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The Impact of the Introduced *Hydrellia* spp. on the Fluridone-Resistant Biotype of *Hydrilla verticillata*

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PURPOSE: The objective of these studies was to determine if leaf-mining flies in the genus *Hydrellia* (*H. pakistanae* Deonier and *H. balciunasi* Bock) introduced as biological control agents would impact the fluridone-resistant biotype of *Hydrilla verticillata* (L.f.) Royle (hydrilla). This was accomplished using a combination of small-scale container bioassays and by examining establishment and impact at field locations.

BACKGROUND: With the development of fluridone resistance in hydrilla in the United States (MacDonald et al. 2001, Michel et al. 2004, Poovey et al. 2005), questions have been raised concerning whether or not genetic changes in the plant would influence the application and impact of other management techniques used for its control. One management method that may be particularly sensitive to genetic changes is the use of introduced hydrilla leaf-mining flies. Fly feeding and development are directly linked to a variety of physiological plant properties including nutrition, gustatory chemicals, olfactory cues, etc. All of these properties may be influenced by genetic changes that led to the development of a fluridone-resistant biotype.

MATERIALS AND METHODS

Bioassays. A series of bioassays were conducted at the U.S. Army Engineer Research and Development Center (ERDC), Vicksburg, MS, from February to June 2006. The experiments used 3.5-L polycarbonate containers (18-cm diameter by 21-cm height) filled with 50 g wet weight (towel-blotted) hydrilla. Two biotypes of hydrilla were used; non-fluridone-resistant (or susceptible) and fluridone-resistant. Both biotypes of hydrilla were obtained from cultures maintained at ERDC. Non-fluridone-resistant hydrilla was obtained from long-term cultures that had never been exposed to fluridone. Fluridone-resistant hydrilla was collected from sites in Florida where hydrilla was known to be highly resistant to fluridone applications.[6] Both biotypes of hydrilla were inoculated by addition of ca. 3 to 5 g of hydrilla containing 50 *Hydrellia pakistanae* eggs. The eggs were obtained from the ERDC greenhouse-based colonies grown on hydrilla that had never been exposed to

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fluridone. As fly emergence occurred, the number of adults was enumerated and percent emergence determined by dividing the number of adults over 50 (the number of eggs originally added) times 100. The number of days to first adult emergence, an indicator of developmental time, was also recorded. The experiment was repeated seven times with each set containing one replication of each biotype and was accomplished on separate dates. Therefore, date was used as the blocking factor. Blocking on date allowed a larger number of replications when fly numbers from research colonies were low. The data were analyzed using Analysis of Variance (ANOVA) in a randomized complete block design (Statistica version 8.0, Statsoft, Tulsa, OK). Significant differences were noted at a = 0.05 unless noted otherwise.

Field Releases. Lake Okahumpka, FL is a small (approximately 500-acre) lake located in the central portion of the Florida peninsula (Figure 1). Hydrilla has been a constant problem for many years and was the target of a series of whole lake fluridone treatments. Over time, these multiple whole lake fluridone treatments resulted in the development of fluridone resistance, which was discovered in 2003 (Michel et al. 2004). The lake was selected for field release of the leaf-mining flies since it contains a high proportion of the resistant hydrilla biotype, is easily accessible, and is relatively small, allowing for better whole lake monitoring of the fly population and associated impact across the entire lake.

Figure 1. Satellite image of Lake Okahumpka, FL (Google Earth Pro 2009).

Beginning in August 2005, releases of both *H. pakistanae* and *H. balciunasi* immatures were made in from one to three different locations on Lake Okahumpka, FL. The majority of the releases were composed of *H. pakistanae* (Table 1) based mainly on periodic collection of adults. Flies were obtained from two fluridone-free sources: (1) colonies maintained in outdoor ponds at ERDC, Vicksburg, MS, and (2) the Lewisville Aquatic Ecosystem Research Facility (LAERF), Lewisville, TX. For each release *Hydrellia* spp. were harvested by collecting the canopied portion of the plant, packaged in insulated coolers, and shipped overnight express to minimize the time spent out of water. The hydrilla containing immature *Hydrellia* spp. was placed into floating screened cages approximately 78 cm by 78 cm to prevent release of cultured hydrilla into the lake. Hydrilla was removed from the cages within three weeks to prevent escape of turions. Such methods were employed because of a concern by the Florida Department of Environmental Protection of introducing a new and potentially more damaging form of hydrilla into Florida systems. From 2005 to 2008 over 3,000,000 immature flies were shipped and released in this manner.

