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# Note

# Potential of *Myrothecium* species as bioherbicides for giant salvinia (*Salvinia molesta*)

MARK A. WEAVER, JUDY F. SHEARER, MICHAEL J. GRODOWITZ, AND C. DOUGLAS BOYETTE*

## INTRODUCTION

Giant salvinia, *Salvinia molesta* Mitchell, is a floating macrophyte native to South America, but since the 1930s it has been spread to areas in Africa, Asia, Australia, and the warmer temperate and subtropical areas of the United States (Jacono and Pitman 2001). This fern has a tremendous growth rate in eutrophic water, forming dense floating mats with biomass production of up to 100 tons $ha^{-1}$ $yr^{-1}$ (Mitchell and Tur 1975, McFarland et al. 2004). Water resources can be degraded by giant salvinia through the competitive exclusion of native vegetation, depletion of dissolved oxygen, and the impeding of navigable waterways (McFarland et al. 2004, Knutson and Nachtrieb 2012). Control measures with limited efficacy include drawdown of the infested waterbody and mechanical control. In-water or foliar application of registered aquatic herbicides can provide effective control (Mudge and Haller 2012, Mudge et al. 2016), but can be difficult to implement effectively in complex infested systems, and selectivity of control and herbicide resistance management must be considered as part of long-term integrated strategies. The release of the weevil, *Cyrtobagous salviniae* Calder & Sands (Coleoptera: Curculionidae) has demonstrated large reductions in giant salvinia biomass in at least 12 countries, including Australia, Fiji, India, Kenya, Namibia, South Africa, Sri Lanka, Zambia, and Zimbabwe (Joy et al. 1986), and more recently in the United States, including the states of Texas and Louisiana (Tipping et al. 2008). However, weevil efficacy is limited in more temperate infested sites (Sullivan et al. 2011).

A recent survey for fungi with potential utility as biocontrol agents discovered three isolates of *Myrothecium roridum* as pathogens on Eurasian milfoil (*Myriophyllum spicatum* L.) in Vermont and dioecious hydrilla [*Hydrilla verticillata* (L.f.) Royle] in Florida and Texas. Several isolates of *M. roridum* and *Myrothecium verrucaria* have been evaluated as bioherbicides against a range of invasive weed species (Hoagland et al. 2007, Lee et al. 2008, Okunowo et al. 2010, Weaver et al. 2016), including ferns (Clarke et al. 2007). We describe here a series of mesocosm experiments to evaluate the virulence of these *Myrothecium* isolates against salvinia.

## MATERIALS AND METHODS

Three isolates of *M. roridum* (WES28809, WES15910, and WES5810) and one isolate of *M. verrucaria* (IMI 361390) were grown at 28 C in the dark on potato dextrose agar (PDA) or a modified Vogels–glucose medium (VMG) known to inhibit production of trichothecene mycotoxins in *M. verrucaria* (Weaver et al. 2009b). After incubation for 10 d, condia were collected in a 0.1% triton solution. Conidia were counted with a hemacytometer and adjusted to $1 \times 10^8$ condia $ml^{-1}$ before application. Condia from the Vogels–dextrose medium were washed by centrifuging and resuspending in sterile water, a process previously shown to remove trichothecene mycotoxins (Weaver et al. 2009b). Giant salvinia was produced outdoors with ambient temperature and light in fiberglass cylindrical tanks (ca. 120 cm tall, 70 cm diameter) with deionized, reverse-osmosis, carbon-filtered water supplemented with 16 g Everris 15–5–15 Cal Mag fertilizer and 4 g iron chelate. For biocontrol assays, 200 g of secondary- and tertiary-growth–stage giant salvinia was transferred to a fresh tank of water with fertilizer and allowed to acclimate for 1 d before bioherbicide application. Conidial suspensions were applied by spraying 15 ml of a $1 \times 10^8$ ($ml^{-1}$) conidia in 0.25% nonionic surfactant (Induce, proprietary blend of alkyl aryl polyoxylkane ethers, free fatty acids, and dimothyl polysiloxane), except where noted otherwise. The tanks of salvinia were visually rated on a 10-point scale after 4 d and compared to an Induce-only control. To measure the efficacy of each bioherbicide in the reduction in green biomass the green biomass was manually separated from the necrotic tissue 6 d after bioherbicide application. The green biomass was oven dried to determine dry weight. Each isolate, produced on each medium, was applied to two tanks, with 200 g of fresh giant salvinia, and the experiment was repeated in July 2016 and May 2017. In another experiment the surfactant Induce was compared with Silwet L-77 (polyalkyleneoxide modified heptamethyltrisiloxane), methylated seed oil, and a no-surfactant

*First, third, and fourth authors: U.S. Department of Agriculture, Agricultural Research Service, Biological Control of Pests Research Unit, Stoneville, MS 38776. Second author: U.S. Army Engineer Research and Development Center, Vicksburg, MS 39180. Corresponding author's E-mail: Mark.Weaver@ars.usda.gov. Received for publication September 1, 2017 and in revised form May 16, 2018.

