J. Aquat. Plant Manage. 58: 112–117

The suppressive effects of aquatic foliar herbicide prescriptions on nontarget panicgrass (*Paspalidium geminatum*)

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ABSTRACT

Panicgrass [*Paspalidium geminatum* (Forssk.) Stapf] is a wetland habitat species native to Florida. A mass decline of this grass species was observed starting in 2010 with the cause unknown. Invasive plants, namely water hyacinth [*Eichhornia crassipes* (Mart.) Solms] and water lettuce (*Pistia stratiotes* L.), colonize these grass beds and are controlled year round as part of a preemptive maintenance program to protect these panicgrass habitats from being displaced. Out of concern that collateral herbicide injury may be a potential cause for this decline, a series of mesocosm and field trials tested the sensitivity of panicgrass to prescriptive foliar herbicide treatments applied with a single dose or multiple, sequential doses. Mesocosm trials measured sublethal growth suppression on panicgrass with diquat and the combination of 2,4-D + flumioxazin with both single and sequential applications, while the imazamox + carfentrazone combination measured no suppression in the mesocosm. In the field trial, single and sequential applications of all three herbicide treatments measured reduction in green canopy at 7 d after treatment (DAT), followed by full recovery within 40 DAT. All trials were initiated in late summer into fall, where seasonality may have elicited phenological traits in panicgrass reducing susceptibility to herbicide. Therefore, timing of a prescriptive herbicide treatment may have important consequences on nontarget sensitivity. Overall, the results of these trials demonstrated operational aquatic herbicide prescriptions to yield transitory, suppressive effects on nontarget panicgrass, followed by rapid posttreatment recovery. This highlights how discriminant management against invasive species is being deployed to select for panicgrass habitat. However, it is advised that in order to maintain this selectivity, applicators should become more aware of their retreatment schedules in order to mitigate collateral suppression that could increase vulnerability of panicgrass to other stressors.

Key words: carfentrazone, 2 4-dichlorophenoxyacetic acid, diquat, field trial, flumioxazin, fractional green canopy cover, imazamox, mesocosm.

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INTRODUCTION

Panicgrass [*Paspalidium geminatum* (Forssk.) Stapf] is a pantropical, wetland species native to several regions including North and South America (Clayton et al. 2006). It was originally vouchered in 1761 in the Nile Delta and thought to have evolved as an emergent aquatic species with the creation of large, shallow lake systems during the last ice age (Stapf 1934, Friis 1983, Boulos and Fahmy 2007). In Florida, it occupies the shoreline and littoral zones of lentic systems and the Everglades (Busch et al. 1998). It can buffer wave action, creating an environment conducive to the establishment of other aquatic plants (Welsh and Denny 1978, Billore and Vyas 1981). It often dominates the emergent zone in the summer monsoon and has been shown to remain rooted after hurricanes (Welch 2009). It has a strong association with maidencane (*Panicum hemitomon* Schult.), another native grass species, and is also a key habitat species for macroinvertebrates, fish, and water avifauna (Schramm et al. 1987, Havens et al. 2005). Panicgrass also provides structural habitat to exotic and native apple snails (*Pomacea* spp.), which in turn are an important food source for the endangered snail kite (*Rostrhamus sociabilis* Vieillot) (Monette et al. 2017).

Several restoration projects have established this native grass in lake systems with varying success (Pouder et al. 2006, Mallison and Thompson 2010). However, in the last decade, declines in health and abundance of panicgrass have been noted on Lake Tohopekaliga, Lake Kissimmee, Lake Jackson, and the Everglades. Surveys conducted in 2010 and 2015 estimated 27 to 55% population reductions on Lake Tohopekaliga, and 22 to 51% reductions on Lake Kissimmee (Anonymous 2016). The cause of these declines remains unknown, but private stakeholders and public agencies alike have expressed concerns that they may be linked to management of invasive plants congregating within the swards of panicgrass.

