Invasive Plant Science and Management 2012 5:477–486

Repeated Herbicide Application for Control of Old World Climbing Fern (*Lygodium microphyllum*) and the Effects on Nontarget Vegetation on Everglade Tree Islands

Jeffrey T. Hutchinson and Kenneth A. Langeland*

The effects of annual, aerial and ground, herbicide treatments with glyphosate and metsulfuron were evaluated for control of Old World climbing fern (OWCF) and effects on native plants on tree islands in Arthur R. Marshall Loxahatchee National Wildlife Refuge during 2006 to 2009. Initial aerial herbicide treatments reduced OWCF cover by greater than 98% on metsulfuron-treated islands and greater than 88% on glyphosate-treated islands, but there was a concomitant decrease in native ground cover with both herbicides. Follow-up ground treatments, during years two and three of the study, were effective at maintaining low levels of OWCF. OWCF cover at the end of the study was 1 to 2% of pretreatment cover on metsulfuron-treated islands and 8 to 10% on glyphosate-treated islands. At the end of the study (3 yr after treatment), species richness was dominated by ruderal native species not typically found on tree islands. The survival rate of tree and shrubs was 65 to 93% on islands treated with metsulfuron and 6 to 20% on islands treated with glyphosate. Minimum effects were recorded for canopy cover on tree islands treated with metsulfuron compared with glyphosate. These data indicate that the aerial application of metsulfuron can be used for control of OWCF on tree islands. Follow-up ground treatments will be required for OWCF regrowth and new sporelings and should be conducted within 1 yr of the aerial application.

Nomenclature: Glyphosate; metsulfuron; Old World climbing fern, *Lygodium microphyllum* (Cav.) R. Br.

Key words: Everglade tree islands, Florida, herbicide applications, invasive species, nontarget damage.

Old World climbing fern [*Lygodium microphyllum* (Cav.) R. Br.; OWCF] is a nonnative fern currently found in central and southern Florida within the continental United States (Hutchinson and Langeland 2010). OWCF transforms Everglade tree islands in Arthur R. Marshall Loxahatchee National Wildlife Refuge (LNWR) from an open community with a ground cover of native ferns and a shrub layer to the nearly impenetrable thicket of broken limbs and closed canopy of OWCF rachis (Brandt and Black 2001; Ugarte et al. 2006). Northern Everglade tree islands number in the thousands and are a unique upland feature of the Everglades that provide habitat for wildlife in a wetland ecosystem (Brandt et al. 2000, 2003). OWCF is known to decrease native vegetation cover, evenness, and richness on tree islands (Brandt and Black 2001). Ugarte et al. (2006) suggested that OWCF, because of its twining growth into the upper portions of the canopy, acts in synergy with wind events, such as hurricanes, to alter the structure and composition of tree islands often causing total collapse of trees. Recruitment of OWCF is positively correlated with those disturbances (Lynch et al. 2009).

Infestations of OWCF often occur in natural areas that are remote and inaccessible to vehicles and personnel, making treatment difficult. This is especially true in the Florida Everglades. The rapid spread of OWCF across the landscape of southern Florida can be attributed to its small spores (approximately 65 μm), which disperse in normal wind events, tropical storms, and hurricanes (Hutchinson et al. 2006). Spore production can occur throughout the year in south Florida, and each fertile leaflet is capable of producing as many as 28,600 spores (Volin et al. 2004).

Within the LNWR, OWCF was first noted in the interior in 1989 and was documented from a few tree islands in 1992 and 1993 (Thomas 2006). By 1995, OWCF covered an estimated 7,284 ha (18,000 ac) of LNWR. The cover increased to 19,433 ha in 2003 based

DOI: 10.1614/IPSM-D-12-00015.1

* Postdoctoral Assistant and Professor, Agronomy Department, Center for Aquatic and Invasive Plants, University of Florida, 7922 NW 71st Street, Gainesville, FL 32653. Corresponding author's current address: National Fish Hatchery and Technology Center, U.S. Fish and Wildlife Service, 500 East McCarty Lane, San Marcos, TX 78666. Corresponding author's E-mail: jeffrey_hutchinson@fws.gov

Management Implications

The use of herbicides can effectively control Old World Climbing Fern (OWCF) on Everglade tree islands but results in substantial nontarget damage to ground cover. Aerial applications of metsulfuron limited shrub and tree mortality and reductions in canopy cover compared with glyphosate treatments. Monitoring and follow-up treatments on an annual basis would be required for control of OWCF. Without herbicide management, OWCF will continue to form monocultures on tree islands and other habitats resulting in lower native-species richness, evenness, and canopy cover. Follow-up treatments will reduce OWCF growth and spore production, limiting the ability of OWCF to infest new sites from wind blown spores. It is likely that OWCF cover can return to pretreatment levels less than 6 yr following a single herbicide treatment. The best management strategy for OWCF on tree islands is an initial aerial application with metsulfuron over large infestations and annual monitoring and follow-up ground treatments as needed. Managers using aerial application of herbicides for control of OWCF can expect to observe shifts from late-successional vegetation, typical of tree islands, to ruderal species, typical of disturbed sites.

on aerial transects conducted by the South Florida Water Management District (Thomas 2006). The most recent estimate of the coverage of OWCF in the LNWR was 25,200 ha (Woodmansee et al. 2005). Most of the OWCF coverage in the LNWR occurs in tree islands.

