Final Report

Evaluation of Current and Potential New Herbicides to Control *Lygodium microphyllum*

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Abstract

Based of greenhouse and field trails from 2005-2007, we found that glyphosate, metsulfuron methyl, triclopyr, and imazapic are effective for control of *L. microphyllum*. Imazapyr was also effective in controlling *L. microphyllum* but resulted in greater damage to non-target plants and lower species richness 6 months post-treatment. For ground treatment of *L. microphyllum* infestations, we recommend glyphosate (2.0 – 4.0 % product), metsulfuron methyl (2 oz. product /100 gal.), triclopyr (2% product), and imazapic (1.5% product). Field trails indicated that tank mixes of these herbicides in any two mixtures at the above rates are also effective in controlling *L. microphyllum*. Follow-up treatments will be required as no herbicide or tank mix tested was 100% effective on a consistent basis in controlling *L. microphyllum*. Regardless of the herbicide used, non-target damage was high in field trials, especially if *L. microphyllum* was entangled with non-target vegetation. Results indicate that triclopyr and glyphosate treatments result in less non-target damage to ground cover vegetation relative to other herbicides evaluated in this project. Additional field testing is needed on the efficacy of increased rates of triclopyr, imazamox, penoxsulam, and aminopyralid in controlling *L. microphyllum* and the subsequent effects on non-target plants. Field trials with fluroxypyr are also warranted since it exhibited excellent initial control of *L. microphyllum* in the greenhouse.

Introduction

Old World climbing fern (*Lygodium microphyllum*) is considered one of the worst non-native invasive plants of seasonal hydric habitat in southern Florida. Currently, glyphosate and metsulfuron methyl herbicides have been shown to be the most effective thus far for *L. microphyllum* control, but results are inconsistent with regard to long-term management. Another concern with the use of a single herbicide is development of resistance. Plant resistance to herbicides was first reported in 1970 (Ryan, 1970), but is increasing in frequency each year and there are currently 291 biotypes of plants resistant to herbicides (Heap, 2004). The development of resistance to herbicides by plants appears to be following trends of resistance development of insecticides by insects which have a history > ca. 80 years of insecticide spraying (Georghiou, 2000). The acetolactate synthase (ALS) inhibiting herbicide class, which includes metsulfuron methyl, was introduced commercially in 1982 and there are already more weed species resistant to this class of herbicides than any other class (Tranel and Wright, 2002).

The issue of resistance development and the evaluation of other herbicides besides glyphosate and metsulfuron methyl to treat *L. microphyllum* was one of main issues brought up at the 2004

Lygodium Research Review meeting in West Palm Beach. Being a relatively recent invader to natural areas in south Florida, *L. microphyllum* has been exposed to repeated herbicide treatments for < 15 years, a threshold well below the time frame at which some weedy plants have developed herbicide resistance in agricultural systems (Primiani et al., 1990; LeBaron, 1991; Perez and Kogan, 2003). A single population of Old World climbing fern in Polk County has received 11 treatment applications of glyphosate indicating that this population may already be resistant. Testing and the eventual approval of other herbicides currently on the market will add additional herbicides that can be used in rotation and in various combinations to control *L. microphyllum*.

The objectives of this research were to test herbicides under greenhouse conditions (single herbicides) and in the field (single herbicides and tank mixes) to identify additional herbicides and combinations of herbicides that most effectively control *L. microphyllum*.

Methods

Greenhouse Herbicide Trials

Lygodium microphyllum rhizomes were excavated from Allapattah Flats in Martin County. The rhizomes, with soil intact, were potted in 3.8 l pots on-site and propagated vegetatively under greenhouse conditions at the University of Florida's Center for Aquatic and Invasive Plants. The ferns were grown to 1 m in height.

Upon reaching 1 m in height, the ferns were foliar sprayed with eight herbicides (Table 1) using the maximum label rate. Visual observations of percent dead based on a scale of 1-100% were taken weekly. At 2.5 months post treatment, the plants were cut to soil level and allowed to re-grow for an additional 2.5 months. At 2.5 months following cutting, all *L. microphyllum* re-growth was cut, dried for 7 days, and weighed to the nearest 0.01 gram. Controls were included in the experiment and treated with 0.05% DyneAmic surfactant (Helena Chemical Co.). There were 16 ferns per treatment and the project was repeated. Treatments were arranged in a completely randomized design. Analysis of variance and Duncan's Multiple Range Test were used to test for treatment differences ($p < 0.05$) among dry weights.

