J. Aquat. Plant Manage. 62: 7–14

Evaluation of florpyrauxifen-benzyl for control of Old World climbing fern

JONATHAN S. GLUECKERT, JAMES K. LEARY, AND STEPHEN F. ENLOE*

ABSTRACT

Old World climbing fern [*Lygodium microphyllum* (Cav.) R. Br.; OWCF] is an invasive vine in many Florida wetlands. Management involves poodle cutting the climbing rachis at approximately 120 cm, with a subsequent foliar application of glyphosate to the remaining rooted vines. Recently, there has been increasing interest in synthetic auxin herbicides for OWCF control. Florpyrauxifen-benzyl is a new auxin mimic that was developed for weed control in aquatics and is selective at low use rates. There is limited information regarding its activity on OWCF. We conducted field studies at four wetland sites in south Florida from 2017 to 2020 to evaluate the effectiveness of florpyrauxifen-benzyl for OWCF control. In small-plot studies at two locations, florpyrauxifen-benzyl applied at 0.08 and 0.16 g L^{-1} reduced OWCF cover to $\leq$ 4% at 3 mo after treatment (MAT) and $\leq$ 21% at 6 MAT. However, florpyrauxifen-benzyl at these concentrations did not consistently control OWCF at 12 MAT. An additional study evaluated banded applications of florpyrauxifen-benzyl or glyphosate compared to poodle cutting for control of the climbing rachis. For both herbicides, banded applications did not control the climbing rachis, whereas poodle cutting provided complete control of all climbing rachis. Finally, an operational field study demonstrated florpyrauxifen-benzyl applied by professional applicators at 0.16 g L^{-1} reduced OWCF cover to 9% at 17 MAT and was not different from glyphosate applied at 14.4 g L^{-1}. These studies indicate florpyrauxifen-benzyl is active on OWCF and future research should evaluate the effectiveness of repeat applications for longer-term control.

Key words: chemical control, invasive vines, natural areas, wetlands, *Lygodium micropyhllum* (Cav.) R. Br.

INTRODUCTION

Old World climbing fern [*Lygodium microphyllum* (Cav.) R. Br.; OWCF] is an indeterminately climbing and twining fern that is one of the most troublesome invasive plants in Florida. It invades numerous wetland, mesic, and hydric upland habitats, displaces native vegetation, and alters natural fire regimes (Hutchinson et al. 2006). Native to tropical and subtropical regions in Africa, Australia, and southeast Asia, OWCF was first introduced into the United States as an ornamental plant in the nurseries of southeast Florida in the late 1950s (Pemberton and Ferriter 1998). OWCF quickly escaped cultivation and was reported as naturalized and spreading rapidly throughout natural areas in Palm Beach and Martin Counties in southeast Florida in 1965 (Beckner 1968). In less than 50 yr from its introduction, OWCF had expanded to cover more than 70,000 ha throughout south and central Florida (Ferriter and Pernas 2006). Currently, its range continues to expand northward, varying from dense infestations on tree islands of the Everglades in the south of the state to isolated populations in Duval County near Jacksonville, FL with an additional verified report of a single plant found by the Georgia Department of Natural Resources in Long County, GA in 2023 (EDDMapS 2023). The fern has been predicted to continue expansion to other suitable regions in southern Georgia, the Gulf Coast of Texas, the Caribbean, and South and Central America (Goolsby 2004).

In its native range, OWCF is rarely found to be more than a few meters tall and does not displace the native plant community (Goolsby et al. 2003). Alternatively, in its novel range in Florida, it aggressively smothers native vegetation in the understory under rachis mats that can be up to 1 m thick and climbs rapidly into the crown of trees, sometimes collapsing entire forest canopies under the weight of the biomass (Pemberton and Ferriter 1998, Hutchinson et al. 2006). This alteration of the forest structure creates homogenous monocultures of OWCF and reduces the overall biodiversity of the plant community (Brandt and Black 2001, Clark 2002), has negative implications on wildlife (Call et al. 2007, Hutchinson 2006), and can carry fire into tree crowns in fire-intolerant habitats (Pemberton and Ferriter 1998, Hutchinson et al. 2006).

OWCF is common in wetland and mesic ecosystems, including seasonally inundated pine flatwoods, cypress swamps, and tree islands of the Everglades, all of which are common habitats in Florida (Volin et al. 2004, Hutchinson et al. 2006). Spores can germinate and produce gametophytes on a wide variety of natural substrates, including standing water (Call et al. 2007). Sporophytes readily establish in any moist substrate such as tussocks of grasses and native ferns, decaying stumps and logs, or banks of drainage canals (Call et al. 2007, Hutchinson and Langeland 2010). Its rapid spread can be attributed to a prolific mixed mating

*First author: Biological Scientist, Center for Aquatic and Invasive Plants, University of Florida, Gainesville, FL 32653. Second and third authors: Assistant Professor and Professor, Department of Agronomy, Center for Aquatic & Invasive Plants, University of Florida, Gainesville, FL 32653. Corresponding author's E-mail: sfenloe@ufl.edu. Received for publication November 5, 2023 and in revised form January 31, 2024.