Short-term management efforts throughout southern Florida using glyphosate and metsulfuron have not been successful for long-term control of OWCF; vigilant, annual retreatments are required to keep the fern suppressed (Hutchinson et al. 2006). Glyphosate is a nonselective herbicide that damages or kills many types of plants (i.e., grasses, herbs, ferns, shrubs, trees). Graminoids. such as maidencane (*Panicum hemitomon* Schultes) and sawgrass [*Cladium mariscus* (L.) Pohl ssp. *jamaicense* (Crantz) Kukenth.] have shown tolerance to metsulfuron applied to control OWCF (Langeland and Link 2006), but it is unknown whether any shrubs or trees on Everglade tree islands are tolerant to metsulfuron.

Natural areas present a complicated situation for invasive plant treatment while protecting native, nontarget plant species. Selective herbicides have traditionally been developed to control multiple weeds in agricultural areas with no effect on the crop (Hobbs and Humphries 1995). However, in natural areas, the goal of management is to control a single weed while protecting or limiting damage to many nontarget species. Evidence suggests that aerial herbicide treatment of OWCF can be selective with minimal effects on nontarget, deciduous trees during winter application in Florida (Hutchinson et al. 2006); however, evergreen nontarget ground vegetation is often greatly reduced (Hutchinson et al. 2006). This is also true during retreatment of OWCF because of its twining and climbing nature when it grows over and around nontarget plants. The mission of the U.S. Fish and Wildlife Service (USFWS) refuge system is to manage lands and water for the conservation of fish, wildlife, and plant resources and their habitats; thus, herbicides that can effectively control OWCF while limiting nontarget damage to nontarget ground and canopy cover would comply with their mission.

Tree islands in this study were in the late stages of invasion by OWCF at the time of this study. Because of the isolation of tree islands, aerial application of herbicide was the most effective and cost-efficient method for initial control of OWCF. The objectives of this study were to evaluate the effectiveness of consecutive, annual herbicide applications of glyphosate and metsulfuron using aerial and ground treatments for control of OWCF and to examine the effects on native ground and canopy vegetation in the northern Everglade's tree islands in the LNWR.

Materials and Methods

Study Area. Studies were conducted on tree islands in the LNWR (26°N, 80°W) in western Palm Beach County, Florida, from 2006 to 2009. The LNWR encompasses 59,464 ha and represents the last remnants of the northern Everglades. The Everglades ecosystem is listed as an International Biosphere Reserve, World Heritage Site, and a Wetland of International Importance (National Park Service 2011). It is a peat-based wetland that comprises sawgrass strands, wet prairies, sloughs, and tree islands (Loveless 1959). Elevation on tree islands range from 0.2 to 1.0 m (0.7 to 3.3 ft) above the surrounding marsh (Wetzel et al. 2005). Tree islands selected for this study were located in the north portion of the LNWR (26°35′21″N, 80°20′33″W) and were less than 0.13 ha.

Evaluation Periods. Pretreatment evaluation periods were conducted December 2005 to January 2006 (Pretrt). Posttreatment evaluation periods were conducted in December 2006 to January 2007 (1YPT), December 2007 to January 2008 (2YPT), and December 2008 to January 2009 (3YPT). For each evaluation period, the percentage of cover by the vegetation was determined in two strata: soil level to 1.5 m and greater than 1.5 m above the soil. Additionally, the status (live or dead) of dominant shrubs and trees was recorded.

Herbicide Treatment. One of the four treatments with glyphosate at 53.8% ai (Rodeo glyphosate herbicide, DowAgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268) or metsulfuron at 60% ai (Escort XP metsulfuron methyl herbicide, E. I. DuPont de Nemours and Company, Crop Protection, Wilmington, DE 19898), as listed in Table 1, was applied to the tree islands. All tree islands had greater than 46% OWCF cover, and no tree islands used in this study had been previously treated with

https://doi.org/10.1614/IPSM-D-12-00015.1 Published online by Cambridge University Press

Table 1. Aerial and ground herbicide treatments and treatment frequency on tree islands in the A.R.M. Loxahatchee National Wildlife Refuge for control of *Lygodium microphyllum*.

	Application technique/rate		
	Aerial[a]	Ground[b]	
Herbicide	February 2006	February 2007	February 2008
	$kg\ ai\ L^{-1}\ ha^{-2}$	$g\ ai\ L^{-1}$	
Metsulfuron	0.08	0.03	0.03
Metsulfuron	0.16	0.06	0.06
Glyphosate	2.80	5.20	5.20
Glyphosate	5.60	10.40	10.40
Untreated check	No treatment	No treatment	No treatment

[a] Broadcast application.

[b] Spray to wet spot-treatment application.

herbicide. Each treatment was replicated on five individual islands. An additional five tree islands were randomly selected as untreated checks. For each treatment, five tree islands were clustered within 400 to 500 m of each other to facilitate aerial treatment. Tree islands for each treatment were randomly selected within the cluster. For this study, the initial aerial application was followed by two consecutive, annual ground treatments (Table 1). Aerial treatments were applied by broadcast application from a Bell 206 L4 helicopter (Helicopter Applications, Inc., 1670 York Road, Gettysburg, PA 17325) with a Thru Valve (Boom Thru Valve Boom, Waldrum Specialties Inc., P.O. Box 1146, Southampton, PA 18966). Ground treatments were applied on a spray to wet basis using 1.5-L, handheld, pressurized spray bottles. Aerial applications contained 0.5% v/v DLZ adjuvant (DLZ adjuvant, Helena Chemical Company, 225 Schilling Blvd., Suite 300, Collierville, TN 38017), and ground applications contained 0.5% v/v Sunwet adjuvant (Sunwet adjuvant, Brewer International, P.O. Box 690037, Vero Beach, FL 32969), both of which are a proprietary blend of methylated seed oil and nonionic surfactants. Spray volume was 187 L ha^{-1} (20 gal ac^{-1}) for the aerial application and approximately 232 L ha^{-1} for ground treatments.

