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Mosquito fern (*Azolla caroliniana*) response to submersed and foliar contact herbicide applications

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ABSTRACT

Mosquito fern (*Azolla caroliniana*) is a free-floating aquatic fern capable of covering water bodies and outcompeting submersed macrophytes, potentially leading to a loss of biodiversity. Limited evidence suggests that mosquito fern can be controlled with the contact herbicide diquat; however, peer-reviewed literature regarding effects of other contact herbicides labeled for use in aquatic environments on mosquito fern is lacking. The purpose of this work was to conduct two trials to determine the effects of foliar (trial 1) and submersed (trial 2) applications of contact herbicides on mosquito fern. In trial 1, foliar applications of the contact herbicides flumioxazin (0.42 and 0.21 kg ai ha^{-1}), carfentrazone-ethyl (0.21 and 0.11 kg ai ha^{-1}), endothall (2.39 and 1.20 kg ai ha^{-1}), diquat (4.52 and 2.26 kg ai ha^{-1}), and copper (1.47 and 0.74 kg ai ha^{-1}) were administered and biomass assessed 8 wk after treatment (WAT). In trial 2, submersed applications of flumioxazin (0.4 and 0.2 mg ai L^{-1}), carfentrazone-ethyl (0.2 and 0.1 mg ai L^{-1}), endothall (5.0 and 2.5 mg ai L^{-1}), diquat (0.37 and 0.19 mg ai L^{-1}), and copper (1.0 and 0.5 mg ai L^{-1}) were administered and assessed 8 WAT. Foliar treatments were applied at a target diluent rate of 935.4 L ha^{-1}; all foliar herbicide treatments included a 1% v:v nonionic surfactant. At 8 WAT, all foliar treatments reduced mosquito-fern biomass compared with nontreated plants, but only high rates of flumioxazin, carfentrazone-ethyl, and both diquat rates reduced biomass 100%. All submersed herbicide treatments except copper reduced mosquito-fern biomass by 8 WAT, but diquat was the only treatment to provide 100% biomass reduction. To our knowledge, this is the only work to document mosquito fern biomass reduction by the herbicides flumioxazin, copper, carfentrazone-ethyl, or endothall. This work should be validated on field populations of mosquito fern before recommendation for operational use.

Key words: aquatic nuisance species, *Azolla* spp., chemical control, contact herbicide.

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INTRODUCTION

Free floating aquatic plants are becoming more problematic in many shallow nutrient-rich water bodies of the southern United States (Barret 1989). However, some species, like mosquito fern (*Azolla* spp.), are capable of surviving in less productive water bodies (Meeks 2009). *Azolla* species host nitrogen-fixing cyanobacteria (*Nostoc* or *Anabaena* species, potentially both simultaneously), which have been shown to provide as much as 40% of their captured nitrogen to the plant host (Meeks 2009). This ability to capitalize on an internal nitrogen source has made *Azolla* species attractive as an organic fertilizer source in India and parts of Asia (Wagner 1997), but it also can make nuisance populations of these species difficult to manage.

Although mosquito fern (*Azolla caroliniana* Willd.) is considered native in the southeastern United States, this species can grow to nuisance levels in many water bodies, in part due to its internal nitrogen-fixing symbionts (Meeks 2009). *Azolla caroliniana* has a rapid growth rate (doubling time of 6.1 d at 30 C), and infested water bodies often become completely covered by this plant, sometimes to the extent that individual plants overlap one another owing to the physical morphology of the plants and the ability of mosquito fern populations to rapidly expand through vegetative means (Arora and Singh 2003). Nuisance populations of mosquito fern can cover water bodies and block penetration of light into the water column, killing aquatic macrophytes and reducing biodiversity (Satapathy and Singh 1985). Dense mosquito-fern mats also reduce gas exchange between water and atmosphere and, when coupled with depleted dissolved oxygen from decomposing plants, can potentially lead to hypoxia and fish kills that further reduce aquatic biodiversity (Arora and Singh 2003, Gettys et al. 2021).

Some evidence suggests that mosquito fern can be controlled with the contact herbicide diquat (Blackburn and Weldon 1965, Westerdahl and Getsinger 1988, Wersal and Turnage 2021). However, other contact herbicides registered for aquatic use in United States (Gettys et al. 2021) have not been evaluated for control of mosquito fern. Other *Azolla* species have been reduced by U.S. registered and non-U.S.-registered contact herbicides in the protoporphyrinogen oxidase (PPO)- and photosynthesis-inhibiting classes of herbicides (Hill and Cilliers 1999, Singh et al. 2009, Silva et al. 2012). There are currently five herbicides registered for general aquatic use in the United States that are classified as contact herbicides: diquat, carfentrazone-ethyl, copper, endothall, and flumioxazin (Gettys et al. 2021).