Table 1. Numbers of *Hydrellia pakistanae* and *H. balciunasi* released in Lake Okahumpka, FL beginning in summer 2005.

Date	Number Released
8/8/2005	369,120
8/29/2005	1,521,300
7/31/2006	788,911
6/24/2008	141,808
7/8/2008	252,947
8/11/2008	106,400
11/3/2008	111,500
8/17/2009	121,691
TOTAL	**3,413,677**

Monitoring. Random collections of approximately 2 kg wet weight of hydrilla were made from each release site on the lake at various periods, mainly during the active growing season (June to November). Hydrilla was shipped either to the ERDC or LAERF where stem pieces ranging in length from 10 cm to 20 cm were chosen at random and weighed, and stem length was measured. The number of immatures and associated fly-damaged leaves were counted using a stereo microscope at 7X to 10X magnification. Differences were determined using Analysis of Variance (ANOVA) and a Newman-Keuls post-hoc test was used to determine significant differences between means (Statistica version 8.0, Statsoft, Tulsa, OK). Significant differences were made at a = 0.05 unless noted otherwise.

In addition, a point-intercept sampling method was employed to visually determine hydrilla coverage and fly distribution throughout the entire lake. A grid of points was developed based on spacing of approximately 130 m, providing a total of about 200 points, depending on sampling date. The grid spanned the entire lake. Hydrilla coverage, fly numbers, and associated damage were determined at each point for various time periods, usually twice per year. Spatial analysis was used in conjunction with ArcGIS (ESRI, Redmond, CA) to visually portray hydrilla coverage and fly distribution. ArcGIS visual representation was used through April 2007. The point intercept sampling method was continued through 2008 with more continuous sampling accomplished mainly with random collection procedures. Differences were determined using ANOVA and the Scheffe test based on its conservatism in determining significant differences (Statistica Version 8.0, Statsoft, Tulsa, OK). Significant differences were made at a = 0.05 unless noted otherwise.

RESULTS AND DISCUSSION: Bioassays under greenhouse conditions did not reveal differences in leaf-mining fly populations between the hydrilla biotypes. Percent emergence as a measure of developmental success was not significantly different and averaged from 47 percent to 55 percent on fluridone-resistant and susceptible hydrilla, respectively (Figure 2a). Similarly, days-to-first-emergence as an indicator of developmental time was not significantly different, averaging about 25 days for susceptible and 26 days for resistant (Figure 2b). These values are similar to that reported previously for *Hydrellia* spp. reared under greenhouse conditions (Freedman et al. 2001) and indicate that there are no major physiological differences between the biotypes that impact fly development and emergence.

The real test of differences between the hydrilla biotypes was in actual field establishment, population increase, and impact. Large numbers of individuals (both *H. pakistanae* and, to a lesser extent, *H. balciunasi*) were introduced at Lake Okahumpka, FL, in multiple episodes over a 4-year period beginning in August 2005 (Table 1). During the first visit to the lake in 2004, fly populations appeared low to non-existent despite previous releases (Figure 3; Center et al. 1997). Initial sampling occurred during early winter and low populations were expected based on years of sampling at other sites throughout the country (unpublished data). At a subsequent visit in July 2005, fly populations were detected prior to additional introductions. However, numbers of individuals and associated damage were low, averaging less than 200 immatures per kilogram with corresponding damage of close to 1 percent damaged leaves and less than 20 percent of stems containing at least some form of leaf-mining (Figure 3).

Beginning in August 2005 and continuing to August 2009, fly introductions were made mainly during the active growing season for hydrilla. Following the introductions, populations and associated damage remained constant at less than 200 immatures per kilogram with damage averaging about 1 percent leaf damage and from 20 percent to 30 percent of the stems containing damaged leaves (Figure 3).

In the spring of 2008 significant increases were observed in immatures/kilogram, damaged leaves, and percent of stems containing damaged leaves. For example, numbers of immatures/kilogram were greater than 1200 immatures/kilogram toward the latter part of the 2008 growing season (Figure 3a). Percent damaged leaves also increased eight-fold from prior years to about 8 percent (Figure 3b) with percent of stems damaged peaking in August 2008 to almost 55 percent (Figure 3c). Based on this information, the flies appeared to be well established and were evidently not impacted by the fluridone-resistant hydrilla. The advent of additional releases over multiple years evidently allowed for increases in fly numbers and associated damage to hydrilla.