DOI: 10.57257/JAPM-D-23-00011

sexual reproduction system (Lott et al. 2003). Each fertile leaflet can contain up to approximately 28,000 wind dispersed spores (Volin et al. 2004) and a single isolated spore can result in a viable population far from its parent plant through intragametophytic selfing (Lott et al. 2003). The dispersal rate of its spores, facilitated by its propensity to climb to the top of tree canopies, has been estimated to be up to 724 spores m^3 hr^{-1} (Pemberton and Ferriter 1998).

Throughout the entire Everglades watershed, 74% of the OWCF infestations occur within the Arthur R. Marshall Loxahatchee National Wildlife Refuge (LNWR; Rodgers et al. 2014). The LNWR is a 58,755-ha impounded peat wetland that is a historical remnant of the northern Everglades in western Palm Beach County, FL. Dense OWCF infestations within the refuge are significantly correlated to the tree and shrub vegetation found on them (Wu et al. 2006, Fujisaki et al. 2010) and the species is most successful in the transitional area where wet and dry conditions meet (Hutchinson and Langeland 2006). Some tree islands are completely blanketed by OWCF to the extent that no other vegetation can be seen beneath it (Pemberton and Ferriter 1998).

The most common method for controlling OWCF is a cut and spray technique known as poodle cutting. This involves cutting the climbing rachis at approximately 120 cm and then applying a foliar herbicide treatment to the lower portion of the climbing rachis below the cutting height (Thomas and Brandt 2003, Hutchinson et al. 2006). Previous studies have demonstrated that poodle cutting is required to control climbing vines as complete foliar treatment of climbing vines from the ground is generally not possible (Hutchinson et al. 2006). Furthermore, banded sprays that treat only the lower portion of the climbing vines do not translocate herbicide sufficiently to the higher unsprayed portions for complete control. However, Stocker et al. (1997) found limited evidence of upward translocation of the auxin triclopyr following a banded application. Additionally, treatments with glyphosate, triclopyr, and metsulfuron-methyl will all control aboveground biomass but regrowth from the rhizomes can result in rapid recovery of OWCF, revealing limited basipetal translocation of the herbicide into the rhizomes (Hutchinson et al. 2010). Ground treatments that require poodle cutting are often very expensive because of increased effort of applicators. In 2021, the South Florida Water Management District (SFWMD) reported a total of 655 ground-treated hectares of OWCF in LNWR, amounting to nearly $438 dollars per hectare (E. Allen, SFWMD, pers. comm.).

Additionally, nonselective herbicides like glyphosate have the potential to cause nontarget damage to desirable vegetation (Stocker et al. 1997, Hutchinson et al. 2006, Hutchinson and Langeland 2007). There are no known instances of long-term control using any herbicide without repeated applications (Hutchinson and Langeland 2006). In greenhouse and field trials, glyphosate reduced OWCF cover by over 92%, and despite mortality of native plants and frequent OWCF resprouting it remains the most common management option (Thomas and Brandt 2003, Hutchinson and Langeland 2006, Hutchinson and Langeland 2007). Applications of metsulfuron-methyl resulted in similar efficacy on OWCF and many trees and shrubs were less susceptible to inadvertent damage; however, native ferns frequently found in the understory are highly sensitive to the herbicide (Hutchinson and Langeland 2007, Hutchinson and Langeland 2012).

In 2018, a new active ingredient, florpyrauxifen-benzyl, was developed and registered for aquatic use (Anonymous 2018). Florpyrauxifen-benzyl represents a new class of synthetic auxins in the arylpicolinate family, exhibiting a different binding affinity than other synthetic auxins that are currently available, including triclopyr (Netherland and Richardson 2016). Florpyrauxifen-benzyl was developed for the selective control of weeds in rice and aquatic areas and has demonstrated systemic activity at low use rates on numerous aquatic weeds with limited nontarget damage (Netherland and Richardson 2016, Richardson et al. 2016, Beets et al. 2019, Sperry et al. 2021). Florpyrauxifen-benzyl has had limited testing in wetlands and riparian areas where OWCF is problematic. Recent studies have indicated that florpyrauxifen-benzyl is successful at controlling barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.] and several broadleaf weeds commonly found in rice (Miller et al. 2018, Miller and Norsworthy 2018). However, foliar application of florpyrauxifen-benzyl to Brazilian peppertree (*Schinus terebinthifolia* Raddi) and four mangrove species failed to control the invasive tree and resulted in considerable nontarget injury to all four mangrove species (Enloe et al. 2020). Regardless, given its low use rates, selectivity, and utility in aquatic settings, florpyrauxifen-benzyl is an ideal candidate for efficacy testing on OWCF.

Given the need for additional selective tools for OWCF management, the objectives of this study were 1) to evaluate the efficacy of backpack foliar application of florpyrauxifen-benzyl at three different concentrations on OWCF and compare them to glyphosate, which is the commercial standard; 2) to determine if a banded application of florpyrauxifen-benzyl to the lower portion of the climbing rachis will control the entire climbing portion of the fern without poodle cutting; and 3) to evaluate the performance of florpyrauxifen-benzyl compared to glyphosate in an operational setting. A better understanding of florpyrauxifen-benzyl efficacy on OWCF could provide land managers with a more selective, lower-use-rate herbicide that is already approved for use in wetland and aquatic sites where this troublesome invasive plant is commonly present.

