

Response of Old World climbing fern and native vegetation to repeated ground herbicide treatments

JEFFREY T. HUTCHINSON AND KENNETH A. LANGELAND*

ABSTRACT

Old World climbing fern [*Lygodium microphyllum* (Cav.) R. Br.; OWCF] is an invasive vine in Florida that alters natural area structure and composition, displacing native vegetation and altering fire regimes. In this study, we examined the use of repeated ground foliar herbicide treatments using glyphosate, metsulfuron methyl, triclopyr, and imazapic alone or in combinations with treatments applied at 6-mo intervals over 2 yr. OWCF was controlled to $\leq 4.4\%$ cover for all treatments after four treatments over 2 yr. No treatment completely eliminated OWCF, indicating that follow-up treatments will be required for long-term control of this fern. OWCF can be managed at low levels with two herbicide treatments 6 mo apart, with monitoring and retreatment required as needed every 12 mo thereafter. Following herbicide treatment of OWCF, there was a shift toward early-successional, ruderal native species such as graminoids and forbs and a decline in native ferns. These results indicate that at least two initial herbicide treatments 6 mo apart followed by long-term spot treatments are required for greater than 95% control of OWCF, and native plant community recovery in heavily infested areas is slow and may take more than 2 yr to recover.

Key words: Florida, glyphosate, imazapic, *Lygodium microphyllum* (Cav.) R. Br., metsulfuron, triclopyr.

INTRODUCTION

Old World climbing fern [*Lygodium microphyllum* (Cav.) R. Br.; OWCF] is one of the most invasive plants in natural areas of central and south Florida and is expanding into northern Florida (Pemberton and Ferriter 1998, Hutchinson and Langeland 2010). First documented in Florida in 1959 from two herbarium records from nurseries in central and southern Florida, OWCF has spread across the landscape of central and south Florida very rapidly. OWCF alters natural communities by displacing native species and changing community structure and ecological functions (Gordon 1998, Brandt and Black 2001). Outside its native range in Southeast Asia and Northern Australia, OWCF is

known to be invasive only in Florida, but has the potential to spread into Central and South America due to wind-blown spores (Goolsby 2004). Estimated spore dispersal in OWCF has been recorded to be $724 \text{ spores m}^3 \text{ h}^{-1}$, which explains how OWCF has rapidly invaded natural areas of southern and central Florida (Pemberton and Ferriter 1998).

Herbicides are currently the primary technique used to control OWCF in Florida's natural areas (Hutchinson et al. 2006). OWCF invades seasonally inundated forested natural areas such as mesic pine flatwoods, bay swamps, cypress swamps, and Everglade tree islands. Historically, glyphosate was the most frequently used herbicide for OWCF control (Stocker et al. 1997). Some of the initial herbicide screenings to control OWCF under field conditions were conducted by Stocker et al. (1997) using glyphosate, triclopyr, glyphosate + triclopyr, 2,4-D, and glufosinate. All herbicides resulted in 100% top-kill of OWCF, but regrowth occurred from rhizomes (Stocker et al. 1997). Thomas and Brandt (2003) reported that the cut-and-spray method using glyphosate, with an initial and follow-up treatment, was very effective as long as all parts of the fern were treated. In a follow-up to the Thomas and Brandt (2003) study, the median OWCF ground and midcanopy cover were both less than 5% (Barrett et al. 2006). Metsulfuron methyl has shown to be effective in control of OWCF (Langeland and Link 2006). Control of OWCF using two applications of metsulfuron methyl (84 g ha^{-1}) was 94% at 23 mo posttreatment, with 64% control reported for plots treated twice with metsulfuron methyl at 42 g ha^{-1} (Langeland and Link 2006). Triclopyr used at 2-mo and 6-mo intervals over 22 mo resulted in greater than 99% control of OWCF (Stocker et al. 2008). Repeated use of triclopyr over 22 mo did not result in a permanent decrease in native species cover, richness, evenness, or diversity (Stocker et al. 2008). Hutchinson and Langeland (2012) reported a reduction in OWCF cover to 1 to 2% and 8 to 10% pretreatment cover using metsulfuron and glyphosate, respectively, following an initial aerial treatment and two subsequent ground spot treatments on Everglade tree islands. However, there was a large decrease in native ground cover at the end of the study with a concomitant increase of ruderal species not typically found on Everglade tree islands (Hutchinson and Langeland 2012).

Of the studies above, only those in which there were two or more herbicide applications resulted in acceptable control of less than 5% cover of OWCF. Stocker et al. (2008) described the difficulties and inability to achieve complete control of OWCF after observing 0.7% OWCF

*First and second authors: Postdoctoral Assistant and Professor, University of Florida, Center for Aquatic and Invasive Plants, 7922 NW 71st S, Gainesville, FL 32653. Current address of first author: U.S. Fish and Wildlife Service, San Marcos Aquatic Resources Center, 500 E McCarty Lane, San Marcos, TX 78666. Corresponding author's E-mail: jeffrey_hutchinson@fws.gov. Received for publication December 4, 2013 and in revised form August 31, 2014

cover in plots evaluated and treated with triclopyr up to 12 times over a 2-yr period. Additionally, Langeland and Link (2006) reported that OWCF dry weight biomass in plots treated with metsulfuron (42 and 84 g ha⁻¹) was not significantly different from the OWCF dry weight biomass in untreated plots 28 mo posttreatment. Hutchinson and Langeland (2012) found that herbicide control of OWCF was effective, but that long-term monitoring and spot treatments are required for long-term management. It is likely that established, spore-producing populations of OWCF in natural areas will require multiple herbicide applications and long-term management to maintain OWCF at less than 5% cover. With the exception of Stocker et al. (2008) and Hutchinson and Langeland (2012), no other studies have evaluated the response of native plants to greater than two repeated herbicide applications for control of OWCF.

The objectives of this study were to evaluate the effectiveness of glyphosate, metsulfuron, triclopyr, and imazapic alone and in combinations using repeated ground treatments for control of OWCF and examine the effects on native plant cover. These herbicides were chosen because glyphosate, metsulfuron, and triclopyr are the herbicides most commonly used to treat OWCF in Florida and exhibited high efficacy in greenhouse trials on OWCF (Hutchinson and Langeland 2006; Hutchinson et al. 2006). Imazapic was selected because it showed high efficacy in greenhouse trials (J. T. Hutchinson and K. A. Langeland, unpub. data) but has not been tested on OWCF under field conditions.