Lake Okahumpka was periodically subjected to chemical applications throughout the fly introduction period, with treatments confined mainly to the middle of the lake and access points. Chemical applications consisted of various formulations of endothall. A large treatment was made in March 2006, where hydrilla in the middle of the lake and beyond was heavily impacted as indicated by Figure 4. Regrowth of hydrilla occurred through November 2006, although some areas remained free of hydrilla. In January 2007, personnel at the U.S. Army Engineer District, Jacksonville, contacted ERDC regarding an almost complete disappearance of hydrilla for unknown reasons. In April 2007, a point-intercept sampling was conducted that confirmed substantial reduction of hydrilla throughout the lake. Based on personal communication with Dr. Michael Netherland

(ERDC, Gainesville, FL), endothall did not reach beyond the middle of the lake and impact was non-existent throughout the lake at the time of hydrilla disappearance.

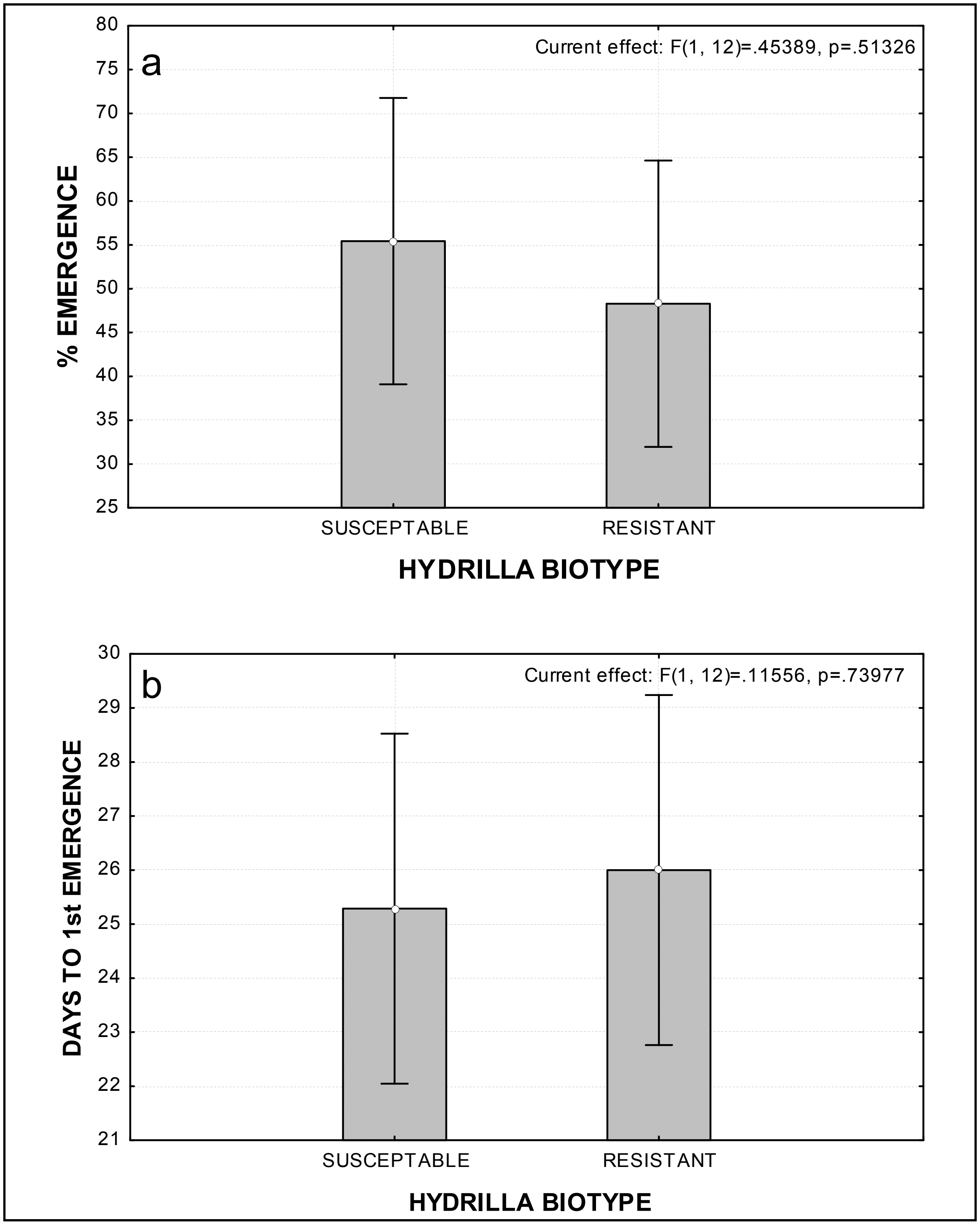

Figure 2. Percent emergence (a) and days to first emergence (as an indicator of developmental time) of *H. pakistanae* under greenhouse bioassay conditions for both the fluridone-susceptible and fluridone-resistant biotypes of hydrilla. Bars represent the 95-percent confidence interval. No significant differences were noted with $p > 0.05$. Actual p-values and df are displayed on each graph.

