Combining Plant Pathogenic Fungi and the Leaf-Mining Fly, *Hydrellia pakistanae*, Increases Damage to Hydrilla

YASSER M. SHABANA[1], J. P. CUDA[2], AND R. CHARUDATTAN[3]

ABSTRACT

Four fungal species, F71PJ *Acremonium* sp., F531 *Cylindrocarpon* sp., F542, *Botrytis* sp., and F964 *Fusarium culmorum* [Wm. G. Sm.] Sacc. were recovered from hydrilla [*Hydrilla verticillata* (L. f.) Royle] shoots or from soil and water surrounding hydrilla growing in ponds and lakes in Florida and shown to be capable of killing hydrilla in a bioassay. The isolates were tested singly and in combination with the leaf-mining fly, *Hydrellia pakistanae* (Diptera: Ephydridae), for their capability to kill or severely damage hydrilla in a bioassay. For the fungus plus insect treatments, two ready-to-hatch *H. pakistanae* (HP) eggs were placed on hydrilla shoot in each assay tube. The shoots were rated for insect damage 21 days later and grouped into two damage levels, 15% (HP1) and 25% (HP2). The insect-damaged hydrilla shoots were then exposed to each fungus added to the water in the assay tube at a final concentration of 6×10^4 to 1×10^6 propagules per ml. In two of the fungus-insect combinations (HP2 plus *Acremonium* sp. and HP2 plus *F. culmorum*), the level of damage on hydrilla was increased in comparison with the damage caused by either agent alone. Among the isolates tested alone, the *F. culmorum* isolate was the most effective. The maximum damage to the shoots by this isolate was achieved at 20 to 30 C as compared to 15 or 35 C. The synergistic effect of combining *F. culmorum* and *H. pakistanae* was 1.22 to 1.56 times greater, respectively, for the two herbivory levels of HP1 and HP2, than the effect of the fungus alone. The damage levels were 3.21 to 2.78 times greater for the *F. culmorum* plus *H. pakistanae* treatments compared to the insect alone at the respective levels of herbivory. Thus, the combined use of this pathogenic fungus and the fly appears a promising approach for integrated control of hydrilla.

Key words: Hydrilla verticillata, Hydrellia pakistanae, Fusarium culmorum, Acremonium sp., *Botrytis* sp., fungi, biocontrol, insect-pathogen synergism, integrated control.

INTRODUCTION

Hydrilla is a submersed freshwater macrophyte that belongs to the family Hydrocharitaceae. It is widely distributed in the African-Asian region (Pieterse 1981) and has become one of the most invasive weeds of waterways in tropical and subtropical regions of the world (Langeland 1996). Hydrilla was first reported on the west coast of Florida in 1958 (Blackburn et al. 1969). Since then, it has spread throughout Florida and to many other states (Langeland and Burks 1998). Two factors enable hydrilla to out-compete other submersed aquatic plants: it thrives under low light conditions and it produces an abundance of viable vegetative propagules (Bowes et al. 1977). Hydrilla forms dense surface mats that can severely reduce water flow, interfere with boating, water sports, fishing, and navigation. It can also significantly reduce the water holding capacity of storage ponds. From 1980 to 1993, approximately $39 million was spent in Florida to manage hydrilla in the state's public waters (Schardt 1997), mainly for chemical herbicides containing copper, diquat, endothall, or fluridone as an active ingredient (Langeland 1996).

Biological control of hydrilla has been a high priority in Florida since the 1970s. Among the biocontrol agents released to control hydrilla is *Hydrellia pakistanae* Deonier (Diptera; Ephydridae [HP]), a small fly native to tropical and temperate regions of Asia (Deonier 1993). The leaf-mining larvae of this fly cause extensive damage to hydrilla (Baloch and Sana-Ullah 1974, Deonier 1978, Baloch et al. 1980, Buckingham et al. 1989). Host-range tests conducted in Pakistan (Baloch and Sana-Ullah 1974) and in Florida (Buckingham et al. 1989) demonstrated that *H. pakistanae* is highly specific to hydrilla. The fly was released in Florida in 1987, but to date its population density has never exceeded more than 15 adults per m² of hydrilla stands and the level of damage has not been more than one-fifth the level estimated to be necessary to produce a significant impact on the plant (Wheeler and Center 2001).

