BioOne® RESEARCH EVOLVED

Absorption and Translocation of Glyphosate, Metsulfuron, and Triclopyr in Old World Climbing Fern (*Lygodium microphyllum*)

Author(s): Jeffrey T. Hutchinson, Kenneth A. Langeland, Gregory E. MacDonald, and Robert Querns
Source: Weed Science, 58(2):118-125. 2010.
Published By: Weed Science Society of America
DOI: 10.1614/WS-D-09-00046.1
URL: http://www.bioone.org/doi/full/10.1614/WS-D-09-00046.1

BioOne (www.bioone.org) is an electronic aggregator of bioscience research content, and the online home to over 160 journals and books published by not-for-profit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Web site, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/page/terms_of_use.

Usage of BioOne content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Weed Science 2010 58:118–125

Absorption and Translocation of Glyphosate, Metsulfuron, and Triclopyr in Old World Climbing Fern (*Lygodium microphyllum*)

Jeffrey T. Hutchinson, Kenneth A. Langeland, Gregory E. MacDonald, and Robert Querns*

Old World climbing fern is one of the most invasive plants in natural areas of central and southern Florida. The fern spreads across the landscape by wind-blown spores and invades isolated and undisturbed habitats such as interior portions of the Florida Everglades. Land managers in Florida have reported that multiple herbicide treatments are required to control the fern, which could indicate that herbicides do not translocate throughout the plant in long-established populations. We conducted a greenhouse study to determine the absorption and translocation patterns in Old World climbing fern using the three herbicides most commonly used for management of this plant by land managers in Florida. Using ^{14}C-labeled herbicides, we evaluated absorption and translocation of glyphosate (2.25 kg ai ha^{-1}), metsulfuron (0.10 kg ai ha^{-1}), and triclopyr (1.68 kg ai ha^{-1}) in Old World climbing fern using five different application scenarios (cut-and-spray, basal spray, 25% foliar spray, 50% foliar spray, and 100% foliar spray). Triclopyr was absorbed to the greatest extent (60.3%) of applied radioactive compounds compared to glyphosate (31.2%) and metsulfuron (19.8%). The majority of radioactivity remained in treated leaves for all herbicides with only small percentages of the absorbed radioactivity being detected in other plant parts. All three herbicides translocated acropetally and basipitally to some extent. Radioactivity, for the most part, translocated evenly throughout the plants but the greatest amount of radioactivity derived from triclopyr occurred in rhizomes when the cut-and-spray and basal applications were used. The radioactivity in rhizomes derived from glyphosate was greater in those treated using cut-and-spray. Based on autoradiographs, there was limited horizontal movement of any herbicide in the rhizomes of Old World climbing fern which could explain why resprouts are observed several weeks following treatment.

Nomenclature: Glyphosate; metsulfuron; triclopyr; Old World climbing fern, *Lygodium microphyllum* (Cav.) R. Br.
Key words: Absorption, translocation, invasive ferns, natural areas, Florida.

Nonnative ferns (Pteridophyta) have become problematic in natural areas in many parts of the world (Langeland et al. 2008; Wilson 2002), and some native ferns are considered invasive (De la Cretaz and Kelty 1999; LeDuc et al. 2000). Old World climbing fern (OWCF) is a highly invasive plant that alters the structure and composition of hydric natural areas in central and southern Florida (Brandt and Black 2001; Nauman and Austin 1978). It exhibits indeterminate vining growth and grows into to the canopy of forest wetlands such as cypress and bayhead swamps. The fern is highly pyrogenic and burns rapidly with extreme temperatures, often scorching the cambium layer of larger trees. In addition, OWCF acts as a fire ladder, carrying the fire into the canopy of trees such as bald cypress [*Taxodium distichum* (L.) Rich.] and slash pine (*Pinus elliottii* Engelm.). Because of all these issues, this plant has altered fire management plans on some public lands in Florida (Roberts et al. 2006).

This invasive fern was first discovered in natural areas of southeastern Florida in 1966 (Beckner 1968) and has spread rapidly across the landscape of south Florida in less than 40 yr (Ferriter and Pernas 2006). This spread is due to the production of millions of wind-blown spores that potentially can travel many km (Pemberton and Ferriter 1998). New sporophytes can develop in 8 to 10 wk following spore germination in moist conditions (Mueller 1982). This species is now considered one of the worst threats to natural areas by land managers in central and southern Florida (Hutchinson and Langeland 2006).

OWCF produces spores year-round but spore production is highest during the fall (Volin et al. 2004). These spores, which require moist conditions to germinate, develop into haploid gametophytes with three potential types of fertilization (Lott et al. 2003). Sperm require moist conditions to swim to the egg and fertilization can occur through either crossing or selfing (Lott et al. 2003). Sexual determination of the gametophyte is determined by an antheridiogen system (Kurumatani et al. 2001; Lott et al. 2003). Following fertilization, sporophytes develop rhizomes and fronds with an indeterminate growth pattern.

Mechanical control of OWCF is unfeasible due to access, hydric soils, damage to native vegetation, and the spread of additional spores. Insects have been introduced for biocontrol of OWCF in south Florida with additional species being evaluated (Goolsby et al. 2003). However, it could be several years or even decades before biological control agents become effective for overall OWCF management. Chemical control of OWCF in natural areas can be achieved, but multiple consecutive treatments are required to maintain populations at low levels (Hutchinson and Langeland 2006; Langeland and Link 2006). Presently, there are no known cases where OWCF has been eradicated from a natural area, although control has been achieved with annual treatment (Hutchinson et al. 2006). In some cases, multiple retreatments might not be possible due to funding, limited personnel, and inaccessibility. Consequently, the fern has proven to be extremely difficult to control once it has become established and produces spores.