Ground Cover. Ground vegetation shorter than 1.5 m was estimated along three randomly placed transect lines for the entire width of each tree island (Canfield 1941). The beginning and end of each line were permanently marked with polyvinyl chloride pipe. All ground-cover estimates were recorded along the transect line individually by plant species. Ground cover was calculated for all plants by species along the transect line by dividing the total distance of each species overlapping the line by the length of the transect line. The three lines on each tree island were averaged to obtain the mean ground cover of each species per tree island.

Plants were classified as late succession (species typical of tree islands with canopy intact), ruderal (species not typically present on tree islands with canopy intact), and exotic (nonnative species not indigenous to the area). Late-succession species were based on the 16 most-common, native plant species documented during pretreatment evaluation and those recorded by Brandt et al. (2003).

Species richness patterns were determined by counting the number of plant species that intersected each transect line. Species evenness patterns were determined using the methods of Williams (1964). Evenness (*E*) patterns were calculated as

$$E = 1/DS \quad [1]$$

where *D* is the Simpson's Diversity Index

$$\sum (n/N)^2 \quad [2]$$

and *S* is species richness (Williams 1964). The three lines on each tree island were averaged to obtain the mean species richness and evenness per tree island.

Canopy Cover. The percentage of canopy cover was estimated at the beginning, middle, and end of every transect with a concave densiometer (Forest Densiometers, 5733 SE Cornell Drive, Bartlesville, OK 74006). Densiometer readings were taken in each cardinal direction at each point following the methods of Lemmon (1957). The percentage of canopy cover was measured for live and dead vegetation greater than 1.5 m above the ground. Canopy cover along all three lines was averaged to obtain the mean canopy cover per tree island.

Native Shrubs and Trees. For each treatment, dahoon (*Ilex cassine* L.; $n = 50$), swamp bay [*Persea palustris* (Raf.) Sarg.; $n = 60$], southern waxmyrtle [*Morella cerifera* (L.) Small; $n = 60$], and myrsine [*Rapanea punctata* (Lam.) Lundell; $n = 20$] individuals were randomly selected and tagged within 5 to 10 m on either side of the transect line among the five tree islands per treatment. Randomly selected shrubs and trees were documented as alive if any live foliage was present at 3YPT.

Statistical Analysis. Data were analyzed to determine the most effective aerial treatment (Pretrt to 1YPT) and three consecutive, annual treatments (Pretrt to 3YPT) for control of OWCF. The separate analysis of data was conducted because no previous study, to our knowledge, had examined the combined effects of an aerial herbicide treatment with follow-up ground treatments for control of OWCF and the effects to native plants. A linear mixed-effect ANOVA was used to assess differences in OWCF cover following aerial herbicide treatments (1YPT). The

https://doi.org/10.1614/IPSM-D-12-00015.1 Published online by Cambridge University Press

Table 2. Effects of aerial herbicide applications on Old World climbing fern ground cover ≤1.5 m aboveground level 1 yr after treatment (1YPT)[a] with pretreatment cover as the covariate and at 3 yr after treatment (3YPT)[b] with pretreatment cover as the covariate.

	OWCF[c]			
	Evaluation period			
Treatment: aerial (ground)	% ground cover[d,e]			
kg ai ha^{-1} (g ai L^{-1})	Pretrt	1YPT	2YPT	3YPT
Metsulfuron, 0.08 (0.03)	59.8 (3.8)	0.6 (0.2) a	3.6 (1.2)	0.4 (0.1) a
Metsulfuron, 0.16 (0.06)	54.3 (3.9)	0.4 (0.2) a	3.5 (0.9)	1.2 (0.9)a
Glyphosate, 2.80 (5.20)	66.3 (3.5)	9.0 (3.0) b	16.6 (2.8)	5.7 (1.2)b
Glyphosate, 5.60 (10.40)	72.5 (2.1)	9.0 (1.7) b	17.5 (4.6)	9.2 (2.1)b
Untreated check	46.0 (5.1)	57.0 (4.8) c	60.0 (3.0)	65.0 (4.2)c

[a] Mixed linear model ANOVA: $F = 16.8$, df = 4, $P < 0.0001$.

[b] Repeated-measures ANCOVA: $F = 100.2$, df = 4, $P < 0.0001$.

[c] Abbreviations: OWCF, Old World climbing fern; pretrt, pretreatment; YPT, year posttreatment.

[d] Mean (SE) of five tree islands.

[e] Different letters in last column indicate significant differences among treatments at $P < 0.05$ for the 3-yr evaluation period based on Tukey's adjusted least-square means.

Proc Mixed procedure in SAS software (Version 9.2; SAS Institute, Inc., 100 SAS Campus Drive, Cary, NC 27513-2414) was used with the initial cover at Pretrt as a covariate of 1YPT and random effects of tree islands. Means were separated using Tukey's multiple comparison test ($P < 0.05$). Data were transformed using arcsine square-root transformation to improve homogeneity of variance assumptions (Zar 1999) by visual inspection of the plotted residuals.

Repeated-measures analysis of covariance (ANCOVA) was used with Pretrt conditions as covariates for three consecutive, annual treatments (1YPT to 3YPT) for control of OWCF. Data were analyzed using Proc Mixed repeated-measures ANCOVA (SAS) with time as the repeated measure, treatment as a fixed factor, and tree islands as a random factor. Variables were analyzed by treatment, time, and the treatment by time interaction with pretreatment conditions as covariates. Means were separated using Tukey's multiple comparison test ($P < 0.05$). Data were transformed using arcsine square-root transformation to improve the homogeneity of variance assumptions (Zar 1999) by visual inspection of the plotted residuals.