Panicgrass beds are often colonized by free-floating water hyacinth [*Eichhornia crassipes* (Mart.) Solms] and water lettuce (*Pistia stratiotes* L.), among other species. Experience in Florida has demonstrated that preemptive maintenance control is the best strategy for keeping these invasive populations from achieving harmful levels of infestation (Schmitz et al. 1993, Florida Fish and Wildlife Conservation Commission 2012). Foliar herbicide treatments applied from an airboat is the standard practice for treating these invading plants and can involve frequent interventions in an area to treat missed plants and new recruits (University of Florida 2018). Diquat and 2,4-D are the most widely used herbicides for maintenance control of water lettuce and water hyacinth, respectively, but other herbicides (i.e., flumioxazin, imazamox, and carfentrazone) have also been shown to provide moderate to effective control (Koshnick et al. 2004, Richardson et al. 2008, Mudge and Netherland 2014a,b).

Herbicide treatments directed at the target invasive species may also contact nontarget panicgrass in close vicinity. The declining grass beds, described above, have exhibited symptoms of necrosis and lack of vigor that could be associated with herbicide injury. To address this concern expressed by stakeholder groups, there is a need to determine the effect of maintenance control herbicide applications on nontarget panicgrass. A series of mesocosm experiments and a complementing field trial were designed to specifically determine if operational herbicide prescriptions suppress panicgrass and if so, how long the effect is observed over time.

MATERIALS AND METHODS

Mesocosm trials

A total of four mesocosm trials were performed in 2016 and 2017 in outdoor facilities at the University of Florida Center of Aquatic and Invasive Plants in Gainesville, FL (GNV; 29°43′38.45″N, 82°25′2.05″W) and the Florida Fish and Wildlife Conservation Commission Facility in Tallahassee, FL (TLH; 30°28′28.01″N, 84°21′31.58″W). Gainesville and Tallahassee are in the 9a and 8b USDA Plant Hardiness Zones, respectively, with mean monthly high temperatures of 26.7 and 26.4 C.

Stolons from healthy panicgrass stock cultures were cut to 10-cm lengths and planted into 100-cm^3 square pots filled with washed builder's sand amended with a polymer-coated, slow-release fertilizer[1] (15–9–12) at 1.4 g kg^{-1}. Potted plants were maintained in saturated conditions in subirrigation trays under 60% shade, in ambient outdoor conditions. Four weeks later, actively growing plants were transferred into larger 3-L pots (17.1-cm diameter by 13.3 cm deep) filled with commercial potting soil mix[2] amended with the same fertilizer described above. All pots were then placed in 900-L concrete tanks (approximately 2.5 by 0.6 by 0.6 m) filled to a depth of 20 cm (i.e., 50% submerged) with water sourced from a well on site with pH ~ 7.5 to 8.0. Potted plants were acclimated for another 4 wk in the new conditions before herbicide treatments were administered.

Seven herbicide treatment combinations plus a nontreated control were used. The following herbicide active ingredients and combinations were applied as single or sequential applications: diquat[3] alone at 1,121 g ai ha^{-1} and in combination with 2,4-D[4] at 560 g ai ha^{-1}; 2,4-D at 2,130 g ai ha^{-1} in combinations with diquat and flumioxazin[5]; flumioxazin at 214 g ai ha^{-1} in combination with 2,4-D; and imazamox[6] at 280 g ai ha^{-1} in combination with carfentrazone[7] at 67 g ai ha^{-1}. All treatments included a nonionic surfactant[8] at 0.25% v/v. The rates listed above were for each single application. Diquat (DQ) treatments included single (×1), double (×2) or triple (×3) sequential applications, while the 2,4-D + flumioxazin (24DF) and imazamox + carfentrazone (IC) treatments were applied as single (×1) or double (×2) sequential applications. Sequential applications were administered approximately 3 wk apart. Foliar treatments were applied with a carbon dioxide–pressurized backpack sprayer[9] equipped with a single 8002 flat fan nozzle[10] calibrated to deliver a total volume of 935 L ha^{-1} with a single pass over the top.