Control of OWCF with herbicides is conducted year-round in southern Florida by multiple local, state, federal, and private land management agencies. Except when occasional freezing temperatures occur, OWCF is an evergreen that grows vigorously throughout the year in southern Florida, where the average annual temperature is 23 C with < 2 d/yr when temperatures drop below 0 C (Henry et al. 1994). The most frequently used herbicides for OWCF management in Florida are glyphosate, metsulfuron, and triclopyr (Hutch-

DOI: 10.1614/WS-D-09-00046.1
* First and second authors: University of Florida, Agronomy Department, Center for Aquatic and Invasive Plants, 7922 NW 71st St., Gainesville, Florida 32653; third and fourth authors: University of Florida, Agronomy Department, P.O. Box 110500, Gainesville, Florida 32611-0500. Corresponding author's E-mail: jthutchinson@ifas.ufl.edu

inson and Langeland 2006). Application rates for glyphosate and metsulfuron reported by land managers vary from 0.05 to 7.0 to 17.5 g ai L^{-1} and 0.1 to 0.2 g ai L^{-1} water, respectively, for spot treatments to individual infestations. Land managers use triclopyr less frequently to control OWCF in Florida, but reported rates varied from 22.0 to 55.0 g ai L^{-1}. Glyphosate and triclopyr have been used to manage OWCF for more than 15 yr. Metsulfuron only has been used for ca. 4 yr since a Special Local Need 24c Label was obtained for use on OWCF in Florida (EPA SLN No. FL-030010).

Despite the fact that OWCF has spread across the landscape of central and southern Florida, relatively little research has been conducted to determine the most effective treatment method for control. Stocker et al. (1997) reported that treatments of glyphosate and triclopyr resulted in 100% aboveground mortality of OWCF at rates as low as 1.5% product, but neither herbicide completely eradicated the plant because regrowth was observed for both treatments after 1 yr. In the Arthur R. Marshall Loxahatchee National Wildlife Refuge in Palm Beach County, effective control of OWCF has been reported in research plots by cutting the vertically ascending rachis at 1.5 m aboveground level and foliar spraying the lower portion with glyphosate (Thomas and Brandt 2003). OWCF plots foliar-sprayed with metsulfuron had the same aboveground dry weight biomass as control plots at 2.5 yr posttreatment (Langeland and Link 2006). Glyphosate and metsulfuron have been the most effective herbicides to date to control OWCF, but results are inconsistent with regard to long-term management (Hutchinson et al. 2006). Triclopyr is one of the most common herbicides used in natural areas for management of invasive plants, but it is unknown if this herbicide can be used effectively for control of OWCF.

In addition to spreading long distances by wind-blown spores that are capable of intragametophyte selfing or intergametophyte crossing (Lott et al. 2003), OWCF can also spread by extensive rhizomes and stolons that grow just below or at ground surface. New fronds emerge from rhizomes at irregular intervals and rapidly grow both vertically and horizontally. When OWCF grows vertically into trees and other vegetation, the upper portion of the fern can be killed by cutting all raches at soil level. However, resprouting occurs within 6 to 10 d from rhizomes (J. T. Hutchinson, unpublished data).

The ability of OWCF to resprout from rhizomes following herbicide treatment is an indication that herbicides have limited translocation to the rhizomes or throughout the entire rhizome of an individual fern. Some ferns have high root-to-shoot ratios (LeDuc et al. 2003), and ca. 50% of the carbon sequestered by OWCF is allocated to belowground root mass (J. Volin, personnel communication). This indicates that application techniques that maximize herbicide translocation to the rhizomes of OWCF should result in greater or complete control of the fern.

Current management of OWCF is limited to herbicide treatment, but there is no information available relative to the absorption and translocation of any herbicide in OWCF. Studies to determine herbicide uptake and translocation in OWCF should provide a better understanding of management strategies for long-term control of OWCF. The objective of this research was to determine herbicide uptake and translocation patterns as a function of herbicide placement in OWCF for glyphosate, metsulfuron, and triclopyr using ^{14}C-labeled compounds.

Materials and Methods

Plant Material. Experiments were conducted two times using OWCF plants grown from two different sources. For the first experiment, OWCF was grown from rhizomes in Okeelanta muck soil collected form Allapattah Flats, Martin County, Florida. Rhizome sections of 103 cm^2 were cut with a machete from under mature OWCF, and placed in 0.5 L plastic pots (12.7 cm in depth). Rhizomes were excavated with native soil intact. All fronds (live and dead) from the rhizomes were cut at soil level, and the rhizomes allowed to resprout. Plants were grown for 90 d to a height of 1 m prior to treatment. The experiment was repeated with OWCF grown from spores. Soil excavated below mature OWCF plants was placed in shallow 2.5 L tubs and maintained under saturated conditions. Soil was collected from the same site as rhizomes in Martin County, Florida. The spores were allowed to germinate and form gametophytes; fertilization to production of sporophytes. Approximately 6 mo after germination, the sporophytes, 7.6 cm in height, were transferred into 0.5 L pots in Okeelanta muck soil and grown to 1 m in height prior to treatment.

Plants were propagated in a greenhouse at the University of Florida campus in Gainesville, FL, and exposed to a natural photoperiod (10.5 to 14.0 h sunlight) under a 50% shade cloth with a temperature range of 21 to 37 C. The first experiment was conducted from March to June 2005, and the second experiment was conducted from January to October 2005. No additional nutrient supplements were added to the ferns over the duration of the study. Pots were placed in 7.5 L rectangular tubs with water depth maintained at 2 to 3 cm below soil level and watered 3 to 4 times per wk.