An additional experiment was conducted in December 2006 using the methods and herbicides rates described above. *L. microphyllum* plants were foliar sprayed in December 2006 and all *L. microphyllum* and native vegetation re-growth was evaluated at 6 months. All vegetation was cut at soil level and identified to species. All plant species were separated and dried for 7 days.

Dry weights of *L. microphyllum* and native vegetation were weighed to the nearest 0.01 gram. Native species richness (number of species) per pot was also recorded and the mean taken per treatment to analyze the response of native species to herbicide treatment. Analysis of variance and Duncan's Multiple Range Test were used to test for treatment differences ($p < 0.05$). For native vegetation, all plants were combined for final analysis.

Additional Greenhouse Herbicide Trials

Additional herbicides were tested on *L. microphyllum* under greenhouse conditions in 2006 at 1/8, 1/4, 1/2, and 1 times the maximum label rate including two herbicides with different modes of action than those previously tested (Table 2). Propagation, methods, and statistical analysis were the same as described above for the greenhouse trials. Imazamox (Clearcast) was tested at a rates up to 8.5 higher than the initial greenhouse experiment with imazamox (Raptor).

Field Herbicide Trials

Field herbicide screening trials were conducted at six locations in central and south Florida and an additional field site was added in early 2007 (Table 3). Plots were randomly placed at all sites and marked at the beginning and end with 2 m PVC pipes. At each site, 10 m cover transect lines (Canfield, 1941) were set up for each treatments in areas with ca. 40% or more coverage of *L. microphyllum*. The distant (to the nearest 0.5 cm) of each plant along the transect line was recorded. Percent coverage of each species was determined by dividing the distance of each plant species along the line by the distance of the line. Herbicide rates for field treatments were mixed as percent product and ranged from 1-4%. The active ingredient of each herbicide per dilution are listed in Table 4. For metsulfuron methyl and quinclorac, rates were mixed as ounces / 100 gallons. Pre-treatment monitoring was conducted during September/October 2006 and the plots were sprayed immediately following pre-treatment evaluation. During February-March of 2007, all plots were re-evaluated documenting all plants along the transect lines. All live *L. microphyllum* was re-treated at 6 months. Additional evaluations are planned at 12 and 24 months post-treatment at which time all live *L. microphyllum* will be treated.

Comparisons of the treatments were analyzed for percent change in *L. microphyllum*, native vegetation (all plants combined), and species richness at pre-treatment versus post-treatment evaluation at 6 months using an ANOVA with means separated using Duncan's Multiple Range test ($p<0.10$).

Results and Discussion

Greenhouse Trials

Greenhouse trials form 2005-2006 indicated that triclopyr exhibited 100% necrosis at 10 weeks post-treatment (Figure 1). Glyphosate and metsulfuron methyl also exhibited high rates of necrosis at 92.5% and 87.5%, respectively. Imazapyr and imazapic exhibited intermediate necrosis rates relative to the other herbicides tested, while quinclorac, penoxsulam, and imazamox exhibited ≤ 50% necrosis. New growth was observed for *L. microphyllum* treated with penoxsulam and imazamox at seven weeks post-treatment indicating that the low rates used for these two treatments were not effectively translocated.

Based on dry weight biomass from re-growth, triclopyr and glyphosate were significantly different ($P < 0.05$) from the other herbicides tested in controlling *L. microphyllum* under greenhouse conditions after 2.5 months post-treatment (Table 5). However, the rate for triclopyr in this study was extremely high and additional testing needs to be conducted on lower rates of triclopyr to control *L. microphyllum*. Metsulfuron methyl, imazapyr, and imazapic also provided excellent control of *L. microphyllum* in this experiment. Results for imazapyr and imazapic were variable with some replicates exhibiting no re-growth of *L. microphyllum*.

