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Compatibility of an insect, a fungus, and a herbicide for integrated pest management of dioecious hydrilla

JAMES P. CUDA, JUDY F. SHEARER, EMMA N. I. WEEKS, EUTYCHUS KARIUKI, JULIE BANISZEWSKI, AND MIHAI GIURCANU*

ABSTRACT

During the past 15 yr dioecious hydrilla (*Hydrilla verticillata*) in Florida developed resistance to fluridone and endothall, two registered herbicides approved for aquatic use. An integrated pest management approach could mitigate the effects of herbicide resistance and improve the sustainability of dioecious hydrilla management in Florida. In this study, we tested a reduced-risk method for dioecious hydrilla control by integrating selective insect herbivory with a disease organism or low concentrations of a new herbicide recently registered for aquatic use. Two rates of the fungal pathogen *Mycoleptodiscus terrestris* (Mt) and the acetolactate synthase-inhibiting herbicide imazamox, and two densities of the hydrilla tip-mining midge *Cricotopus lebetis* alone and in combination were randomly applied to aquaria containing established hydrilla plants and replicated three times. Hydrilla shoots in each tank were harvested ~30 d after the treatments were applied. Hydrilla biomass produced in each treatment was compared. Results showed that combining the hydrilla tip-mining midge *C. lebetis* with either the Mt fungus or herbicide imazamox significantly reduced hydrilla growth and the effects in some treatments were synergistic. Furthermore, *C. lebetis* was compatible with the herbicide imazamox; adult emergence of *C. lebetis* was similar in aquaria treated with imazamox compared with untreated controls. Incorporating biological control agents like Mt and the tip-mining midge *C. lebetis* into an integrated weed-management strategy could reduce overreliance on herbicides and provide a more sustainable solution to Florida's dioecious hydrilla problem.

Key words: *Cricotopus lebetis*, herbicide-resistance management, *Hydrilla verticillata*, imazamox, integrated weed management, *Mycoleptodiscus terrestris*.

*First, third, fourth, and fifth authors: Professor, Assistant Research Scientist, Graduate Student, and Undergraduate Student, Entomology & Nematology Department, University of Florida, Gainesville, FL 32611-0620. Second author: Research Plant Pathologist, Environmental Laboratory, U.S. Army Engineer Research & Development Center, Waterways Experiment Station, Vicksburg, MS 39180-6199. Sixth author: Coordinator, IFAS Statistics, University of Florida, Gainesville, FL 32611-0339. Current address of fifth author: Plant and Soil Sciences Department, University of Kentucky, Lexington, KY 40546-0312. Corresponding author's E-mail: jcuda@ufl.edu. Received for publication October 22, 2014 and in revised form October 2, 2015.

INTRODUCTION

Hydrilla verticillata (L.f.) Royle, Hydrocharitaceae (hereafter hydrilla) is a federally listed noxious weed and one of the worst invasive aquatic plants in the United States, with millions of dollars spent annually to control large infestations in all types of water bodies. A dioecious female strain of hydrilla was introduced into Florida from Sri Lanka in the early 1950s through the aquarium trade (Schmitz et al. 1991, Langeland 1996). This aggressive submersed plant was spread intentionally by growers of aquarium plants and unintentionally by boaters from one watershed to another (Balciunas et al. 2002). Florida currently spends approximately $15 million annually controlling dioecious hydrilla in its public waters (Haller 2014). Nationally, established populations of monoecious or dioecious hydrilla biotypes occur in 28 states as far north as Maine on the Atlantic coast, and Washington on the Pacific coast (Lietze and Weeks 2014). Balciunas and Chen (1993) predicted that hydrilla could colonize any water body in North America. Their prediction was validated when hydrilla infestations were recently discovered in the Midwest, as far north as Wisconsin (EDDMaps 2014).

A major factor contributing to the negative impacts of dioecious hydrilla is its pattern of growth. This submersed weed grows as a sparsely branched erect rooted plant until it reaches the water surface, where it forms numerous side branches. Dense surface mats that are produced comprise up to 20% of the plant's biomass (Haller and Sutton 1975). These mats not only displace native vegetation, which affects native fish and zooplankton communities and alters water temperature and chemistry, but also interfere with navigation and flood control (Haller and Sutton 1975, Colle and Shireman 1980, Canfield et al. 1983, Schmitz and Osborne 1984). Furthermore, hydrilla is a major substrate for a new species of cyanobacterium that produces a neurotoxin that causes avian vacuolar myelinopathy in birds (Wilde et al. 2005).