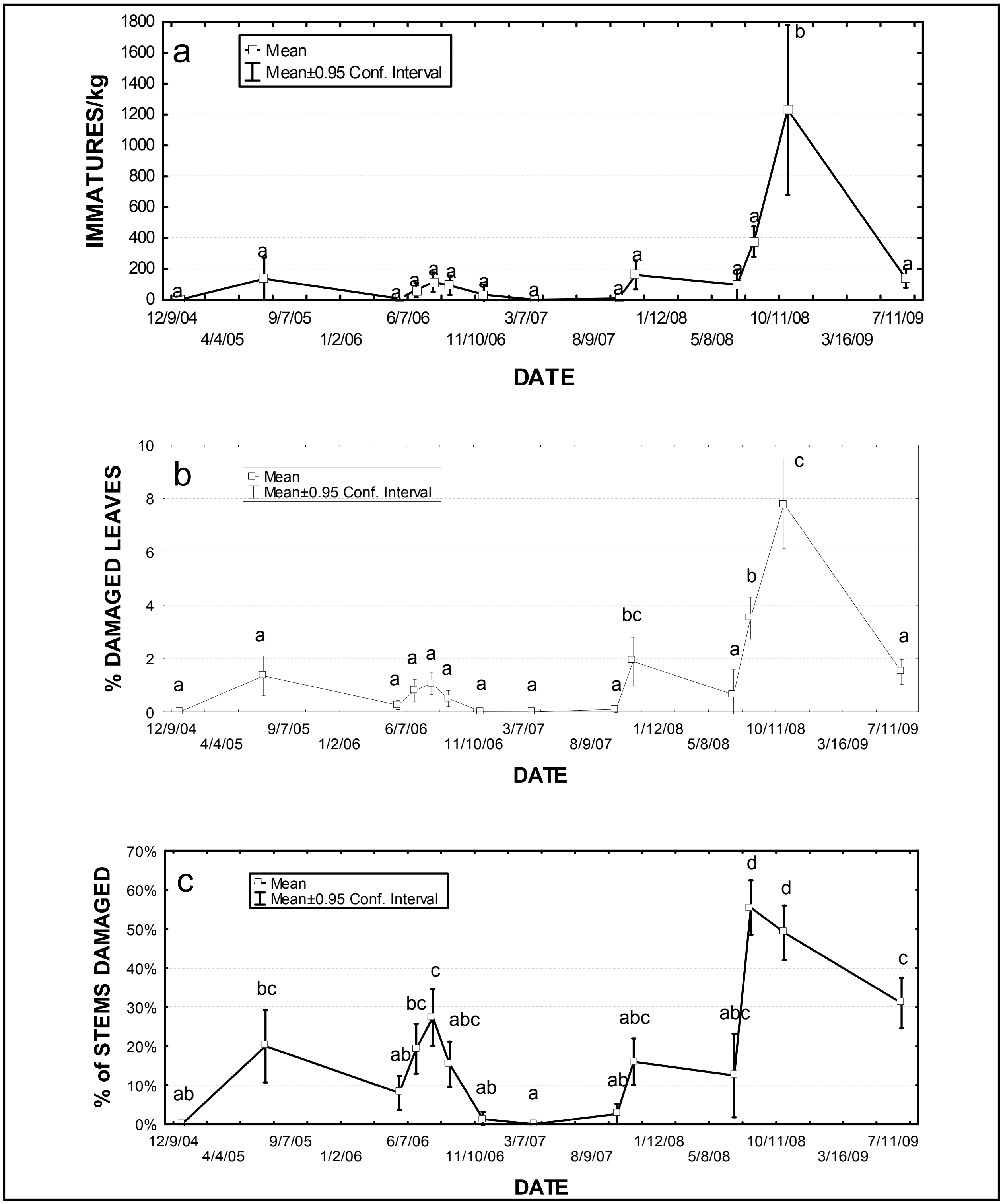

Figure 3. Numbers of immatures per kilogram fresh weight of hydrilla (a), percent damaged leaves (b), and percent of stems containing some form of damage (c) based on stem sampling procedures. Means followed by the same letter are not significantly different at p = 0.05 with df of 9, 2025. Mean comparisons performed using Newman-Keuls.