While penoxsulam and imazamox did not effectively control *L. microphyllum* in our trials, the rates of each herbicide was low relative to the other herbicides tested. Additional trials should be conducted with these herbicides at higher rates to determine if they are effective in controlling *L. microphyllum*. Successful aerial application of high rates of imazamox has provided excellent control of Chinese tallow with minimal non-target damage in forested swamps in Louisiana (Todd Horton, Pers. Comm., BASF).

In an additional herbicide screening, triclopyr, glyphosate, imazapyr, imazapic, and metsulfuron methyl were significantly different ($P < 0.05$) than the other herbicides tested 6 months post-treatment (Table 6). The dry weight biomass of native vegetation was significantly different ($P < 0.05$) for triclopyr than the dry weights of native vegetation from other treatments. The group of imidazolinone herbicides (imazapyr, imazapic, and imazamox) tested had the lowest native vegetation biomass, possibly an indication of their soil residual activity. Native species richness was significantly different ($P < 0.05$) after six month post-treatment for triclopyr and glyphosate than the species richness for other herbicide treatments. Native species richness was lowest for imazapyr and imazapic treatments.

Based on these results, triclopyr and glyphosate which were applied at higher rates, resulted in excellent control of *L. microphyllum*, higher native species dry weight biomass, and higher species richness. Metsulfuron methyl also provided excellent control of *L. microphyllum*, and had high native species biomass and richness 6 months post treatment. Additional research is needed to determine the effects of triclopyr, glyphosate, metsulfuron methyl, and imazapic on native species at various rates under field conditions.

<u>Additional Greenhouse Trails</u>

A single greenhouse trials in 2006 indicated that fluroxypyr exhibited 95% and 85% necrosis at 10 weeks post-treatment for rates of 0.48 lbs a.e. / acre (42 oz product / 20 gal.) and 0.24 lbs a.e. / acre (21 oz product / 20 gal.), respectively (Figure 2). Imazamox, penoxsulam, aminopyralid, and bispyribac-sodium all exhibited ≤ 50% necrosis at 10 weeks.

Results from greenhouse trials in 2006 indicate that imazamox at 0.20 lbs a.e. / acre (25.6 oz product / 20 gal.) and 0.40 lbs a.e / acre (51.2 oz product / 20 gal.) was significantly different ($P < 0.05$) from the other herbicides tested (Table 7). This experiment will be replicated in the summer of 2007. The rates of imazamox in this experiment were 4-8 times greater than the rates used for imazamox in the greenhouse trials above. This is an indication that the low rates of many of the ALS herbicides used typically for seedlings in agriculture environments may not be effective on mature plants in natural areas. Thus, we suggest more research on increased rates of imazamox, penoxsulam, and aminopyralid to control *L. microphyllum* and their effects on native plants.

Based on one trial, fluroxypyr exhibited excellent initial control of *L. microphyllum* at 0.48 and 0.24 lbs a.e. / acre, but re-growth was observed indicating that fluroxypyr may not be effectively translocating into the rhizomes. At 10 weeks, imazamox at 0.40 and 0.20 lbs a.e. / acre exhibited 50% necrosis, but there was < 0.01 g of re-growth was observed indicating that imazamox is translocating to the rhizomes.

Field Herbicide Trials

Six month evaluation post-treatment of field herbicide trials indicated no significant ($P < 0.10$) different in the application of single herbicides or tank mixes in controlling *L. microphyllum* (Table 8). Total percent reduction of *L. microphyllum* for all treatments ranged from 78.1 to 94.2, but there was large variation between treatments and sites. Environmental factors such as changes in humidity, soil moisture, and rainfall < 24 hours post-treatment may have adversely affected treatments at some sites.

There was no significant difference ($P < 0.10$) detected among treatments for change in cover of native plants. All treatments resulted in large reduction of native plants in all treated plots. Glyphosate and imazapic resulted in greater native plant mortality, though it was not significant compared to other treatments. This is an indication of the twining, twisting nature of *L. microphyllum* in which this plant wraps around and covers all non-target vegetation making selectivity and herbicide placement difficult.

No significant difference ($P < 0.05$) was detected among changes in species richness for treatments at six months. The general trend was a loss of species richness at 6 months post-treatment. Glyphosate (2% product) and metsulfuron methyl (2 oz. product/100 gal.) resulted in slight increases in species richness, but all other treatments resulted in a decline of species richness.