Management of dioecious hydrilla is difficult because of its growth rate, which may exceed 30 cm per day (Glomski and Netherland 2012), and its ability to regenerate from fragments (Silveira et al. 2009). Because of the diversity of water resource uses (e.g., fishing, hunting, recreation, flood control, aquaculture, and crop irrigation), effective hydrilla control is difficult to achieve because of a limited number of environmentally sound options for integrated pest management (Hoyer et al. 2005). Current efforts for controlling

dioecious hydrilla in Florida rely primarily on herbicides (FWC 2011) and nonselective biological control using grass carp *Ctenopharyngodon idella* Val. (Cassani 1996, Sutton and Vandiver 1998, Dibble and Kovalenko 2009). Although various chemical, mechanical, and biological methods have been investigated for managing hydrilla infestations to control the explosive growth of the weed (Gettys et al. 2014, Weeks and Lietze 2014), none was as effective as the herbicide fluridone. Until recently, the herbicides fluridone and endothall formed the basis of most publicly funded hydrilla control programs in Florida and elsewhere (MacDonald 2012, Netherland 2014).

In 2000, aquatic plant researchers discovered that dioecious hydrilla in Florida was developing resistance to fluridone in some water bodies (MacDonald et al. 2001). This finding confirmed field observations of declining hydrilla control by public and private aquatic plant managers after large-scale and repeated use of fluridone for hydrilla control in the Kissimmee Chain of Lakes in Osceola County, Florida through the 1990s. This is the first case of a plant developing resistance to a carotenoid biosynthesis inhibitor, or bleaching-type herbicide (Michel et al. 2004, Dayan and Netherland 2005). Fluridone resistance in Florida was not anticipated because of the naïve assumption that dioecious hydrilla could not develop resistance. Nevertheless, at least six clones have been identified with a two- to sevenfold increase in resistance to fluridone (Puri et al. 2006), and the level of resistance appears to be stable over time, even in the absence of fluridone selection pressure (Puri et al. 2007).

The discovery of fluridone resistance in Florida dioecious hydrilla led to several local and national workshops/meetings with concerned researchers, aquatic plant managers, and other stakeholders to establish priorities for future research directions (Hoyer et al. 2005, Netherland et al. 2005, Cuda et al. 2008, Systma 2008). One of the priority areas from these workshops was improving integration of chemical control technology with other aquatic plant management practices, e.g., biological control.

Cricotopus lebetis Sublette (Diptera: Chironomidae) is a herbivorous midge whose larvae mine apical meristems of hydrilla, using living plant material as a food source (Epler et al. 2000, Cuda et al. 2002). Feeding damage by larvae of *C. lebetis* stunts the growth of hydrilla and changes the plant's architecture (Cuda et al. 2011). *Cricotopus lebetis* is widely distributed in peninsular Florida, albeit at relatively low densities (Stratman et al. 2013b). Recent studies have shown that *C. lebetis* is not a hydrilla specialist in laboratory tests (Stratman et al. 2013a), but it has been collected only from hydrilla in field samples. The insect's short generation time, high reproductive rate, and ease of mass rearing (Cuda et al. 2002, Baniszewski et al. 2015) make it an ideal candidate for an augmentation program (Cuda et al. 2008). Using both niche and physiological modeling approaches, Stratman et al. (2014) predicted that *C. lebetis* would complete up to 11 generations per year in Florida, and that much of the southeastern United States was climatically suitable for establishment of the midge.

The indigenous fungus *Mycoleptodiscus terrestris* (Gerd.) Ostazeski (incertae sedis: Magnaporthaceae) (hereafter Mt), isolated in Texas in the 1980s, is pathogenic to hydrilla (Joye 1990). A virulent strain of Mt has been studied extensively as an inundative biological control agent (Shearer 1996, 1998). During the past 10 yr, Mt has been under development as a mycoherbicide by the National Center for Agricultural Utilization Research (NCAUR), U.S. Department of Agriculture–Agricultural Research Service (USDA-ARS) Laboratory, Peoria, IL (Shearer and Jackson 2006), and the SePRO Corporation, Carmel, IN (Heilman 2012).