MATERIALS AND METHODS

Field studies were conducted at four sites in south Florida from 2017 to 2019. These included a florpyrauxifen-benzyl concentration study near Fort Pierce, FL (27°32′25.191″N, 80°22′42.5388″W) and along the Nubbin Slough near Okeechobee, FL (27°15′10.1448″N, 80°41′30.123″W), a poodle cutting/florpyrauxifen benzyl banded treatment study near Taylor Creek, FL (27°16′36.5016″N, 80°38′58.1136″W), and an operational florpyrauxifen-benzyl study on Everglades tree islands in the LNWR (26°30′10.947″N, 80°19′58.2378″W). All sites contained mature infestations with dense, homogenous patches of OWCF climbing into the tree canopies and smothering native understory vegetation under rachis mats up to 0.8 m deep.

Florpyrauxifen-benzyl concentration studies

In March of 2017, a study was initiated in Fort Pierce, FL in St. Lucie County. At this location, OWCF had formed dense patches at the base of a constructed berm surrounding a stormwater retention pond. The habitat consisted of an overstory with an open canopy of slash pine (*Pinus elliottii* Engelm.) and melaleuca [*Melaleuca quinquenervia* (Cav.) S.F. Blake] trees that were treated in the prior year, leaving snags covered with vertically ascending OWCF. Native understory species included saw palmetto [*Serenoa repens* (Bartram) J. K. Small], chain fern [*Woodwardia virginica* (L.) Sm.], swamp fern (*Blechnum serrulatum* Rich.), and bracken fern [*Pteridium aquilinum* (L.) Kuhn] which were growing underneath the rachis mats. The soil is composed of Lawnwood and Myakka sands (Soil Survey Staff 2022). The study was initiated before the peak of the wet season in March and no standing water was present in any of the plots at the time of treatment.

The study was repeated at a second site along the Nubbin Slough near Okeechobee, FL. The Nubbin Slough is a forested intermittent stream that collects water from the Nubbin Slough Basin and discharges it into northeast Lake Okeechobee. OWCF at this site has replaced much of the native plant community in the understory, forming monotypic patches that would have historically consisted mainly of saw palmetto, native ferns, and other woody and herbaceous species. The dominant tree species is slash pine, of which a majority are affected by the climbing rachis up to the canopy. Basinger and Placid sand make up the soil composition at this site and exhibit ponding during frequent rainfall (Soil Survey Staff 2022). Throughout the duration of the study, occasional inundation of up to 15 cm occurred, but did not persist for extended periods in any of the experimental units.

At both sites, 20 plots, each 25 m^2 (5 m by 5 m) in size, were established in dense, horizontally growing OWCF. Vertically ascending OWCF was excluded from the experiment and prevented the need for poodle cutting. Plots were marked with a single polyvinyl chloride (PVC) pole at the center point with a buffer of at least 1 m between plots to prevent overspray into neighboring plots. A total of five treatments were applied, including florpyrauxifen-benzyl[1] at three concentrations of 0.08 g ae L^{-1}, 0.16 g ae L^{-1}, 0.32 g ae L^{-1}, glyphosate[2] at the commercial standard concentration of 14.4 g ae L^{-1} (Hutchinson et al. 2006), and a nontreated control. Treatments were assigned to experimental units in a completely randomized design and each treatment was replicated four times. A nonionic surfactant (NIS)[3] was added to each treatment at 0.5% volume to volume (v/v). In Ft. Pierce, FL, plots were treated on 5 April 2017, and the Nubbin Slough applications were made on 26 May 2017, and completed on 27 May 2017. The minor delay was due to the threat of forecasted storms later in the day on 26 May. The treatments at both sites were applied using a carbon dioxide (CO_2) pressurized backpack sprayer with a single adjustable cone nozzle at an application volume of 374 L ha^{-1}. The sprayer was calibrated before each treatment to ensure that a consistent volume of the solution was applied to each plot.

A visual estimate of percent OWCF cover was collected using a 1-m^2 quadrat placed at four locations within the entire 25-m^2 plot. Visual estimations from these subplots were averaged to obtain the percent cover for the whole plot. Most resprouting observed within the plots throughout the duration of the study was from the rachis, and few new sporophytes were observed. Baseline data were collected prior to the herbicide application and posttreatment cover evaluations were collected at 1, 3, 6, and 12 MAT.

Poodle cutting/florpyrauxifen-benzyl banded treatment study

This study was initiated near Taylor Creek, FL in August 2018 to investigate the effectiveness of florpyrauxifen-benzyl using banded treatments with and without poodle cutting. The site was located on the margin of a cypress dome, where OWCF was ascending vertically into the canopy of bald cypress [*Taxodium distichum* (L.) Rich.] and red maple (*Acer rubrum* L.). Soil composition consisted of Samsula muck and Lawnwood and Myakka sands which are poorly drained and exhibit frequent ponding during the wet season (Soil Survey Staff 2022).