The first series of mesocosm trials at GNV and TLH were treated on 14 September 2016 with second and third treatments administered 21 and 43 d after treatment (DAT; 5 October and 27 October 2016), respectively. Harvests occurred 3 (6 December 2016) and 8 (16 May 2016) mo after treatment (MAT) at both locations. The second series of mesocosm trials were treated on 27 September 2017 with second and third treatments administered on 21 and 42 DAT (18 October and 8 November 2017), respectively. Harvests occurred 3 (8 December 2017) and 6 MAT (4 April 2018). For all mesocosm trials, experimental plant units were separated into aboveground (shoot) and belowground (root) biomass and oven-dried to constant weights at 60 C before final measurement.

Field trial

A field trial was installed at Lake Pierce, FL (27°58′25.06″N, 81°31′16.48″W), located in the 9b USDA Plant Hardiness Zone with a mean monthly high temperature of 29.3 C. Treatment plots (~ 0.1 ha) were established in healthy, dominant panicgrass grass beds occupying the southern and eastern shorelines. There was a total of six herbicide treatments, including DQ, 24DF, and IC at the same concentrations described above, with each administered as single (×1) or sequential (×2) applications. Each herbicide treatment had a corresponding nontreated control plot as a reference. Single and sequential treatments were administered on 29 June 2017 and 3 August 2017 (35 DAT), respectively, by Polk County Invasive Plant Management staff. Treatments were delivered as foliar spray-to-wet, broadcast applications with a high-pressure, single adjustable orifice sprayer from an airboat traversing the plots to achieve uniform foliar coverage estimated at 935 L ha^{-1}.

Plot surveys were conducted before and after each application to assess plant health based on visual assessments of three randomly placed 1-m^2 quadrats within each plot. Twelve-megapixal images were recorded with a digital camera[13] over each quadrat, in the nadir position, ~ 1.5 m above the canopy. The RGB images were processed via application software[14] (Patrignani and Ochsner 2015) to estimate the fractional green canopy cover (FGCC), using the methods adopted by others (Goodwin et al. 2018, Shepherd et al. 2018). Surveys were conducted at –7, 7, 33, 42, 63, 89, and 118 DAT, ending 25 October 2017. Relative green cover difference (RGCD) was calculated as the proportion of the difference in FGCCs of the treatment to the nontreated control, relative to the control.

Statistics

The replicated mesocosm ($n = 5$) and field ($n = 3$) trials were completely randomized. There were no differences in effects observed between GNV and TLH mesocosm locations, but there were differences observed between years 2016 and 2017. Thus, data from GNV and TLH were pooled in the analyses for 2016 and 2017 separately. Each of the harvest and field observation dates of these respective trials were subjected to a one-way analysis of variance. Data transformations were performed as necessary to adjust for normality and homogeneity of variances. In the mesocosm trials, square root transformations were performed on 2016 shoot biomass and 2017 root biomass data. In the field trial, square root transformations were made to RGCDs recorded on 7 and 89 DAT and log transformations to RGCDs recorded on −7, 33, 42, 63, and 118 DAT. Means with 95% confidence intervals were back transformed in graphical presentations. A Dunnett's post hoc test was used to compare the multiple treatments with nontreated control references ($\alpha = 0.05$) for each date. The statistical software XLSTAT[14] was used for all analyses.