MATERIALS AND METHODS

This study occurred in six locations infested with OWCF in central and southern Florida from September 2006 to October 2008 (Figure 1). Habitat types included cypress swamp (Jonathan Dickinson State Park, Pacific Tomato Growers Inc.), bay swamp (Lakeland, Balm Scrub Natural Area, Highlands Hammock State Park), and Everglade tree islands (Loxahatchee National Wildlife Refuge). Different habitats were selected for this study to represent the most common types of habitat invaded by OWCF (Langeland and Hutchinson 2013). Treatment methods for OWCF are generalized and often not specific to habitat type and limited information exists on the effects of different herbicides on native vegetation.

Each treatment was replicated at the six study locations and included control plots. Control plots were selected on the edges of OWCF infestations and contained lower cover of OWCF to evaluate if OWCF was expanding in untreated plots over the 2-yr evaluation period. Plots were 2 × 10 m and randomly placed at all sites and marked at the beginning and end with tagged 2-m polyvinyl chloride pipes. At each site, 10-m cover transect lines (Canfield 1941) were set up for each treatment in areas with greater than 40% coverage of OWCF. The distance, to the nearest 0.5 cm, of each plant along the transect line was recorded. Percentage of coverage of each species was determined by dividing the summed distance of each plant species along the line by the distance of the line. Pretreatment monitor-

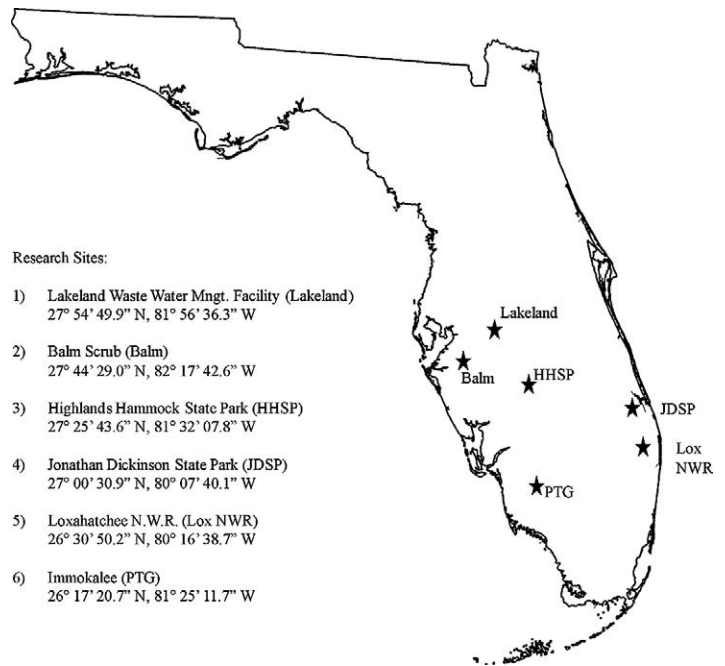


Figure 1. Location of Old World climbing fern ground herbicide research sites in Florida.

ing was conducted prior to treatment in September and October 2006 and the plots were sprayed immediately following evaluation. All plots were reevaluated in February and March 2007, September and October 2007, and February and March 2008. All live OWCF was spot-treated with the same treatments for corresponding plots immediately after the 6-, 12-, and 18-mo evaluations. A final posttreatment evaluation was conducted at 24 mo (September to October 2008).

Herbicide treatments included glyphosate,¹ metsulfuron,² triclopyr,³ and imazapic,⁴ representing three modes of action used alone or in combination at maximum label rates (Table 1). Herbicides were applied to OWCF on a spray-to-wet basis with a 5.7-L hand-pressurized backpack sprayer with a flat-fan nozzle; treatments represent the maximum label rate. Treatments were directed at OWCF to avoid nontarget damage and areas within plots that were not covered with OWCF were not treated. Because of the climbing and twining nature of OWCF over native vegetation, some nontarget damage was unavoidable. Treatments were designed to follow the methods of field applicators treating OWCF in natural areas of Florida. All treatments included nonionic surfactant⁵ at 0.5 v/v with a spray volume of 1 L.

Plots were analyzed for OWCF and native percentage of cover (all species combined), species richness, and evenness at 0 (pretreatment), 6, 12, 18, and 24 mo after treatment (MAT). Data for native plant cover were combined into one composite sample because some species were not present in all sites and many species had < 1% cover at 24 MAT. All native species with ≥ 5% cover at pretreatment and 24 MAT in at least two plots were compiled to document the

TABLE 1. HERBICIDE TREATMENTS USED FOR CONTROL OF OLD WORLD CLIMBING FERN FIELD TRIAL STUDY DURING 2006 TO 2008.

Treatment	Active Ingredient (g L ⁻¹)	Mode of Action
Glyphosate	12.9 and 25.8	Inhibits aromatic amino acid synthesis (AAAS)
Metsulfuron methyl	0.03 and 0.06	Inhibits branched-chain amino acid synthesis (BCAAS)
Triclopyr	10.0	Growth regulator (GR)
Imazapic	3.9	Inhibits BCAAS
Metsulfuron methyl + glyphosate	0.03 + 12.9	BCAAS + AAAS
Metsulfuron methyl + triclopyr	0.03 + 10.0	BCAAS + GR
Metsulfuron methyl + imazapic	0.03 + 3.9	BCAAS + BCAAS
Glyphosate + imazapic	12.9 + 3.9	AAAS + BCAAS
Glyphosate + triclopyr	12.9 + 10.0	AAAS + GR
Glyphosate + metsulfuron methyl + triclopyr	4.3 + 0.015 + 3.33	AAAS + BCAAS + GR
Control		No treatment

most common species at 0 and 24 MAT. All OWCF was treated at 0, 6, 12, and 18 mo and the final evaluation occurred at 24 mo. Species richness was determined by counting the number of plant species that intersected the transect line. Evenness patterns were calculated as $E = 1/DS$, where E is evenness, D is Simpson's Diversity Index [$\sum(n/N)^2$], and S is species richness (Williams 1964).