Imazamox is a systemic herbicide registered in 2008 for aquatic use (Netherland 2014). This herbicide targets the plant-specific enzyme acetolactate synthase (ALS), which plays a critical role in the production of amino acids required for protein synthesis (Netherland 2014). Treating hydrilla with imazamox reduces the plant's biomass and suppresses growth for up to 7 mo (Netherland 2014). More important, there are no restrictions for drinking water and minimal restrictions for irrigation (Netherland 2014).

In this study, we assessed the efficacy of integrating herbivory by the naturalized meristem-mining midge *C. lebetis* with either the native fungal pathogen Mt or the ALS-inhibiting herbicide imazamox for controlling hydrilla.

MATERIALS AND METHODS

The hydrilla tip-mining midge *C. lebetis* was collected at Lake Rowell, Bradford County, FL (29°55′16.96″N; 82°09′32.85″W) and reared according to procedures described by Cuda et al. (2002). The fungal inoculum of Mt[1] (USDA ARS Culture Collection [NRRL] #30559) was prepared using protocols described by Shearer and Jackson (2006) and shipped to the Engineer Research and Development Center (ERDC), Vicksburg, MS. Appropriate 100-ml dilutions of imazamox[2] were mixed with distilled water in 125 ml-screw-cap bottles[3] at the University of Florida, Gainesville, FL. Eggs/neonates of *C. lebetis* were transferred to plastic screw-cap scintillation vials (20 ml) containing water from the aquaria at ERDC before the experiments. Bottles containing the insects and the imazamox dilutions were delivered to the ERDC via ground transportation 24 h before initiating the aquarium tests.

Compatibility of the hydrilla tip-mining midge *C. lebetis* with the fungus Mt

Experiments were conducted in 55-L aquaria located in a controlled-environment growth chamber at the ERDC, Vicksburg, MS. Growth chamber conditions were maintained for optimal hydrilla growth: 25 C ± 1 C and a 14 : 10-h light : dark photoperiod. Aquaria (0.9 m tall × 0.09 m^2) were filled with a water-based culture solution (Smart and Barko 1985). Plastic cups (946 ml) containing fertilized topsoil were drenched with reverse-osmosis water and four 15-cm apical cuttings from dioecious hydrilla were planted in each cup and placed in the aquaria (four cups per aquarium). Aquaria were gently aerated to provide circulation. Plants were allowed to grow in the aquaria for approximately 28 d, by which time they had formed a canopy. Dry inoculum of the fungus Mt was applied by scattering it evenly onto the water surface and allowing it to naturally dissipate over the hydrilla. As the rehydrated