Separate analyses were conducted to evaluate the responses of native species ground cover, canopy cover, late succession species richness, ruderal species richness, and species evenness to experimental treatments. For ground cover, separate analyses were conducted for the five most-common late-succession species that occurred on tree islands throughout the 3-yr study. All other late succession, ruderal, and nonnative species occurred too infrequently to be analyzed individually for cover.

Data were analyzed using Proc Mixed ANCOVA (SAS) with time as the repeated measure, treatment as a fixed factor, and tree islands as a random factor. Variables were analyzed by treatment, time, and the treatment by time interaction with pretreatment conditions as covariates. Data were transformed using arcsine square-root transformation to improve homogeneity of variance assumptions (Zar 1999) by visual inspection of the plotted residuals.

Analysis of shrub and tree survival by species were assessed at 3YPT by logistic regression to model the dichotomous variable as 0 for dead and 1 for live. Proc Logistics (SAS) was used to compare treatments based on χ^2 values with $P < 0.05$.

Results

OWCF Ground Cover. All aerial herbicide treatments reduced OWCF ground coverage to 9% or less at 1YPT, an 88 to 99% decrease compared with the untreated checks (Table 2). Metsulfuron reduced OWCF ground coverage greater than glyphosate did, but the effect was independent of herbicide use rate. At 3YPT, following aerial and ground herbicide treatments, OWCF coverage was reduced compared with untreated checks. Islands treated with metsulfuron provided greater control of OWCF than did glyphosate

Numerous new sporophytes less than 1.5 cm (0.5 in) in height and some regrowth of OWCF were observed on all treated islands during the final evaluation at 3YPT. OWCF was not completely eliminated by any combination of treatments during this study. Although not tested statistically

Table 3. Percentage of change in ground cover ≤1.5 m aboveground level from pretreatment (Pretrt) to 3 yr posttreatment (3YPT) of the most-common, late-succession, native plant species.

	Species			
Treatment, Aerial (ground)	Swamp fern	Bamboovine	Cinnamon fern	Muscadine
kg ai ha^{-1} (g ai L^{-1})	% change in ground cover[a,b] from Pretrt to 3YPT			
Metsulfuron, 0.08 (0.03)	−55 a	−95 a	−60 a	+ 282 a
Metsulfuron, 0.16 (0.06)	−64 a	−99 a	−23 a	+ 250 ab
Glyphosate, 2.80 (5.20)	−75 a	−90 a	+ 29 b	+ 482 b
Glyphosate, 5.60 (10.40)	−78 a	−98 a	+ 24 b	+ 353 b
Untreated checks	−5 b	−31 b	+ 33 b	+ 47 a

[a] Change in percentage of ground cover represents the means of five tree islands.

[b] Different letters in species columns indicate significant differences among treatments at P < 0.05 at yr 4. based on Tukey's adjusted least-square means.

because of multiple treatments and the lack of statistical independence, OWCF coverage continued to expand with a 41% increase in ground cover from Pretrt (at 46% cover) to 4YPT (at 65% cover) on untreated checks.

Native Plant Ground Cover. Both Swamp fern (*Blechnum serrulatum* Rich.) (F = 9.4, df = 4, P = 0.0002) and bamboovine (*Smilax laurifolia* L.) (F = 12.5, df = 4, P < 0.0001) cover were reduced by 55% or more on all herbicide-treated islands 3YPT compared with the untreated island (Table 3). There were no observed differences in the effect of herbicide type or rate in cover of these species.

Cinnamon fern (*Osmunda cinnamomea* L.) cover was reduced by both metsulfuron treatments compared with glyphosate treatments and untreated islands (F = 6.2, df = 4, P = 0.002), but showed no response to glyphosate (Table 3). Muscadine (*Vitis rotundifolia* Michx.) cover increased overall from pretreatment levels (Table 3). This species became the most-common native plant based on cover at 3YPT. Cover of muscadine on metsulfuron-treated islands was not different than untreated islands but was higher on those islands treated with glyphosate (F = 6.0, df = 4, P = 0.0024).

Canopy Cover. At 3YPT, canopy cover was lower on all herbicide treated islands compared with untreated islands and lower on glyphosate treated islands than on those treated with metsulfuron (P < 0.05; Table 4). Canopy cover was reduced 88 to 71% on glyphosate-treated islands compared with untreated islands but only 31 to 48% on metsulfuron treated islands 3YPT.

Shrub and Tree Survival. There were no differences (P = 0.0963; Table 5) at 3YPT in the survival of dahoon between herbicide-treated islands and untreated islands, indicating this species, though damaged, will survive the

Table 4. Effects of aerial and two annual ground treatments of herbicide on tree canopy cover >1.5 m aboveground level at 3 yr posttreatment (3YPT)[a] with pretreatment (Pretrt) canopy cover as the covariate.

	Canopy[b,c]			
	Evaluation period			
Treatment, aerial (ground)	Pretrt	1YPT	2YPT	3YPT
kg ai ha^{-1} (g ai L^{-1})	% (SE)			
Metsulfuron, 0.08 (0.03)	51 (9)	51 (6)	44 (11)	31 (6) b
Metsulfuron, 0.16 (0.06)	44 (8)	52 (8)	43 (8)	40 (6) b
Glyphosate, 2.80 (5.20)	55 (5)	44 (4)	21 (5)	7 (3) a
Glyphosate, 5.60 (10.40)	42 (4)	33 (6)	13 (3)	16 (4) a
Untreated check	69 (5)	71 (4)	65 (8)	58 (7) c

[a] Repeated-measures ANCOVA, F = 7.5, df = 4, P < 0.0001)

[b] Mean (SE) of five tree islands.