Absorption and Translocation. OWCF plants were treated with glyphosate,[1] metsulfuron,[2] and triclopyr[3] herbicides at rates of 1.67 kg ae ha^{-1}, 0.10 kg ai ha^{-1}, and 1.20 kg ae ha^{-1}, respectively. Five different application scenarios were used for each herbicide as follows: (1) plants were cut 6 cm from the base of the plant and the remaining foliage was sprayed (denoted as cut-and-spray); (2) foliage was treated from 0 to 6 cm above the base of the plant (denoted as basal spray); (3) foliage was treated from 0 to 25 cm above the base of the plant (denoted as 25% foliar); (4) foliage was treated 0 to 50 cm above the base of the plant (denoted as 50% foliar); and (5) 100% of the plant was treated (denoted as 100% foliar). Herbicides were applied with a CO_2 sprayer at 172 kPa with a single TeeJet 11003 flat fan nozzle.[4] All treatments included nonionic surfactant,[5] 0.5 v/v, at a spray volume of 370 L ha^{-1}. Uptake and translocation were determined using ^{14}C-labeled glyphosate[6] (specific activity 1998.0 kBq mg^{-1}), metsulfuron[7] (specific activity 1845.2 kBq mg^{-1}), and triclopyr[8] (specific activity 895.4 kBq mg^{-1}). Immediately after spraying, application of 5 µL droplets of ^{14}C-radiolabeled material was made to the adaxial surface of five separate pinnae or the rachis on each plant immediately after treatment. Spotting of radiolabeled material was made at 6 cm (cut-and-spray, and basal), 25 cm (25% foliar), 50 cm (50% foliar), and 100 cm (100% foliar). This resulted in a total of 12.12 kBq mg^{-1} (0.33 µCi) ^{14}C glyphosate, 11.84 kBq mg^{-1} (0.32 μCi) ^{14}C metsulfuron, and 12.95 kBq mg^{-1} (0.35 μCi) ^{14}C triclopyr per plant.

At 9 d after treatment (DAT), plants (including rhizomes) were removed from pots, radiolabeled leaflets excised and the soil washed from roots using detergent and water. Treated areas on leaflets or rachis were excised and washed in five sequential 1-ml aliquots of deionized water applied to each radiolabeled spot to determine percent uptake. A total of 25 ml of rinsate (combined total for all five spots) was obtained for each treatment. Unabsorbed ^{14}C from the leaf wash solution was quantified with liquid scintillation spectrometry (LSS).[9]

Whole plants were placed on blotter paper in plant presses and oven-dried at 70 C for 72 h. Dried plants were covered with X-ray film[10] and stored 40 d at room temperature to obtain autoradiographs. Following autoradiography, the plants were sectioned into rhizomes and stolons, lower 50% rachis and leaves, and upper 50% rachis and leaves. Radioactivity was determined by LSS. Translocation was determined by measuring radioactivity in individual plant parts. Plant tissue were finely ground through a 0.5 mm screen using a Wiley Mill,[11] and 0.15 to 0.20 g of ground tissue was oxidized to recover ^{14}C using a Harvey OX-500 biological oxidizer[12] with a proprietary ^{14}C-trapping liquid scintillation cocktail.[13] Absorbed radioactivity is defined as the total ^{14}C-kBq obtained from all plant parts and presented as the percent of applied radioactivity for each herbicide. Percent recovery is the absorbed radioactivity plus the amount recovered from leaf rinses. Translocated radioactivity is reported as the percent of absorbed radioactivity in plant parts (top, bottom, rhizomes) and presented as a percent of absorbed radioactivity.

Experimental Design and Data Analysis. Treatments were arranged in a completely randomized design. Each treatment was replicated five times and the experiment was repeated. There was no significant ($P > 0.05$) treatment by experiment interaction; therefore, data represent the average of both experiments. Total recovery, leaf wash, absorbed, and translocated radioactivity were combined among treatment scenarios for each herbicide and subjected to analysis of variance (ANOVA) using Proc GLM (SAS[14]) procedure, and means were separated using Fisher's protected LSD test at the 5% level of probability.

Radioactivity in plant parts was analyzed by herbicide and treatment scenario separately to determine patterns of translocation for each herbicide. Data were subjected to ANOVA and means were separated using Fisher's protected LSD test at the 5% level of probability. Translocation was analyzed for each treatment method for percentage radioactivity in top 50%, bottom 50%, and rhizomes using an ANOVA with means separated using Fisher's protected LSD test at the 5% level of probability. For translocation data with the cut-and-spray method, a paired *t* test using SAS was used to compare bottom 50% and rhizomes at the 5% level of probability.

Results and Discussion

Visual Effects of Herbicides on OWCF. OWCF treated with triclopyr exhibited chlorosis, epinasty, and necrosis nine DAT on 30–40% of OWCF leaflets for all treatments scenarios. There were no observable effects on OWCF treated with glyphosate or metsulfuron at 9 DAT. This might indicate that triclopyr is absorbed or translocated at a faster rate relative than glyphosate or metsulfuron, or that the rate at which symptoms of the different mechanisms of actions are expressed are different. In field trials with triclopyr at similar rates, we have observed necrosis in < 4 h in OWCF. Rapid burning and tissue necrosis by triclopyr in OWCF might limit translocation by limiting entry of the herbicide into vascular tissue. It was discovered that rapid wilting and yellowing in purple loosestrife treated with triclopyr stimulated new growth (Gardner and Grue 1996). Hofstra et al. (2006) suggested that lower concentrations of triclopyr might result in more effective translocation in parrotsfeather compared to higher rates and prevent basal stem resprouts.

Autoradiography. The general pattern observed from autoradiographs indicated most of the ^{14}C material remained in the treated leaflets or rachis. For all three herbicides, some basipetal movement was observed for cut-and-spray and basal spray (Figure 1). However, basipetal movement with 100% foliar spray for any herbicide was minimal with almost no movement of ^{14}C material into the rhizomes. With 25% and 50% foliar spray, herbicide movement was primarily acropetal with some basipetal movement for all three herbicides.