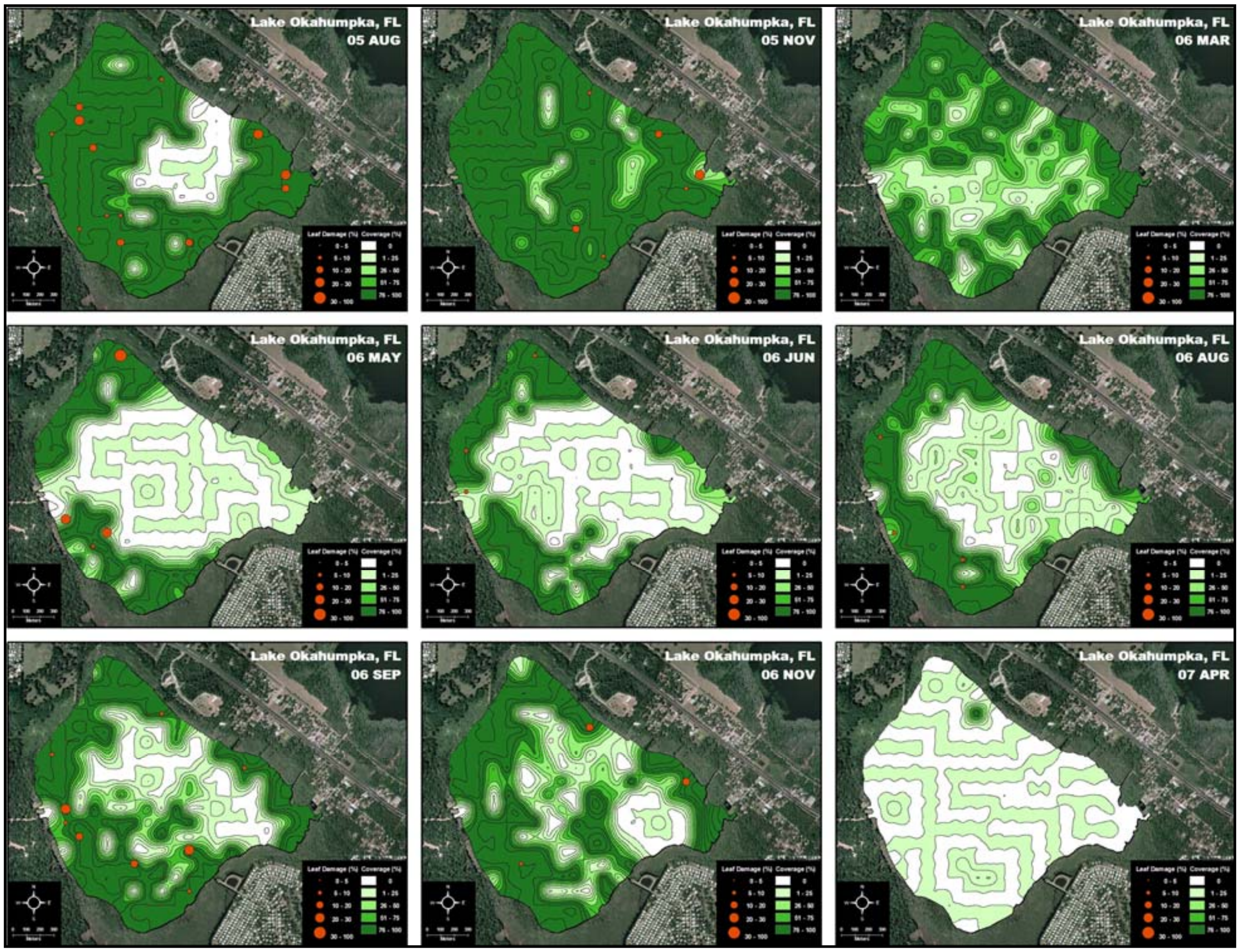

Figure 4. Spatial analysis of Lake Okahumpka from August 2005 through April 2007 showing estimated hydrilla coverage (various shades of green where darker greens indicate higher coverage values) and percent leaf damage by *Hydrellia pakistanae* (as indicated by red circles where larger circles are higher leaf damages). Maps were drawn based on a point sampling method of from 150 to 250 points. Note decreases in hydrilla coverage following endothall application in May 2006 followed by rapid regrowth by November 2006. Note almost complete disappearance of hydrilla between November 2006 and April 2007 for unknown reasons.

An alternative way of examining changes in hydrilla coverage is by exploring the point-intercept data directly. In this regard, the percentage of points with hydrilla present was at its highest (~ 98 percent) during 2005 (Figure 5). In May 2006, hydrilla coverage in the lake was reduced to < 40 percent of the points sampled due to the chemical application during March 2006. Hydrilla quickly increased almost linearly from May 2006 through November 2006 followed by reductions to almost zero during the period November 2006 through the next sampling in April 2007. This is similar to what was shown visually in Figure 4. Note that hydrilla coverage increased dramatically to about 50 percent of the points sampled during the July 2008 sampling.