Based on preliminary results from field trials, glyphosate, metsulfuron methyl, triclopyr, and imazapic (alone or in tank mixes) all provided adequate control of *L. microphyllum*. However, there was substantial decline in native plants. The long term objective of this project is to evaluate the effects of repeated ground treatments on controlling *L. microphyllum*, increasing native vegetation cover and richness. Additional evaluations and re-treatment of *L. microphyllum* in field plots will be conducted at 12 and 24 months.

Conclusion

Our results indicate that glyphosate, metsulfuron methyl, triclopyr, and imazapic are effective in controlling *L. microphyllum* under greenhouse and field trials, but additional treatments will be required. No herbicide tested in both greenhouse and field trials resulted in complete elimination of *L. microphyllum* on a consistent basis. In greenhouse trials, imazapyr was effective in controlling *L. microphyllum*, but results were highly variable and many treatments with imazapyr exhibited no plant growth of any kind between 2.5 - 6.0 months.

Triclopyr at very high rates of 6.0 lbs a.e. / acre in greenhouse trials exhibited excellent control of *L. microphyllum* and there was an increase in native vegetation and species richness six months post-treatment. Similar results were observed in the field for triclopyr using 2% product, indicting that additional research is needed to determine the most effective rate of triclopyr to control *L. microphyllum*. Further research is also needed on the effects of increased rates of imazamox, penoxsulam, and aminopyralid on controlling *L. microphyllum* and their effects on native plants in Florida's natural areas.

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Figure 1. Mean percent necrosis from week 1–10 post-treatment for first greenhouse herbicide trials 2005-2006 (n = 32 plants / treatment) using maximum label rates and 20 gallons diluent.