Basipetal movement into the rhizomes was observed for cut-and-spray and basal spray treatments of triclopyr and glyphosate. However, there was no evidence of horizontal movement for triclopyr or glyphosate along rhizomes in autoradiographs. Basal treatment with triclopyr also indicated some acropetal translocation (Figure 1c). Autoradiographs of OWCF treated with metsulfuron indicated minimal translocation of the herbicide into the rhizomes. These results corroborate field observations of all three herbicides in which OWCF is highly susceptible to herbicide treatment, but resprouting occurs within several weeks to months posttreatment. Resprouts from OWCF treated with herbicide is likely due to limited translocation into the rhizomes and no horizontal translocation within the rhizomes.

Absorption. There was no significant difference in total recovery between treatments (Table 1). Total recovery was 67% for metsulfuron, 65% for glyphosate, and 69% for triclopyr. However, differences were observed among herbicides in leaf wash with only the lowest percentage, 9%, washed from leaves treated with triclopyr followed by 34% for glyphosate and 47% for metsulfuron. Accordingly, differences in absorption were observed among herbicides (Table 1). The highest level of absorption was observed for triclopyr (60%) followed by glyphosate (31%) and metsulfuron (20%). Total translocation was different for triclopyr (14%) compared to glyphosate (6%) and metsulfuron (5%).

Overall, more of the applied triclopyr (87%) was absorbed compared to only 48% of glyphosate and 30% of metsulfuron. However, 23% of the applied metsulfuron was translocated out of the treated leaflets compared to only 23% of triclopyr and 20% of glyphosate. There was no difference in treatment scenarios for absorption of metsulfuron and triclopyr, but we observed differences between the cut-and-spray (38%) and 100% foliar spray (27%) with glyphosate (Tables 2–4).

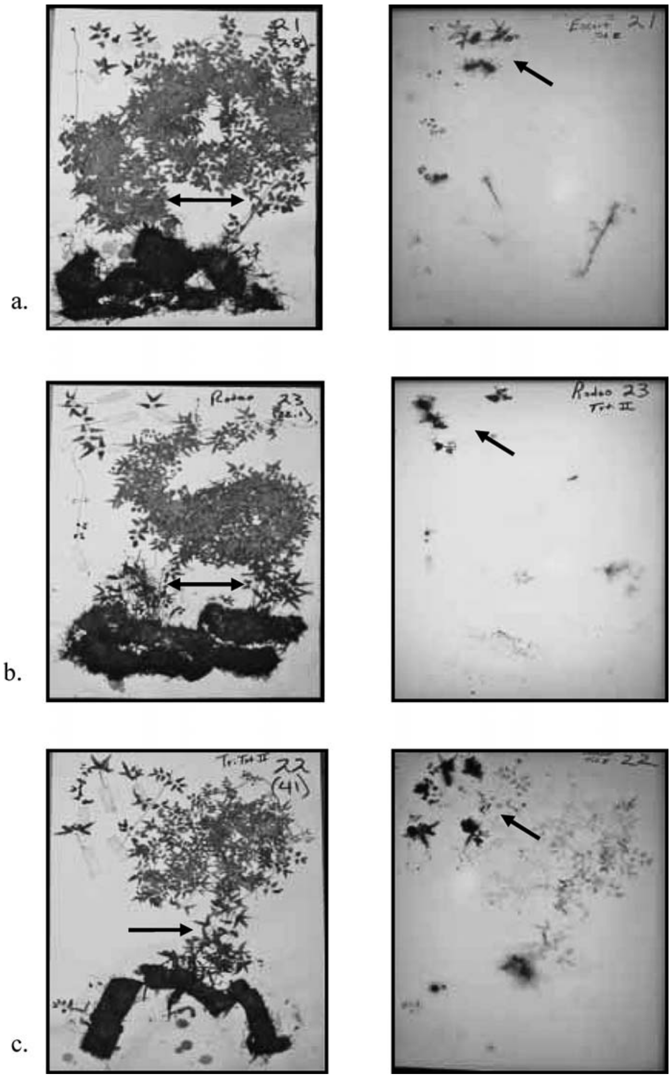

Figure 1. Dried plants (left) and autoradiographs (right) of Old World climbing fern (OWCF) treated basally with (a) metsulfuron methyl, (b) glyphosate, and (c) triclopyr. Arrows on left indicate site of treatment and arrows on right indicate spotted pinnae.

Reddy (2000) reported 22% absorption of glyphosate in redvine [*Brunnichia ovata* (Walt.) Shinners] and Chachalis and Reddy (2004) reported 20% absorption of glyphosate in trumpetcreeper [*Campsis radicans* (L.) Seem. ex Bureau]. These values are similar to the 31% absorption of glyphosate we observed in OWCF. Although our data indicate that triclopyr and glyphosate are absorbed in greater amounts than metsulfuron by OWCF, increased rates of herbicide absorption do not necessarily mean better control, but the relationship is plant-specific (Chachalis and Reddy 2004; Norsworthy et al. 2001). Norsworthy et al. (2001) found that some species have more tolerance for glyphosate even at higher absorption rates.

Translocation. Radioactivity was detected in all portions of treated plants for all herbicide treatments, indicating that all herbicides were translocated to some extent (Tables 2–4). The majority of radioactivity remained in treated leaves for all herbicides with only small percentages of the absorbed radioactivity being detected in other plant parts (top 50%, bottom 50%, and rhizomes). Averaged over all treatment methods for each herbicide, more radioactivity remained in the triclopyr- (47%) compared to metsulfuron methyl- (15%) and glyphosate-treated leaves (25%) (Table 1). Necrosis at 9 DAT and the large amount of radioactivity remaining in treated leaves indicates that translocation might have been limited with triclopyr. In a review of phytotoxic action on herbicide translocation, it was suggested that herbicides might inhibit phloem translocation and herbicide distribution (Geiger and Bestman 1990). Our results indicate that this might have been the case in this study where inhibition of normal carbon metabolism and phloem transport occurred in OWCF.

Table 1. Radioactivity (percent of applied) and standard error for total recovery, leaf wash, absorbed, treated leaf, and translocation following application of ^{14}C-labeled metsulfuron, glyphosate, and triclopyr to Old World climbing fern (all treatments combined).