The study was a completely randomized design (CRD). Eight treatments were applied to 5 by 5–m plots, with each plot centered on an infested tree with dense OWCF cover ascending vertically above 5 m in height. Each treatment was replicated four times for a total of 32 plots. Treatments included florpyrauxifen-benzyl[1] at 0.08 g ae L^{-1} and 0.16 g ae L^{-1}, glyphosate[2] at 14.4 g ae L^{-1}, and a nontreated control, each with and without poodle cutting. A nonionic surfactant[3] was applied with each herbicide treatment at 0.5% v/v. In plots with poodle cutting, the climbing fern was cut at 1.2 m, which separated the upper climbing portion from the rooted vine. The intact lower portion of the vines were then pulled away from the tree and treated with herbicide. In plots without poodle cutting, a band of herbicide was applied to the climbing OWCF from the ground up to 1.2 m around the circumference of the tree centered in the plot. Additionally, all OWCF ground cover within each 5 by 5–m plot was also foliar treated with the same herbicide treatment as the banded treatment. Plots receiving the control treatments were either poodle cut or were not poodle cut but did not receive any herbicide treatment.

Visual percent climbing cover was recorded by estimating OWCF climbing cover on two opposite sides of the tree in the center of each plot and averaging them for a single value for the plot. Ground cover was eliminated by the foliar treatment and resprouts did not influence the climbing cover. Data were collected just prior to treatment (baseline) and at 1, 3, 6, and 12 MAT. These sample dates allowed us to separate both short and longer-term impacts of both the herbicide and poodle cutting treatments.

Operational florpyrauxifen-benzyl study

The final study was established on tree islands in the LNWR in 2018. Twenty tree islands ranging from 0.07 to 0.37 ha (average 0.18 ha) were randomly selected within a 1-km^2 grid in the central region of the LNWR. The landscape in the LNWR consists of a mosaic of sloughs, wet prairies, and sawgrass ridges, and contains a high density of tree islands (Brandt and Black 2001). Tree islands, composed of Loxahatchee Muck (Soil Survey Staff 2022) contained mixed stands of moderate or dense OWCF in the understory with ascension into the canopy of most trees, which included swamp bay [*Persea palustris* (Raf.) Sarg], wax myrtle [*Morella cerifera* (L.) Small], and dahoon holly (*Ilex cassine* L.). Common native vegetation growing in association with OWCF in the understory included cinnamon fern (*Osmunda cinnamomea* L.), sword fern [*Nephrolepis exaltata* (L.) Schott], swamp fern, hottentot fern [*Thelypteris interrupta* (Willd.) K. Iwats.], chain fern, and other herbaceous species. The fringes of the tree islands were generally comprised of sawgrass (*Cladium jamaicense* Crantz.) just before the transition to deeper water and open marsh.

Individual tree islands served as experimental units. Islands were randomly assigned to one of five treatments, with four replicate islands per treatment. A total of five treatments were applied and included florpyrauxifen-benzyl[4] at 0.08 g ae L^{-1} or 0.16 g ae L^{-1}, triclopyr acid[5] at 5.4 g ae L^{-1}, glyphosate[2] at 14.4 g ae L^{-1}, and a nontreated control.

Treatments were applied by a commercial applicator (Aquatic Vegetation Control Inc., Riviera Beach, FL, USA) in December 2018. The expected application volume for treatments was 374 L ha^{-1}, but across experimental units, the actual application volume was 524 L ha^{-1}. This was likely because of the heavy OWCF cover over both the ground and many shrubs, creating a variable three-dimensional canopy structure that was inherently more difficult to treat with backpack sprayers. Triclopyr acid was also included in the study because previous studies found triclopyr reduced OWCF cover and resulted in an increase in native ferns and herbaceous species in the understory (Glueckert et al. 2023). Research by Hutchinson and Langeland (2007) also resulted in an increase in native vegetation following treatment with triclopyr formulations when compared with glyphosate.

A six-person crew initiated the treatments on 10 December 2018. The 16 tree islands that received an herbicide treatment were first poodle cut by the applicator crew at a height from the ground of approximately 1.2 m to disconnect the climbing rachis from the rooted vines. The four nontreated control islands were not poodle cut. Herbicide applications occurred from 17 December to 19 December 2018. The herbicide treatments were mixed in a 378-L tank and distributed to 15-L backpack sprayers with single adjustable cone nozzles. A methylated seed oil (MSO)[6] was included in the tank mix at 1% v/v. Applicators treated the lower, intact portion of the climbing rachis and all other OWCF cover below the poodle cutting height across each island.

For data collection, three 25-m^2 (5 by 5 m) subplots were placed within dense patches of OWCF on each island, totaling 60 subplots across the 20 tree islands. Plots were marked at their center with PVC poles to ensure their location at each sampling date. Baseline data were collected prior to the initiation of the treatments. An estimate of visual percent cover of OWCF was collected using a 1-m^2 quadrat that was placed at four locations within each of the 25-m^2 subplots. Percent cover was then averaged among each of the three subplots on the island to obtain the estimate for the entire tree island. Essentially all climbing cover was eliminated on herbicide-treated islands after poodle cutting. As a result, percent cover evaluations for each treatment are a cumulative measure of ground cover and climbing cover within the plot, but are based almost entirely on ground cover, except for the nontreated controls, which were not poodle cut. Sample dates included baseline, 3, 6, 13, and 17 MAT.

