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Do hydrilla leaf-mining flies contribute to increased hydrilla fragmentation?

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PURPOSE: Unpublished data and field observations suggest that fragmentation occurs more frequently in hydrilla stems damaged by the introduced leaf-mining flies in the genus *Hydrellia* especially at the point of larval feeding. This study focuses on fragmentation of hydrilla with and without *Hydrellia* spp. damage. Observations from the field (unpublished data) suggest that fly damage can increase hydrilla fragmentation; this study examines and validates these observations.

INTRODUCTION: Hydrilla (*Hydrilla verticillata* (L.f.) Royle) is an invasive, nonindigenous aquatic plant that was first discovered in the United States in the late 1960's (Blackburn et al. 1969). Current distribution includes the northern states of Maine and Washington; the Gulf and Atlantic coastal states; the western states of Arizona, Idaho and California; Tennessee, and recently Arkansas (U.S. Geological Survey (USGS) 2008), Oklahoma (Smart pers. comm.), Indiana (Lembi 2006), and Kentucky (Oster et al. 2008). Hydrilla spreads primarily through vegetative structures. Lateral spread over short distances occurs through development of rhizomes and tubers (localized), while spread over long distances is made possible by turion production, and dispersal of fragments (Madsen and Smith 1999, Madsen and Owens 1998). Hydrilla fragments are often generated by outside factors, such as recreational activities (Owens et al. 2001), wildlife, flooding events, boating traffic (Sculthorpe 1985) or possibly through herbivory.

Two introduced host-specific leaf-mining flies have shown some success for long-term management of hydrilla in controlled experimentation and field sites (Doyle et al. 2002, Grodowitz et al. 2003a). The two introduced agents include the Australian and Asian hydrilla leaf-mining flies, (*Hydrellia balciunasi* Bock) and (*H. pakistanae* Deonier), respectively. The three larval instars damage the plant by penetrating and mining internal leaf tissues (Balciunas et al. 2002, Buckingham and Grodowitz 2004).

Research has shown that moderate-to-high levels of herbivory by *Hydrellia* spp. can impact hydrilla biomass production and reduce tuber number and size (Doyle et al. 2002, 2007; Grodowitz et al. 2003a, 2007). By damaging hydrilla leaves through the removal of internal tissues, flies reduce the ability of hydrilla to photosynthesize (Doyle et al. 2002), weakening the plant but also increasing the potential to form fragments. By opening up dense hydrilla canopies through increased fragmentation, conditions may become more favorable for native plant establishment either through natural recruitment or deliberate introduction. Field observations from Lake Seminole, Florida showed that with several years of sustained fly feeding, hydrilla canopies became less dense and apparently allowed native pondweeds to increase (Grodowitz et al. 2003b).

An earlier study (Owens et al. 2006) found that high levels of fly herbivory on hydrilla significantly reduced all parameters measured including biomass (above and belowground), stem length, stem and tuber number when compared to fragments with low levels of herbivory. Further, leaf-mining significantly reduced the ability of hydrilla fragments to settle to sediments, produce roots, and anchor (Owens et al. 2008). These previous studies focused on viability and establishment of fly-damaged hydrilla fragments; this study, however, focuses on the production of hydrilla fragments with and without fly-mining damage.

METHODS: The study was conducted at the US Army Corps of Engineers' Lewisville Aquatic Ecosystem Research Facility (LAERF), in Lewisville, Denton County, TX over four months (June-September) in 2008. Three apical stem segments (10 cm) of dioecious hydrilla were planted into each of twenty 5.5-L containers filled with heat-sterilized LAERF pond sediment (Smart et al. 1995) amended with 1.4 g ammonium sulfate per container. After hydrilla began producing a surface canopy, 10 tanks were each stocked with approximately 50 *Hydrellia* spp. larvae via infested hydrilla stems. The infested stems were attached via cable ties to rooted hydrilla plants close to the water surface approximately three weeks after planting. The remaining 10 tanks received ABATE ® 4E (Clarke Mosquito Control Products, Inc., Roselle, IL) weekly at maximum label rate to prevent establishment of leaf-mining flies in the no herbivory tanks. Treatments included 10 replicates each of hydrilla infested with flies (herbivory) and hydrilla without flies (no herbivory).