Herbicide	Total recovery	Leaf wash	Absorbed	Treated leaf	Translocated
	% Recovered (SE)				
Metsulfuron	66.7 (4.9)	46.9 (4.7)	19.8 (0.6)	15.2 (0.9)	4.6 (0.8)
Glyphosate	65.2 (4.7)	34.0 (5.5)	31.2 (1.8)	25.0 (1.3)	6.2 (0.4)
Triclopyr	69.0 (4.3)	8.7 (1.4)	60.3 (3.4)	46.7 (2.0)	13.6 (1.1)
LSD (0.05%)	NS[a]	6.2	4.4	4.1	2.9

[a] Abbreviation: NS, not significant.

Radioactivity was detected in the upper plant portions for all three herbicides (top 50%) when lower portions of plants (basal, 25% foliar, 50% foliar) were treated, indicating that all three herbicides moved acropetally (Tables 2–4). Radioactivity was also detected in rhizomes in all treatment scenarios for all herbicides, indicating that all three herbicides moved basipitally. Although some significant differences in radioactivity in plant parts (other than treated leaves) were observed, these differences were small, and radioactivity, for the most part, was evenly distributed among top 50%, bottom 50%, and rhizomes. It is noteworthy that the greatest amount of radioactivity derived from triclopyr occurred in rhizomes when the cut-and-spray and basal applications were made and the radioactivity in rhizomes derived from glyphosate was greater in those treated by cut-and-spray compared to foliar-applied treatements of 25%, 50%, and 100%. This might be explained because the herbicide is applied to older leaves at the base. In many plant species, photoassimilates are translocated from older leaves into sinks (rhizomes, tubers, bulbs, etc.) (Khan 1981; Williams 1964; Wolf 1993). Veerasekaran et al. (1977) found that asulam applied to mature fronds of bracken fern resulted in greater translocation into the rhizomes. In addition, when using the cut-and-spray method to treat OWCF, herbicides can diffuse basipitally in the rhizomes through cuts in the rachis. In contrast, translocation of ^{14}C glyphosate in pitted morningglory (*Ipomoea lacunosa* L.) indicated that herbicide movement did not differ whether the herbicide was applied to the top, middle, or bottom portion of the plant (Koger and Reddy 2005).

Translocation of a given herbicide varies among species. Basipetal movement was reported to be 49% for ^{14}C-glyphosate in trumpetcreeper (Chachalis and Reddy 2004), which is substantially greater than the 4% that we observed with OWCF. Similar to our observations for OWCF, limited basipetal movement was reported for stoloniferous ground ivy (*Glechoma hederacea* L.) treated with 2,4-D (Kohler et al. 2004) and ivyleaf morningglory [*Ipomoea hederacea* (L.) Jacq.] treated with glyphosate, imazethapyr, or glufosinate (Hoss et al. 2003).

Table 2. Percent ^{14}C-metsulfuron methyl absorption, translocation, and distribution in Old World climbing fern for five treatment methods. Values represent the means of 10 replications.

Treatment	^{14}C-metsulfuron methyl recovered							LSD[a] (0.05%)
					Movement out of treated leaves			
	Leaf wash	Absorbed	Treated leaves	Translocated	Top 50%	Bottom 50%	Rhizomes	
	%							
Cut-and-spray	32.2	19.1	15.8	3.3	N/A	0.9	2.4	P = 0.03
Basal	41.4	20.5	16.4	4.1	0.6	1.3	2.2	1.5
25% Foliar	49.4	21.8	16.1	4.7	0.7	2.5	1.5	1.5
50% Foliar	51.6	17.9	10.3	7.6	3.2	2.4	2.0	NS[b]
100% Foliar	60.1	20.7	17.3	3.4	0.6	1.3	1.5	NS
LSD (0.05%)	14.4	NS	5.8	3.1	1.7	NS	NS	

[a] Significance test comparing % disintegrations per minute (DPM) in top 50%, bottom 50%, and rhizomes within rows. For cut-and-spray treatment, significance test was a paired *t* test ($P < 0.05$).
[b] Abbreviation: NS, not significant.

Our results indicate that translocation into the rhizomes is greater with the cut-and-spray and basal treatment methods using triclopyr and glyphosate where herbicide is concentrated on the older leaves at the base of the plant. The cut-and-spray method is the most commonly used method for treating OWCF growing high up into trees (Hutchinson and Langeland 2006).

OWCF has extensive rhizomes, especially in long-established populations. It can be assumed that translocation of herbicide through the rhizomes to prevent resprouting is needed for long-term control. Veerasekaran et al. (1977) reported that asulam (4.4 to 8.8 kg ai ha^{-1}) eliminated 90% of the shallow active frond buds in bracken fern [*Pteridium aquilinum* (L.) Kuhn], but only eliminated 52% of the dormant buds on deeper rhizome branches. Translocation of herbicide from the treated leaves might not be indication of the efficacy of the herbicide (Kalnay and Glenn 2000). This is especially true with low volume, high-potency, acetolactate synthase-inhibiting herbicides such as metsulfuron, in which small amounts of herbicide are toxic to plants (Fairbrother and Kapustka 2001). Based on autoradiographs of OWCF, once herbicide translocates to the rhizomes, there is little or no horizontal translocation along the rhizome. Koger and Reddy (2005) suggested that control of pitted morningglory is more likely affected by rate than spray coverage. Reddy (2000) suggested that higher rates of glyphosate will be required for effective control of redvine due to its deep-rooted rootstocks from where new sprouts emerge following treatment. Likewise, the use of higher herbicide rates for cut-and-spray and basal treatments might be required for greater translocation into the rhizomes of OWCF.