Table 1. Contact herbicides, rates used, and modes of action; all treatments included a 1% v:v nonionic surfactant; PPO is protoporphyrinogen oxidase, STP is serine–threonine phosphatase, and PS is photosystem; the mode of action for copper is suspected to be PS 2 electron diversion but this has not been definitively confirmed.

Treatment	Foliar Rate (kg ai ha^{-1})	Submersed Rate (mg ai L^{-1})	Mode of Action
Reference	—	—	—
Flumioxazin[3]	0.42	0.4	PPO inhibitor
	0.21	0.2	
Carfentrazone-ethyl[4]	0.21	0.2	PPO inhibitor
	0.11	0.1	
Endothall[5]	2.39	5.0	STP inhibitor
	1.20	2.5	
Diquat[6]	4.52	0.37	PS 1 electron diversion
	2.26	0.19	
Copper[7]	1.47	1.0	PS 2 electron transport (?)
	0.74	0.5	

Reduction of other aquatic ferns (*Salvinia* spp.) by contact herbicides labeled for aquatic use in the United States (Nelson et al. 2001, Glomski et al. 2003, Glomski and Getsinger 2006, Mudge et al. 2014, Mudge and Sartain 2018, Sartain and Mudge 2019) suggests that a comprehensive screening of these chemicals for mosquito-fern control would benefit stakeholders. However, evidence exists that endothall may have systemic activity on aquatic plants (Ortiz et al. 2019). These five herbicides represent multiple modes of action (Shaner 2014) and likely have differing levels of efficacy on target plants. Diquat's mode of action is photosystem (PS) 1 electron diversion, carfentrazone-ethyl and flumioxazin are PPO inhibitors, and endothall is a serine–threonine phosphatase inhibitor (Shaner 2014, Tresch et al. 2021). Copper may inhibit PS 2 electron transport, but little is known about its mode of action in plants (Shaner 2014). The purpose of the present work thus was to identify contact herbicide active ingredients and rates that show potential for mosquito fern control as foliar or submersed injection treatments.

MATERIALS AND METHODS

Foliar herbicide applications

Mosquito fern was established in 18.9-L buckets in a greenhouse at the Mississippi State University (MSU) R. R. Foil Plant Research Center (33°28′9.696″N; 88°47′4.1994″W). This study was initiated in March 2019, with two full experimental trials performed 2 wk apart for temporal replication (second trial initiated in early April). Buckets were filled with well water and amended with a slow-release fertilizer[1] to stimulate plant growth. Plants were given 2 wk to establish before applying herbicides and plants had covered 100% of the water surface in buckets. Before herbicide application, pretreatment specimens were harvested from each bucket using a 3.2-cm polyvinyl chloride sampling device (Wersal and Madsen 2009, Wersal and Turnage 2021). Pretreatment samples were placed in labeled paper bags, dried in a forced-air oven at 70 C until water content was removed, and then weighed; biomass weights were measured and recorded.

There was a nontreated reference and 10 herbicide treatments (Table 1). Each treatment was replicated three times for a total of 33 buckets (per trial run). Each herbicide treatment contained a 1% v:v nonionic surfactant[2]. After pretreatment harvest, herbicide treatments were administered as foliar applications using a pressurized CO_2 backpack sprayer at a pressure of 276 kPa (40 psi) and a TeeJet 8002 nozzle. Spray solution was applied at a volume of 935.4 L ha^{-1}. Before mixing herbicide solutions, water was amended with muriatic acid to lower the pH to a range of 6.5 to 7.0 to prevent breakdown of PPO-inhibiting herbicides (flumioxazin and carfentrazone-ethyl). Eight weeks after treatment (WAT), biomass specimens were harvested and processed from each bucket in the same manner as pretreatment specimens.

Data were analyzed using a mixed-model ANOVA, with herbicide treatment as a fixed effect and trial run as a random effect. Differences in means detected by ANOVA were further separated using a Fisher's LSD test. All statistical tests were conducted at the alpha = 0.05 significance level in the R statistical software package (R Core Team 2022).

Submersed herbicide applications

Mosquito fern plants were established in the same manner as those used for foliar applications. Well water used to fill buckets had a pH range of 7.5 to 7.8; water was not buffered in buckets to lower the pH. The first run of this trial was conducted in 2019 and then repeated in 2023. Pretreatment specimens were harvested in the same manner as plants receiving foliar herbicide applications. After pretreatment harvest, herbicides were administered as high- and low-dose submersed injections using a pipettor (Table 1); each treatment was replicated four times per trial. Eight WAT, plants were harvested and processed in the same manner as pretreatment specimens.