Data were analyzed at pretreatment and 6, 12, 18, and 24 MAT using an ANOVA ($P \leq 0.05$) and means compared with Tukey's Multiple Comparison test ($P \leq 0.05$) if differences were detected with the ANOVA.⁶ There were no treatment by site interactions and all data from the sites were combined for final analysis. Data were transformed if needed using an arcsine square root transformation to improve homogeneity of variance assumptions (Zar 1999) by visual inspection of the plotted residuals.

RESULTS AND DISCUSSION

Old World climbing fern

At pretreatment, there was a significant difference ($P = 0.021$) between treatment plots and controls (Table 2), with control plots containing less OWCF. At 6 MAT, seven plots

treated with herbicide contain less OWCF cover than control plots ($P = 0.0031$). During the 12- ($P = 0.0005$), 18- ($P = 0.0017$), and 24-MAT evaluations, all herbicide-treated plots contained significantly less OWCF cover than control plots. Comparison of OWCF cover among controls from 0 to 24 MAT was nonsignificant ($P = 0.894$), indicating that over the 2-yr period, OWCF did not increase in area cover. At 24 MAT, the percentage of reduction in OWCF cover was greater than 95% for all treatments. The mean cover of OWCF for all treatments combined at the 24-MAT evaluation was 2.1% (SE = 0.4; 95% confidence interval = 1.3 to 2.9).

The 12 treatments used in this study, four herbicides used alone or in combination, applied four times each at 6-mo intervals, were equally effective for control of OWCF. These results are comparable to those reported for four applications of triclopyr over a 2 yr period in which OWCF cover was reduced to less than 0.7% using prescribed fire and triclopyr in pine flatwoods (Stocker et al. 2008). As reported by Stocker et al. (2008) and documented in this study, OWCF was still present after four applications, and increased in some plots during this study from 18 to 24 mo. During the second herbicide application at 6 mo, the majority of the OWCF present was rhizome sprouts or foliage missed during the initial treatment. During the third

TABLE 2. TREATMENT, MEAN PERCENTAGE OF COVER, AND STANDARD ERROR OF OLD WORLD CLIMBING FERN AT 0, 6, 12, 18, AND 24 MO AFTER TREATMENT.

Herbicide (g L ⁻¹)	Old World Climbing Fern (% cover) ¹				
	Time (mo after treatment)				
	0 (pretreatment)	6	12	18	24
Glyphosate (12.9)	66.3 (8.8) a	5.8 (2.4) b	4.1 (2.6) b	1.7 (1.1) b	1.5 (0.7) b
Glyphosate (25.8)	68.8 (9.7) a	1.9 (1.1) b	1.0 (0.6) b	2.1 (1.8) b	3.3 (2.7) b
Metsulfuron (0.03)	71.7 (11.8) a	16.8 (12.6) ab	7.3 (4.5) b	1.4 (0.7) b	4.3 (3.0) b
Metsulfuron (0.06)	69.3 (9.5) a	13.9 (8.6) ab	3.5 (1.9) b	2.8 (1.3) b	3.6 (2.1) b
Triclopyr (10.0)	70.8 (7.1) a	4.6 (1.4) b	0.7 (0.3) b	0.8 (0.7) b	0.7 (0.5) b
Imazapic (3.9)	70.5 (3.9) a	11.1 (7.2) ab	2.3 (1.3) b	1.3 (0.8) b	4.4 (3.0) b
Metsulfuron (0.03) + glyphosate (12.9)	65.7 (9.4) a	10.2 (6.4) ab	1.1 (0.7) b	2.4 (2.1) b	2.4 (1.1) b
Metsulfuron (0.03) + triclopyr (10.0)	67.2 (8.1) a	5.8 (2.9) b	2.4 (1.8) b	1.6 (0.7) b	1.2 (0.8) b
Metsulfuron (0.03) + imazapic (3.9)	64.9 (8.1) a	3.3 (1.8) b	2.1 (1.1) b	2.4 (1.8) b	0.4 (0.2) b
Glyphosate (12.9) + imazapic (3.9)	72.3 (7.8) a	4.8 (4.4) b	0.0 (0.0) b	0.8 (0.6) b	1.7 (1.0) b
Glyphosate (12.9) + triclopyr (10.0)	64.0 (8.9) a	7.7 (4.3) b	2.3 (1.3) b	0.9 (0.8) b	1.6 (1.1) b
Glyphosate (4.3) + metsulfuron (0.015) + triclopyr (3.3)	59.0 (9.2) a	9.3 (4.6) ab	1.6 (0.8) b	2.4 (2.4) b	0.6 (0.5) b
Control	28.8 (13.6) b	28.2 (14.6) a	33.2 (15.6) a	29.2 (14.9) a	32.7 (15.1) a

¹Different letter in columns represent significant treatment differences based on Tukey's Multiple Comparison test ($P < 0.05$). Values represent the means of six replications.

TABLE 3. TREATMENT, MEAN PERCENTAGE OF COVER, AND STANDARD ERROR OF NATIVE VEGETATION AT 0, 6, 12, 18, AND 24 MO AFTER TREATMENT.