[c] Different letters in the last column indicate significant differences among treatments at P < 0.05 for the 3-yr evaluation period based on Tukey's adjusted least square means.

https://doi.org/10.1614/IPSM-D-12-00015.1 Published online by Cambridge University Press

Table 5. Survival rates of the four most-common shrub and tree species (*Ilex cassine*, *Persea palustris*, *Myrica cerifera*, and *Rapanea punctata*) at 3 yr after aerial herbicide treatment. Herbicide rates represent aerial treatment.

	Tree survival[a,b]			
	Three years post-treatment			
Herbicide	Dahoon holly	Swamp bay	Wax myrtle	Myrsine
kg ai ha^{-1}	% (SE)			
Metsulfuron, 0.08	80 (7)	77 (6) b	65 (7) b	12 (8) a
Metsulfuron, 0.16	89 (4)	93 (3) bc	81 (4) ab	18 (8) a
Glyphosate, 2.80	75 (6)	6 (3) a	18 (6) c	88 (13) bc
Glyphosate, 5.60	70 (7)	20 (6) a	17 (5) c	58 (10) b
Untreated check	100 (0)	100 (0) c	93 (5) a	100 (0) c

[a] Mean (SE) of 50 dahoon, 60 swamp bay, 60 wax myrtle, and 20 myrsine plant replications per treatment.

[b] Different letters in columns for tree species indicates significant difference at $P < 0.05$ for the 3-yr evaluation period based on logistic regression analysis.

treatment rates of metsulfuron and glyphosate tested. Swamp bay was highly susceptible to glyphosate but exhibited a high tolerance to metsulfuron ($P < 0.0001$). Wax myrtle was also highly susceptible to glyphosate and exhibited a high tolerance to metsulfuron ($P < 0.0001$). In contrast, myrsine was highly susceptible to metsulfuron but exhibited tolerance to glyphosate, especially at the lower rate. The highest survival percentage for myrsine was 88% for the low and high rates of glyphosate.

Richness and Evenness. General trends from Pretrt to 3YPT indicated no loss of late-successional species for any treatment, large increases in ruderal species for all treatments, and small increases in other nonnatives for glyphosate-treated tree islands. A total of 16 late-succession species, 34 ruderal species, and 5 exotic (nonnative) plant species were documented on tree islands during the 3-yr duration of this project (data not shown).

Lower late-succession species richness was observed on tree islands treated with the low rate of metsulfuron compared with all other treatments and untreated islands ($P < 0.05$; Table 6). There was no difference in late-succession species richness among metsulfuron (high rate), glyphosate (low or high rate), and untreated tree islands.

Ruderal species richness was higher on those islands treated with the high rate glyphosate compared with islands treated with metsulfuron and untreated islands ($P < 0.05$; Table 6). There were no differences in the number of ruderal species on islands treated with different rates of glyphosate. The largest increase in ruderal species was found on tree islands treated with the high rate of glyphosate, and the lowest number of ruderal species was found on untreated checks. Ruderal species richness on tree islands treated with metsulfuron was sixfold higher compared with untreated islands, but twofold to threefold lower than islands treated with glyphosate.

Species evenness was lower on glyphosate-treated islands compared with metsulfuron-treated and untreated tree islands ($P < 0.05$; Table 6). The low evenness values for the glyphosate-treated islands are attributed to large increases in muscadine cover at 3YPT.

Discussion

OWCF Management. These results indicate that metsulfuron provided significantly better control of OWCF than did glyphosate for a single aerial followed by two annual ground herbicide treatments. However, hundreds of new OWCF sporophytes were observed on tree islands for all glyphosate- and metsulfuron-treated islands at 3YPT. This indicates that even with total eradication of mature OWCF, spores in the soil and ground surface debris as well as those blown in by wind currents will require long-term management. Stocker et al. (2008) continued to find OWCF regrowth from rhizomes at less than 1% cover 2 yr after treatment with triclopyr following bimonthly and biannual treatments but reported no new growth from spores. This suggests that unless infestations of OWCF are only a few square meters in size, retreatment will be required for some regrowth and new growth from spores.

In this study, aerial treatment of OWCF with metsulfuron reduced cover to less than 1.5%, which is similar to the results observed for aerial treatment of brackenfern [*Pteridium aquilinum* (L.) Kuhn] with asulam (Pakeman et al. 2005). Both species are rhizomatous and can resprout from surviving stems and fronds. For control of brackenfern, multiple treatments were more effective than one to two treatments (Stewart et al. 2007).

Without follow-up treatments, OWCF will likely eventually recover on the tree islands to pretreatment levels, possibly returning to pretreatment levels within 5 to 6 yr after treatment. In this study, OWCF increased in

Table 6. Effects of aerial and two annual ground treatments of herbicide on late succession species richness,[a] ruderal species richness,[b] and species evenness[c] ≤1.5 m aboveground level at 3-yr posttreatment (3YPT) based on a repeated-measures ANCOVA with pretreatment (Pretrt) as the covariate.