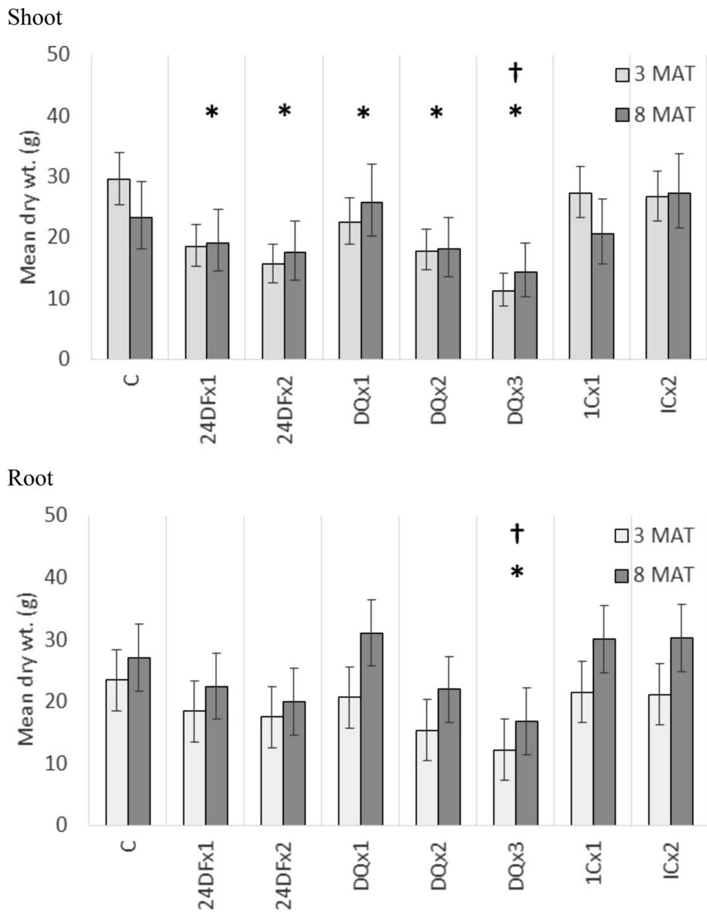

Figure 1. 2016 Mesocosm trials mean panicgrass shoot and root dry weights (± confidence intervals at 95%) at 3 and 8 mo after treatment (MAT). Foliar treatments included: 2,4-D + flumioxazin (24DF), diquat (DQ), and imazamox + carfentrazone (IC). All herbicides were applied as single (×1) or sequential (×2 or ×3) application treatments, applied with second and third treatments administered 21 and 43 d after the initial treatment, respectively. All foliar applications included a nonionic surfactant at 0.25% v/v. A Dunnett's post hoc test compared treatment means against the control ($\alpha = 0.05$). The asterisk over each treatment designates significant difference from the control at 3 MAT. The dagger over each treatment designates significant difference from the control at 8 MAT.

RESULTS AND DISCUSSION

Mesocosm trials

None of the herbicide treatments were lethal to panicgrass in any of the mesocosm trials. In 2016, the single and sequential application treatments of IC×1 and IC×2 did not suppress shoot growth at 3 or 8 MAT (Figure 1). However, single and sequential application treatments of 24DF×1 and 24DF×2 measured shoot suppression at 3 MAT, but not at 8 MAT. The diquat treatments resulted in shoot suppression with all three sequential applications (DQ×1, DQ×2, and DQ×3) at 3 MAT. Both 24DF and DQ treatments showed increased shoot growth suppression with sequential applications, but it was only the DQ×3 treatment that suppressed shoot growth out to 8 MAT. It was also the only treatment to result in root suppression at 3 and 8 MAT.

Consistent with 2016 trials, the IC×1 and IC×2 treatments again did not result in suppression on shoots nor roots in 2017 (Figure 2). However, unlike the 2016 trials, only DQ×2 resulted in shoot suppression at 3 MAT, while four treatments (i.e., 24DF×1, DQ×1, DQ×2, and DQ×3) resulted in shoot suppression at 6 MAT. Inexplicably, the concomitant increase in shoot suppression with sequential applications was not observed in 2017; for example, DQ×2 was suppressive at 3 MAT, while DQ×3 was not. This was also the case for 24DF×1 being suppressive at 6 MAT, while 24DF×2 was not. There were no measured root suppressions at 3 or 6 MAT.

Field trial

All single-application (×1) treatments and sequential application treatments 24DF×2 and DQ×2, reduced FGCC of panicgrass at 7 DAT (Figure 3). Only the sequential treatment IC×2 was not significantly suppressed at 7 DAT ($P = 0.107$). Each of the single-application treatments rapidly recovered with new foliar growth and with no measured reductions in FGCC for the remaining observations (i.e., 33 to 118 DAT). All plants treated with sequential applications recovered at 33 d after the initial treatment but showed reduced green cover at 7 d after the second treatment application (i.e., 42 d after the initial treatment; Figure 2). Again, there were no measured reductions in FGCC for the remaining observations (i.e., 63 to 118 DAT). Here, we observed a 51% mean reduction in FGCC among all treatments after the first application, while the mean reduction after two sequential applications was substantially greater at 79%, suggesting a compounding effect similar to what was observed in the 2016 mesocosm trial. Furthermore, IC×2 exhibited a mean reduction in FGCC (> 50%) 28 d after the sequential treatment (63 DAT); this reduction was not significant ($P = 0.098$) but suggestive of slower recovery. Interestingly, there were no significant differences between herbicide active ingredients, including the IC