Herbicide (g L ⁻¹)	Native Vegetation (% cover) ¹				
	Time (mo after treatment)				
	0 (pretreatment)	6	12	18	24
Glyphosate (12.9)	63.0 (11.2) b	35.3 (11.9) b	38.8 (8.8) b	55.4 (10.3) bc	53.8 (4.6) bc
Glyphosate (25.8)	72.0 (10.6) ab	31.3 (9.7) b	34.5 (8.5) b	43.3 (6.4) bc	53.3 (5.9) bc
Metsulfuron (0.03)	52.0 (13.6) b	29.8 (8.0) b	50.5 (13.8) b	49.2 (6.4) bc	67.1 (9.9) ab
Metsulfuron (0.06)	45.5 (9.1) b	28.5 (11.6) b	24.1 (9.1) b	37.7 (9.7) c	33.4 (5.4) c
Triclopyr (10.0)	46.5 (9.0) b	36.4 (7.8) b	43.2 (10.6) b	55.0 (14.2) bc	55.3(10.3) bc
Imazapic (3.9)	62.1 (16.4) b	18.6 (7.6) b	21.5 (7.1) b	36.7 (13.7) c	50.3 (14.1) bc
Metsulfuron (0.03) + glyphosate (12.9)	60.2 (15.7) b	23.7 (9.1) b	28.3 (8.5) b	32.3 (8.9) c	40.8 (6.8) bc
Metsulfuron (0.03) + triclopyr (10.0)	63.7 (12.1) b	28.6 (5.9) b	41.2 (12.4) b	54.1 (11.3) bc	54.4 (8.9) bc
Metsulfuron (0.03) + imazapic (3.9)	63.1 (13.0) b	30.7 (10.4) b	29.2 (10.4) b	38.4 (11.2) c	40.9 (8.9) bc
Glyphosate (12.9) + imazapic (3.9)	57.9 (12.4) b	24.8 (6.8) b	24.7 (5.7) b	52.7 (9.9) bc	53.3 (4.7) bc
Glyphosate (12.9) + triclopyr (10.0)	56.9 (11.9) b	38.7 (15.3) b	49.2 (9.5) b	52.3 (10.5) bc	62.7 (10.6) b
Glyphosate (4.3) + metsulfuron (0.015) + triclopyr (3.3)	64.2 (17.9) b	36.7 (12.9) b	48.3 (12.4) b	72.9 (12.7) ab	64.7 (13.9) ab
Control	106.5 (26.0) a	83.5 (13.8) a	90.4 (17.9) a	94.9 (18.4) a	92.7 (15.6) a

¹Different letter in columns represent significant treatment differences based on Tukey's Multiple Comparison test ($P < 0.05$). Values represent the means of six replications.

(12-mo) and fourth (18-mo) applications, the majority of live OWCF observed was from new growth from spore germination, with less than 5% from rhizome regrowth. These results indicate that a minimum of two treatments (initial and retreatment at 6 mo) are required to reduce OWCF cover to less than 5%, but additional treatments will be required for long-term control of new growth from spore germination.

Management of OWCF with herbicides needs to include a follow-up treatment at 6 mo posttreatment, with additional follow-up treatments at 12 mo following the second treatment for control of new growth from spores. Stocker et al. (2008) reported that OWCF recorded at 2 yr following four treatments with triclopyr was from rhizome sprouts. Regardless, an initial treatment and a follow-up treatment at 6 mo are required for greater than 95% reduction of OWCF. Additional treatments will be required as funding and personnel allow and should be incorporated into management plans where OWCF is a problematic species.

The initial herbicide treatment applied by ground applicators is difficult due to the twining and climbing nature of OWCF, which forms a matrix through and over native vegetation. With the characteristic overlapping layers of OWCF rachis intermixed with native vegetation, dense ground cover, fallen debris, and hydric conditions, it is often difficult to apply a complete foliar spray to dense infestations of OWCF in which every rachis is treated. These conditions make it imperative that follow-up treatments occur at 6 mo following the initial treatment. There is evidence that OWCF ground cover can be controlled to less than 5% cover on tree islands after two treatments with glyphosate for up to 4.5 yr (Barrett et al. 2006). In this study, minimal OWCF was observed at 12, 18, and 24 MAT. This indicates that initial and follow-up treatments from 0 to 6 mo are important for control of established plants, and additional follow-up treatment is needed for new OWCF growth developed from spores.

Native vegetation

At pretreatment, control plots had significantly ($P = 0.045$) greater native vegetative cover than all herbicide-treated plots except the plot in which glyphosate was applied at 25.8 g L⁻¹ (Table 3). During the 6- ($P = 0.017$), 12- ($P = 0.004$), and 18- ($P = 0.026$) MAT evaluations, the control plots contained significantly greater area cover of native vegetation compared to herbicide-treated plots. Native vegetation cover in all herbicide-treated plots decreased 60 to 80% at 6 MAT compared to pretreatment cover, but increased in native vegetation cover at 18 MAT. At 24 MAT, the control plots contained significantly greater native vegetation cover than all herbicide-treated plots except metsulfuron (0.03 g L⁻¹) and glyphosate + metsulfuron + triclopyr plots. At 24 MAT, the highest native vegetation cover was recorded in plots treated with glyphosate + triclopyr, metsulfuron (0.03 g L⁻¹), and glyphosate + metsulfuron + triclopyr. Plots treated with metsulfuron (0.03 g), triclopyr, and glyphosate + triclopyr exhibited higher percentage of native cover at 24 MAT relative to pretreatment (0 mo) cover. At 24 MAT, the percentage of change in native cover was highly variable and ranged from a 20% increase for metsulfuron (low rate) to a 42% decrease for metsulfuron + glyphosate. Overall, there was a general trend of increasing cover in native species from 6 to 24 MAT. Comparison of native vegetation in control plots from 0 to 24 MAT was nonsignificant ($P = 0.453$), indicating that native vegetation patterns were similar over the duration of the study.

It is doubtful that residual herbicide in the soil from treatments affected native plant recruitment or resprouting since recruits of ruderal species were common at 24 MAT. OWCF was spot-treated and limited herbicide runoff was observed. Imazapic has the longest half-life of the herbicides tested (Vencill 2002) and plant species richness in the imazapic treatment was the fourth highest among the 12 herbicide treatments.

Native plant cover exhibited an increase from 6 to 24 MAT, but the composition of native plants changed from

TABLE 4. TREATMENT, MEAN, AND STANDARD ERROR OF SPECIES RICHNESS AT 0, 6, 12, 18, AND 24 MO AFTER TREATMENT.