Data were analyzed using a mixed-model ANOVA with herbicide treatment as a fixed effect and trial run as a random effect. Differences in means detected by ANOVA were further separated using a Fisher's LSD test. All statistical tests were conducted at the alpha = 0.05 significance level in the R statistical software package (R Core Team 2022).

RESULTS AND DISCUSSION

Foliar herbicide applications

All herbicide treatments reduced mosquito-fern biomass 8 WAT compared with nontreated reference plants ($P < 0.0001$; Figure 1). There was no difference in mosquito-fern biomass

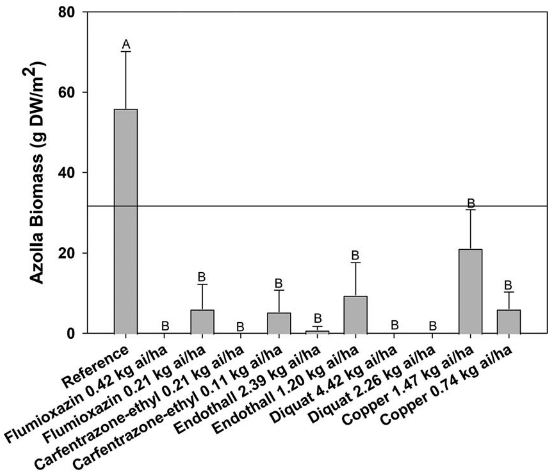

Figure 1. Response of *Azolla caroliniana* biomass 8 wk after treatment with foliar contact herbicide applications; solid line is pretreatment biomass; error bars are 1 standard error of the mean; bars sharing the same letter are not different from one another at the $\alpha = 0.05$ significance level ($n = 6$).

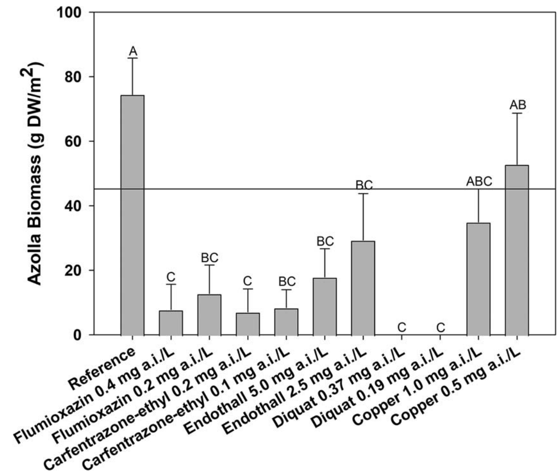

Figure 2. Response of *Azolla caroliniana* biomass 8 wk after treatment with submersed contact herbicide applications; solid line is pretreatment biomass; error bars are 1 standard error of the mean; bars sharing the same letter are not different from one another at the $\alpha = 0.05$ significance level ($n = 8$).

among the herbicide-only treatments ($P < 0.0001$; Figure 1). Flumioxazin (0.42 kg ai ha^{-1}), carfentrazone-ethyl (0.21 kg ai ha^{-1}), and both rates of diquat (4.52 and 2.26 kg ai ha^{-1}) controlled mosquito fern 100% in experimental units 8 WAT (Figure 1). Low rates of flumioxazin (0.21 kg ai ha-1) and carfentrazone-ethyl (0.11 kg ai ha^{-1}) provided 89 and 90% (respectively) mosquito-fern biomass reduction 8 WAT compared with references (Figure 1). Endothall (2.39 and 1.20 kg ai ha^{-1}) reduced biomass 83 to 99%, whereas copper (1.47 and 0.74 kg ai ha^{-1}) reduced mosquito fern 62 to 89% compared with reference plants 8 WAT (Figure 1).

Submersed herbicide applications

Diquat (0.37 and 0.19 mg ai L^{-1}) applications were the only treatments to reduce azolla biomass 100% compared with reference plants 8 WAT ($P < 0.0001$; Figure 2). Flumioxazin (0.4 and 0.2 mg ai L^{-1}) reduced mosquito fern 83 to 90%, whereas carfentrazone-ethyl (0.2 and 0.1 mg ai L^{-1}) reduced plants 89 to 91% compared with reference plants (Figure 2). Endothall (5.0 and 2.5 mg ai L^{-1}) reduced mosquito fern 61 to 80% compared with references 8 WAT (Figure 2). Copper treatments (1.0 and 0.5 mg ai L^{-1}) did not reduce mosquito-fern biomass compared with reference plants 8 WAT (Figure 2). However, mosquito-fern biomass treated with high rates of copper (1.0 mg a.i. L^{-1}) was not different from any noncopper herbicide treatment (Figure 2). Biomass in mesocosms treated with low copper rates (0.5 mg ai L^{-1}) were similar to biomass of plants treated with either endothall (5.0 and 2.5 mg ai L^{-1}), low rates of flumioxazin (0.2 mg ai L^{-1}), and low rates of carfentrazone-ethyl (0.1 mg ai L^{-1}).