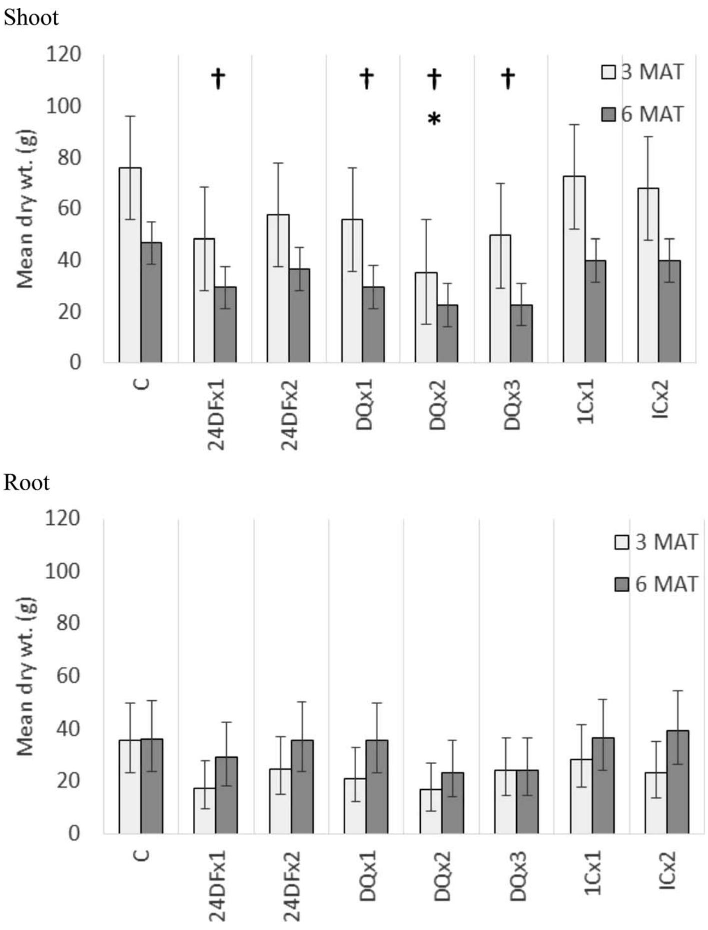

Figure 2. 2017 Mesocosm trials mean panicgrass shoot and root dry weights (± confidence intervals at 95%) at 3 and 6 mo after treatment (MAT). Foliar treatments included: 2,4-D + flumioxazin (24DF), diquat (DQ), and imazamox + carfentrazone (IC). All herbicides were applied as single (×1) or sequential (×2 or ×3) application treatments, applied with second and third treatments administered 21 and 43 d after the initial treatment, respectively. All foliar applications included a nonionic surfactant at 0.25% v/v. A Dunnett's post hoc test compared treatment means against the control ($\alpha = 0.05$). The asterisk over each treatment designates significant difference from the control at 3 MAT. The dagger over each treatment designates significant difference from the control at 6 MAT.