Herbicide (g L ⁻¹)	Species Richness ¹				
	Time (mo after treatment)				
	0 (pretreatment)	6	12	18	24
Glyphosate (12.9)	7.8 (0.9) abc	6.5 (0.5) abcd	8.0 (1.0) ab	8.3 (0.8) ab	9.5 (1.4)
Glyphosate (25.8)	6.7 (0.7) bc	6.0 (1.6) abcd	6.8 (1.3) ab	7.8(1.1) ab	11.0 (2.1)
Metsulfuron (0.03)	6.5 (0.4) c	6.0 (0.9) abcd	6.8 (1.1) ab	8.2 (1.2) ab	8.8 (1.6)
Metsulfuron (0.06)	7.0 (0.4) bc	5.8 (0.8) abcd	6.0 (0.4) ab	8.2 (1.2) ab	8.8 (1.7)
Triclopyr (10.0)	7.8 (0.9) abc	7.2 (1.1) ab	9.2 (1.1) a	8.3 (1.1) ab	8.5 (1.2)
Imazapic (3.9)	8.6 (0.8) abc	3.8 (0.7) d	6.3 (0.9) ab	6.5 (1.1) b	9.8 (1.4)
Metsulfuron (0.03) + glyphosate (12.9)	8.0 (0.5) abc	5.5 (1.0) abcd	7.5 (1.8) ab	9.0 (1.3) ab	9.2 (1.2)
Metsulfuron (0.03) + triclopyr (10.0)	9.8 (0.9) a	8.0 (0.9) a	8.8 (1.5) a	10.3 (1.7) a	10.5 (1.8)
Metsulfuron (0.03) + imazapic (3.9)	7.3 (0.7) bc	5.2 (0.6) bcd	5.3 (1.2) b	8.7 (0.7) ab	9.5 (2.2)
Glyphosate (12.9) + imazapic (3.9)	6.8 (0.9) bc	4.3 (1.2) cd	4.7 (0.9) c	9.2 (1.6) ab	8.3 (1.5)
Glyphosate (12.9) + triclopyr (10.0)	7.8 (0.6) abc	6.0 (0.7) abcd	7.3 (1.0) ab	7.7 (1.2) ab	8.5 (1.6)
Glyphosate (4.3) + metsulfuron (0.015) + triclopyr (3.3)	7.5 (0.7) bc	7.5 (1.1) ab	8.5 (1.2) ab	8.7 (1.1) ab	10.0 (1.1)
Control	8.8 (1.2) ab	6.8 (0.9) abc	8.0 (1.0) ab	8.5 (0.6) ab	8.8 (0.7)

¹Different letter in columns represent significant treatment differences based on Tukey's Multiple Comparison test ($P < 0.05$). Values represent the means of six replications.

typical late-successional species dominated by native ferns to a composition dominated by generalists and early-successional species. Stocker et al. (2008) reported that the cover of graminoids, which are primarily early-successional, open-canopy species, increased following treatment with triclopyr in pine flatwoods, while native fern cover decreased significantly with time. A comparison of tree islands at 3.0 and 4.5 yr posttreatment treated twice with glyphosate to control OWCF indicated that native plants did not increase for ground or overstory cover, but increased from 10 to 24% for midstory (i.e., shrub layer) (Barrett et al. 2006). Hutchinson and Langeland (2012) found that Everglade tree islands treated with aerial and ground applications of glyphosate or metsulfuron for control of OWCF over a 4-yr period resulted in an influx of ruderal native species not typical of tree islands. The results from this study indicate that native plant recovery in natural areas will require greater than 2 yr to succeed from generalist, early-successional plants to one of native ferns, provided that OWCF cover is managed at levels less than 5%.

Bay swamp, cypress swamp, and tree islands are important for providing habitat heterogeneity for many taxa in the landscape matrix of southern Florida. It is unknown how large-scale alteration of these habitats into ruderal vegetation will affect species composition over many years. The increase in ruderal species in all three habitats is not reflective of their typical vegetation, but more reflective of a habitats modified by OWCF and subsequent herbicide treatment. The transition from late-successional to early-successional native species following herbicide application resulted in a few recruits of native late-successional species and a large influx of ruderal species recruits. As suggested by MacDougall and Turkington (2005), there were shifts from species dominants (i.e., late-successional species) to annual and perennial forbs, and graminoids that are functionally distinct (i.e., ruderal) in disturbed ecosystems.

Species richness and evenness

Species richness ranged from 6.5 to 8.8 at pretreatment (0 MAT) and was significantly different ($P = 0.041$) in five of the herbicide-treated plots (Table 4). At 6 MAT, species richness was lowest in plots treated with imazapic ($P = 0.017$). Species richness increased in all plots from 6 to 24 MAT with the exception of glyphosate + imazapic at 24 MAT, where there was a decline in species richness. Species richness was highest in plots treated with glyphosate + triclopyr ($n = 11$) and metsulfuron + imazapic ($n = 10.5$), and lowest in plots treated with metsulfuron (0.06 g; $n = 6.3$) and glyphosate + imazapic ($n = 6.3$). Species richness in control plots from 0 to 24 MAT was not significantly ($P = 0.062$) different at pretreatment and at 6, 12, 18, and 24 MAT, indicating that species richness remain relatively stable throughout the study.

At pretreatment, 27 native species with $\geq 5\%$ ground cover were recorded in at least two plots across time and location, with 37.0% of these species being ferns (Table 6). During the 24-mo evaluation period, 63 species with $\geq 5\%$ ground cover were recorded in at least two plots, with only 6.4% of these species being ferns. At 24 MAT, the dominant native ground cover shifted from being dominated by ferns to being dominated by graminoids and forbs. The number of nonnative species with $\geq 5\%$ ground cover increased from four during pretreatment to seven at 24 MAT.

There were no significant differences in species evenness at pretreatment (0 MAT) ($P = 0.897$; Table 5). Species evenness was variable at 6, 12, 18, and 24 MAT with no discernable pattern. However, species evenness was higher or equal to pretreatment values at 24 MAT. At 6, 12, and 24 MAT, species evenness was higher in plots treated with metsulfuron + imazapic. Changes in evenness were due to a decline in OWCF and native ferns and a concomitant increase in generalist and early-successional species. In control plots, no generalist or early-successional species were observed during the study and the control plots were dominated by two to four native ferns. Comparison of species evenness in control plots from 0 to 24 MAT was

TABLE 5. TREATMENT, MEAN, AND STANDARD ERROR OF EVENNESS AT 0, 6, 12, 18, AND 24 MO AFTER TREATMENT.