Several plant pathogens have been discovered and shown to be capable of killing hydrilla under controlled conditions (Charudattan 1990, Verma and Charudattan 1993, Shearer 1998, Nachtigal and Pitelli 1999, Shabana et al. 2003). A multicomponent, integrated control approach rather than a single control tactic offers the best prospect for long-term management of aquatic weeds (Pieterse 1977; Charudattan 2001). In this respect, the potential to exploit synergistic interactions between insects and plant pathogens or a pathogen and a herbicide has been suggested as an option (Charudattan et al. 1978, Charudattan 1986, Netherland and

[1]Corresponding author: Plant Pathology Department, Faculty of Agriculture, Mansoura University, El-Mansoura, Egypt, ymsh@mans.edu.eg & yassershabana@hotmail.com.

[2]Entomology and Nematology Department, University of Florida, Gainesville, FL, USA.

[3]Plant Pathology Department, University of Florida, Gainesville, FL, USA. Received for publication October 16, 2002 and in revised form June 11, 2003.

Shearer 1996, Caesar 2000). However, no previous studies have attempted to develop an integrated control system for hydrilla by combining pathogens and insects as biocontrol agents. The specific objective of this study was to evaluate the feasibility of integrating fungal pathogens indigenous to Florida with *H. pakistanae* for the management of hydrilla.

MATERIALS AND METHODS

Evaluation of four fungal species and H. pakistanae *for controlling hydrilla in a bioassay*. Four fungal species, F71PJ *Acremonium* sp., F531 *Cylindrocarpon* sp., F542, *Botrytis* sp., and F964 *Fusarium culmorum* [Wm. G. Sm.] Sacc. were recovered from hydrilla shoots or from soil and water surrounding hydrilla growing in ponds and lakes in Florida. Each fungal species was capable of killing hydrilla in a bioassay (Shabana et al. 2003). The isolates were tested singly and in combination with the leaf-mining fly, *H. pakistanae*, for their ability to kill or damage hydrilla in a test-tube bioassay. The bioassay system consisted of glass tubes with dimensions of 22 mm diameter by 150 mm in length. Each tube contained 49 ml of sterile tap water and one healthy, terminal shoot of dioecious female hydrilla 9 cm in length. Two 1- to 4-day-old eggs of *H. pakistanae*, collected from a colony maintained at the University of Florida, Gainesville, were transferred with a fine brush onto the top leaves of the hydrilla shoot in each assay tube. The tubes were then covered with sterile clear plastic caps and placed under diurnal light (12 h; 137 µE/m².s) at 25 ± 2 C for 3 weeks. After 3 weeks, the hydrilla shoots were separated according to the level of *H. pakistanae* damage into two groups with 15 and 25% damage (percentage of stem tissue mined and turned chlorotic). Sufficient numbers of shoots were infested with *H. pakistanae* to obtain the required number of replicates for this experiment. For the fungus plus insect treatments, hydrilla shoots at each level of insect damage were inoculated with each fungus. One ml of a suspension of fungal propagules was added to the contents of a tube to give a final inoculum concentration ranging from 6×10^4 to 1×10^6 propagules per ml of water in each tube. Three sets of controls [hydrilla tubes with no *H. pakistanae* or fungus, HP1 (15% *H. pakistanae* damage), HP2 (25% *H. pakistanae* damage)] were maintained under the same conditions. Six replicates, arranged in a completely randomized design, were used per treatment. Hydrilla was rated for disease and/or damage severity 7 days after inoculation with the fungi.

Further evaluation of F. culmorum *plus* H. pakistanae *combinations for controlling hydrilla in 3.7-L jars*. Healthy dioecious female hydrilla were collected from the Manatee Springs, Levy County, Florida. Ten apical cuttings of shoots, approximately 12-cm long, were planted in surface-sterilized 3.7-L glass jars containing a 10-cm layer of washed, sterilized sand and filled with 3.0 L of sterilized 5% Hoagland's solution (Hoagland and Arnon, 1950) amended with 0.1% KHCO₃ and adjusted to pH 6.5 with 1 N HCl or 1 N NaOH. Each jar was then covered with a sterile 15-cm-diam plastic petri plate cover and placed under diurnal light at 12 h, 190 µE/m².s and a temperature of 25 ± 2 C in a plant growth room. Cuttings in jars were allowed to acclimate for 2 days before the *H. pakistanae* eggs were placed on them. Twenty 1- to 4-day-old eggs of *H. pakistanae*, collected from the colony in Gainesville, were released carefully onto the top leaves of hydrilla shoots in the jars. After day 11