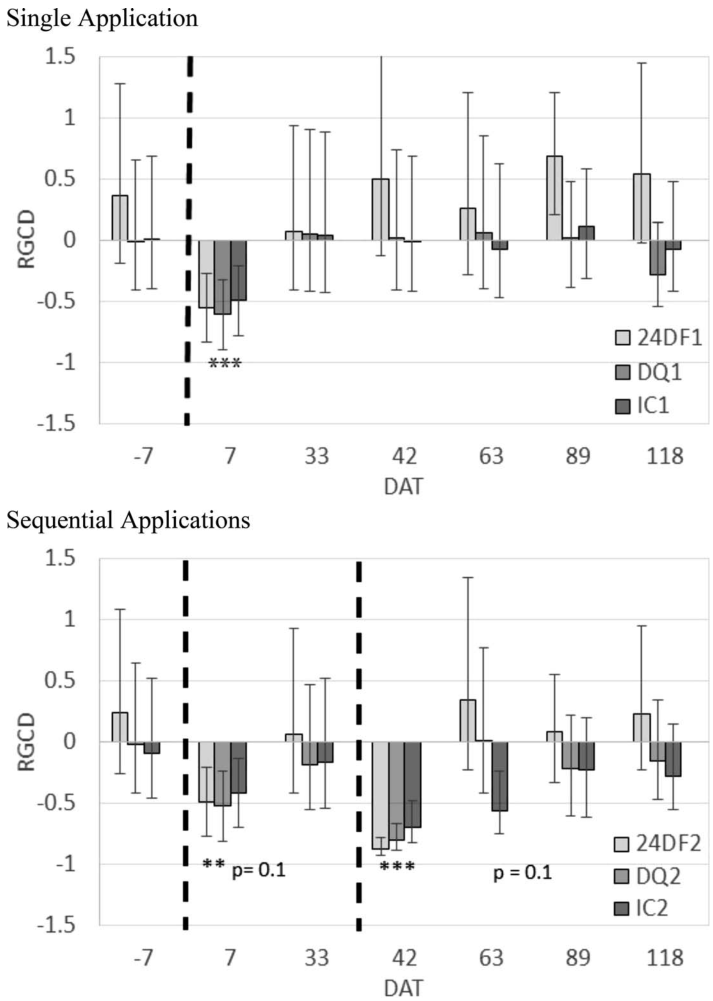

Figure 3. The relative green cover difference (RGCD) of naturalized panicgrass in the field affected by single (×1) or sequential (×2) application treatments during the time period −7 to 118 d after treatment (DAT). Foliar treatments included: 2,4-D + flumioxazin (24DF), diquat (DQ), and imazamox + carfentrazone (IC). Single and sequential applications are designated by vertical dash lines at 0 and 35 DAT, respectively. RGCD is the proportion of the difference between treatment and nontreated reference fractional green canopy cover over the reference. The asterisk over each treatment designates significant difference from the nontreated control (RGCD = 0) via Dunnett's post hoc test control ($\alpha = 0.05$).

combination, which showed suppressive effects in the field despite a lack of observed suppression in the mesocosm trials. In the field, all treatments were most suppressive after two applications, although panicgrass fully recovered within 40 d of the final application. This recovery again appears to be more accelerated than what was observed in the mesocosms.

Koschnick et al. (2007) measured no growth reductions to panicgrass in mesocosms treated with a submersed application of imazamox at 300 $\mu g\ L^{-1}$. However, imazamox is noted to be an effective option in controlling another monocot, southern cattail (*Typha domingensis* Pers.) (Rodgers and Black 2012). Carfentrazone is a contact broadleaf herbicide that is effective on water lettuce and water hyacinth but is unlikely to suppress grasses alone (Dayan et al. 1997, Durgan et al. 1997, Koschnick et al. 2004). Here, the IC combination was benign in the mesocosm trials but performed equally to the other treatments suppressing panicgrass in the field.

Flumioxazin alone does not control grasses or sedges and has specifically been shown to be ineffective on the native monocots maidencane (*Panicum hemitomon* Schult.) and bulrush (*Schoenoplectus* spp.) (Grichar and Colburn 1996, Askew et al. 1999, Koschnick et al. 2007, Mudge and Netherland 2014b). However, the combination of 2,4-D and flumioxazin has previously been shown to be effective on *Schoenoplectus* spp. (Glomski et al. 2009). In this research, the 24DF treatments consistently suppressed panicgrass in the mesocosm and field trials. There were no other reports found on 2,4-D injury to other *Paspalidium* spp.