After four month's growth, all tanks were exposed to five seconds of high pressure spraying (700 ml/5sec) evenly around the tank to agitate the water column, thereby inducing weakened hydrilla stems to fragment. Hydrilla fragments in the field are generally created by outside environmental factors including recreational activities (Owens et al. 2001), wildlife, flooding events, boating traffic (Sculthorpe 1985), or possibly through herbivory. Resultant fragments were collected and counted, and percent leaf damage was calculated (fly damaged leaf number per sprig divided by total leaf number per sprig times 100). Treatments were compared using a one-way analysis of variance (ANOVA) and the Tukey's test at ($p = 0.05$ level) of significance (Statistica 9, Tulsa, OK).

RESULTS AND DISCUSSION: Leaf mining had a significant impact on the number of hydrilla fragments produced by experimental manipulation. Plants with moderate to heavy mining damage had a greater than two-fold higher fragmentation rate compared to non-herbivory treatments when exposed to high-pressure spraying (Figure 1). Average number of fragments collected from the herbivory treatments was 24 stem fragments compared to only 11 fragments for no herbivory treatments and was statistically different at the $p < 0.05$ level.

Reasons for increased stem fragmentation are not completely understood, and current published literature on stem fragmentation is minimal at best. Larval damage is confined to the leaves (leaf mining) so stem involvement causing increased fragmentation might not be expected. The weakening of the stem near leaf damage may be a function of physiological processes attempting to seal off the damage (or reduced photosynthesis, loss of nutrients, or release of secondary compounds to combat herbivory) or may simply be a physical reaction to the leaf damage. Further research is warranted.