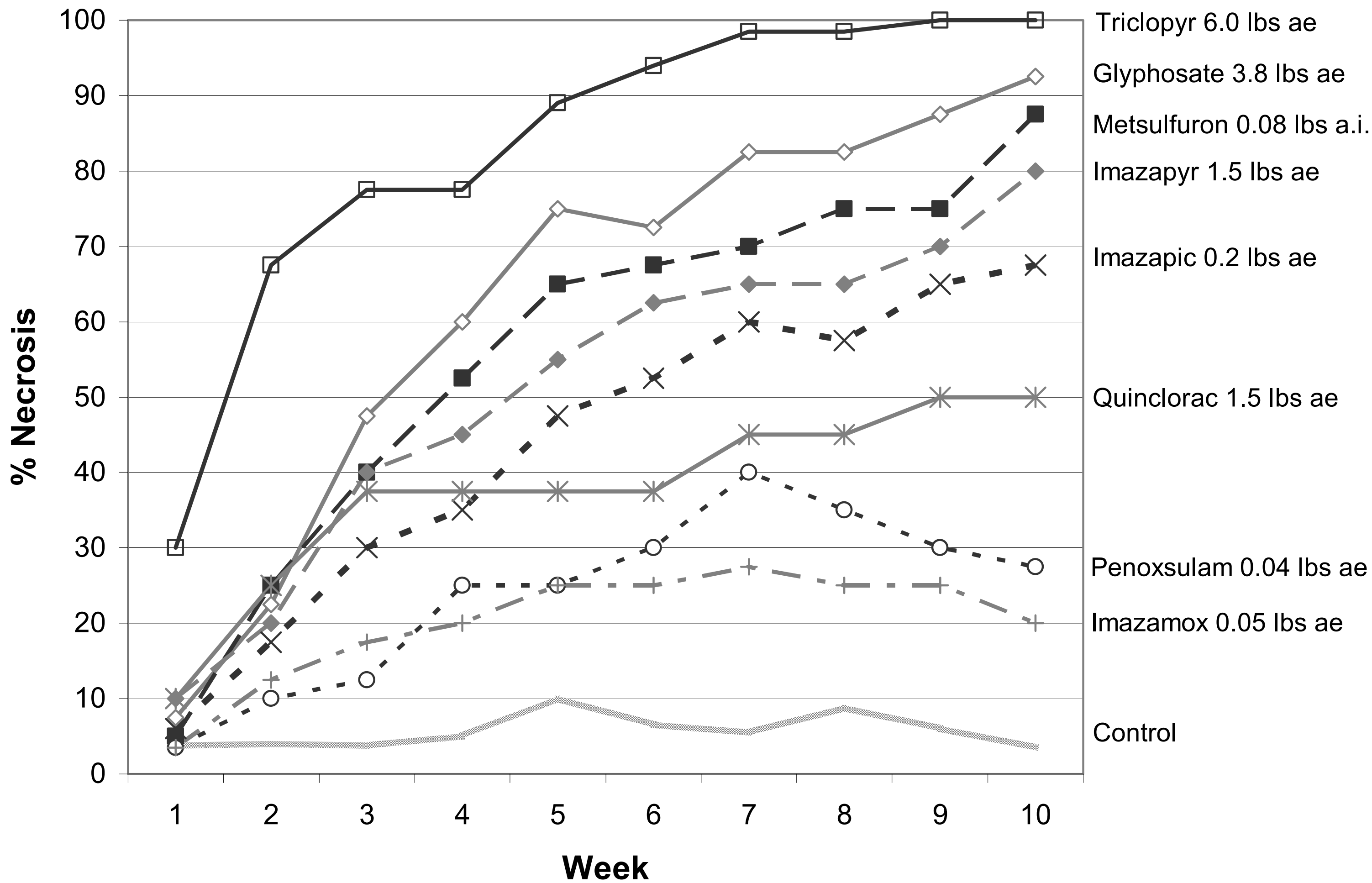

Figure 2. Percent necrosis at 2.5 months for 1/8, 1/4, 1/2, and maximum label rate (20 gallon diluent) for additional greenhouse herbicide trials in 2006 (n = 4 plants / treatment).