Herbicide (g L ⁻¹)	Evenness ¹				
	Time (mo after treatment)				
	0 (pretreatment)	6	12	18	24
Glyphosate (12.9)	0.38 (0.05)	0.54 (0.08) ab	0.50 (0.03) bcd	0.44 (0.06) ab	0.47 (0.03) ab
Glyphosate (25.8)	0.46 (0.05)	0.47 (0.06) ab	0.43 (0.05) cd	0.48 (0.07) ab	0.46 (0.08) ab
Metsulfuron (0.03)	0.34 (0.04)	0.48 (0.05) ab	0.46 (0.06) cd	0.41 (0.06) b	0.43 (0.05) ab
Metsulfuron (0.06)	0.37 (0.06)	0.61 (0.08) ab	0.52 (0.06) bcd	0.44 (0.03) ab	0.51 (0.05) a
Triclopyr (10.0)	0.36 (0.06)	0.47 (0.09) ab	0.53 (0.05) abc	0.48 (0.07) ab	0.48 (0.06) ab
Imazapic (3.9)	0.35 (0.04)	0.63 (0.9) ab	0.57 (0.07) abc	0.58 (0.06) a	0.55 (0.03) a
Metsulfuron (0.03) + glyphosate (12.9)	0.36 (0.05)	0.62 (0.6) ab	0.61 (0.06) abc	0.48 (0.02) ab	0.44 (0.07) ab
Metsulfuron (0.03) + triclopyr (10.0)	0.37 (0.05)	0.47 (0.04) ab	0.57 (0.01) abc	0.53 (0.04) ab	0.49 (0.04) ab
Metsulfuron (0.03) + imazapic (3.9)	0.41 (0.07)	0.65 (0.07) a	0.70 (1.0) a	0.50 (0.05) ab	0.51 (0.05) a
Glyphosate (12.9) + imazapic (3.9)	0.42 (0.04)	0.63 (0.11) ab	0.67 (0.09) ab	0.40 (0.06) b	0.46 (0.05) ab
Glyphosate (12.9) + triclopyr (10.0)	0.38 (0.04)	0.62 (0.08) ab	0.56 (0.02) abc	0.45 (0.06) ab	0.46 (0.07) ab
Glyphosate (4.3) + metsulfuron (0.015) + triclopyr (3.3)	0.40 (0.05)	0.47 (0.08) ab	0.46 (0.08) cd	0.45 (0.05) ab	0.47 (0.07) ab
Control	0.39 (0.04)	0.43 (0.04) ab	0.34 (0.04) d	0.40 (0.05) b	0.35 (0.03) b

¹Different letter in columns represent significant treatment differences based on Tukey's Multiple Comparison test ($P < 0.05$). Values represent the means of six replications.

nonsignificant ($P = 0.215$) indicating evenness patterns were similar in control plots over the duration of the study.

Herbicide treatments did not result in the loss of most native species in this study, but greatly reduced the cover of native ferns. Many of the native ferns and shrubs that survived exhibited etiolated or chlorotic leaves from 6 to 24 MAT. Richness and evenness for native ground cover increased from 0 to 24 MAT for 83 and 75%, respectively, of the treatments, but these results are somewhat ambiguous. The effects of OWCF management with herbicides on native ground cover are comparable to secondary succession. Herbicide treatments resulted in a shift of ground cover dominated by OWCF and native ferns to one dominated by patches of early-successional, generalist species. The increase in generalist species shifts the vegetative composition of these habitats to one that is more cosmopolitan and ruderal rather than the flora composition that is unique to habitats in south Florida. Based on Simpson's Reciprocal Index (1/D), the mean number of common species per treatment increased from 2.5 (SE = 0.1) at pretreatment (0 mo) to 4.4 (SE = 0.4) at 24 MAT.

Stocker et al. (2008) reported there was no significant difference in native species cover, richness, evenness, or diversity at 2 yr posttreatment of OWCF using triclopyr for biannual treatments, but documented an increase in cover of native grasses and one exotic herb. Herbicide treatment of Russian knapweed [*Acroptilon repens* (L.) DC.] with glyphosate resulted in an increase in diversity, but this was due to an increase in nonnative grasses and forbs (Sheley et al. 2007). Herbicide treatment was successful in reducing cover of sweet fennel [*Foeniculum vulgare* P. Mill.], but resulted in an increase of nonnative invasive grasses with no significant increase in native vegetation (Ogden and Rejmanek 2005). This study and those cited above appear to indicate that changes in cover caused by herbicide treatment result in an influx of early-successional species following treatment.

In this study, the loss of native fern cover from 0 to 24 MAT indicates a shift in ground cover from late-successional to early-successional, generalist species at 2 yr

posttreatment. Infestations of OWCF are known to decrease native vegetation cover (Brandt and Black 2001), which may also result in decreased propagules being produced by native ferns. In adjacent sites where no OWCF was present and the upper canopy was intact, the ground cover was visually assessed to be greater than 50% native ferns and less than 5% graminoids. The increase in early successional species at 24 MAT is likely due to decreased ground cover with a concomitant increase in sunlight to the soil; the low tolerance of native ferns to herbicides, which limited their reestablishment after 2 yr; an increase in soil moisture due to a decrease in transpiration; and a seed bank or influx of propagules from graminoids and herbaceous plants.

Species richness does not always correspond to species evenness and diversity (Whittaker 1965, Stirling and Wilsey 2001). Species richness may be more influenced by propagule pressure and seedling emergence whereas evenness and diversity are more influenced by competition (Wilsey and Stirling 2007). In the 2-yr duration of this study, it appears that propagule pressure is the driving factor in the establishment of vegetation following repeated herbicide applications. The decrease of native plants due to OWCF cover combined with the additional stress from repeated herbicide treatments severely impacted the remaining native plants in the treatment sites.

Conclusion

Management of OWCF will require two consecutive treatments 6 mo apart for 95% control. Additional follow-up treatments at > 12 MAT for control of new growth from spores and some resprouts from rhizomes will be required. Managers can expect some variation in the response of OWCF and native vegetation to herbicide treatments based on herbicide, number of treatments, and the habitat type. Following two to four treatments, managers can expect a decline in late-successional native plants followed by an increase in ruderal native plant cover. Long-term research greater than 10 yr may be needed to determine the recovery time for late-successional species to return to natural

TABLE 6. PLANT SPECIES IN A MINIMUM OF TWO PLOTS WITH GROUND COVER $\geq 5\%$ AT PRETREATMENT AND 24 MO FOLLOWING HERBICIDE APPLICATION ACROSS TREATMENT SITES.