and 28 following egg placement on shoots, plants grouped in two levels of *H. pakistanae* damages (HP1, 15% damage and HP2, 25% damage) and were inoculated with conidia of *F. culmorum* (isolate F964). For the *F. culmorum* plus *H. pakistanae* treatments, hydrilla shoots at both HP1 and HP2 damage levels were inoculated with *F. culmorum*. A 10-mL conidial suspension was added to a hydrilla-containing jar to give a final concentration of 1×10^6 conidia per mL of liquid in the jar. Three sets of controls, namely hydrilla with no *H. pakistanae* or fungus, with HP1 and HP2 level of damage were used and maintained under the same conditions. The jars, with four replicates per treatment, were arranged in a completely randomized design and maintained for 4 weeks under the same conditions as described above. Hydrilla was rated for disease/damage severity two weeks after inoculation with the fungus.

Effect of temperature on F. culmorum *plus* H. pakistanae *combinations*. Hydrilla in tubes, inoculated with *F. culmorum* alone, the *H. pakistanae* alone, and the *F. culmorum* plus *H. pakistanae* combination, were incubated at constant temperatures of 15, 20, 25, 30, and 35 C. These tubes were prepared as described previously in the bioassay experiment and the tubes were placed under diurnal light (12 h; 210 µE/m².s) in five growth chambers. Two 1- to 4-day-old eggs of *H. pakistanae* were carefully placed on the top leaves of hydrilla shoot in each tube. *H. pakistanae* eggs were placed on the leaves 11 and 28 days prior to fungal inoculation to create HP1 and HP2 levels of damage on hydrilla shoots. The hydrilla tubes were returned to the respective temperatures and the damage levels from herbivory were recorded at the time of fungal inoculation (time zero). *H. pakistanae* damage was visually rated on each hydrilla shoot for each release time, as mean percent shoot damage from 12 replicates (six replicates of *H. pakistanae* only and six replicates of *H. pakistanae* to be inoculated with the fungus).

Inoculation with the fungus was carried out by adding a conidial suspension of *F. culmorum* isolate F964 to the water in the tube to give a final inoculum concentration of 1×10^6 conidia/ml. After inoculation, the tubes were returned to their respective incubation temperatures. Hydrilla was rated for disease-damage severity 15 days after fungal inoculation. Four sets of controls (with no *H. pakistanae*, or fungus, HP1, HP2 and fungus alone) were maintained at each temperature condition. The replicates were arranged in a completely randomized design within each temperature (incubator). Damage was rated as percent shoot biomass affected by chlorosis, lysis, and disintegration.

Statistical analysis. The data were analyzed using Statistical Analysis System (SAS Institute, 2000). All multiple comparisons were first subjected to ANOVA, and Least Significant Difference (LSD) separation method was used for pairwise comparisons. Ratings of pathogen and/or insect damages at a given temperature were pooled and analyzed. Tukey's Studentized Range test was used for comparison of damage at a given temperature. Contrast statements were used for pairwise comparisons in the study evaluating the four fungi and *H. pakistanae*.

RESULTS

Effects of four fungal species and H. pakistanae *on hydrilla shoots in the test-tube assay*. Hydrilla shoots inoculated with the

fungal species F71PJ *Acremonium* sp., F531 *Cylindrocarpon* sp., and F964 *Fusarium culmorum* developed disease symptoms in 6 to 8 days after inoculation and after 8 to 10 days when inoculated with F542 *Botrytis* sp. Reddening of leaf margins and decline of chlorophyll were characteristic symptoms associated with *Acremonium* sp. (F71PJ) while severe chlorosis and tissue lysis were typical symptoms induced by *Cylindrocarpon* sp. (F531) and *F. culmorum* (F964). Browning of hydrilla shoots was a distinctive symptom of *Botrytis* sp. (F542). Loss of leaf tissue due to larval mining and chlorosis was the typical type damage caused by *H. pakistanae* (Figure 1).

