Impact of Insect Herbivory on the Establishment of *Hydrilla verticillata* (L.f.) Royle Fragments

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INTRODUCTION

Hydrilla (*Hydrilla verticillata* [L.f.] Royle) is an invasive, nonindigenous aquatic plant first discovered in the United States in the late 1960s (Blackburn et al. 1969). Current distribution includes the northern states of Maine and Washington, the Gulf and Atlantic coastal states, California, Tennessee, and recently Arkansas (USGS 2007), Oklahoma (Smart pers. comm.), Indiana (Lembi 2006) and Wisconsin (Netherland 2007). Once hydrilla invades an aquatic system, the plant can rapidly spread locally through rhizome expansion or over longer distances through generation of fragments (Sculthorpe 1985, McFarland and Barko 1996) and/or turions. For example, in the San Marcos River, Texas, Owens et al. (2001) found that healthy, undamaged hydrilla fragments generated by recreational usage, harvesting, and environmental factors eventually led to creation of new colonies downriver (some several miles) as fragments settled.

Two host-specific leaf-mining flies, introduced beginning in 1987 (Grodowitz et al. 2007), have shown success in long-term management of hydrilla in controlled experimentation and field sites (Doyle et al. 2002, Grodowitz et al. 2003). The two introduced agents, the Australian leaf-mining fly (*Hydrellia balciunasi* Bock) and the Asian leaf-mining fly (*H. pakistanae* Deonier), have larval life stages (three-instars) that damage the plant by penetrating, mining, and destroying hydrilla leaves (Balciunas et al. 2002, Buckingham and Grodowitz 2004). Past research has shown that moderate-to-high levels of herbivory can impact hydrilla biomass production and reduce tuber numbers and size (Doyle et al. 2002, 2007, Grodowitz et al. 2003). Doyle et al. (2002) reported that when 10 to 30% of leaves were damaged, the maximum rate of photosynthesis was reduced by almost 40%. When leaf damage reached 70%, photosynthetic rates were reduced by up to 60%.

Based on field observations, fragmentation appears to be higher in hydrilla stems damaged by fly mining (Grodowitz, unpubl. data); however, only limited information is available concerning fragment establishment following varying levels of fly leaf-mining. In an earlier study, when fragments were physically planted in container sediment, Owens et al. (2006) determined that high levels of leaf damage (70 to 100%) were associated with significantly reduced above and belowground biomass, stem length and number, rhizome number, and tuber production in comparison to fragments with low levels of herbivory. This study examines establishment success of hydrilla fragments exposed to four different levels of herbivory as measured by percent leaf damage by quantifying fragment settling, rooting, change in length, branching, and aboveground biomass accumulation.

MATERIALS AND METHODS

The study was conducted in an outdoor mesocosm facility at the U. S. Army Engineer Research and Development Center, Lewisville Aquatic Ecosystem Research Facility (LAERF) in Lewisville, Texas, over a 30-day period during August 2006. Apical fragments measuring approximately 20 cm were collected from fly-damaged hydrilla plants at the LAERF (33°04'45"N, 96°57'30"W). Although both *Hydrellia* fly species occur on hydrilla at the LAERF, *H. pakistanae* composes over 90% of the *Hydrellia* population. The fragments (n = 20 per treatment) were separated into four categories of percent leaf damage, where 0 = control, 1-30% = low, 40-60% = medium, and 70-100% = high. Three initial (n = 3 each treatment) fragments (20 cm) were collected and dry weighted to determine averages. Eighty 7.6-L containers (25 cm tall and 22 cm wide) holding 0.24 L of LAERF pond sediment (Smart et al. 1995) overlain with 0.48 L of pea gravel were filled with alum-treated Lake Lewisville water. A single fragment was added to each container. After 30 days growth, each fragment was collected, change in length was calculated, and new branches were counted. We also noted if fragments had settled to the substrate, developed roots, and/or anchored (having roots penetrating the sediment). Following collection, plant biomass was dried to a constant weight at 55°C using a Blue M forced air oven (General Signal, Atlanta, GA). Statistical differences between settling and anchoring percentages were determined using Cross Tabulation Tables and Pearson Chi-Square test for significance of categorical variables to determine differences in expected frequencies. Percentage statistics were performed using Statistica 7.1 (StatSoft, Inc., Tulsa, OK). Statistical differences between dry weights change in length, and branching were determined using one-way ANOVA. Significant differences between means were determined using Tukey's level of significance. Statistics were performed using Statistix (Analytical Software, Tallahassee, FL, 1996).