Species	Common name	Pretreatment	24 mo after Treatment
<i>Andropogon</i> spp.	Broomsedge grass		X
<i>Acer rubrum</i> L.	Red maple	X	X
<i>Apios americana</i> Medik.	Groundnut		X
<i>Blechnum serrulatum</i> Rich.	Swamp fern	X	X
<i>Boehmeria cylindrica</i> (L.) Sw.	Smallspike false nettle	X	X
<i>Carex longii</i> Mack.	Long's sedge		X
<i>Cephalanthus occidentalis</i> L.	Buttonbush	X	X
<i>Chamaecrista</i> spp.	Partridge pea		X
<i>Cissus verticillata</i> (L.) Nicolson & Jarvis	Possum grape		X
<i>Cladium jamaicense</i> Crantz.	Jamaica swamp sawgrass	X	X
<i>Clematis virginiana</i> L.	Virgin's bower		X
<i>Coelorachis tuberculosa</i> (Nash) Nash	Jointtailgrass		X
<i>Commelina</i> spp.	Dayflower		X
<i>Cuphea carthagenensis</i> (Jacq.) Macbr. ¹	Tarweed cuphea		X
<i>Cyperus haspan</i> L.	Haspan flatsedge		X
<i>Cyperus lanceolatus</i> Poir. ¹	Lance sedge		X
<i>Cyperus odoratus</i> L.	Fragrant flatsedge		X
<i>Cyperus polystachyos</i> Rottb.	Manyspike flatsedge		X
<i>Cyperus strigosus</i> L.	Strawcolored flatsedge		X
<i>Daucus</i> spp.	Wild carrot		X
<i>Echinochloa walteri</i> (Pursh) Heller	Coast cocksbur		X
<i>Erechtites hieraciifolius</i> (L.) Raf. ex DC.	American burnweed		X
<i>Eupatorium capillifolium</i> (Lam.) Small	Dogfennel		X
<i>Helenium pinnatifidum</i> (Nutt.) Rydb.	Southeastern sneezeweed		X
<i>Hydrocotyle</i> spp.	Pennywort		X
<i>Hyptis alata</i> (Raf.) Shinners	Clustered bushmint		X
<i>Ipomoea</i> spp.	Morningglory		X
<i>Juncus effuses</i> L.	Soft rush	X	X
<i>Lachnanthes caroliniana</i> (Lam.) Dandy	Carolina redroot		X
<i>Ludwigia repens</i> Forst.	Floating waterprimrose		X
<i>Ludwigia octovalvis</i> (Jacq.) Raven	Longfruited primrose-willow		X
<i>Lygodium microphyllum</i> (Cav.) R. Br. ¹	Old World climbing fern	X	X
<i>Melaleuca quinquenervia</i> (Cav.) Blake ¹	Melaleuca	X	X
<i>Mikania scandens</i> (L.) Willd.	Climbing hempweed		X
<i>Momordica charantia</i> L. ¹	Balsamapple		X
<i>Myrica cerifera</i> L.	Wax myrtle	X	X
<i>Myrsine cubana</i> A. DC.	Myrsine	X	X
<i>Nephrolepis exaltata</i> (L.) Schott	Sword fern	X	
<i>Nephrolepis</i> \times <i>averyi</i> C.E. Nauman	Avery's sword fern	X	
<i>Oldenlandia uniflora</i> L.	Clustered mille grains		X
<i>Osmunda cinnamomea</i> L.	Cinnamon fern	X	X
<i>Osmunda regalis</i> L.	Royal fern	X	X
<i>Panicum hemitomon</i> Schultes	Maidencane	X	X
<i>Parthenocissus quinquefolia</i> (L.) Planch.	Virginia creeper	X	X
<i>Persea palustris</i> (Raf.) Sarg.	Swamp bay	X	X
<i>Phytolacca americana</i> L.	Common pokeweed		X
<i>Pluchea rosea</i> R.K. Godfrey	Rosy camphorweed		X
<i>Polygonum hydrophiperoides</i> Michx.	Mild smartweed		X
<i>Polygonum punctatum</i> Elliot	Dotted smartweed		X
<i>Pontederia cordata</i> L.	Pickelweed		X
<i>Rhexia mariana</i> L.	Pale meadowberry		X
<i>Rhynchospora chalarocephala</i> Fernald & Gale	Loosehead beaksedge		X
<i>Rhynchospora inundata</i> (Oakes) Fernald	Narrowfruit horned beaksedge		X
<i>Rhynchospora microcarpa</i> Baldw. ex. A. Gray	Southern beaksedge		X
<i>Rubus trivialis</i> Michx.	Southern dewberry	X	X
<i>Salix caroliniana</i> Michx.	Carolina willow	X	X
<i>Saururus cernuus</i> L.	Lizardstail	X	X
<i>Schinus terebinthifolius</i> Raddi. ¹	Brazilian pepper	X	X
<i>Scleria reticularis</i> Michx.	Netted nutrush		X
<i>Scoparia dulcis</i> L.	Licoriceweed		X
<i>Solanum americanum</i> P. Mill.	American black nightshade		X
<i>Spermacoce verticillata</i> L. ¹	Shrubby false buttonweed		X
<i>Thelypteris interrupta</i> (Willd.) K. Iwats	Hottentot fern	X	
<i>Thelypteris</i> spp.	Maiden fern	X	
<i>Toxicodendron radicans</i> (L.) Kuntze	Eastern poison-ivy		X
<i>Urena lobata</i> L. ¹	Cadillo	X	X
<i>Woodwardia areolata</i> (L.) T. Moore	Netted chainfern	X	
<i>Woodwardia virginica</i> (L.) Sm.	Virginia chainfern	X	
<i>Vitis rotundifolia</i> Michx.	Muscadine	X	X

¹Indicates nonnative species.

communities where multiple herbicide treatments have been applied for control of OWCF. Additional research is needed to test novel herbicides for control of OWCF and their impacts to nontarget vegetation, and examine if specific native plants can outcompete OWCF following herbicide application.

SOURCES OF MATERIALS

¹Rodeo, Monsanto Agricultural Company, 800 N Lindbergh Blvd, St. Louis, MO 63167.