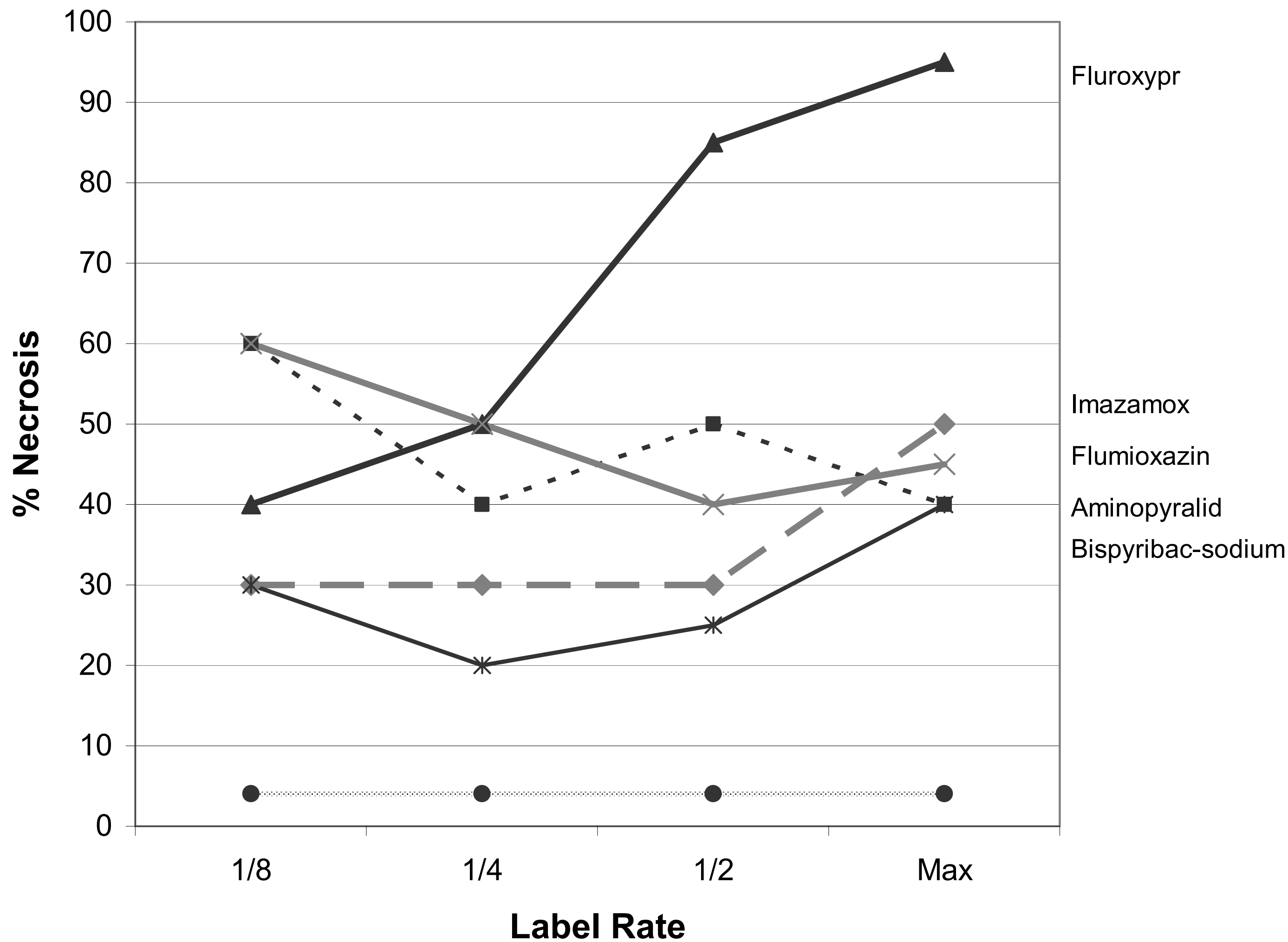

Table 1. Herbicides (product name and mode of action) evaluated in greenhouse trials 2005-2006 to control *Lygodium microphyllum*.

Name	Product Name	Mode of Action
Metsulfuron methyl	Escort XP	Inhibits Branched Chain Amino Acid Synthesis
Glyphosate	Rodeo	Inhibits Aromatic Amino Acid Synthesis
Imazapyr	Habitat	Inhibits Branched Chain Amino Acid Synthesis
Imazapic	Plateau	Inhibits Branched Chain Amino Acid Synthesis
Triclopyr	Renovate 3	Growth Regulator
Imazamox	Raptor	Inhibits Branched Chain Amino Acid Synthesis
Penoxsulam	Grasp	Inhibits Branched Chain Amino Acid Synthesis
Quinclorac	Drive	Growth Regulator

Table 2. Herbicides (product name and mode of action) evaluated in greenhouse trials 2006-2007 to control *Lygodium microphyllum*.

Name	Product Name	Mode of Action
Imazamox	Clearcast	Inhibits Branched Chain Amino Acid Synthesis
Aminopyralid	Milestone	Growth Regulator
Fluroxypyr	Vista	Photosynthetic Inhibitor
Flumioxazin	Sureguard	Cell Membrane Disruptor
Bispyribac-sodium	Regiment	Amino Acid Inhibitor

Table 3. Site name, location and managing agency of field sites where herbicide field trials on *Lygodium microphyllum* have been conducted. Field trails were initiated in September 2006 except where noted.

Site name	Location / County	Managing Agency
Balm Scrub	Balm (Hillsborough)	Hillsborough County Parks, Recreation, and Conservation Dept.
Indian River Community College	Ft. Pierce (St. Lucie)	State of Florida
Lakeland Artificial Wetland System	Lakeland (Polk)	City of Lakeland
Jonathan Dickinson State Park	Hobe Sound (Martin)	Florida Department of Environmental Protection
Highlands Hammock State Park	Sebring (Highlands)	Florida Department of Environmental Protection
Pacific Tomato Growers Inc.	Immokalee (Collier)	Private Business
[1]Loxahatchee National Wildlife Refuge	Boynton Beach (Palm Beach)	U.S. Fish and Wildlife Service

[1] – Field trials initiated in March, 2007

Table 4. Herbicide (product name, amount active ingredient) and percent solution used in *Lygodium microphyllum* field trials 2006-2008.

