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# Performance of Two Established Biological Control Agents on Hydrilla Genotypes Susceptible and Resistant to Fluridone Herbicide

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## ABSTRACT

Hydrilla (*Hydrilla verticillata* [L.f.] Royle: Hydrocharitaceae) is a submersed aquatic plant widely distributed in most tropical and temperate zones worldwide. A dioecious female strain of hydrilla was introduced into Florida from Sri Lanka in the early 1950s through the aquarium trade. This aggressive submersed plant spread rapidly and now occurs in all states bordering the U.S. coastline except New Mexico and Oregon. The recent development of fluridone herbicide resistance in Florida hydrilla populations has resulted in the inability to economically control large infestations of this invasive aquatic weed. The objective of this study was to compare the performance of two established insect biological control agents, a stem-mining midge (*Cricotopus lebetis* Sublette: Chironomidae) and a leaf-mining fly (*Hydrellia pakistanae* Deonier: Ephydridae), on a fluridone susceptible and two fluridone-resistant genotypes. Terminal shoots of susceptible, moderately resistant, and highly resistant hydrilla genotypes were obtained and placed in individual culture tubes. Two neonate larvae of each biocontrol agent were transferred to culture tubes containing the three hydrilla genotypes. Survival and sex of the emerged adults were recorded. Results showed that the two biocontrol agents differed in their acceptance of the hydrilla genotypes. Survival of the leaf-mining fly was similar on all three Florida genotypes tested. In contrast, the stem-mining midge exhibited significantly lower emergence when reared on the medium and highly resistant genotypes compared to the fluridone susceptible hydrilla genotype. These findings highlight the importance of examining insect performance on all plant genotypes present in the invaded range when selecting effective biological control agents.


*Key words*: biological control, *Cricotopus lebetis*, herbicide resistance, *Hydrellia pakistanae, Hydrilla verticillata.*

## INTRODUCTION

Hydrilla (*Hydrilla verticillata* (L.f.) Royle) is one of the worst invasive aquatic weeds in the United States, with millions of dollars spent annually to control large infestations in all types of water bodies. Various mechanical, chemical, and biological methods have been investigated for managing hydrilla infestations. The herbicide fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1*H*)-pyridinone) was found to be the most effective for treating large-scale infestations (Fox et al. 1994). Recently, it was discovered that hydrilla has developed resistance to fluridone (Michel et al. 2004), the only systemic herbicide for aquatic systems approved by the U.S. Environment Protection Agency for managing this submersed invasive aquatic weed. Because the resistance problem is increasing in scope, one strategy to cope with this problem currently entails higher application rates of fluridone or alternative herbicides. Consequently, the spread of fluridone-resistant hydrilla biotypes to other water bodies within the United States is inevitable due to several factors, including similar growth and reproductive patterns between the susceptible and resistant biotypes (Puri et al. 2007).

The discovery of fluridone resistance is cause for concern. The resistance problem will make it difficult for aquatic plant managers in Florida to control hydrilla in a cost-effective and selective manner. This can lead to the eventual spread and establishment of the resistant dioecious biotypes throughout hydrilla's introduced range. In addition, the loss

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of fluridone as a major tool for hydrilla control is forcing aquatic plant managers and researchers to consider evaluating new herbicides, evaluation of new use patterns for registered contact herbicides, and developing novel biological controls (Cuda et al. 2008).

Recently, Cuda et al. (2008) recommended that testing of established hydrilla biological control agents, specifically the stem-mining midge *Cricotopus lebetis* Sublette (Diptera: Chironomidae; Cuda et al. 2002) and the two leaf-mining flies *Hydrellia pakistanae* Deonier and *H. balciunasi* Bock (Diptera: Ephydridae; Center et al. 1997, Grodowitz et al. 1997) should be completed as soon as possible to assess their developmental and reproductive performance on the fluridone-resistant hydrilla biotypes.

