. microphyllum* ground cover to ≤2%, while glyphosate-treated islands contained an average of 4% ground cover. All herbicide treatments were different from the nontreated reference. This pattern of control continued through 3 MAT, as all herbicide treatments reduced *L. microphyllum* cover to less than 1%.

At 6 MAT (August 2018), new sporophytes began to appear in moist, exposed substrate, but overall *L. microphyllum* percent cover on the tree islands remained low, with an average of 3% across all treatments. Sporophyte flushes have been observed following treatments in other locations and are likely due to the opening of the understory and the germination of spores (Hutchinson et al. 2006). *Lygodium microphyllum* spores and new gametophytes were likely not affected by the herbicide treatments. Spores and gametophytes have been shown to be sensitive to metsulfuron-methyl but are highly tolerant to triclopyr and glyphosate (Hutchinson and Langeland 2011). The survival of these sporophyte flushes is variable and depends on the environmental conditions following their germination (Hutchinson et al. 2006).

Climbing rachis above 120 cm were eliminated before herbicide application by poodle cutting in all experimental units until 12 MAT, when a small amount of regrowth (4% cover) on the triclopyr acid–treated islands began to ascend vertically (Figure 2). This verifies the absolute effectiveness of poodle cutting to initially control all climbing rachis.

Overall percent cover of *L. microphyllum* on the nontreated reference islands was reduced to 35% at 12 MAT in February 2019 and did not differ from percent cover on herbicide-treated islands. One nontreated reference island experienced an outbreak of *N. conspurcatalis* in December 2018, which led to a reduction of percent cover of *L. microphyllum* across the island. Additionally, reductions in *L. microphyllum* cover may be attributed to the dry season and colder conditions in the winter. Although *L. microphyllum* can grow throughout the year, it does experience seasonal variations in growth, with the peak of its growth and sexual reproduction occurring in the warmer wet season (Philippi and Richards 2007; Volin et al. 2004).

Given the whole-island treatments and larger experimental units, additional data collection was possible at 20 and 28 MAT. By 20 MAT, *L. microphyllum* on nontreated reference islands had regrown, with a percent cover of 73%. On herbicide-treated islands, new sporophytes and rachis resprouts were prevalent,

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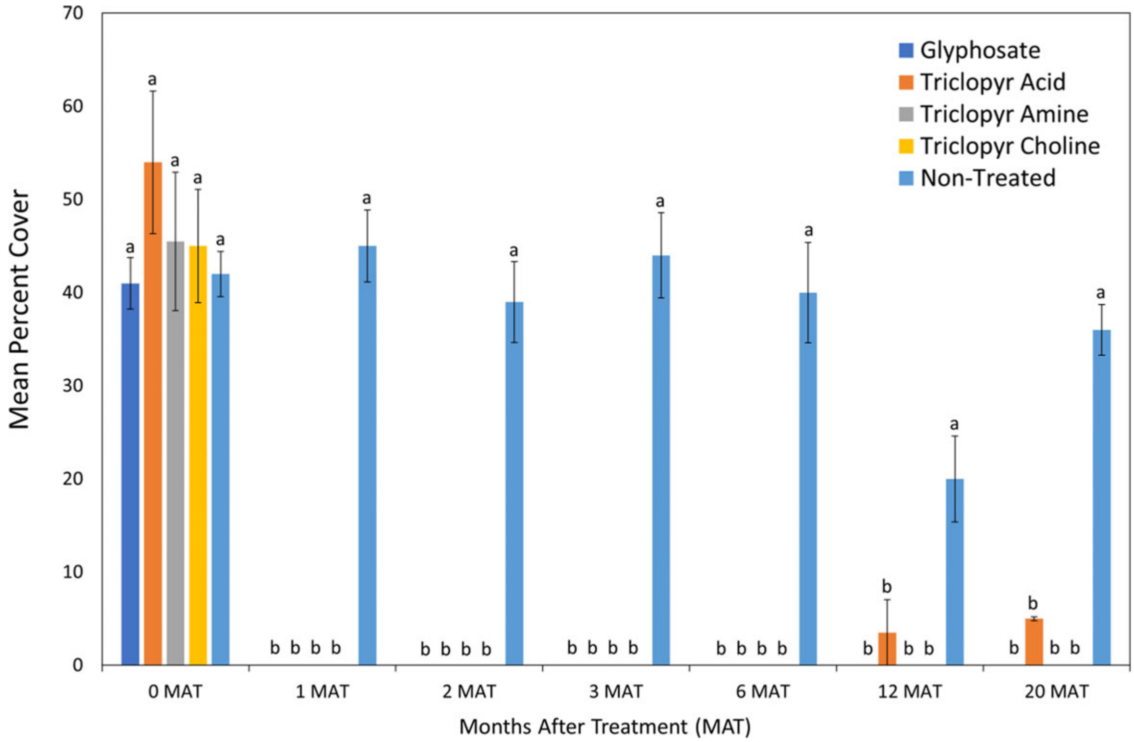

Figure 2. Mean percent cover ± SE of climbing rachis greater than 120 cm in height by treatment over time in the operational tree island study. There was no significant difference between any treatment at 0 months after treatment (MAT). *Lygodium microphyllum* climbing cover was eliminated after 0 MAT by poodle cutting. At 20 MAT, all treatment plots were significantly different from the nontreated reference, which was not poodle cut (36% cover). Bars within sample dates with the same letter are not different ($P > 0.05$).

but percent cover of *L. microphyllum* continued to be suppressed below 19%, and there were no significant differences among treatments. This pattern continued through 28 MAT, with each treatment suppressing regrowth to less than 25% cover.

Overall, the three triclopyr formulations provided some control of *L. microphyllum* at 28 MAT and were comparable to the commercial standard glyphosate. These results indicate that triclopyr can be an effective alternative tool for land managers who are managing *L. microphyllum* infestations. However, it is important to mention that at all three sites, limited regrowth began to occur from 6 to 12 MAT and beyond. The regrowth in these studies illustrates the need for repeated applications, as there are currently no known instances of long-term control after a single treatment (Hutchinson and Langeland 2006). Future research should examine the effects of repeat applications and retreatment intervals for triclopyr treatments on *L. microphyllum*. In addition, triclopyr is commonly used for the control of woody plants, so non-target damage to native trees and shrubs that are growing below *L. microphyllum* can occur (Hutchinson and Langeland 2015). Research examining directed applications of triclopyr amine, triclopyr acid, and triclopyr choline to non-target plants should be conducted to understand the tolerance of common native species to these formulations, which could minimize the risk of damaging desirable species during *L. microphyllum* treatments.

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