Herbicide (product name)	% Product
Glyphosate (Rodeo, 4 lbs a.e./gallon)	2.0
Glyphosate (Rodeo, 4 lbs a.e./gallon)	4.0
Metsulfuron methyl (Escort XP, 60% a.i.)	2 oz./100 gal
Metsulfuron methyl (Escort XP, 60% a.i.)	4 oz./100 gal
Triclopyr (Renovate 3, 3 lbs a.e./gallon)	2.0
Imazapic (Plateau, 2 lbs a.e./gallon)	1.5
Metsulfuron methyl (60% a.i.) + glyphosate (4 lbs a.e./gallon)	2 oz/100 gal + 2.0
Metsulfuron methyl (60% a.i.) + triclopyr (3 lbs a.e./gallon)	2 oz/100 gal + 2.0
Metsulfuron methyl (60% a.i.) + imazapic (2 lbs a.e./gallon)	2 oz/100 gal + 1.5
Glyphosate (4 lbs a.e./gallon) + imazapic (2 lbs a.e./gallon)	2.0 + 1.5
Glyphosate (4 lbs a.e./gallon) + triclopyr (3 lbs a.e./gallon)	2.0 + 2.0
Glyphosate (4 lbs a.e./gallon) + metsulfuron methyl (60% a.i.) + triclopyr (3 lbs a.e./gallon)	1.0 + 1.0 + 1.0
Control	Surfactant + water

Table 5. Mean dry weight (grams) and standard error of *Lygodium microphyllum* re-growth following 2.5 months following herbicide treatment (n = 32 plants per treatment) under greenhouse conditions using 20 gallons diluent.

Herbicide	Rate	Dry Weight [1] (grams)	S.E.
Glyphosate (Rodeo)	3.75 lbs a.e./acre	0.13 [a]	0.07
Metsulfuron methyl (Escort XP)	1.20 oz a.i./ acre	0.61 [a, b]	0.42
Imazapyr (Habitat)	1.50 lbs a.e./acre	4.07 [b]	1.18
Imazapic (Plateau)	0.19 lbs a.e./acre	3.58 [a, b]	0.97
Quinclorac (Drive)	1.5 lbs a.i. / acre	8.01 [c]	1.38
Penoxsulam (Grasp)	0.04 lbs a.i. / acre	7.71 [c]	1.09
Imazamox (Raptor)	0.05 lbs a.e./acre	10.37 [d]	1.16
Triclopyr (Renovate 3)	6.0 lbs a.e./acre	0.00 [a]	0.00
Control (surfactant only)	N/A	9.68 [d]	0.65

[1] - Letters in column with different letters indicate significant differences ($p < 0.05$)

Table 6. Native species richness, *Lygodium microphyllum* dry weight, and native vegetation dry weight for greenhouse pots at 6 months post-treatment. Herbicide rates are same as in Table 4 (n = 15 plants per treatment).

	Means (S.E.) [1]		
Herbicide	*Lygodium microphyllum* Dry Weight (g)	Native Vegetation Dry Weight (g)	Species Richness
Glyphosate	0.52 [a] (0.25)	5.50 [d] (0.76)	5.27 [e] (0.53)
Metsulfuron methyl	2.08 [a] (1.57)	4.52 [c,d] (0.77)	4.93 [d,e] (0.51)
Imazapyr	1.78 [a] (0.88)	0.64 [a] (0.25)	1.40 [a] (0.35)
Imazapic	0.69 [a] (0.42)	1.73 [a,b] (0.64)	1.87 [a,b] (0.39)
Quinclorac	6.33 [b] (1.28)	4.05 [b,c,d] (1.18)	3.67 [c,d] (0.61)
Penoxsulam	10.00 [c] (0.87)	2.07 [a,b] (0.54)	2.93 [b,c] (0.48)
Imazamox	11.07 [c,d] (1.24)	1.43 [a] (0.40)	3.20 [b,c] (0.45)
Triclopyr	0.35 [a] (0.09)	9.06 [e] (1.36)	6.33 [e] (0.63)
Control	13.41 [d] (1.34)	2.22 [a,b,c] (0.47)	3.67 [c,d] (0.51)

[1] – Letters in column with different letters indicate significant differences ($p < 0.05$)

Table 7. Mean dry weight (grams) and standard error of *Lygodium microphyllum* re-growth following 2.5 months following herbicide treatment (n = 4 plants per treatment).