²Escort, DuPont Corporation, 1007 Market St, Wilmington, DE 19898.

³Renovate 3, SePRO Corporation, 11550 N Meridian St, Suite 600, Camel, IN 46032.

⁴Plateau, BASF Corporation, 26 Davis Dr, Research Triangle Park, NC 27709.

⁵Dyne-Amic nonionic surfactant, Helena Chemical Company, 225 Schilling Blvd, Suite 300, Collierville, TN 38017.

⁶SAS Institute, 2002–2003, SAS software, Version 9.1, SAS Institute, SAS Campus Drive, Cary, NC 27513.

ACKNOWLEDGEMENTS

Financial support for this project was provided by the Florida Fish and Wildlife Conservation Commission - Bureau of Invasive Plant Management and the University of Florida - Center for Aquatic and Invasive Plants. We are grateful to all the people and management agencies in Florida that allowed access to their property for this study which include: Bill Anderson, Ken Geoli, Jennifer Roberts, Gayle Martin, Barry Daniels, Mark Barrett, Rob Rossmanith, Ken Alvarez, Elizabeth Gandy, St. Lucie County Extension Agency, Highlands Hammock State Park, A.R.M. Loxahatchee National Wildlife Refuge, Pacific Tomato Growers Inc., Jonathan Dickinson State Park, City of Lakeland Wetlands Treatment System, and the Hillsborough County Department of Parks, Recreation, and Conservation.

LITERATURE CITED

Barrett MA, Brandt LA, Thomas B, Jr. 2006. Monitoring ground treatments of Old World climbing fern (*Lygodium microphyllum*) on the Arthur R. Marshall Loxahatchee National Wildlife Refuge: A follow-up report. *Wildland Weeds* 10(4):4–7.

Brandt LA, Black DW. 2001. Impacts of the introduced fern, *Lygodium microphyllum*, on the native vegetation of tree islands in the Arthur R. Marshall Loxahatchee National Wildlife Refuge. *Fla. Sci.* 64:191–196.

Canfield RH. 1941. Application of the line interception method in sampling range vegetation. *J. For.* 39:388–394.

Goolsby JA. 2004. Potential distribution of the invasive Old World climbing fern, *Lygodium microphyllum* in North and South America. *Nat. Areas J.* 24:351–353.

Gordon DR. 1998. Effects of invasive, non-indigenous plant species on ecosystem processes: Lessons from Florida. *Ecol. Appl.* 8:975–989.

Hutchinson J, Ferriter A, Serbesoff-King K, Langeland K, Rodgers L (eds.). 2006. Old World climbing fern (*Lygodium microphyllum*) management plan for Florida. South Florida Water Management District. West Palm Beach, FL. <http://www.fleppc.org>. Accessed November 11, 2013.

Hutchinson JT, Langeland KA. 2006. Survey of control measures on Old World climbing fern (*Lygodium microphyllum*) in southern Florida. *Fla. Sci.* 69:217–223.

Hutchinson JT, Langeland KA. 2010. Review of two non-native, invasive climbing ferns (*Lygodium japonicum* and *L. microphyllum*), sympatric records and additional distribution records from Florida. *Am. Fern J.* 100:57–66.

Hutchinson JT, Langeland KA. 2012. Repeated herbicide application for control of Old World climbing fern (*Lygodium microphyllum*) and the effects on nontarget vegetation on Everglade tree islands. *Invasive Plant Sci. Manag.* 5:477–486.

Langeland KA, Hutchinson JT. 2013. Natural area weeds: Old World climbing fern (*Lygodium microphyllum*). University of Florida, Institute of Food and Agricultural Science Publication SS-AGR-21. <http://edis.ifas.ufl.edu/pdffiles/AG/AG12200.pdf>. Accessed November 11, 2013.

Langeland KA, Link ML. 2006. Evaluation of metsulfuron methyl for selective control of *Lygodium microphyllum* growing in association with *Panicum hemitomon* and *Cladium jamaicense*. *Fla. Sci.* 69:149–156.

MacDougall AS, Turkington R. 2005. Are invasive species the drivers or passengers of change in degraded ecosystems? *Ecology* 86:42–55.

Ogden JAE, Rejmanek M. 2005. Recovery of native plant communities after control of a dominant invasive plant species, *Foeniculum vulgare*: Implications for management. *Biol. Conserv.* 125:427–439.

Pemberton RW, Ferriter AP. 1998. Old World climbing fern (*Lygodium microphyllum*), a dangerous invasive weed in Florida. *Am. Fern J.* 88:165–175.

Sheley RL, Laufenberg SM, Jacobs JS, Borkowski J. 2007. Restoring species richness and diversity in a Russian knapweed (*Acroptilon repens*)-infested riparian plant communities using herbicides. *Weed Sci.* 55:311–318.

Stirling G, Wilsey BJ. 2001. Empirical relationships between species richness, evenness and proportional diversity. *Am. Nat.* 158:286–300.

Stocker RK, Ferriter A, Thayer D, Rock M, Smith S. 1997. Old World climbing fern hitting south Florida below the belt. *Wildland Weeds* 1(1):6–10.

Stocker RK, Miller RE, Jr., Black DW, Ferriter AP, Thayer DD. 2008. Using fire and herbicide to control *Lygodium microphyllum* and effects on a pine flatwoods plant community in south Florida. *Nat. Areas J.* 28:144–145.

Thomas B, Jr., Brandt LA. 2003. Monitoring ground treatments of Old World climbing fern (*Lygodium microphyllum*) on the Arthur R. Marshall Loxahatchee NWR. *Wildland Weeds* 6 (1):9–11.

Vencill WK (ed.). 2002. *Herbicide handbook*. 8th ed. Weed Science Society of America. Lawrence, KS. 493 pp.

Whittaker RH. 1965. Dominance and diversity in land plant communities. *Science* 147:250–260.

Williams CB. 1964. *Patterns in the balance of nature*. Academic, London. 324 pp.

Wilsey B, Stirling G. 2007. Species richness and evenness respond in a different manner to propagule density in developing prairie microcosm communities. *Plant Ecol.* 190:259–273.

Zar JH. 1999. *Biostatistical analysis*. 4th ed. Prentice Hall International, Englewood Cliffs, NJ. 663 pp.