The levels of damage caused by fungal isolates F531 and F964 alone, were greater than that caused by *H. pakistanae* alone (Table 1). Six of eight fungus-*H. pakistanae* combinations produced higher levels of damage than that caused by *H. pakistanae* alone. Two treatments, involving fungus-*H. pakistanae* combinations (HP2 plus *Acremonium* sp. and HP2 plus *F. culmorum* produced more damage than the corresponding fungus-alone treatments. The most damaging combination was HP2 plus *F. culmorum*, which caused 88% shoot damage within 1 week after inoculation (Table 1). The hydrilla shoots treated with this combination were completely killed after 12 days in this bioassay.

Evaluation of F. culmorum *plus* H. pakistanae *combinations for controlling hydrilla in the jar assay.* *Fusarium culmorum* was proven to be the most effective fungus capable of killing hydrilla shoots (Table 1) and hence was selected for further evaluation. It rapidly sporulated and produced a large quantity of spores in laboratory cultures, which facilitated its use in these studies. The glass jar based bioassay provided a larger test system allowed root and shoot growth of hydrilla.

The treatment combination HP2 plus *F. culmorum* produced the highest level (97.5%) of hydrilla damage within two weeks after the addition of the fungus to the jar (*P* = 0.0001) (Table 2, Figure 2). The second and third best treatments were HP1 plus *F. culmorum* (76% shoot damage) and the fungus *F. culmorum* alone (62.5% shoot damage), respectively (Table 2).

Effect of temperature on F. culmorum *plus* H. pakistanae *treatments.* At the time of fungal inoculation (time zero), average damage ratings (*n* = 12) of shoots in the HP1 treatment at
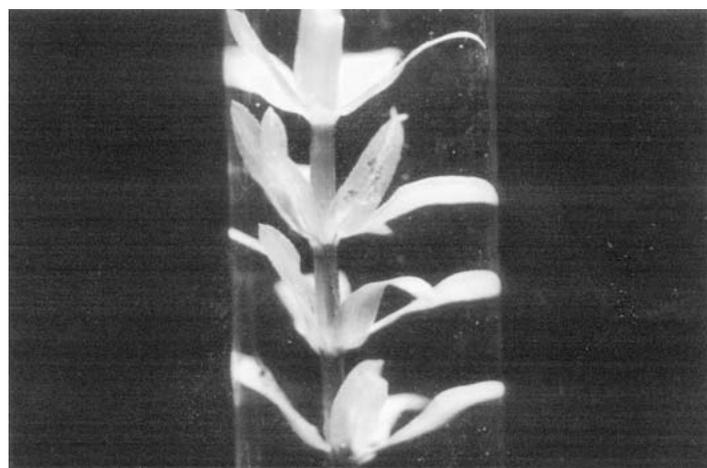

Figure 1. Leaf mining damage caused by *Hydrellia pakistanae* on hydrilla.

TABLE 1. DISEASE/DAMAGE RATING OF INSECT-FUNGUS COMBINATIONS ON HYDRILLA AT 1 WEEK AFTER INOCULATION WITH THE FUNGI IN A TEST-TUBE BIOASSAY.

Treatment	% Damage[a]
Control I (no fungus/no insect)	2.20 i
Control II (*Hydrellia pakistanae* alone)[b]:	
HP1 (15% *H. pakistanae* damage)	20.0 gh
HP2 (25% *H. pakistanae* damage)	31.3 efgh
Control III (fungus alone):	
Acremonium sp. (F71PJ)	31.3 efgh
Cylindrocarpon sp. (F531)	52.5 bcd
Botrytis sp. (F542)	33.3 defgh
Fusarium culmorum (F964)	55.8 bcd
Insect-fungus combinations:	
HP1 + *Acremonium* sp. (F71PJ)	48.3 cdef
HP1 + *Cylindrocarpon* sp. (F531)	61.6 bc
HP1 + *Botrytis* sp. (F542)	48.3 cdef
HP1 + *F. culmorum* (F964)	55.8 bcd
HP2 + *Acremonium* sp. (F71PJ)	61.6 bc
HP2 + *Cylindrocarpon* sp. (F531)	78.3 ab
HP2 + *Botrytis* sp. (F542)	52.5 bcd
HP2 + *F. culmorum* (F964)	88.3 a