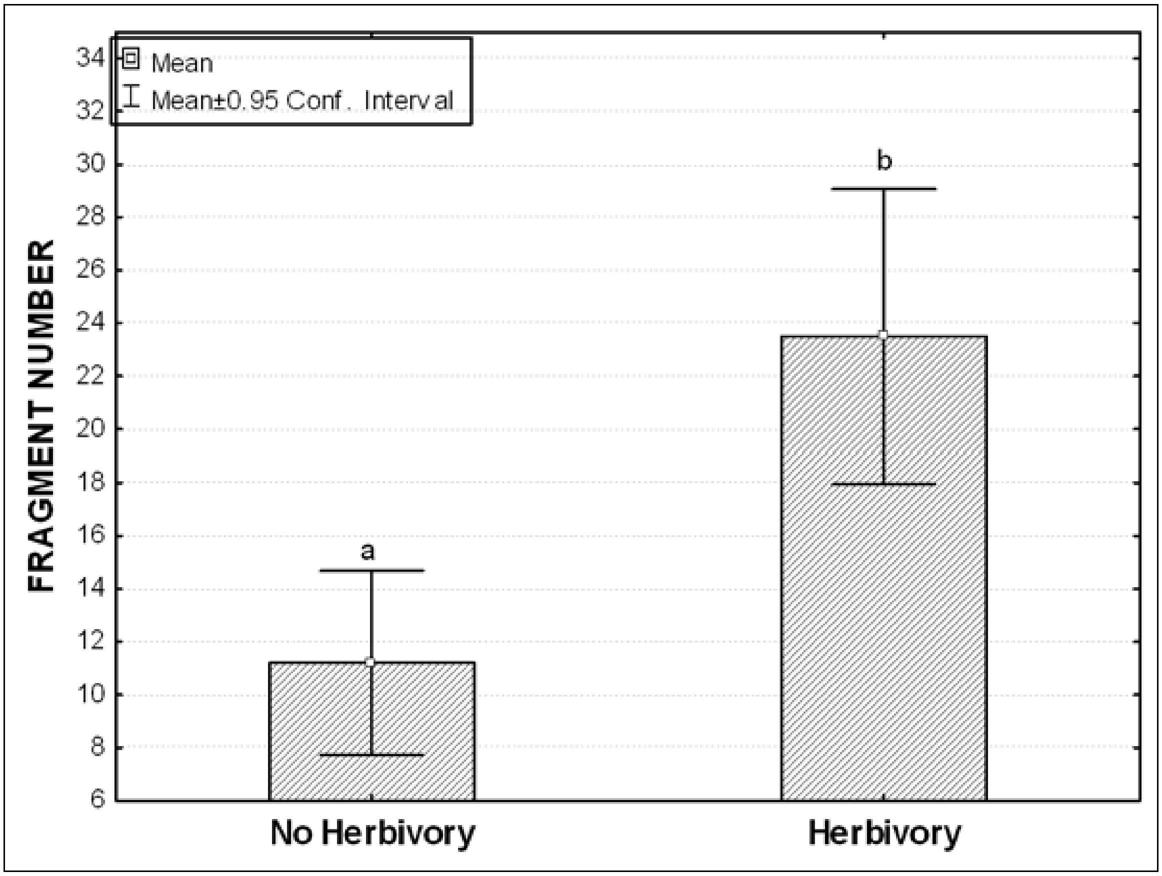

Figure 1. Fragmentation rates, herbivory versus non-herbivory treatments (F=13.51, df=1, p=0.0017; mean and 0.95 conF. interval).

No signs of leaf mining or flies were observed on the no herbivory treated tanks in this study. However, hydrilla exposed to herbivory had approximately 25% leaf mining damage (average) with many individual fragments having greater than 50% damage (Figure 2). No larvae were found in the hydrilla fragments, although mining was observed in leaves and pupae cases. This indicated that larvae could have already mined leaves, pupated, or moved onto other rooted hydrilla plants.

While leaf-mining damage apparently causes increased fragmentation, previous studies (Owens et al. 2006) found that fragments with high levels of leaf damage (70-100%) planted into sediment were associated with significantly reduced biomass, stem length and number, rhizome production and tuber production in comparison to fragments with lower levels of leaf damage. Further, leaf mining significantly impacted the ability of hydrilla stem fragments to settle to the sediment and produce roots and anchor. Fragments with high levels of damage (70-100%) had a four-fold decrease while medium damaged fragments (40-60%) had two-fold decreased settling rates (Owens et al. 2008). The stems, especially at the highly damaged rates, were structurally impaired overall as compared to control or low damaged fragments.