Statistical analysis

Data were analyzed in RStudio[7] using "base, and "agricolae packages (de Mendiburu and Yaseen 2020, R Core Team 2023) There was a significant interaction between sites in the florpyrauxifen-benzyl concentration study conducted at Ft. Pierce, FL and Nubbin Slough, likely caused by differences in hydrology throughout the duration of the study. Therefore, each site and sampling date were analyzed separately. Herbicide treatment was a fixed effect in all studies. For each location, a normal Q–Q plot was used to check for normality and residuals were not normally distributed. Therefore, mean percent cover was transformed using the arcsin–square root transformation to improve homogeneity of variance. Transformed means were used for all analyses and untransformed means are presented for clarity. For each study, an analysis of variance (ANOVA) was performed on mean percent cover of OWCF at all sampling dates. Treatment means were compared using Tukey's honest significant difference (HSD) test at the 5% level of significance.

RESULTS AND DISCUSSION

Florpyrauxifen-benzyl concentration study, Ft. Pierce

Visual percent cover of OWCF at the baseline sampling date ranged from 71 to 85% (Table 1). By 1 MAT, all concentrations of florpyrauxifen-benzyl reduced OWCF cover to 20 to 23% and were not different. Glyphosate reduced OWCF cover to less than 2% at 1 MAT, which was significantly lower than in plots treated with florpyrauxifen-benzyl at 0.08 and 0.16 g ae L^{-1}.

At 3 MAT, all three concentrations of florpyrauxifen-benzyl reduced OWCF cover to 1 to 4 percent and were not different. These were also not different from glyphosate, which reduced OWCF cover to zero. At 3 MAT, we observed some new resprouts on the above ground rachis within the treated plots for all three concentrations of florpyrauxifen-benzyl. Resprouting from the rachis and rhizomes following an herbicide treatment may indicate limited basipetal translocation. Studies conducted by Hutchinson et al. (2010) examined absorption and movement of triclopyr, glyphosate, and metsulfuron-methyl in OWCF and concluded that there was very little movement of the chemical out of the leaf tissue and into the rhizomes. Treatments with triclopyr, a synthetic auxin mimic with a similar mode of action to florpyrauxifen-benzyl, have resulted in rapid necrosis of the leaf tissue in under 4 h, potentially limiting the amount of herbicide which is absorbed into the plant (Hutchinson et al. 2010). Although we did not observe rapid necrosis from florpyrauxifen-benzyl treatments, we did

Table 1. Old World climbing fern cover response to herbicide treatment over time in the florpyrauxifen-benzyl concentration study in Ft. Pierce, FL.

Herbicide	Concentration g ae L^{-1}	0 MAT[1]	1 MAT	3 MAT	6 MAT	12 MAT
		% Cover [2]				
Florpyrauxifen-benzyl	0.08	74 a	21 b	4 b	21 b	39 b
Florpyrauxifen-benzyl	0.16	85 a	23 b	1 b	9 b	28 b
Florpyrauxifen-benzyl	0.32	73 a	20 bc	2 b	4 b	12 bc
Glyphosate	14.4	83 a	1 c	0 b	4 b	6 c
Nontreated	–	71 a	71 a	69 a	68 a	73 a

[1]MAT= mo after treatment.
[2]Means within columns followed by the same letter are not significantly different according to Tukey's honest significant difference ($P = 0.05$).

observe onset of auxin herbicide symptoms including epinasty and pinnae curling at the meristems within 24 h.

By 6 MAT, all herbicide treatments reduced OWCF cover compared to the nontreated control (Table 1). OWCF percent cover in plots treated with all concentrations of florpyrauxifen-benzyl ranged from 4 to 21% and were not different. OWCF cover in the glyphosate-treated plots was 4% and not different from any of the florpyrauxifen-benzyl treatments.

By 12 MAT, all herbicide treatments reduced OWCF cover compared to the nontreated control. OWCF cover in plots treated with florpyrauxifen-benzyl ranged from 12 to 39% and were not different (Table 1). Glyphosate reduced OWCF cover to six percent and was not different from the highest concentration of florpyrauxifen-benzyl (12%). The lack of significant treatment differences between the three florpyrauxifen-benzyl treatments was driven by highly variable OWCF cover within treatments, especially for the two lower concentrations. This is not surprising, as, observationally, variation in herbicide efficacy among treatment plots generally increases when a given treatment begins to fail over time, especially for perennial invasive plants.

Florpyrauxifen-benzyl concentration study, Nubbin Slough

Across sample dates, herbicide efficacy observed at the Nubbin Slough site was generally similar to the Fort Pierce site (Table 2). The baseline percent OWCF cover ranged from 77 to 93% and was not different between plots. At 1 MAT, all herbicide treatments reduced OWCF cover to between 7 and 26% and were not different. Symptoms such as chlorosis and epinasty were present in the florpyrauxifen-benzyl treated plots within a few days of application, but OWCF was slow to defoliate.

At 6 MAT, all herbicide treatments reduced OWCF cover compared to the nontreated control. However, the nontreated plots exhibited lower cover than expected and was 45%. This was due to an outbreak of the biological control agent, the brown *Lygodium* moth [*Neomusitima conspurcatalis* (Warren) (Lepidoptera: Crambidae)]. The moth feeding resulted in localized brown out of OWCF patches and damage was evident across the study site. The life cycle of the moth is approximately 30 d from egg to adult (Boughton and Pemberton 2009) and OWCF generally recovers between population booms (Jones and Lake 2020).