Conversely, it is also possible that the herbicides we tested might interfere with phloem transport at the junction of the rachis and rhizome, limiting horizontal movement of herbicides through the rhizome, and increased rates might not result in greater translocation in to rhizomes. Pakeman et al. (2000) reported variable control of bracken fern with aerial application of asulam in which ca. 75% of the sites sprayed exhibited bracken recovery. This corresponds with reports from land managers in Florida who have reported inconsistent results when treating OWCF with glyphosate and metsulfuron (Hutchinson et al. 2006). Research has shown that glyphosate inhibits phloem transport in plants 24 to 48 h after treatment (Geiger and Bestman 1990; Kirkwood et al. 2000; Walker and Oliver 2008). Our results, which show limited translocation in OWCF, could indicate that triclopyr and metsulfuron also interfere with physiological processes that block phloem transport in OWCF. In autoradiographs, we observed limited or no horizontal movement of herbicide through the rhizomes.

There are no studies of herbicide translocation in OWCF, but radio-labeled asulam accumulated in rhizomes of bracken fern (Veerasekaran et al. 1977). With OWCF, we did not observe accumulation of radio-labled material in the rhizomes with the three herbicides tested. Based on autoradiographs, 2,4-D movement into the rhizomes of bracken fern was greatest when the herbicide was applied to immature fronds (McIntyre 1962). Treatment of immature fronds of OWCF

Table 3. Percent ^{14}C-glyphosate absorption, translocation, and distribution in Old World climbing fern for five treatment methods. Values represent the means of 10 replications.

Treatment	^{14}C-glyphosate recovered							LSD[a] (0.05%)
					Movement out of treated leaves			
	Leaf wash	Absorbed	Treated leaves	Translocated	Top 50%	Bottom 50%	Rhizomes	
	%							
Cut-and-spray	15.4	37.5	31.3	6.2	N/A	1.8	4.4	P = 0.06
Basal	30.4	28.8	22.5	6.3	1.4	1.8	3.1	NS[b]
25% Foliar	48.4	32.2	25.0	7.2	1.7	3.3	2.2	NS
50% Foliar	38.2	30.6	24.5	6.1	2.8	1.1	2.2	NS
100% Foliar	37.4	26.7	21.7	5.0	0.4	1.3	3.3	1.4
LSD (0.05%)	19.1	10.1	8.0	NS	1.8	NS	1.8	

[a] Significance test comparing % disintegrations per minute (DPM) in top 50%, bottom 50%, and rhizomes within rows. For cut-and-spray treatment, significance test was a paired *t* test ($P < 0.05$).
[b] Abbreviation: NS, not significant.

Table 4. Percent ^{14}C-triclopyr absorption, translocation, and distribution in Old World climbing fern for five treatment methods. Values represent the means of 10 replications.

Treatment	^{14}C-triclopyr recovered							LSD[a] (0.05%)
					Movement out of treated leaves			
	Leaf wash	Absorbed	Treated leaves	Translocated	Top 50%	Bottom 50%	Rhizomes	
	%							
Cut-and-spray	5.8	51.1	38.4	12.7	N/A	4.6	8.1	P = 0.003
Basal	6.9	53.3	36.2	17.1	2.8	7.0	7.3	4.1
25% Foliar	11.1	64.6	52.0	12.6	4.1	5.9	1.9	NS[b]
50% Foliar	12.8	64.3	49.4	14.9	5.5	6.3	1.5	NS
100% Foliar	6.8	68.3	57.7	10.6	4.2	9.7	0.7	NS
LSD (0.05%)	5.9	NS	11.1	NS	NS	NS	3.3	

[a] Significance test comparing % disintegrations per minute (DPM) in top 50%, bottom 50%, and rhizomes within rows. For cut-and-spray treatment, significance test was a paired *t* test ($P < 0.05$).
[b] Abbreviation: NS, not significant.

could only be accomplished where the fern has first invaded or on new sprouts after a herbicide treatment or fire. Main et al. (2006) found that triclopyr displayed excellent basipetal translocation in Chinese yam (*Dioscorea oppositifolia* L.), resulting in the elimination of tubers. In pitted morning glory, movement of ^{14}C-glyphosate to the roots was highest for 100% foliar treatment (Koger and Reddy 2005). Other authors have reported limited basipetal movement of herbicides in other vines (Hoss et al. 2003; Kohler et al. 2004; Unland et al. 1999), but there is little information on herbicide movement available for most ferns and none for climbing ferns (*Lygodium* spp.). Control of OWCF is dependent on controlling the rhizomes, and all three herbicides used in this study exhibited some basipetal movement into the rhizomes. However, autoradiographs revealed limited movement through the rhizomes, indicating that all raches must be treated to prevent resprouts. Control of OWCF rhizomes would prevent resprouts, which are commonly observed after herbicide treatment of the fern. In field sites infested with OWCF, coverage is often > 75% over several ha and the fern can grow > 15 m into the canopy, which makes treatment difficult. In field studies using several herbicides and tank mixes, it took two to three herbicide applications every 6 mo to eliminate OWCF from the study plots (J. T. Hutchinson and K. A. Langeland, unpublished data).

Limited field research on OWCF is available for the three herbicides tested in this study. Thomas and Brandt (2003) stated that spot treatments using the cut-and-spray method with glyphosate (0.12 kg ai 3.8 L^{-1}) provided control of OWCF > 3 yr posttreatment with limited resprouts. Our work agrees with the above results in which greater basipetal movement into the rhizomes of OWCF was observed for OWCF treated with the cut-and-spray treatment. Metsulfuron at rates of 0.04 and 0.08 kg ai ha^{-1} have been shown to provide control of OWCF for 12 mo using 100% foliar treatment (Langeland and Link 2006). Based on our results, metsulfuron could be effective for ground treatments using the cut-and-spray, basal, or 100% foliar treatments. We have observed excellent results (90–100% mortality) 1 yr post-treatment with aerial applications of metsulfuron at rates of 0.08 and 0.16 kg ai ha^{-1} over OWCF (J. T. Hutchinson and K. A. Langeland, unpublished data). Stocker et al. (1997) reported that spot treatments with triclopyr (0.13 kg ai 3.8 L^{-1}) foliar sprayed and band-sprayed from the base of OWCF to 1.2 m above ground resulted in 100% browning of OWCF, but regrowth was observed; they suggested limited translocation. Using prescribed fire and/or triclopyr, it was found that bimonthly and biannual treatments of OWCF were successful in reducing OWCF cover to < 1% but resprouts were still documented after 3 yr (Stocker et al. 2008). Our results suggest that cut-and-spray and basal treatments with triclopyr should result in greater basipetal movement into the rhizomes.