Herbicide	Rate [1]	Mean Dry Weight (grams)[2]
Imazamox	0.40 lbs a.e./acre	0.01 [a]
	0.20 lbs a.e./acre	0.03 [a]
	0.10 lbs a.e./acre	0.35 [a, b]
	0.05 lbs a.e./acre	2.27 [a, b, c, d, e]
Aminopyralid	0.10 lbs a.e./acre	3.84 [c, d, e, f, g]
	0.05 lbs a.e./acre	0.48 [a, b]
	0.25 lbs a.e./acre	5.35 [e, f, g]
	0.13 lbs a.e./acre	2.09 [a, b, c, d]
Fluroxypyr	0.48 lbs a.e./acre	1.00 [a, b, c]
	0.24 lbs a.e./acre	3.51 [b, c, d, e, f]
	0.12 lbs a.e./acre	8.63 [h]
	0.06 lbs a.e./acre	6.34 [f, g]
Flumioxazin	0.38 lbs a.i./acre	5.37 [e, f, g]
	0.19 lbs a.i./acre	0.91 [a, b, c]
	0.10 lbs a.i./acre	6.87 [g, h]
	0.05 lbs a.i./acre	2.92 [a, b, c, d, e]
Bispyribac-sodium	0.13 lbs a.i./acre	2.62 [a, b, c, d, e]
	0.07 lbs a.i./acre	3.98 [c, d, e, f, g]
	0.04 lbs a.i./acre	5.44 [e, f, g]
	0.02 lbs a.i./acre	2.06 [a, b, c, d]
Control	Surfactant + water	4.92 [d, e, f, g]

[1] – Amount active ingredient was rounded to the nearest 1/100 lb.

[2] – Letters in column with different letters indicate significant differences ($p < 0.05$)

Table 8. Percent change at 6 months post-treatment in *Lygodium microphyllum*, native vegetation, and species richness based on herbicide field trials at six locations in central and south Florida.

Herbicide (rate)	% Change (SE)[1] *L. microphyllum*	% Change (SE)[1] Native Plants	% Change (SE)[1] Species Richness
Glyphosate (2% product)	- 84.1 [a] (8.1)	- 56.87 [a,b] (13.8)	+ 2.78 [b] (12.1)
Glyphosate (4% product)	- 87.3 [a] (9.8)	- 66.2 [a] (10.9)	- 15.5 [a,b] (18.9)
Metsulfuron methyl (2 oz. / 100 gal.)	- 74.6 [a] (13.5)	- 31.6 [a,b] (23.6)	+ 2.5 [b] (9.9)
Metsulfuron methyl (2 oz. / 100 gal.)	- 78.2 [a] (10.8)	- 31.4 [a,b] (18.9)	-23.9 [a,b] (13.4)
Triclopyr (2% product)	- 91.5 [a] (2.4)	- 31.4 [a,b] (8.1)	- 5.1 [b] (9.7)
Imazapic (1.5% product)	- 82.8 [a] (9.8)	- 71.5 [a] (7.1)	-48.5 [a] (4.1)
Metsulf. methyl (2 oz) + Glyphosate (2%)	- 81.9 [a] (8.5)	- 53.8 [a,b] (19.2)	- 27.3 [a,b] (15.7)
Metsulf. methyl (2 oz) + Triclopyr (2%)	- 91.5 [a] (3.8)	- 43.6 [a,b] (31.7)	- 24.3 [a,b] (7.7)
Metsulf. methyl (2 oz) + Imazapic (1.5%)	- 94.2 [a] (3.5)	- 50.4 [a,b] (9.2)	- 21.9 [a,b] (6.1)
Glyphosate (2%) + Imazapic (1.5%)	- 92.4 [a] (6.9)	- 55.3 [a,b] (11.3)	- 30.1 [a,b] (15.1)
Glyphosate (2%) + Triclopyr (2%)	- 89.6 [a] (6.6)	- 41.9 [a,b] (22.5)	- 27.2 [a,b] (18.5)
Glypho. (1%) + metsulf methyl (1 oz) + triclopyr(1%)	- 78.1 [a] (10.1)	- 56.4 [a,b] (12.9)	-14.1 [a,b] (17.1)
Control (surfactant only)	- 4.6 [b] (7.1)	- 16.0 [b] (14.9)	- 24.3 [a,b] (12.9)

[1] - Letters in column with different letters indicate significant differences ($p < 0.10$).