Old World climbing fern cover in the nontreated plots recovered from the biological control damage at 12 MAT to 66% and was not different from cover in all three florpyrauxifen-benzyl treatments, which ranged from 30 to 54%. Regrowth of OWCF in glyphosate treated plots was 6% and was different from the two lower concentrations of florpyrauxifen-benzyl and the nontreated control.

Poodle cutting/florpyrauxifen-benzyl banded treatment study, Taylor Creek, FL

Florpyrauxifen-benzyl band treatments applied at either concentration without poodle cutting did not reduce OWCF climbing cover above the herbicide-treated band at any posttreatment sample date compared to the nontreated control treatment that was poodle cut (Table 3). The same held true for glyphosate. Poodle cutting followed by florpyrauxifen-benzyl band treatment at either concentration reduced OWCF cover to $\leq$ 3% at 1, 3, and 6 MAT and $\leq$ 24% at 12 MAT. These results were not different from poodle cutting followed by a glyphosate band treatment. No herbicide treatment reduced OWCF climbing cover without poodle cutting compared to the nontreated control without poodle cutting. These results indicate poodle cutting is necessary for OWCF control if florpyrauxifen-benzyl is only

Table 2. Old World climbing fern cover response to herbicide treatment over time in the florpyrauxifen-benzyl concentration study at Nubbin Slough.

Herbicide	Concentration g ae L^{-1}	0 MAT[1]	1 MAT	3 MAT	6 MAT	12 MAT
		% Cover [2]				
Florpyrauxifen-benzyl	0.08	77 a	26 b	2 b	15 b	53 a
Florpyrauxifen-benzyl	0.16	90 a	19 b	1 bc	6 bc	54 a
Florpyrauxifen-benzyl	0.32	93 a	12 b	0 c	3 c	30 ab
Glyphosate	14.4	93 a	7 b	1 bc	2 c	6 b
Nontreated	–	86 a	93 a	100 a	45 a	66 a

[1]MAT = months after treatment.
[2]Means within columns followed by the same letter are not significantly different according to Tukey's honest significant difference ($P = 0.05$).

Table 3. Old World climbing fern (OWCF) climbing cover response to herbicide band treatment and poodle cutting near Taylor Creek, FL.

Herbicide band treatment[1]	Concentration g ae L^{-1}	Poodle cut	0 MAT[2]	1 MAT	3 MAT	6 MAT	12 MAT
			% Cover [3]				
Florpyrauxifen-benzyl	0.08	No	78 a	78 a	78 a	69 a	79 a
Florpyrauxifen-benzyl	0.08	Yes	74 a	0 b	3 b	3 b	24 b
Florpyrauxifen-benzyl	0.16	No	79 a	77 a	79 a	74 a	89 a
Florpyrauxifen-benzyl	0.16	Yes	58 a	0 b	0 b	0 b	8 b
Glyphosate	14.4	No	74 a	54 a	55 a	46 a	76 a
Glyphosate	14.4	Yes	63 a	0 b	0 b	0 b	13 b
Nontreated	–	No	76 a	55 a	73 a	75 a	89 a
Nontreated	–	Yes	74 a	1 b	5 b	4 b	11 b

[1]Herbicide band treatments were applied to the climbing rachis below 1.2 m and all OWCF ground cover in each 25-m^2 plot.
[2]MAT= months after treatment.
[3]Means within columns followed by the same letter are not significantly different according to Tukey's honest significant difference ($P = 0.05$).

applied to the lower 120-cm band of climbing vines. Additionally, we did not observe herbicide symptoms above the treated band at any posttreatment sample date. This would indicate there is insufficient acropetal translocation to climbing vines above the treated zone. Although the pinnae within the herbicide-treated band defoliated, the wiry stems were not killed within the treated band and eventually resprouted. This also suggests a potential lack of herbicide absorption through the wiry stems and mandates good foliar coverage for complete control. This study also confirms the necessity of poodle cutting when glyphosate is used for ground-based backpack treatments for OWCF control (Stocker et al. 1997).

Operational florpyrauxifen-benzyl study

At LNWR, baseline cover of OWCF ranged from 63 to 79% and was not different between treatments (Table 4). Following treatment, site access was limited because of hydrologic conditions, and posttreatment sampling is reported beginning at 3 MAT. Across all herbicide-treated islands, all OWCF climbing cover was eliminated by poodle cutting for the duration of the study (data not shown). At 3 MAT, triclopyr and the florpyrauxifen-benzyl treatments applied at 0.08 and 0.16 g ae L^{-1} reduced OWCF cover to 4% or less and were not different. Glyphosate reduced OWCF cover to zero, which was significantly lower than cover in the florpyrauxifen-benzyl treatment at 0.08 g ae L^{-1}. Very similar results were observed across all treatments at both 6 and 13 MAT.

At 17 MAT, all herbicide treatments reduced OWCF cover compared to the untreated control. Florpyrauxifen-benzyl applied at 0.16 g ae L^{-1}, triclopyr acid applied at 5.4 g ae L^{-1}, and glyphosate resulted in OWCF cover of less than 10% and these were not different. All three provided commercially acceptable control, while significantly higher OWCF cover (25%) was observed in the florpyrauxifen-benzyl treatment applied at 0.08 g ae L^{-1}.