	Species richness[d,e]					
	Late succession		Ruderal		Species evenness[d,e]	
Treatment, aerial (ground)	Pretrt	3YPT	Pretrt	3YPT	Pretrt	3YPT
kg ai ha^{-1} (g ai L^{-1})	mean (SE)					
Metsulfuron, 0.08 (0.03)	7.4 (0.3)	5.6 (0.3) a	0.1 (0.1)	1.8 (0.2) a	0.34 (0.03)	0.44 (0.04) b
Metsulfuron, 0.16 (0.06)	7.6 (0.2)	6.7 (0.3) b	0.1 (0.1)	1.8 (0.3) a	0.40 (0.03)	0.39 (0.03) b
Glyphosate, 2.80 (5.20)	7.3 (0.4)	7.1 (0.4) bc	0.0 (0.0)	3.7 (0.6) ab	0.42 (0.03)	0.21 (0.02) a
Glyphosate, 5.60 (10.40)	7.7 (0.6)	7.0 (0.7) bc	0.3 (0.1)	5.6 (0.9) b	0.38 (0.02)	0.27 (0.03) a
Untreated check	7.8 (0.4)	8.1 (0.6) c	0.0 (0.0)	0.3 (0.1) c	0.41 (0.03)	0.37 (0.04) b

[a] $F = 6.9$, df = 4, $P < 0.0001$.

[b] $F = 19.9$, df = 4, $P < 0.0001$.

[c] $F = 5.0$, df = 4, $P = 0.0012$.

[d] Mean (SE) of five tree islands.

[e] Different letters for 3YPT indicate significant differences among treatments at $P < 0.05$ for the 3-yr evaluation period based on Tukey's adjusted least-square means.

cover 1.4- to 17.5-fold on tree islands receiving two annual treatments (one aerial and one ground treatment) compared with three annual treatments (data not shown). This indicates the need for monitoring and follow-up treatments as wells as the difficulty in finding regrowth and new sporophytes during retreatment, especially in thick OWCF rachis mats. Research by Stocker et al. (2008) indicated that retreatments less than 6 mo apart may be more beneficial to native species because OWCF can be spot-treated with herbicide before it has the opportunity to climb onto and twine around native vegetation.

Tree islands are important for providing habitat heterogeneity in the wetland landscape of the northern Everglades and provide food and refuge for multiple taxa of wildlife including migratory birds (Brandt and Black 2001; Brandt et al. 2003). Large-scale, aerial application of glyphosate over hundreds or thousands of tree islands would greatly alter the structure of the tree islands and is not recommended. With the exception of myrsine, aerial application of metsulfuron, which affects ground cover, has minimal effects on the dominant shrubs or trees, preserving the canopy of tree islands. On all tree islands in this study, myrsine was a minor component of the canopy.

In this study, invasions of new OWCF sporophytes were common along the edges and throughout the interior at 2YPT and 3YPT following aerial treatments. The open canopy and increased sunlight likely created favorable conditions for OWCF spore germination. It appears that the combined loss of ground and canopy cover following aerial application creates more suitable, open conditions for OWCF spores to germinate in the interior portions of tree islands compared with ground treatments. With ground treatments, there is minimal tree mortality and loss of canopy cover. Increased spore germination and gametophyte development of ferns can be attributed to increased light intensity and soil disturbance (Watkins et al. 2007). Gaps created from disturbance and loss of canopy can result in increased native and nonnative vine cover in hardwood forest of south Florida (Horvitz et al. 1998). In the Everglade tree islands, a significant number of OWCF sporophytes were found at the base of treefalls with reduced canopy cover following hurricanes (Lynch et al. 2009).

The increase in species richness observed in this study following herbicide treatment is not reflective of the vegetation typical of tree islands (Brandt and Black 2001; Brandt et al. 2003) but is more reflective of a habitat modified by OWCF and subsequent herbicide treatment. The total number of species recorded on tree islands at Pretrt to 1YPT ranged from 15 to 21. Species richness is relatively low on tree islands in the LNWR where OWCF is not present, averaging eight native species with swamp fern being the most abundant ground cover (Brandt and Black 2001). However, at the end of this study (3YPT), the number of species recorded increased to 38 to 42 species on glyphosate-treated tree islands and 28 to 31 species on metsulfuron-treated tree islands.

Tree islands treated with glyphosate exhibited a composition shift from OWCF and late-succession species (primarily native ferns) to muscadine. Muscadine is present as a late-succession species on tree islands but seldom accounts for more than 10% of the cover. In this study, muscadine was suppressed by OWCF, and its coverage was low before treatment. After the decline in OWCF coverage following herbicide treatments, muscadine responded like an early succession, ruderal species and likely increased in cover because of increased sunlight and nutrients and reduced competition. Aerial treatments of brackenfern with asulam resulted in a shift of vegetation changes from bracken and Ericaceae species, although in that case to one dominated by early successional species, such as mosses and grasses (Pakeman et al. 2005).

Evenness values for ground cover were 0.49 or less at all evaluation periods from Pretrt to 3YPT in this study but were lower for glyphosate-treated islands than they were for metsulfuron-treated islands. Brandt et al. (2003) reported the mean evenness value for ground cover was 0.58 on tree islands with minimal OWCF cover. The increase in the number of ruderal species, especially with the high rate of glyphosate, likely did not affect evenness values because the cover of most ruderal species was typically less than 5%. This indicates that as OWCF cover decreased following treatment, there was a concomitant increase in muscadine, especially on glyphosate-treated islands, and a decrease in native ferns.

Native Ground Cover. Both glyphosate and metsulfuron affected the native ground cover on tree islands. Aerial application of glyphosate resulted in more ruderal species at 3YPT. Metsulfuron affected native ferns more than glyphosate did. Native ferns, such as swamp fern and cinnamon fern, have been shown to be sensitive to metsulfuron at rates as low as 0.01 kg ai at 187 L ha^{-1} (Hutchinson and Langeland 2008). With the exception of ruderal species, few recruits of late-succession native species, other than muscadine, were observed on treated islands. At 3YPT, some small native ferns were observed growing on tussocks and at the base of dead trees, but new growth of native ferns were far out-numbered by new OWCF sporophytes.