Diquat and 2,4-D are the most widely used herbicides for water lettuce and water hyacinth control in Florida lakes and have been the operational standards in maintenance control for decades. Over 117,000 ha have been treated with these two herbicides in the last decade, accounting for 92% of applications for floating plant control (Florida Fish and Wildlife Conservation Commission 2018). This combination continues to be highly effective, but nontarget injury is common when applied in mixed emergent plant communities (Mudge and Netherland 2014b). This is further supported by the research here, which found that panicgrass exhibited growth suppression and foliar reduction after treatments with either diquat or 2,4-D. However, this research further showed these symptoms to be transitory and proceeded by full recovery. Mudge and Netherland (2015) also observed similar transitory, posttreatment effects on maidencane. However, here, there is also some evidence of increased suppression with multiple applications administered within a short period of time (e.g., < 50 d). Frequent interventions may be necessary for suppressing recruitment and ingress of new target plants, which could lead to multiple treatment applications in a designated management area (University of Florida 2018). Further research is needed to better understand the effects of application frequency on the severity of nontarget injury.

These mesocosm and field trials were all initiated in late summer and early fall, with monitoring through the winter and spring. As a result, shoot biomass reductions were observed for all treatments including the nontreated control. For example, in the mesocosm trials, shoot biomass for the controls were reduced approximately 20 to 40% over the terms of the experiments. Conversely, root biomass increased during these periods for all treatments. All nontreated reference plots in the field trial showed reductions in FGCC up to the final rating at 118 DAT (i.e., October 2017). This phenological change expressed in these trials were likely determined by seasonal reductions in temperature (Wardlaw 1990), possibly causing plants to become less susceptible to herbicide treatments. Further research on application timing is needed to confirm this phenomenon, which could identify seasonal periods where better selectivity of maintenance control activities can be achieved.

As demonstrated in this research, the herbicides prescribed for controlling floating plants can suppress nontarget panicgrass, but does not implicate this as the sole cause of decline. It is possible for sublethal herbicide treatments to induce stresses that can exacerbate vulnerability to other harmful factors, such as pathogenesis (Canaday et al. 1986, Altman and Rovira 1989, Sanogo et al. 2000). Further research would be needed to evaluate the interaction of chronic, sublethal herbicide effects with other potential stressors on panicgrass.

This research determined that operational herbicide prescriptions were sublethal on panicgrass. However, extra precautions would be warranted if conducting maintenance control activities in panicgrass communities. Considerations for the time of year may limit treatment susceptibility of panicgrass, while extending the time interval between interventions can allow for adequate growth recovery.

SOURCES OF MATERIALS

[1]Osmocote® Plus Smart-release® Plant Food, 15–9–12, The Scotts Company, 14111 Scottslawn Road, Marysville, OH 43041.

[2]Professional Topsoil, Margo™ Garden Products, 50 N. Laura St., Suite 2550, Jacksonville, FL 32202.

[3]Reward®, Syngenta Crop Protection LLC, P.O. Box 18300, Greensboro, NC 27419-8300.

[4]2,4-D amine, Alligare, LLC, 13 N. 8th St., Opelika, AL 36801.

[5]Clipper™, Valent U.S.A. Corporation, P.O. Box 8025, Walnut Creek, CA 94596-8025.

[6]Clearcast®, SePRO Corporation, 11550 N. Meridian St., Suite 600, Carmel, IN 46032.

[7]Stingray®, SePRO Corporation, 11550 N. Meridian St., Suite 600, Carmel, IN 46032.

[8]Induce, Helena Chemical Company, 225 Schilling Blvd., Suite 300, Collierville, TN 38017.

[9]315 Heavy Duty Backpack Frame, Bellspray INC R&D Sprayers, 419 Hwy. 104, Opelousas, LA 70570.

[10]TeeJet, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60187.

[11]iPad Pro (1st generation), Apple Inc., Cupertino, CA 95014.

[12]Canopeo, http://canopeoapp.com, Oklahoma State Univ., 371 Agricultural Hall, Stillwater, OK 74078.

[13]XLSTAT, version 2019.1.2, Addinsoft Inc., 10-34 44th Dr., 2nd Floor, Long Island City, NY 11101.

ACKNOWLEDGEMENTS

We thank Carl Della Torre, Ryan Moore, and Polk County Invasive Plant Management Section for technical support and the Florida Fish and Wildlife Conservation Commission Invasive Plant Management Section for funding and access to the Tallahassee mesocosm facility. We further acknowledge the comments and edits offered by the associate editor and anonymous reviewers to strengthen this manuscript.

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