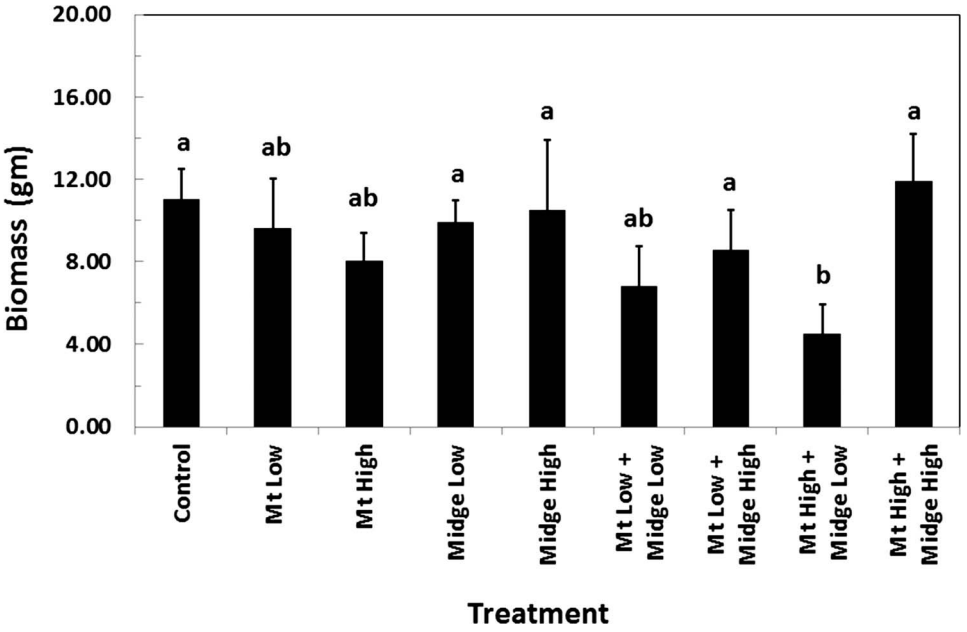

Figure 1. Biomass of hydrilla 28 d after application of different combinations of the plant pathogenic fungus *Mycoleptodiscus terrestris* (Mt) and the hydrilla tip-mining midge *Cricotopus lebetis* in 55-L aquaria compared with untreated controls. Bars with different letters are statistically different (ANOVA, Fisher's LSD test, $\alpha < 0.05$).

granules fell through the water column they became lodged on leaves and in leaf axils. Neonates of *C. lebetis* were applied the same day.

Treatments included effective rates of 0.02 and 0.06 g L^{-1} of Mt alone (Shearer and Nelson 2009), 40 and 80 neonates of *C. lebetis* alone (Cuda et al. 2011), all combinations of Mt and *C. lebetis*, and untreated controls. Aquaria were covered with screens and each treatment was replicated three times. At 28 d after treatment, hydrilla shoot biomass was harvested, dried for 4 d at 60 C, and dry weight (gm) recorded.

Compatibility of the hydrilla tip-mining midge *C. lebetis* with the herbicide imazamox

Using the same experimental setup, a second experiment was conducted to determine if the hydrilla tip-mining midge *C. lebetis* was compatible with the herbicide imazamox, which causes hydrilla to branch at low concentrations (M. D. Netherland, pers. comm.). Treatments included low and high rates of imazamox (10 and 50 µg L^{-1}) alone (Shearer and Nelson 2009), 40 and 80 neonates of *C. lebetis* alone, all combinations of imazamox and *C. lebetis*, and untreated controls. After imazamox was added to the tanks, midge neonates were added 2 wk later to allow the herbicide to induce branching and ensure that the minimum exposure/half-life times were met (Netherland 2014). Each treatment was replicated three times. Hydrilla biomass was harvested 28 d after aquaria were inoculated with midge larvae. Plant material was dried for 4 d at 60 C, and dry weight recorded. The number of adult midges that emerged was monitored in this experiment to determine if exposure of developing larvae to imazamox negatively affected their development and survival.

Data analysis

Data are reported as means ± standard error. Hydrilla biomass and emergence of adult midges in the imazamox tests were subjected to ANOVA (SAS Version 9.2, 2011). When significant treatment effects were detected, means were separated using Fisher's LSD test at the 0.05 significance level. Synergistic effects between midge and Mt treatments and between midge and imazamox treatments were analyzed using a nonlinear mixed-model procedure (Blouin et al. 2004). Significant interactions were indicated when Colby estimates were positive (synergistic effect) or negative (antagonistic effect), and means were separated by NLMIXED *t* tests at the 0.05 significance level.

RESULTS AND DISCUSSION

Compatibility of the hydrilla tip-mining midge *C. lebetis* with the fungus Mt

Results from combining two rates of Mt with two densities of the midge *C. lebetis* are presented in Figure 1. Hydrilla biomass produced in the Mt low or high aquaria (9.65 ± 2.40 g and 8.04 ± 1.36 g, respectively) did not differ statistically from the controls (11.03 ±1.47 g). Similarly, plant biomass in the midge low- and high-treatment aquaria (9.91 ± 1.06 g and 10.51 ± 3.40 g, respectively) was not statistically different from the controls. The nonsignificant increase in biomass observed in the midge high-treatment aquaria was not unexpected as feeding damage by the developing larvae often stimulates the formation of new shoot tips (Buckingham 1994). However, aquaria containing the Mt high and midge low treatment produced significantly less biomass (4.50 ± 1.43 g) compared with the controls (df =