Community-level response to herbicide treatment is difficult to analyze because native species cover has already been reduced by the invasive plant (Laufenberg et al. 2005; Marrs 1985). The presence of OWCF on tree islands before treatment had likely already reduced native ground cover and limited propagule production. On tree islands, the combined effects of OWCF (limited growth and propagule production of natives) before treatment, along with herbicide treatments (further reductions in native plant abundance) and thick rachis mats, will greatly inhibit and limit the recolonization of ground cover.

Native ferns were still present on all tree islands at 3YPT, but the dominant native fern, swamp fern, was reduced by 55 to 77% on treated islands compared with pretreatment conditions. The increase in muscadine cover may have prevented further invasion of OWCF, other nonnative plants such melaleuca [*Melaleuca quinquenervia* (Cav.) Blake] and Brazilian peppertree (*Schinus terebinthifolius* Raddi), as well as limiting recolonization of native vegetation at 3 yr after treatment. Russell et al. (1998) found that Old World forkedfern [*Dicranopteris linearis* (Burm. F.) Underw.] native to the Old World tropics and Polynesia, influenced forest floor light patterns, directed flora development patterns, and possibly prevented invasion of nonnatives in Hawaiian rainforests. However, muscadine is tardily deciduous, which may create suitable conditions for germination of OWCF spores in the winter. This aspect of muscadine may have also limited its exposure to herbicides during winter treatments, which, in turn, allowed it to thrive and become the dominant plant at 3YPT because of increased sunlight. At the conclusion of the study, few native plants were observed in areas where dead OWCF rachis mats and muscadine were present.

As suggested by MacDougall and Turkington (2005), there are shifts from species dominants (i.e., late-succession species) to annual and perennial forbs and graminoid species that are functionally distinct (i.e., ruderal) in disturbed ecosystems. Annual plants respond to the loss of canopy cover and increased sunlight, whereas perennial plants take longer to recover from disturbance because of their interdependence on the previous year's growth (Lindgren and Sullivan 2001).

All native, late-successional species on tree islands are perennials, but they exhibited little recovery by the end of the study. It appears that native, late-succession species, such as ferns, will require longer periods to recover. At 3YPT, native ferns were at approximately 30% of pretreatment level, which may indicate a recovery time of 9 to 10 yr. Spot treatment by ground applicators targeting OWCF resprouts and new growth could limit nontarget damage. Bimonthly and biannual ground treatments of OWCF with triclopyr resulted in no effects to native species cover, richness, evenness, or diversity (Stocker et al. 2008).

Tree Survival and Canopy Cover. Limited tree mortality and preservation of the canopy was achieved with metsulfuron treatments. The dominant shrub and tree species, dahoon, swamp bay, and wax myrtle, all exhibited a high tolerance to metsulfuron. In Everglades National Park, similar results were reported for aerial treatment with metsulfuron with limited damage to swamp bay and wax myrtle but high mortality to native ferns (Taylor 2006). The dramatic reduction in shrubs and trees on tree islands aerially treated with glyphosate completely altered the structure and composition of the islands.

Native shrub and tree species represented 22% of the ground cover on tree islands in this study before treatment. This seems to indicate that the growth habit of OWCF altered the growth form of shrubs and trees because of the twining nature of its rachis over the limbs and boles of shrubs and trees. As suggested by Ugarte et al. (2006), for large trees, the thick rachis mats of OWCF can alter growth patterns and increase the chance of snapping and uprooting.

The dynamics of the tree islands following herbicide treatment are complex. The combined effects of OWCF, hurricanes, altered hydrology, and herbicide treatments greatly alter the structure and composition of tree islands. Multiple disturbances have been suggested to act in synergy to alter habitats (Hobbs and Huenneke 1992), which appears to be the case with OWCF on tree islands. In addition, the size, frequency, and intensity of disturbances may limit native plant recruitment and establishment (Hobbs and Huenneke 1992).

Acknowledgments

We are grateful to the U.S. Fish and Wildlife Service for providing funding for this project. The entire staff at A.R.M. Loxahatchee NWR was extremely helpful in multiple ways over the 4 yr of the project. Gayle Martin and Mark Barrett with the USFWS assisted with data collection throughout the study and their assistance was greatly appreciated. Kevin Maier, George Pelt, and Don Napier were very helpful with the initial setup of plots on tree islands. Pedro Mendez of the University of Florida assisted with field work during the last year of the study. We thank William Haller, Doria Gordon, Robert Stamps, and Greg MacDonald for providing critical comments on an earlier version of this manuscript.

Literature Cited

Brandt, L. A. and D. W. Black. 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.

Brandt, L. A., D. Ecker, I. G. Rivera, A. Traut, and F. J. Mazzotti. 2003. Wildlife and vegetation of bayhead islands in the A.R.M. Loxahatchee National Wildlife Refuge. Southeast. Nat. 2:179–194.

Brandt, L. A., K. M. Portier, and W. M. Kitchens. 2000. Patterns of change in tree islands in the Arthur R. Marshall Loxahatchee National Wildlife Refuge from 1950 to 1991. Wetlands 20:1–14.

Canfield, R. H. 1941. Application of the line interception method in sampling range vegetation. J. Forestry 39:388–394.

Hobbs, R. J. and L. F. Huenneke. 1992. Disturbance, diversity, and invasion: implications for conservation. Conserv. Biol. 6:324–337.

Hobbs, R. J. and S. E. Humphries. 1995. An integrated approach to the ecology and management of plant invasions. Conserv. Biol. 9: 761–770.