The objective of this study was to compare the performance of two of the established insect biological control agents on fluridone susceptible and fluridone-resistant hydrilla genotypes. The stem-mining midge *C. lebetis* is an adventive species that was discovered attacking hydrilla in Crystal River, Citrus County, Florida, in 1992 (Epler et al. 2000), whereas the leaf mining fly *H. pakistanae* was first introduced into Polk County, Florida, in 1987 (Center et al. 1997).

## MATERIALS AND METHODS

The hydrilla midge *C. lebetis* was collected in Crystal River, Citrus County, Florida, on 15 June 2006, from fluridone-susceptible hydrilla. The procedures for collecting the hydrilla samples and rearing the midge were described by Cuda et al. (2002). Terminal stem sections (~5 cm long) of three hydrilla genotypes (susceptible, moderately resistant, and highly resistant) were obtained at the Agronomy Department Weed Science Field Laboratory, University of Florida, Gainesville. Resistance levels of all hydrilla genotypes were determined by biochemical and phenological assays that addressed changes in carotenoid levels and growth compared to susceptible hydrilla genotypes (Puri et al. 2006). A randomized complete block experiment was conducted using terminal shoots of the three hydrilla genotypes in glass culture tubes, and each genotype had three replications.

A single excised stem of hydrilla (n = 10 per genotype) was placed into a glass culture tube (2 cm dia × 15 cm tall) and then submerged in well water with about 1 cm of air remaining at the top of the test tube. The test tubes were covered with perforated plastic caps to allow airflow and confine the emerging adults (Figure 1). Using a small glass pipette, two newly hatched larvae that had not yet fed on hydrilla were transferred to each culture tube. The culture tubes with developing midge larvae were checked daily under a microscope to record development of pupae and adult emergence. After the adult emerged, they were sexed and preserved. The midge bioassay was conducted from 28 June to 8 November 2006 in a Biotronette Mark III Environmental Chamber. The environmental conditions in the growth chamber were 28 ± 4 C and a 15: 9 hr (light:dark) photoperiod.

The hydrilla fly bioassay was conducted from 2 October 2007 to 6 June 2008. Adults of *H. pakistanae* used in this experiment were collected at the UF/IFAS Center for Aquatic and Invasive Plants, Gainesville, Florida, in December 2007. Fluridone-susceptible hydrilla infested with *H. pakistanae* larvae was collected in a 19 L (5 gal) bucket and the top covered with Nitex® screen to trap emerging adult flies. The rearing procedure was similar to the one described by Goodson (1997). Samples of fluridone-susceptible, fly-free hydrilla were collected at the Santa Fe River in High Springs, Alachua County, Florida and placed into 3.8 L (1 gal) jars to establish a colony.

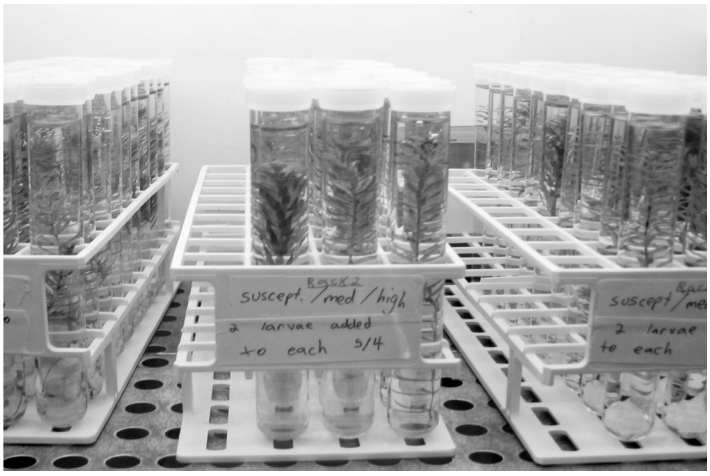


Figure 1. Experimental setup showing test tube racks with the three hydrilla genotypes and 10 replications per resistance level.