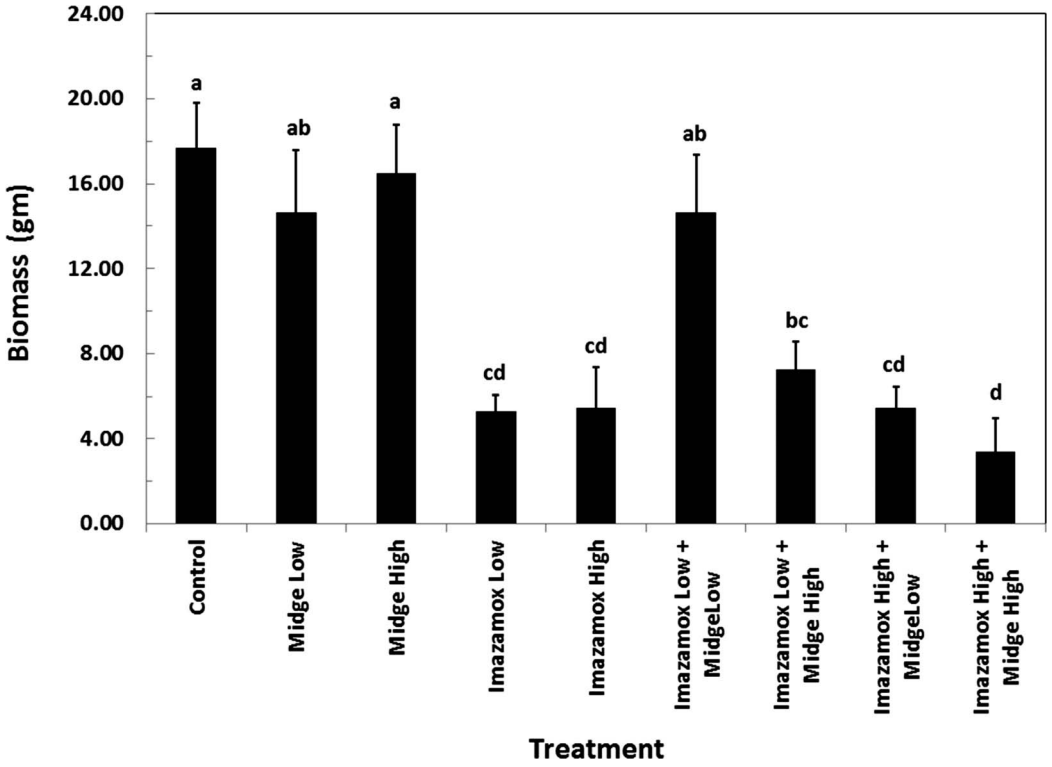

Figure 2. Biomass of hydrilla 28 d after application of different combinations of the acetolactate synthase herbicide imazamox and the hydrilla tip-mining midge *Cricotopus lebetis* in 55-L aquaria compared with untreated controls. Bars with different letters are statistically different (ANOVA, Fisher's LSD test, $\alpha < 0.05$).

8, $F = 5.73$, $P < 0.001$). This treatment combination significantly reduced hydrilla biomass by almost 60% compared with the untreated controls. In addition, a synergistic effect on hydrilla biomass reduction from this fungus–insect combination was indicated (df = 27, $C = 9.7009$, $t = 3.70$, $P < 0.001$). However, for the high Mt and high midge combination, the biomass produced (11.95 ± 2.26 g) was statistically the same as the controls, suggesting an antagonistic effect (df = 27, $C = -1.6755$, $t = -0.7$, $P > 0.05$).

Compatibility of the hydrilla tip-mining midge *C. lebetis* with the herbicide imazamox

Results of combining two midge densities with two imazamox rates are shown in Figure 2. As in the previous experiment, hydrilla biomass produced in the midge low- and high-treatment aquaria (14.65 ± 2.93 g and 16.49 ± 2.30 g, respectively) did not differ statistically from the controls (17.68 ± 2.12 g). However, a significant antagonistic interaction was observed in the imazamox low-midge low aquaria (df = 27, $C = -10.2592$, $t = 4.71$, $P < 0.001$). Hydrilla biomass produced in this treatment combination (14.66 ± 2.70 g) was not statistically different from controls. Subtle midge feeding damage and low herbicide rate probably stimulated the production of new shoots that neutralized the treatment effects. However, the imazamox low- and high-treatment aquaria as well as the remaining imazamox–midge treatment combination aquaria yielded significantly lower hydrilla biomass compared with the controls (df = 8, $F = 6.11$, $P < 0.0001$). Furthermore, the imazamox high-midge high treatment combination reduced hydrilla biomass by 81% and the effect was synergistic (df = 27, $C = 6.4046$, $t = 3.93$, $P < 0.001$).