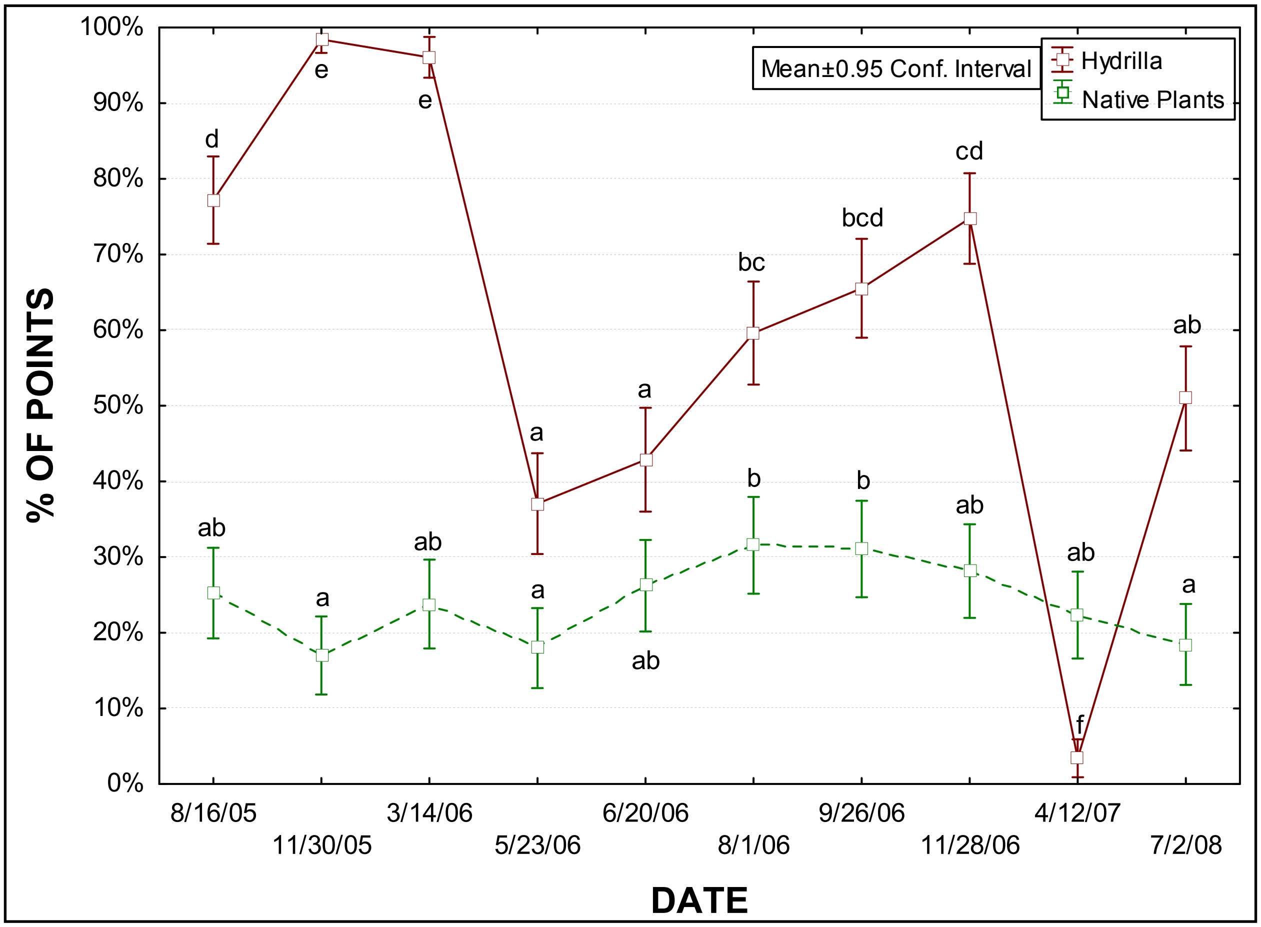

Figure 5. Percentage of points containing either hydrilla or native plants on Lake Okahumpka from August 2006 through July 2008. Means followed by the same letter are not significantly different at p = 0.05 using Scheffe test. Degrees of freedom for the ANOVA were 9, 2025.

Reasons for such a dramatic decline are unknown. Unfortunately, no sampling was conducted during the periods of sharp decline (i.e., November 2006 through January 2007) so whether the decline was sudden or occurred slowly over the entire period cannot be determined. It is important to note that such declines have never been observed previously (unpublished data) on Lake Okahumpka during the winter months. An obvious environmental factor that could explain the decrease would be unusually cold temperatures, especially those in the freezing range. However, temperatures were seasonally within range as indicated by no freezing temperatures achieved throughout the period August 2006 through January 2007 (Figure 6). Hence, cold temperatures were apparently not the cause.

Declines were observed in the hydrilla population with native plants remaining relatively constant throughout the period of decrease (Figure 5). It seems that the factor responsible for the decline only affected hydrilla. Native plant species observed mainly consisted of *Nymphaea odorata* Ait.*,* *Nuphar lutea* (L), *Typha* spp., *Nelumbo lutea* Willd., *Limnobium spongia* (Bosc) Rich. ex Steud., *Hydrocotyle* spp., *Potamogeton illinoensis* Morong, *Utricularia* spp., *Najas* spp., *Vallisneria americana* Michx, and *Azolla* spp. Native plants taken as a single group remained at about 20 percent to 30 percent of the points sampled throughout the period of decline. In fact, native plants increased as hydrilla populations declined during the period of recovery in 2007. As hydrilla populations increased to over 60 percent of points, sampled native plants decreased.