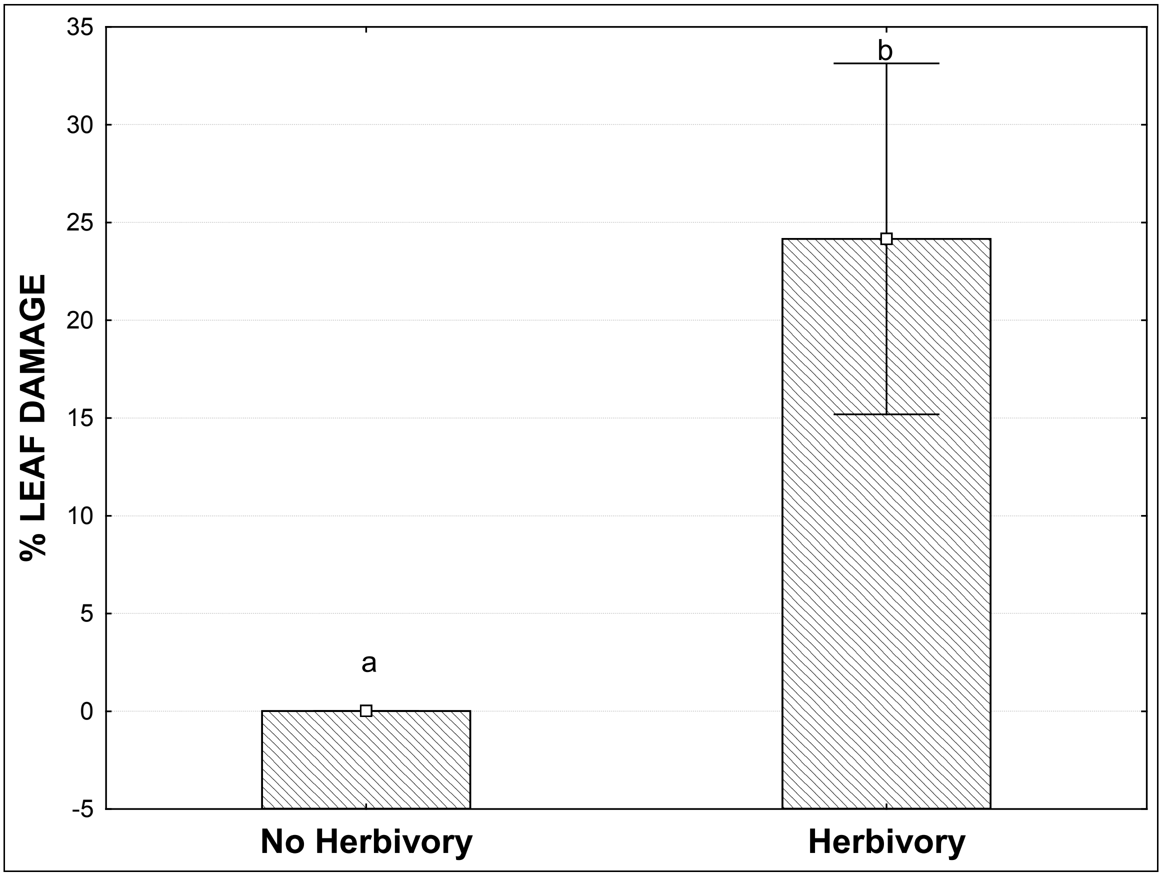

Figure 2. Percent leaf damage, herbivory versus non-herbivory treatments (F=18.30, dF=1, p=0.0000, mean and 0.95 conF. interval).

Based on field observations, fragmentation appears to be higher in hydrilla stems damaged by fly leaf mining (Grodowitz, unpublished data). This study supports field observations and found that herbivory by leaf-mining flies can increase fragmentation two-fold. Importantly, Owens et al. (2006, 2008) observed that *Hydrellia* fly-damaged fragments were less viable and, in most cases not able to settle and establish. The results of the study indicate that increased fragmentation from *Hydrellia* spp. leaf-mining may not provide hydrilla a viable dispersal mechanism. While this study has provided additional information regarding the effects of leaf-mining on hydrilla growth, much remains unknown about the interrelationship of herbivorous insects and their host plants. Further investigations should be conducted to better define these relationships and thereby improve understanding of invasive plant ecology.

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REFERENCES

Balciunas, J. K., M. J. Grodowitz, A. F. Cofrancesco, and J. F. Shearer. 2002. Hydrilla. In *Biological control of invasive plants in the eastern United States*, ed. R. Van Driesche, S. Lyon, B. Blossey, M. Hoddle, and R. Reardon, 91-114. Morgantown, WV: USDA Forest Serv. Publ. FHTET-2002-04.

Blackburn, R. D., L. W. Weldon, R. R. Yeo, and T. M. Taylor. 1969. Identification and distribution of certain similar-appearing submersed aquatic weeds in Florida. *Hyacinth Cont. J*. 8(1):17-21.

Buckingham, G. R., and M. J. Grodowitz. 2004. Hydrilla. In *Biological control of invasive plants in the United States*, ed. E. M. Coombs, J. K. Clark, G. L. Piper, and A. F. Cofrancesco, Jr., 184-195. Corvallis, OR: Oregon State University Press.