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RESULTS AND DISCUSSION

Leaf-mining had a significant impact on the ability of hydrilla stems to settle to the substrate, produce roots and anchor. Fragments having high damage had greatly reduced settling (four-fold) and were overall structurally impaired, breaking up after 30 days, whereas all fragments rated as control and low damaged settled to the substrate (Figure 1A), generally within the first week of the study. Compared to the control, settlement of fragments with medium damage was reduced approximately two-fold. Leaf-mining damage also reduced the ability of the fragments to root (Figure 1B). Fragments with high damage produced no roots, whereas root production and anchoring was reduced approximately four-fold in fragments with medium and low damage compared to the controls. Fragments must ultimately settle and root for new hydrilla plants to establish (McFarland and Barko 1996), but fragments weakened by leaf-mining seem to have little chance of survival.

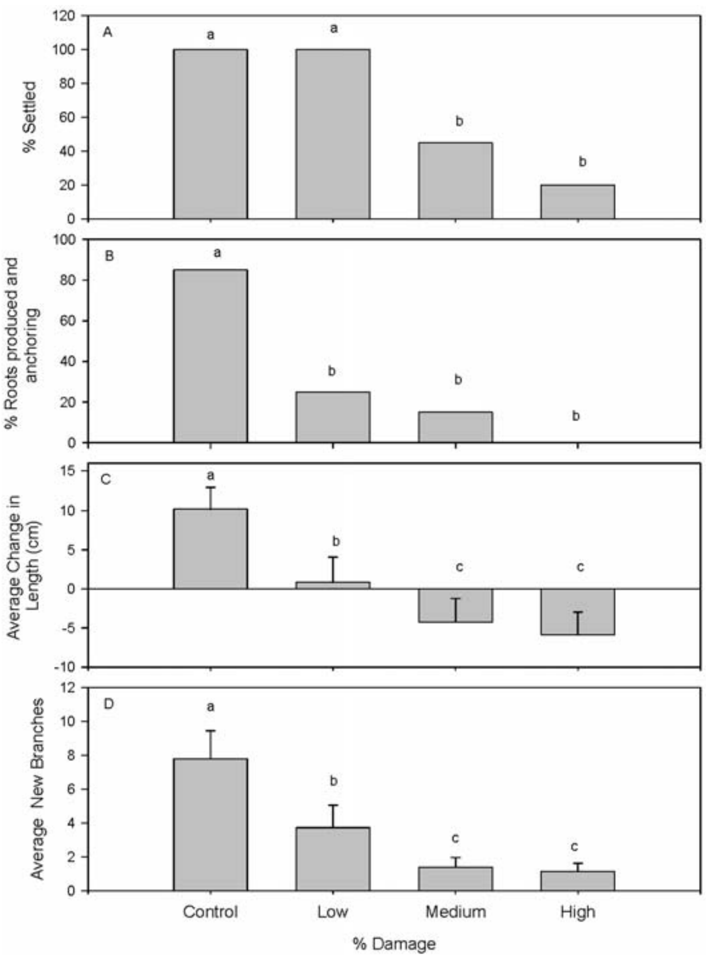

Figure 1. (A) Comparison of hydrilla fragments subjected to high, medium, low and no herbivory damage and % that settled (Pearson Chi-Square: 43.550, df = 3,p = 0.0000): (B) % that produced roots and anchored (Pearson Chi-Square: 35.3276, df = 3,p = 0.0000); (C) increase (or decrease) in length from initial 20 cm; and (D) production of new branches. Error bars represent 95% confidence intervals. Means with the same letter are not significantly different at the p = 0.05 level. Percentages with the same letter are not significantly different. N = 20 for each treatment.