Seventy 3.8 L glass jars were used for colony establishment. Each jar was covered with Nitex® screen for ventilation and securely closed with an adjustable metal ring. A small hole was made in the top of the screen, and a rubber tube (0.5 cm dia) connected to a Top Fin® Air Pump was placed in the jar for aeration. Emerging adults of the parental generation of *H. pakistanae* were collected from the bucket inside a light box and transferred to the jars with a hand held mouth aspirator and allowed to oviposit on the hydrilla. The jars were placed in the same Biotronette Mark III Environmental Chamber used for the *C. lebetis* bioassay.

After the fly population increased, 30 adults were removed from the jars and transferred to a clear plastic container (9 cm tall × 26 cm dia) for mating and oviposition. Two small whorls of fluridone-susceptible hydrilla were transferred to a standard 9 cm plastic petri dish containing enough water to cover the bottom and placed inside the container as an oviposition site. Using this approach, newly hatched larvae could be collected before they were able to mine into the hydrilla. To allow for ventilation, four equally spaced holes (4 cm dia) were cut in the sides of the container and covered with Nitex® mesh. Because the adult flies are nectar feeders, they also were provided with Gatorade® in a 50 ml covered cup fitted with a cotton wick. After 1 week, the hydrilla whorls with eggs were removed from the oviposition chamber and examined under a microscope. Two neonates were transferred with a small camelhair brush to each of the culture tubes described for the hydrilla midge.

For data analysis, the number and sex of the insects that emerged from the three hydrilla genotypes were recorded.

Proportional data (% emergence) were arcsine transformed and analyzed using a one-way analysis of variance (ANOVA; SAS 2003). Tukey's post-hoc multiple comparison procedure was used to separate the means at $P = 0.05$. Data are reported as means ±SD.

## RESULTS AND DISCUSSION

Survival of the leaf mining fly *H. pakistanae* was similar on all three Florida hydrilla genotypes tested. Percent emergence of *H. pakistanae* adults on the susceptible, moderately resistant, and highly resistant genotypes was 46.7 ± 19.9, 56.7 ± 23.7, and 50.0 ± 20.0%, respectively ($F_{2, 27} = 1.01$, $P = 0.37$; Figure 2). The results showed no differences in the emergence of male *H. pakistanae* among the three hydrilla genotypes tested, but survival of females on the moderately resistant genotype was significantly higher (33.3 ± 5.9%) compared to the susceptible and highly resistant hydrilla plants (10.0 ± 4.1 and 16.7 ± 7.2%, respectively). Why females performed better on one of the resistant genotypes needs further investigation.

In contrast, the stem-mining midge *C. lebetis* exhibited significantly lower emergence when reared on the moderately and highly resistant genotypes (3.3 ± 3.2 and 1.7 ± 2.9%, respectively) compared to the susceptible hydrilla genotype (38.3 ± 12.8%; $F_{2, 27} = 20.67$, $P < 0.0001$; Figure 3). Percent emergence of male and female midges also was similar on the susceptible genotype (20.1 and 18.2%, respectively) but only males emerged on the two resistant genotypes.

Overall, the results showed that these two biocontrol agents differed in their developmental performance on the three hydrilla genotypes. In 2000, aquatic plant researchers discovered that the aquatic weed hydrilla appeared to be developing resistance to fluridone in some Florida waterbodies (MacDonald et al. 2002). This finding confirmed field observations by public and private aquatic plant managers that although the same procedures from previously successful fluridone treatments were used, hydrilla control was impaired. The fluridone resistance was unexpected because dioecious hydrilla reproduces vegetatively in Florida.