Blackburn and Weldon (1965) reported approximately 70% mosquito-fern biomass reduction by diquat concentrations > 0.19 mg ai L^{-1} at 7 d after treatment (DAT) in a laboratory trial, which is similar to our findings (Figures 1 and 2). Westerdahl and Getsinger (1988) reported good control of mosquito fern by diquat and diquat + copper mixes but did not test copper alone or endothall alone. Wersal and Turnage (2021) reported a 93% total biomass reduction 4 WAT in a mixed-species assemblage of mosquito fern, watermeal (*Wolffia columbiana* Karst), and duckweed (*Lemna minor* L.) in a Mississippi pond treated with diquat (2.26 kg ai ha^{-1}). Wersal and Turnage (2021) also reported that watermeal was the only plant recorded after the pond diquat treatment, which corroborates our findings of 100% mosquito-fern biomass reduction after diquat treatments (Figures 1 and 2).

Other researchers have reported reduction of *A. caroliniana* and other *Azolla* species by the contact herbicides oxadiazon (PPO inhibitor), oxyfluorofen (PPO inhibitor), and paraquat (photosynthesis disruptor), which are not labeled for aquatic use in the United States (Hill and Cilliers 1999, Singh et al. 2009, Silva et al. 2012). Singh et al. (2009) reported that the PPO-inhibiting herbicide oxadiazon inhibited *Azolla pinnata* R. Br. (feathered mosquito fern) growth up to 15 DAT. Silva et al. (2012) reported a 50% lethal concentration 7 DAT of 80.5 mg ai L^{-1} for *A. caroliniana* exposed to oxyfluorofen. Hill and Cilliers (1999) reported *Azolla filiculoides* Lam. (Pacific mosquito fern) sensitivity to paraquat, a contact herbicide that disrupts photosynthesis.

These results indicate that mosquito fern is sensitive to foliar applications of all five contact herbicides tested and submersed applications of all herbicides except copper, suggesting that aquatic resource managers may have multiple chemical control options to choose from when managing mosquito fern-infested water bodies. The PPO-inhibiting herbicides carfentrazone-ethyl and flumioxazin were not registered for aquatic use until 2004 and 2011, respectively (Gettys et al. 2021) and thus have not been included in herbicide screenings against mosquito fern until after those dates. To our knowledge, this is the first work to document reduction of mosquito-fern biomass by flumioxazin, carfentrazone-ethyl, copper, or endothall (Figures 1 and 2). Because water-body use can vary (e.g., livestock watering vs. irrigation), restrictions surrounding herbicide use also vary; therefore, a plurality of chemical control options ensures that stakeholders and resource managers can control mosquito fern in multiple water-use scenarios. The sensitivity of *Azolla* species to contact herbicides from multiple herbicide families (Figures 1 and 2; Blackburn and Weldon 1965, Westerdahl and Getsinger 1988, Hill and Cilliers 1999, Singh et al. 2009, Silva et al. 2012, Wersal and Turnage 2021) is beneficial for resource managers who want to rotate herbicides annually or seasonally for herbicide stewardship purposes, prevention of herbicide resistance in nuisance weed populations, and also because herbicide costs can change over time. Future work should assess tank mixes of herbicides for control of mosquito fern and assess the rates tested here on field populations before use as an operational control strategy. Future work should also assess the effects of these herbicides on other *Azolla* and floating nuisance plant species.

SOURCES OF MATERIALS

[1]Osmocote® Plus, ICL Fertilizers, 4950 Blazer Memorial Parkway, Dublin OH 43017.

[2]Top Surf®, Winfield Solutions, LLC, P.O. Box 64589 St. Paul, MN 55164.

[3]Clipper® SC Aquatic Herbicide, Nufarm Inc., 11901 S. Austin Ave., Alsip, IL 60803.

[4]Stingray® Aquatic Herbicide, FMC Corporation, 2929 Walnut St., Philadelphia, PA 19104.

[5]Aquathol® K Aquatic Herbicide, UPL, 630 Freedom Business Center, Suite 402, King of Prussia, PA 19406.

[6]Reward® Landscape and Aquatic Herbicide, Syngenta Crop Protection, LLC, P.O. Box 18300, Greensboro, NC 27419.

[7]Harpoon® Aquatic Herbicide, Applied Biochemists, 11550 N. Meridian St., Suite 600, Carmel, IN 46032.

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