Our results revealed limited movement of the herbicides tested through the rhizomes of OWCF, which explains why resprouts from treated OWCF are commonly observed in the field < 12 mo posttreatment. In a greenhouse study on OWCF using twice the labeled rates of metsulfuron (0.16 kg ai) and glyphosate (11.20 kg ai), no resprouts were observed 52 wk posttreatment, indicating that herbicide rates might need to be increased for initial treatments to effectively control the fern (Hutchinson and Langeland 2008). The current treatment methods (cut-and-spray and 100% foliar spray) employed by applicators in Florida should continue to be used to manage OWCF, but retreatment will be required. Additional research is needed to determine if basal treatments (i.e., band spraying) are effective under field conditions because our results revealed herbicide movement to the rhizomes using this method. Glyphosate and metsulfuron are the most commonly used herbicides to treat OWCF in Florida, but our results indicate that triclopyr exhibits greater translocation to the rhizomes. Field research is needed to determine the efficacy of triclopyr and herbicide combinations on OWCF for both ground and aerial treatments. Research on herbicide rates above those used in this project are also needed to determine their efficacy on controlling OWCF.

Sources of Materials

[1] Glyphosate herbicide, Monsanto Agricultural Company, 800 N. Lindbergh Blvd., St. Louis, MO 63167 (Roundup Ultramax, 50.2 ai).

[2] Metsulfuron methyl herbicide, DuPont Corporation, 1007 Market St., Wilmington, DE 19898 (Escort XP, 60% ai).

[3] Triclopyr herbicide, SePRO Corporation, 11550 N. Meridian St., Suite 600, Camel, IN 46032 (Renovate 3, 44.4% ai).

[4] Flat fan nozzle, TeeJet MidTech Southeast, P.O. Box 832, Tifton, GA 31793.

[5] Entry nonionic surfactant, Monsanto Corporation, 800 N. Lindbergh Blvd., St. Louis, MO 63167.

[6] ^{14}C-glyphosate, Amersham Life Sciences Inc., 3350 N. Ridge Ave., Arlington Heights, IL 60004.

[7] ^{14}C-metsulfuron methyl, gift from DuPont Corporation, 1007 Market St., Wilmington, DE 19898.

[8] ^{14}C-triclopyr, gift from Dow AgroSciences LLC, 9330 Zionsville Rd., Indianapolis, IN 46268.

[9] Packard Tricarb 1600CA Liquid Scintillation Analyzer, Packard Instrument Co., 800 Research Parkway, Meriden, CT 06450.

[10] Kodak X-OMAT XAR-5 film, Sigma-Aldrich, 3050 Spruce St., St. Louis, MO 63103.

[11] Wiley Mill, Arthur W. Thomas Company, Philadelphia, PA 19099 (No model number).

[12] R. J. Harvey Biological Oxidizer, Model OX-500, R. J. Harvey Instrument Co., 123 Patterson Street, Hillsdale, NJ 07642.

[13] R. J. Harvey 14-Carbon Cocktail (proprietary blend of biscumene and PPO [diphenyloxazole] in mixed xylenes), R. J. Harvey Instrument Co., 123 Patterson Street, Hillsdale, NJ 07642.

[14] Statistical software, SAS Institute, 2002–2003, SAS software, Version 9.1, SAS Institute, Cary, NC 57513.

Acknowledgments

This project was funded by the Florida Department of Environmental Protection, Bureau of Upland Invasive Plant Management. David Mayo assisted with plant treatment, harvests, and lab work. Steve Smith and David Johnson of the South Florida Water Management District provided site access to collect plant material.

Literature Cited

Beckner, J. 1968. *Lygodium microphyllum*, another fern escaped in Florida. Amer. Fern J. 58:93.

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.

Chachalis, D. and K. N. Reddy. 2004. Pelargonic acid and rainfall effects on glyphosate activity in trumpetcreeper (*Campsis radicans*). Weed Technol. 18:66–72.

De la Cretaz, A. L. and M. J. Kelty. 1999. Establishment and control of hay-scented fern: a native invasive species. Biol. Invasions 1:223–236.

Fairbrother, A. and L. A. Kapustka. 2001. Low-dose, high-potency herbicides: a historical perspective of environmental concerns to frame the issues. Pages 1–17 *in* S. A. Ferenc, ed. Impacts of Low-Dose, High-Potency Herbicides on Nontarget and Unintended Plant Species. Pensacola, FL: SETAC.

Ferriter, A. and T. Pernas. 2006. An explosion in slow motion: tracking the spread of *Lygodium microphyllum* in Florida. Wildland Weeds 9(2):7–9.

Gardner, S. C. and C. E. Grue. 1996. Effects of Rodeo® and Garlon 3A® on nontarget wetland species in Central Washington. Environ. Toxicol. Chem. 15:441–451.

Geiger, D. R. and H. D. Bestman. 1990. Self-limitation of herbicide mobility by phytotoxic action. Weed Sci. 38:324–329.

Goolsby, J. A., A. D. Wright, and R. W. Pemberton. 2003. Exploratory surveys in Australia and Asia for natural enemies of Old World climbing fern, *Lygodium microphyllum*: Lygodiaceae. Biol. Control 28:33–46.

Henry, J. A., K. M. Portier, and J. Coyne. 1994. The Climate and Weather of Florida. Sarasota, FL: Pineapple. 279 p.