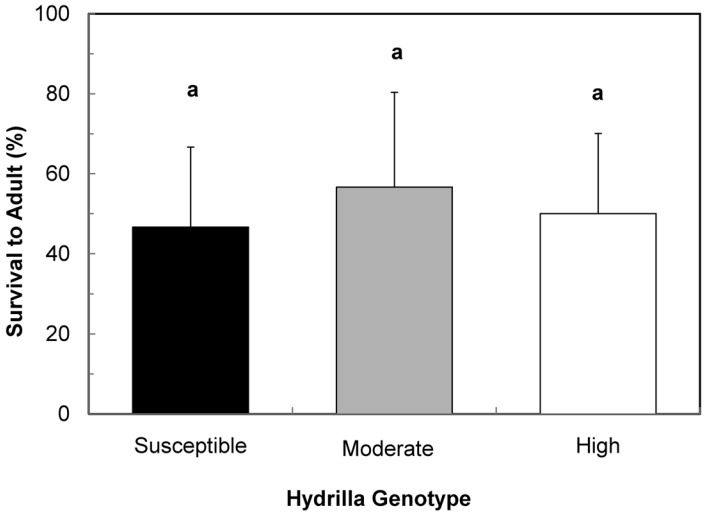


Figure 2. Percent survival of *Hydrellia pakistanae* larvae on three genotypes of hydrilla with varying levels of resistance to fluridone. Bars with the same letter are not statistically different according to Tukey's post-hoc multiple comparison procedure at $\alpha = 0.05$. Mean of three replications with standard deviation of the mean.

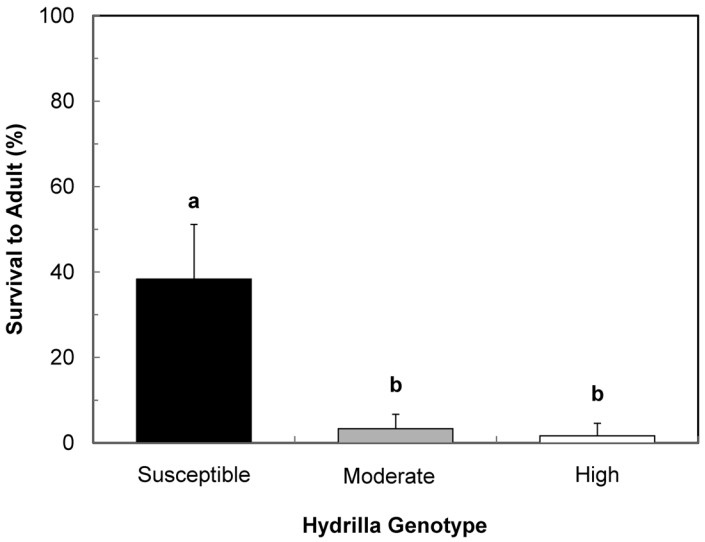


Figure 3. Percent survival of *Cricotopus lebetis* larvae on three genotypes of hydrilla with varying levels of resistance to fluridone. Bars with the same letter are not statistically different according to Tukey's post-hoc multiple comparison procedure at $\alpha = 0.05$. Mean of three replications with standard deviation of the mean.

Fluridone-susceptible hydrilla plants are being replaced by fluridone-resistant plants, which have a higher β-carotene content (Puri et al. 2006). The mutation in the fluridone-resistant genotypes may have conferred resistance to some insect herbivores, as shown by our data. Subtle changes in the pigment levels of the fluridone-resistant plants may alter their palatability or nutritional quality. Therefore, new insects selected as potential biological control agents should be tested against the fluridone-resistant as well as the fluridone-susceptible hydrilla genotypes.

In this study, the leaf-mining fly *H. pakistanae* and stem-mining midge *C. lebetis*, established biological control agents of hydrilla, clearly differed in their ability to complete their development on the susceptible and fluridone resistant hydrilla genotypes. Percent survival of *H. pakistanae* to adulthood was similar on all three genotypes. Because this introduced biological control agent is widely established in Florida and elsewhere, it should adapt quickly to the fluridone-resistant genotypes as they become more widespread (Puri et al. 2007).

In contrast, the stem-mining midge *C. lebetis* also completed its development on the fluridone-resistant hydrilla genotypes, although survival to the adult stage was significantly lower compared to the fluridone susceptible hydrilla. Through natural selection, we expect field populations of the midge *C. lebetis* to eventually adapt to the fluridone-resistant hydrilla. Because the life cycle of the midge from egg to adult is completed in 1 to 2 weeks under laboratory conditions (Cuda et al. 2002), the selection process should occur rapidly.

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