The tree island operational study results from the LNWR generally validate the studies from the Fort Pierce, Nubbin Slough, and Taylor Creek sites. First, they all indicate florpyrauxifen-benzyl has considerable activity on OWCF with ground-based foliar treatments. This is promising, as available tools are limited for OWCF chemical control. Second, short-term control of OWCF at 3 MAT with florpyrauxifen-benzyl was consistent with glyphosate in all studies. Florpyrauxifen-benzyl applied at 0.08 and 0.16 g ae L^{-1} reduced cover to less than 5% at 3 MAT at all sites and was not different from glyphosate. Third, at later sample dates, OWCF cover in plots treated with florpyrauxifen-benzyl at 0.08 g ae L^{-1} was higher than in glyphosate-treated plots. However, it was generally not higher than in plots treated with florpyrauxifen-benzyl at 0.16 or 0.32 g ae L^{-1}. As a result, there was not a clear dose–response relationship established in these studies for florpyrauxifen-benzyl efficacy on OWCF. Future dose–response studies in controlled conditions with a greater range of concentrations could help to clarify this.

Fourth, the study at Taylor Creek indicated florpyrauxifen-benzyl banded treatments applied to the lower 120 cm of climbing OWCF cover without poodle cutting will not control higher OWCF climbing cover, whereas the addition of poodle cutting at the same height does. The poodle cutting followed by florpyrauxifen-benzyl treatment at LNWR

Table 4. Old World climbing fern cover response to herbicide treatment over time in the operational study conducted on tree islands in the Loxahatchee National Wildlife Refuge.

Herbicide	Concentration g ae L^{-1}	0 MAT[1]	3 MAT	6 MAT	13 MAT	17 MAT
		% Cover [2]				
Florpyrauxifen-benzyl	0.08	72 a	4 b	6 b	21 b	25 b
Florpyrauxifen-benzyl	0.16	79 a	1 bc	1 bc	6 bc	9 c
Triclopyr	5.4	68 a	0 bc	1 bc	5 bc	9 c
Glyphosate	14.4	63 a	0 c	0 c	5 c	5 c
Nontreated	–	69 a	76 a	78 a	89 a	59 a

[1]MAT= months after treatment.
[2]Means within columns followed by the same letter are not significantly different according to Tukey's honest significant difference ($P = 0.05$).

confirmed that the combination treatment is operationally effective to control OWCF climbing cover.

Finally, the low use rates associated with florpyrauxifen-benzyl may represent an opportunity to reduce the total amount of herbicide necessary to achieve control. Future research should focus on establishing the proper concentrations necessary to maintain efficacy 12 MAT and beyond and the necessary retreatment intervals required to maintain control long-term. In addition, there is limited information available about the response of many native wetland species to florpyrauxifen-benzyl, so research examining directed applications of florpyrauxifen-benzyl to desirable vegetation could help to minimize potential nontarget damage to the native plant community when making applications to OWCF.

SOURCES OF MATERIALS

[1]ProcellaCOR® EC; SePRO Corporation, 11550 North Meridian Street, Suite 600, Carmel, IN 46032.

[2]Roundup Custom®; Bayer Crop Science LP, 800 North Lindbergh Boulevard, St. Louis, MO 63167.

[3]Induce®; Helena Chemical Company, 225 Schilling Boulevard, Suite 300, Collierville, TN.

[4]ProcellaCOR® SC; SePRO Corporation, 11550 North Meridian Street, Suite 600, Carmel, IN 46032.

[5]Trycera®; Helena Chemical Company, 225 Schilling Boulevard, Suite 300, Collierville, TN.

[6]Dyne-Amic®; Helena Chemical Company, 225 Schilling Boulevard, Suite 300, Collierville, TN.

[7]R software (version 4.3.1), R Foundation for Statistical Computing, Vienna, Austria.

ACKNOWLEDGEMENTS

The authors would like to thank LeRoy Rodgers (SFWMD), Ellen Allen (SFWMD), and Rebekah Gibble (USFWS) for their assistance with the project. The authors would also like to thank Paul Terry (Aquatic Vegetation Control, Inc.) for executing the herbicide treatments in the LNWR, and Garrett Wong and Adam Kobylski for assistance with data collection. Funding was provided by the Florida Fish and Wildlife Conservation Commission and the South Florida Water Management District.

LITERATURE CITED

Anonymous. 2018. ProcellaCOR® SC product label. SePro Publication No. ProcellaCOR SC Spec__3-23-18__PA. SePro Corporation, Carmel, IN. 5 pp.

Beckner J. 1968. *Lygodium microphyllum,* another fern escaped in Florida. Am Fern J 58:93–94.

Beets J, Heilman M, Netherland MD. 2019. Large-scale mesocosm evaluation of florpyrauxifen-benzyl, a novel arylpicolinate herbicide, on Eurasian and hybrid watermilfoil and seven native submersed plants. J. Aquat. Plant Manage. 57:49–55.

Boughton AJ, Pemberton RW. 2009. Establishment of an imported natural enemy, *Neomusitima conspurcatalis* (Lepidoptera: Crambidae) against an invasive weed, Old World climbing fern, *Lygodium microphyllum*, in Florida. Biocontrol Sci. Technol. 19:769–772.