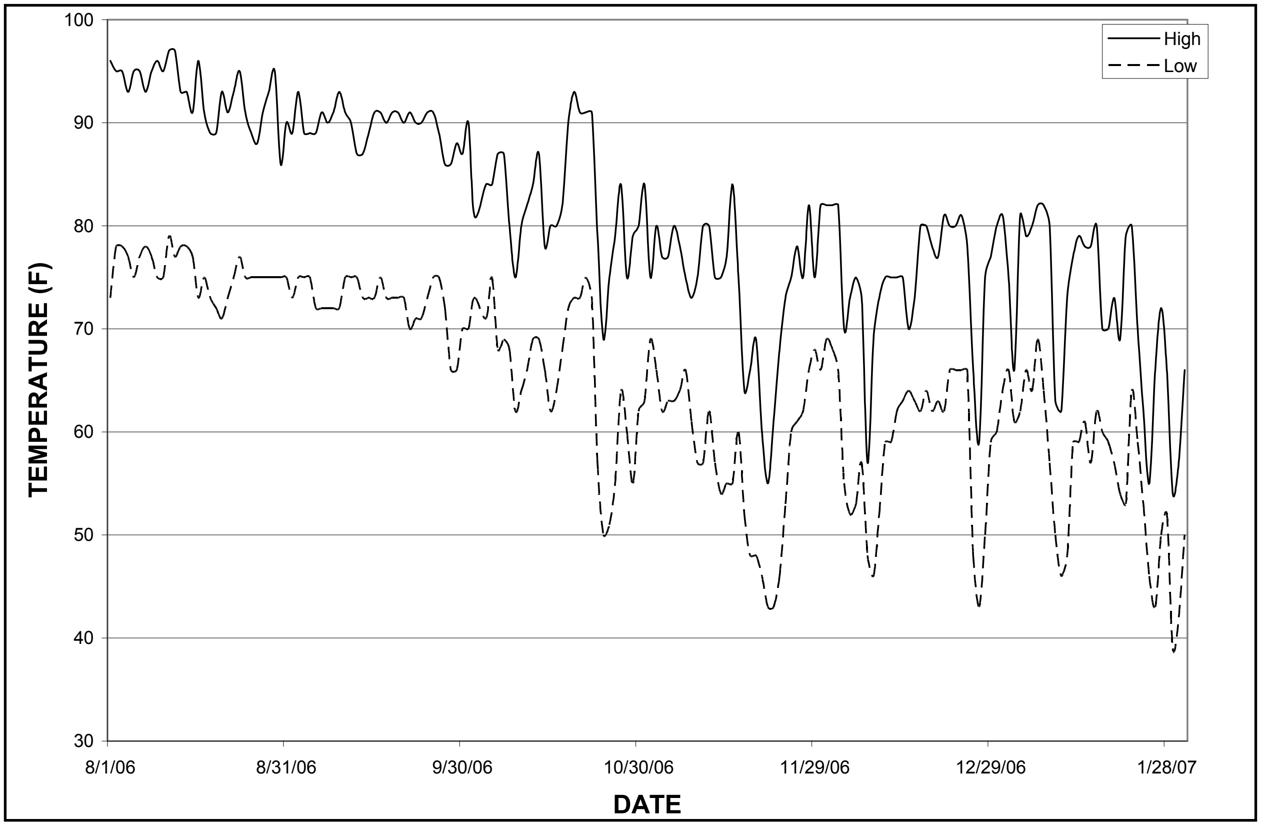

Figure 6. Both high and low temperatures recorded daily near Orlando, FL for the period August 1, 2006 through January 31, 2007. Note that no freezing temperatures were recorded throughout the period, with lowest temperatures never dropping below 39 °F.

The presence of leaf-mining flies is a factor that may have impacted hydrilla on the lake. However, there is little evidence that the flies were responsible for the decline. It is important to note that fly populations remained relatively low throughout the period prior to and right up to the decline, although small but significant increases in percent of stems damaged (~ 8 percent) occurred during 2006 (Figure 3). It is also important to note that fly damage was observed throughout the lake, especially during September 2006, with some areas having high amounts of damage (Figure 4). However, fly populations and associated leaf damage at these levels rarely seem to impact hydrilla to such an extent (unpublished data). Since no information is available from November 2006 until qualitative observations in January 2007 and quantitative sampling in April 2007, fly populations could have conceivably increased to the point of severely impacting the hydrilla during this time. Significant declines in hydrilla populations have been observed previously after release of high numbers of the leaf-mining flies with only limited evidence of increasing fly populations and damage. This has been documented on Lake Ouachita, AR,[1] Coleto Creek Reservoir, TX