Leaf-mining significantly reduced the ability of fragments to grow (change in length). Control fragments grew five-fold more than fragments with low damage and nearly eight times more than fragments with medium or high damaged (Figure 1C). Mean length of highly damaged fragments was significantly reduced due to lack of fragment survival and/or loss of integrity (Figure 1C). The highly damaged, and to some degree the fragments with medium damage, were structurally impaired from leaf-mining activities that probably removed the mesophyll and reduced or limited photosynthesis.

In a study by McFarland and Barko (1996), undamaged hydrilla fragments increased in biomass and branching while floating on the Potomac River. In this study, another impact of leaf mining was decreased branching and reduced biomass production. Nearly four-fold more branching occurred on control and low-damaged fragments compared to medium- and high-damaged fragments (Figure 1D). New growth indicative of branching and increased change in length increased biomass production, especially for control and low-damaged fragments (Figure 2). Mean initial biomass dry weights for all fragments was approximately 0.09g (P = 0.1648, F = 2.88) with no significant differences between treatments. Within 30 days, all control fragments had settled, rooted, and became anchored in the sediment. The dry weight biomass was 14-fold more than the initial biomass and significantly higher than the biomass produced for any other leaf-damage level. Low-damaged fragments increased eight-fold over the initial fragment dry weight and was significantly higher than medium or high damaged fragments, where biomass increases over 30 days was only four-fold and two-fold, respectively (Figure 2). In a previous study where low-, medium-, and high-damaged fragments were deliberately planted, there was an almost three-fold increase in aboveground biomass of fragments having low leaf damage compared to fragments having high leaf damage (Owens et al. 2006).

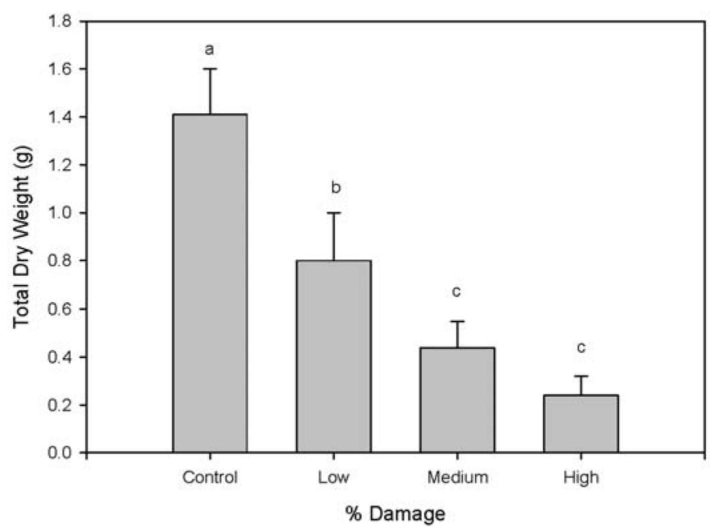

Figure 2. Mean dry weight (g) of hydrilla fragments subjected to high, medium, low and no percent leaf damage pre- and 30 days post treatment. Error bars represent 95% confidence intervals. Means with the same letter are not significantly different at the p = 0.05 level. N = 20 for each treatment.

Past research (Doyle et al. 2002, 2007, Grodowitz et al. 2003) has shown that moderate-to-high levels of herbivory can impact biomass production and reduce flowering and tuber production in rooted hydrilla plants. This study has demonstrated that moderate-to-high levels of herbivory by leaf-mining flies can also severely impact the ability of hydrilla fragments to successfully establish new populations. Fragments increase the ability of hydrilla to colonize new and distant locations (Sculthorpe 1985, McFarland and Barko 1996); however, if hydrilla fragments are damaged by leaf mining (even at fairly moderate levels) and cannot settle, as this study suggests, the ability of hydrilla to establish via fragmentation is severely limited, thus negating the ability to colonize new locations within an aquatic system.

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