Hofstra, D. E., P. D. Champion, and T. M. Dugdale. 2006. Herbicide trials for the control of parrotsfeather. J. Aquat. Plant Manag. 44:13–18.

Hoss, N. E., K. Al-Khatib, D. E. Peterson, and T. M. Loughin. 2003. Efficacy of glyphosate, glufosinate, and imazethapyr on selected weed species. Weed Sci. 51:110–117.

Hutchinson, J., A. Ferriter, K. Serbesoff-King, K. Langeland, and L. Rodgers, 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: December 10, 2008.

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

Hutchinson, J. T. and K. A. Langeland. 2008. Control of Old World climbing fern (*Lygodium microphyllum*) with increased rates of glyphosate and metsulfuron methyl under greenhouse conditions. Fla. Sci. 71:201–207.

Kalnay, P. A. and S. Glenn. 2000. Translocation of nicosulfuron and dicamba in hemp dogbane (*Apocynum cannabinum*). Weed Technol. 14:476–479.

Khan, A. A. 1981. Effect of leaf position and plant age on the translocation of ^{14}C-assimilates in onion. J. Agric. Sci. 96:451–455.

Kirkwood, R. C., R. Hetherington, T. L. Reynolds, and G. Marshall. 2000. Absorption, localization, translocation, and activity of glyphosate in barnyardgrass [*Echinochloa crus-galli* (L) Beauv.]: influence of herbicide and surfactant concentration. Pestic. Manag. Sci. 56:359–367.

Koger, C. H. and K. N. Reddy. 2005. Glyphosate efficacy, absorption, and translocation in pitted morningglory (*Ipomoea lacunose*). Weed Sci. 53:277–283.

Kohler, E. A., C. S. Throssell, and Z. J. Reicher. 2004. 2,4-D rate response, absorption, and translocation of two ground ivy (*Glechoma hederacea*) populations. Weed Technol. 18:917–923.

Kurumatani, M., K. Yagi, T. Murata, M. Tezuka, L. N. Mander, M. Nishiyama, and H. Yamane. 2001. Isolation and identification of antheridiogens in the ferns, *Lygodium microphyllum* and *Lygodium reticulatum*. Biosci. Biotech. Biochem. 65:2311–2314.

Langeland, K. A., H. M. Cherry, C. M. McCormick, and K. A. Craddock Burks. 2008. Identification and Biology of Nonnative Plants in Florida's Natural Areas. Gainesville, FL: IFAS Communication Services, University of Florida. 193 p.

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.

LeDuc, M. G., R. J. Pakeman, and R. H. Marrs. 2003. Changes in the rhizome system of bracken subjected to long-term experimental treatment. J. Appl. Ecol. 40:508–522.

LeDuc, M. G., R. J. Pakeman, P. D. Putwain, and R. H. Marrs. 2000. The variable responses of bracken fronds to control treatments in Great Britain. Ann. Bot. (London) 85 (Suppl. B):17–29.

Lott, M. S., J. C. Volin, R. W. Pemberton, and D. A. Austin. 2003. The reproductive biology of the invasive ferns *Lygodium microphyllum* and *L. japonicum* (Schizaeaceae): implications for invasive potential. Am. J. Bot. 90:1144–1152.

Main, C. L., J. E. Beeler, D. K. Robinson, and T. C. Mueller. 2006. Growth, reproduction, and management of Chinese yam (*Dioscorea oppositifolia*). Weed Technol. 20:773–777.

McIntyre, G. I. 1962. Preliminary studies on the translocation of ^{14}C-labelled herbicides in bracken (*Pteridium aquilinum*). Weed Res. 2:51–59.

Mueller, R. J. 1982. Shoot morphology of the climbing fern *Lygodium* (Schizaeaceae): general organography, leaf initiation, and branching. Bot. Gaz. 143:319–330.

Nauman, C. E. and D. F. Austin. 1978. Spread of the exotic fern *Lygodium microphyllum* in Florida. Am. Fern J. 68:65–66.

Norsworthy, J. K., N. R. Burgos, and L. R. Oliver. 2001. Differences in weed tolerance to glyphosate involve different mechanisms. Weed Technol. 15:725–731.

Pakeman, R. J., M. G. LeDuc, and R. H. Marrs. 2000. Bracken distribution in Great Britain: strategies for its control and the sustainable management of marginal lands. Ann. Bot. (London) 85 (Suppl. B):37–46.

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

Reddy, K. N. 2000. Factors affecting toxicity, absorption, and translocation of glyphosate in redvine (*Brunnichia ovata*). Weed Technol. 14:457–462.

Roberts, R. E., R. O. Woodbury, and J. Popenoe. 2006. Vascular plants of Jonathan Dickinson State Park. Fla. Sci. 69:288–327.

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

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. Areas J. 28:144–145.

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

Unland, D. R., K. Al-Khatib, and D. E. Peterson. 1999. Interactions between imazamox and diphenylethers. Weed Sci. 47:462–466.

Veerasekaran, P., R. C. Kirkwood, and W. W. Fletcher. 1977. Studies on the mode of action of asulam in bracken (*Pteridium aquilinum* L. Kuhn) I. Absorption and translocation of (^{14}C) asulam. Weed Res. 17:33–39.

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.

Walker, E. R. and L. R. Oliver. 2008. Translocation and absorption of glyphosate in flowering sicklepod (*Senna obtusifolia*). Weed Sci. 56:338–343.
Williams, R. D. 1964. Assimilation and translocation in perennial grasses. Ann. Bot. 28:419–426.
Wilson, K. A. 2002. Continued pteridophyte invasion of Hawaii. Am. Fern J. 92:179–183.
Wolf, S. 1993. Effect of leaf age on photosynthesis, carbon transport and carbon allocation in potato plants. Potato Res. 36:253–262.

Received October 2, 2009, and approved January 5, 2010.