Doyle, R., M. Grodowitz, M. Smart, and C. Owens. 2007. Separate and interactive effects of competition and herbivory on the growth, expansion, and tuber formation of *Hydrilla verticillata*. *Biol. Control* 41:327-338.

Doyle, R. D., M. J. Grodowitz, R. M. Smart, and C. S. Owens. 2002. Impact of herbivory by *Hydrellia pakistanae* (Diptera: Ephydriadae) on growth and photosynthetic potential of *Hydrilla verticillata. Biol. Control* 24: 221-229.

Grodowitz, M. J., C. S. Owens, R. M. Smart, and J. M. Graham. 2007. *Impact of herbivory and plant competition on the growth of hydrilla in small ponds*. ERDC/TN APCRP-BC-08. Vicksburg, MS: US Army Engineer Research and Development Center.

Grodowitz, M. J., R. M. Smart, R. D. Doyle, C. S. Owens, R. Bare, C. Snell, J. Freedman, and H. Jones. 2003a. *Hydrellia pakistanae* and *H. balciunasi* insect biological agents of hydrilla: boon or bust? In *Proceedings of the XI International Symposium on Biological Control of Weeds, Canberra, Australia*, ed. J. M. Cullen, D. T. Briese, D. J. Kriticos, W. M. Lonsdale, L. Morin, and J. K. Scott, 529-538.

Grodowitz, M. J., A. F. Cofrancesco, R. M. Stewart, J. Madsen, and D. Morgan. 2003b. *Possible impact of Lake Seminole Hydrilla by the introduced leaf-mining fly Hydrellia pakistanae.* ERDC/EL TR-03-18. Vicksburg, MS: U.S. Army Engineer Research and Development Center.

Lembi, C.A. 2006. Hydrilla found in Indiana. *Aquatic Plant News* 83.

Madsen, J. D., and C. S. Owens. 1998. Seasonal biomass and carbohydrate allocation of dioecious hydrilla. *J. Aquat. Plant Manage*. 36:138-145.

Madsen, J. D., and D. H. Smith. 1999. Vegetative spread of dioecious hydrilla colonies in experimental ponds. *J. Aquat. Plant Manage*. 37:25-29.

Oster, R., K. Frey, and P. Rister. 2008. Hydrilla observations in Kentucky. *Aquatic Plant News* (89) October 2008.

Owens, C. S., M. J. Grodowitz, and R. M. Smart. 2008. Impact of insect herbivory on the establishment of *Hydrilla verticillata* (L.f.) Royle fragments. *J. Aquat. Plant Manage*. 46:199-201.

Owens, C. S., M. J. Grodowitz, R. M. Smart, N. E. Harms, and J. M. Nachtrieb. 2006. Viability of hydrilla fragments exposed to different levels of insect herbivory. *Aquat. Plant Manage*. 44:145-147.

Owens, C. S., J. D. Madsen, R. M. Smart, and R. M. Stewart. 2001. Dispersal of native and nonnative aquatic plant species in the San Marcos River, Texas. *J. Aqua. Plant Manage*. 39:75-79.

Sculthorpe, C. D. 1985. *The biology of aquatic vascular plants*. London, England: Edward Arnold (Publ.) Ltd.

Smart, R. M, J. D. Madsen, G. O. Dick, and D. R. Honnell. 1995. *Physical and environmental characteristics of experimental ponds at the Lewisville Aquatic Ecosystem Research Facility*. Miscellaneous paper A-95-2. Vicksburg, MS: U.S. Army Engineer Waterways Experiment Station.

U.S. Geological Survey (USGS). 2008. USGS-NAS-Nonindigenous Aquatic Species, *Hydrilla verticillata* (L.f.) Royle, March 2006. http://nas.er.usgs.gov/taxgroup/plants/docs/hy_verti.html.