In previous laboratory tests and small-scale field trials, integrating low doses of fluridone, endothall, or imazamox with Mt increased the susceptibility of hydrilla to low doses of these herbicides (Netherland and Shearer 1996, Shearer and Nelson 2002, 2009). For example, combining Mt fungus with fluridone significantly reduced hydrilla biomass by 92% compared with the untreated control and by over 80% when compared with individual treatments (Netherland and Shearer 1996). In this study, we showed that integrating 1) high rates of Mt with low densities of the tip-mining midge *C. lebetis* and 2) low or high rates of imazamox with high densities of *C. lebetis* significantly reduced hydrilla growth. In the first experiment, tissue damage from insect herbivory probably increased the Mt infection process by creating new entry wounds in hydrilla for the fungus to infect, resulting in pathogenesis. Shabana et al. (2003) observed that hydrilla damage was significantly greater when the fungus *Fusarium culmorum* (W. G. Smith) Sacc. (Hypocreales: Nectriaceae) was integrated with the hydrilla leaf-mining fly *Hydrellia pakistanae* Deonier (Diptera: Ephydridae).

In the second experiment, it is unclear how the interaction between insect and herbicide affected hydrilla growth. As expected, midge larvae were not adversely affected by exposure to imazamox. The observed increase

in adult emergence in some of the aquaria may be the result of additional shoot tips created by branching of hydrilla after imazamox treatment (M. D. Netherland, pers. comm.). Imazamox-induced branching of hydrilla could provide additional feeding/development sites for the midge larvae to exploit, which would account for greater plant damage and an increase in the production of adult midges. Further research will investigate whether efficacy of imazamox will be enhanced by applying the herbicide simultaneously or after midge establishment has occurred.

On the basis solely of laboratory host range tests, *C. lebetis* may be unsuitable for redistribution outside the state of Florida, although it currently is established on hydrilla in Louisiana (Epler et al. 2000). The native Canadian waterweed, *Elodea canadensis* Michx., and the introduced Brazilian elodea, *Egeria densa* Planchon, were good laboratory (physiological) hosts for *C. lebetis* (Stratman et al. 2013a), yet the insect only has been field collected from hydrilla in Louisiana and Florida (Epler et al. 2000, Stratman et al. 2013b). It is noteworthy that the Australian hydrilla stemboring weevil *Bagous hydrillae* O'Brien (Coleoptera: Curculionidae) that was released in Florida in 1991 and recently discovered in Louisiana (Center et al. 2013) exhibits a similar field (or realized) host specificity. Both *Elodea canadensis* and *Egeria densa* were suitable laboratory hosts for *B. hydrillae* but were not attacked in the field in Australia (Buckingham 1994, Balciunas et al. 1996). The *B. hydrillae* case study clearly illustrates how laboratory tests often overestimate field host range.

The recent discovery in Florida of endothall resistance in some dioecious hydrilla populations (Berger and MacDonald 2011, Giannotti 2013) provides further evidence that resistance management will require a concerted effort by aquatic plant managers to adopt an integrated approach (Norsworthy et al. 2012, Vencill et al. 2012). Additional studies under field conditions are underway to test the efficacy of combining both biological control agents with the herbicide imazamox for controlling dioecious hydrilla.

SOURCES OF MATERIALS

[1]Fungal pathogen *Mycoleptodiscus terrestris*, USDA-ARS –NCAUR, 1815 North University Street, Peoria, IL 61604-3902.

[2]Clearcast (imazamox 120 g ai L^{-1}). SePro Corporation, 11550 North Meridian Street, Carmel, IN 46032.

[3]Nalgene® plastic vials, Fisher Scientific, 300 Industry Drive, Pittsburgh, PA 15275.

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