[1] Personal communication. 2005. J. Cantrell, Project Biologist, U.S. Army Engineer District, Vicksburg, Lake Greeson Field Office, Mountain Pine, AR.

(unpublished data), Lake Patrick, FL,[1] Lake Raven, TX (unpublished data), and Sheldon Reservoir, TX (unpublished data), among others. Alternatively, low to moderate fly damage may have combined with other unidentified environmental factors and possible pathogen impacts that led to significant hydrilla declines. More research is warranted.

In summary, these results indicate that the introduced leaf-mining flies are not affected physiologically by developing on fluridone-resistant hydrilla. This is evidenced by no differences in developmental time or emergence success using bioassay experimentation. In addition, establishment and increasing population levels of the flies on Lake Okahumpka, a lake known to have one of the highest occurrences of resistant hydrilla, provides further proof that the fluridone-resistant form of hydrilla does not impact leaf-mining flies.

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> Grodowitz, M., C. Ashton, C. DuBose, J. Freedman, J. G. Nachtrieb, and N. Harms. 2010. *The impact of the introduced Hydrellia spp. on the fluridone-resistant biotype of Hydrilla verticillata.* APCRP Technical Notes Collection, ERDC/TN APCRP-BC-18. Vicksburg, MS: U.S. Army Engineer Research and Development Center.

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