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

Call EM, Brandt LA, Deangelis DL. 2007. Old World climbing fern (*Lygodium microphyllum*) spore germination in natural substrates. Fla. Sci. 70:55–61.

Clark DW. 2002. The Effect of Old World Climbing Fern (*Lygodium microphyllum* (Cav.) R. Brown) on South Florida Cypress (*Taxodium distichum* (L.) Rich.) Swamp Plant and Insect Community Structure. Master's thesis. University of Florida, Gainesville, FL. 86 pp.

de Mendiburu F, Yaseen M. 2020. Agricolae: Statistical procedures for agricultural research. R package version 1.4.0. https://CRAN.R-project.org/package=agricolae. Accessed 15 September 2023.

EDDMapS. 2023. Early Detection & Distribution Mapping System. University of Georgia Center for Invasive Species and Ecosystem Health. https://www.eddmaps.org/. Accessed October 12, 2023.

Enloe SF, Leary J, Prince C, Sperry B, Lauer D. 2020. Brazilian peppertree and mangrove species response to foliar-applied novel auxin-type herbicides. Invasive Plant Sci. Manage. 13:102–107.

Ferriter AP, Pernas T. 2006. An explosion in slow motion: Tracking the spread of *Lygodium microphyllum* in Florida. Wildland Weeds 9:7–9.

Fujisaki I, Brandt LA, Chen H, Mazzotti FJ. 2010. Colonization, spread, and growth of *Lygodium microphyllum* on tree islands in a wetland in Florida. Invasive Plant Sci. Manage. 3:412–420.

Glueckert JS, Leary JK, Enloe SF. 2023. Evaluation of novel triclopyr formulations for control of Old World climbing fern (*Lygodium microphyllum*). Invasive Plant Sci. Manage. 16:73–80.

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

Goolsby JA, Wright AD, Pemberton RW. 2003. Exploratory surveys in Australia and Asia for natural enemies of Old World climbing fern, *Lygodium microphyllum*: Lygodiaceae. Biol. Control 28:33–46.

Hutchinson JT. 2006. Additional report of *Lygodium microphyllum* mats as a potential problem for wildlife. Wildland Weeds 9:7

Hutchinson JT, Ferriter AP, Serbesoff King K, Langeland KA, Rodgers L. 2006. Old World climbing fern (*Lygodium microphyllum*) management plan for Florida. Florida Exotic Pest Plant Council Lygodium Task Force. https://floridainvasivespecies.org/. Accessed November 13, 2021.

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

Hutchinson JT, Langeland KA. 2007. Final Report: evaluation of current and potential new herbicides to control *Lygodium microphyllum*. Tallahassee, FL: Florida Department of Environmental Protection Bureau of Invasive Plant Management. 17 p

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

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

Hutchinson JT, Langeland KA, MacDonald GE, Querns R. 2010. Absorption and translocation of glyphosate, metsulfuron, and triclopyr in Old World climbing fern (*Lygodium microphyllum*). Weed Science 58:118–125.

Jones IM, Lake EC. 2020. Defoliation of the invasive fern *Lygodium microphyllum* by *Neomusitima conspurcatalis:* Effects on plant performance across a range of light conditions. Biol. Control 144:104236.

Lott MS, Volin JC, Pemberton RW, Austin DF. 2003. The reproductive biology of the invasive ferns *Lygodium microphyllum* and *L. japonicum* (Schizaeaceae): Implications for invasive potential. Am. J. Bot. 90:1144–1152.

Miller MR, Norsworthy JK. 2018. Florpyrauxifen-benzyl weed control spectrum and tank-mix compatibility with other commonly applied herbicides in rice. Weed Technol. 32:319–325.

Miller MR, Norsworthy JK, Scott RC. 2018. Evaluation of florpyrauxifen-benzyl on herbicide-resistant and herbicide-susceptible barnyardgrass accessions. Weed Technol 32:126–134.

Netherland MD, Richardson RJ. 2016. Evaluating sensitivity of five aquatic plants to a novel arylpicolinate herbicide utilizing an organization for economic cooperation and development protocol. Weed Sci. 64:181–190.

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

R Core Team. 2023. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/. Accessed September 15, 2023.

Richardson RJ, Haug EJ, Netherland MD .2016. Response of seven aquatic plants to a new arylpicolinate herbicide. J. Aquat. Plant Manage. 54:26–31.

Rodgers L, Pernas T, Hill SD. 2014. Mapping invasive plant distributions in the Florida Everglades using the digital aerial sketch mapping technique. Invasive Plant Sci. Manage. 7:360–.374

Soil Survey Staff. 2022. Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm. Accessed May 20, 2022.

Sperry BP, Leary JK, Jones D, Ferrell JA. 2021. Observations of a submersed field application of florpyrauxifen-benzyl suppressing hydrilla in a small lake in central Florida. J. Aquat. Plant Manage. 59:20–26.

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

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

Volin JC, Lott MS, Muss JD, Owen D. 2004. Predicting rapid invasion of the Florida Everglades by Old World climbing fern (*Lygodium microphyllum*). Diversity Distrib. 10:439–446.

Wu Y, Rutchey K, Wang N, Godin J. 2006. The spatial pattern and dispersion of *Lygodium microphyllum* in the Everglades wetland ecosystem. Biol. Invasions 8:1483–1493.