Horvitz, C. C., J. B. Pascarella, S. McMann, A. Freedman, and R. H. Hofstetter. 1998. Functional role of invasive non-indigenous plants in hurricane-affected subtropical hardwood forests. Ecol. Appl. 8: 947–974.

Hutchinson, J. T. and K. A. Langeland. 2008. Response of selected nontarget native Florida wetland plant species to metsulfuron methyl. J. Aquat. Plant Manag. 46:72–76.

Hutchinson, J. T. and K. A. Langeland. 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, J., A. Ferriter, K. Serbesoff-King, K. Langeland, and L. Rodgers, eds. 2006, Old World Climbing Fern (*Lygodium microphyllum*) Management Plan for Florida. http://www.fleppc.org/Manage_Plans/Lygo_micro_plan.pdf. Accessed: February 11, 2011.

Langeland, K. A. and M. L. Link. 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.

Laufenberg, S. M., R. L. Sheley, J. S. Jacobs, and J. Borkowski. 2005. Herbicide effects on density and biomass of Russian knapweed (*Acroptilon repens*) and associated plant species. Weed Technol. 19: 62–72.

Lemmon, P. E. 1957. A new instrument for measuring forest overstory density. J. Forestry 55:667–668.

Lindgren, P.M.F. and T. P. Sullivan. 2001. Influence of alternative vegetation management treatments on conifer plantation attributes: abundance, species diversity, and structural diversity. For. Ecol. Manag. 142:163–182.

Loveless, C. M. 1959. A study of the vegetation in the Florida Everglades. Ecology 40:1–9.

Lynch, R. L., H. Chen, L. A. Brandt, and F. J. Mazzotti. 2009. Old World climbing fern (*Lygodium microphyllum*) invasion in hurricane caused treefalls. Nat. Area J. 29:210–215.

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

Marrs, R. H. 1985. The effects of potential bracken and scrub control herbicides on lowland Calluna and grass heath communities in East Anglia, UK. Biol. Conserv. 32:13–32.

National Park Service. 2011. Everglades: International Designations. http://www.nps.gov/ever/parknews/internationaldesignations.htm. Accessed: February 11, 2011.

Pakeman, R. J., J. L. Small, M. G. Le Duc, and R. H. Marrs. 2005. Recovery of moorland vegetation after aerial spraying of bracken (*Pteridium aquilinum* (L.) Kuhn) with Asulam. Restor. Ecol. 13: 718–724.

Russell, A. E., J. W. Raich, and P. M. Vitousek. 1998. The ecology of the climbing fern *Dicranopteris linearis* on the windward Mauna Loa, Hawaii. J. Ecol. 86:765–779.

Stewart, G. B., A. S. Pullin, and C. Tyler. 2007. The effectiveness of Asulam for bracken (*Pteridium aquilinum*) control in the United Kingdom: a meta-analysis. Environ. Manag. 40:747–760.

Stocker, R. K., R. E. Miller Jr., D. W. Black, A. P. Ferriter, and D. D. Thayer. 2008. Using fire and herbicide to control *Lygodium microphyllum* and effects on a pine flatwoods plant community in south Florida. Nat. Area J. 28:144–145.

Taylor, J. 2006. Management of Old World climbing fern in Everglades National Park. Pages 67–69 *in* J. Hutchinson, A. Ferriter, K. Serbesoff-King, K. Langeland, and L. Rodgers, eds., Old World Climbing Fern (*Lygodium microphyllum*) Management Plan for Florida. http://www.fleppc.org/Manage_Plans/Lygo_micro_plan.pdf. Accessed: February 11, 2011.

Thomas, B. 2006. A.R.M. Loxahatchee National Wildlife Refuge. Pages 73–78 *in* J. Hutchinson, A. Ferriter, K. Serbesoff-King, K. Langeland, and L. Rodgers, eds., Old World Climbing Fern (*Lygodium microphyllum*) Management Plan for Florida. http://www.fleppc.org/Manage_Plans/Lygo_micro_plan.pdf. Accessed: February 11, 2011.

Ugarte, C. A., L. A. Brandt, S. Melvin, F. J. Mazzotti, and K. G. Rice. 2006. Hurricane impacts on tree islands in Arthur R. Marshall Loxahatchee National Wildlife Refuge, Florida. Southeast. Nat. 5: 737–746.

Volin, J. C., M. S. Lott, J. D. Muss, and D. Owens. 2004. Predicting rapid invasion of the Florida Everglades by Old World climbing fern (*Lygodium microphyllum*). Divers. Distrib. 10:439–446.

Watkins Jr., J. E., M. K. Mack, and S. S. Mulkey. 2007. Gametophyte ecology and demography of epiphytic and terrestrial tropical ferns. Am. J. Bot. 94:701–708.

Wetzel, P. R., A. G. van der Valk, S. Newman, D. E. Gawlik, T. T. Gann, C. A. Coronado-Molina, D. L. Childers, and F. H. Sklar. 2005. Maintaining tree islands in the Florida Everglades: nutrient redistribution is the key. Front. Ecol. Environ. 3:370–376.

Williams, C. B. 1964. Patterns in the Balance of Nature. London, United Kingdom: Academic. 324 p.

Woodmansee, S. W., K. Bradley, and S. Hodges. 2005. Systematic Reconnaissance Flights and Exotic Plant Species Mapping at Selected National Wildlife Refuges in Florida. Sanibel, FL: U.S. Fish and Wildlife Service. 34 p.

Zar, J. H. 1999. Biostatistical Analysis. 4th ed. Englewood Cliffs, NJ: Prentice Hall International Inc. 663 p.

Received February 22, 2012, and approved August 14, 2012.