Table 1. Bioherbicidal activity of *Myrothecium* isolates on giant salvinia.

| *Myrothecium* Strain[1] | Grown on Potato Dextrose Agar | | Grown on Vogels–Glucose Medium | |
|---|---|---|---|---|
| | % Reduction in Salvinia Dry Wt.[2] | Median Visual Rating[3] | % Reduction in Salvinia Dry Wt.[2] | Median Visual Rating[3] |
| Control—surfactant only | 0(22) b | 10 | | |
| WES 5810 | 56(9) a | 4 | 26(34) a | 7.5 |
| WES 15910 | 61(10) a | 3.5 | 35(30) a | 7.5 |
| WES 28809 | 67(3) a | 4.5 | 46(6) a | 4 |
| IMI 361390 | 73(8) a | 2.5 | 52(33) a | 4 |
| WES 15910 + no surfactant | 41(1) a | 4.5 | | |
| WES 15910 + Silwet L-77 | 54(18) a | 3.2 | | |
| WES 28809 + Methlyated seed oil | 46(1) a | 3.5 | | |

[1]Bioherbicide applications include 0.25% Induce surfactant unless otherwise noted.
[2]Standard deviation in parentheses. Values with the same letter are not significantly different at the 0.05 level by Tukey's range test.
[3]Visual rating taken 4 d after bioherbicide application. 0 = complete necrosis, no green tissue visible. 10 = no discernible symptoms.

control. Treatment effects were assessed by analysis of variance and Tukey's range test.

## RESULTS AND DISCUSSION

All four of the tested isolates of *Myrothecium* reduced the green biomass of salvinia after 6 d (Table 1). Some treatments exceeded 70% biomass reduction, but there was no significant difference between the four isolates (prob $> F = 0.55$). Visual ratings generally corresponded to the percent biomass reduction ($r^2 = 0.91$). All *Myrothecium* isolates were more effective as a bioherbicides when grown on PDA than on VMG (prob $> F = 0.018$). Previous work with *M. verrucaria* IMI 361390 demonstrated that condia produced on VMG lacked detectable trichothecene mycotoxins, but were still pathogenic on sicklepod (*Cassia obtusifolia* L.). Commercial standards for the trichothecenes are no longer available, so we were unable to determine if the new *M. roridum* isolates are toxigenic. In other plant–pathogen systems, macrocyclic trichothecenes are known to enhance virulence (Kuti et al. 1989). In our study, condia produced in conditions known to support trichothecene production were more effective in reducing salvinia biomass. This may be a result of trichothecenes or other, incidental, virulence factors.

Surfactants and other spray adjuvants can be essential to bioherbicidal efficacy and we have observed improved activity from *M. verrucaria* by adding of Induce surfactant (Weaver et al. 2009a). Here, we tested *M. verrucaria* and the three *M. roridum* isolates with 0.25% nonionic surfactants, Induce, and Silwet L-77 and with 1% methylated seed oil. All of the adjuvants improved bioherbicidal activity compared to the no-adjuvant controls, but there were no significant differences among the adjuvants. Additional experiments indicated that none of these surfactants at the tested concentrations had measurable phytotoxicity. It is possible that the abundant leaf moisture diluted the adjuvants and that a much higher surfactant concentration may be needed. The abundant "eggbeater" trichomes and densely spaced leaves may limit sufficient contact between the conidia and the plant.

The activity described here is limited and we did not include long-term or landscape-scale observations, but there are several opportunities for improvements. Subsequent development of these pathogens for giant salvinia should first formally verify the trichothecene production characteristics and the virulence of trichothecene-deficient formulations. Given the unique morphology of this fern, a higher application volume and/or different surfactants might result in improved efficacy. Glyphosate, even at rates below phytotoxic thresholds, inhibits plant disease responses, and is synergistic with *Myrothecium* in other systems (Boyette et al. 2008). *Myrothecium* may also find a niche with integrated biocontrol. For example, the salvinia weevil does not overwinter in some portions of salvinia's range, cannot be introduced until founder colonies are established in late spring, and requires mature giant salvinia. The results presented here indicate that *Myrothecium* formulations have the potential of being effective bioherbicides of giant salvinia, as they have been other weed systems.

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