[a]Values represent the means of six replicates. Values followed by the same letter(s) are not significantly different according to Fisher's LSD test (P < 0.05).
[b]Two 1- to 4-day-old eggs of *H. pakistanae* collected in Gainesville were released carefully onto the top leaves of the hydrilla shoot per hydrilla tube. The hydrilla tubes were then covered with sterile clear plastic caps and placed under diurnal light of 137 µE/m².s for 12 h held at 25 ± 2 C for 3 weeks. Hydrilla tubes were then classified, according to damage on hydrilla caused by the insect into two groups of 15 and 25% damage levels = HP1 and HP2, respectively.

TABLE 2. DISEASE/DAMAGE RATING OF INSECT-FUNGUS COMBINATIONS ON HYDRILLA 2 WEEKS AFTER INOCULATION WITH THE FUNGUS IN JARS.[a]

Treatment	% Damage
Control I (no insect/no fungus)	3.00 f
Control II (*H. pakistanae* alone)[b]:	
HP1 (15% *H. pakistanae* damage)	23.75 e
HP2 (25% *H. pakistanae* damage)	35.00 d
Control III (fungus alone):	
Fusarium culmorum (F964)	62.50 c
Insect-fungus combinations:	
HP1 + *F. culmorum* (F964)	76.25 b
HP2 + *F. culmorum* (F964)	97.50 a

[a]Values represent the means of four replicates. Values followed by the same letter(s) are not significantly different according to Fisher's LSD test (P < 0.01).
[b]Twenty 1- to 4-day-old eggs of *H. pakistanae* collected in Gainesville were released carefully onto the top leaves of the hydrilla shoots per hydrilla jar. Hydrilla jars were then covered with sterile clear 15-cm-diameter plastic petri plate covers and placed under diurnal light at 190 µE/m².s for 12 h held at 25 ± 2 C in a plant growth room. Two release dates of 11 or 28 days before inoculation with the fungus of *H. pakistanae* eggs were selected to obtain two levels of insect damage at the time of inoculation with the fungus at 15 and 25% damage levels = HP1 and HP2, respectively.

Figure 2. The effects of *Hydrellia pakistanae* and *Fusarium culmorum* on hydrilla shoots, 2 weeks after inoculation with the fungus. Control, no insect or fungus; HP2, *H. pakistanae* alone; F964, *F. culmorum* alone; and F964+HP2, combination of 25% *H. pakistanae* damage plus *F. culmorum*.

TABLE 3. DISEASE/DAMAGE RATINGS OF INSECT-FUNGUS TREATMENT COMBINATIONS ON HYDRILLA MAINTAINED AT FIVE TEMPERATURE LEVELS 2 WEEKS AFTER INOCULATION WITH THE FUNGAL CONIDIA.

Treatment[a]	Temperature	% Damage[b]
Control (no insect/no fungus)	15	0.83 a
	20	0.83 a
	25	0.00 a
	30	0.00 a
	35	0.00 a
Fusarium culmorum alone	15	29.17 b
	20	56.67 a
	25	55.83 a
	30	50.83 a
	35	43.33 ab
H. pakistanae alone (HP1)	15	3.33 a
	20	9.17 a
	25	8.33 a
	30	8.00 a
	35	5.00 a
H. pakistanae alone (HP2)	15	3.33 b
	20	28.33 a
	25	20.00 ab
	30	22.50 a
	35	20.00 ab
HP1 + *F. culmorum*	15	35.83 c
	20	86.67 a
	25	77.50 ab
	30	62.50 b
	35	40.00 c
HP2 + *F. culmorum*	15	38.33 c
	20	91.67 a
	25	84.17 a
	30	66.67 b
	35	55.83 b

[a]See Materials and Methods for details of experimental set up and damage rating.
[b]Values represent the means of six replicates. Values within a given treatment followed by the same letter(s) are not significantly different according to Fisher's LSD test (P < 0.05).

each level of water temperature were 0 at 15 C, 0.8 at 20 C, 6 at 25 C and 30 C, and 3 at 35 C. At the same time, shoots in the HP2 treatment had mean damage levels of 0.16 at 15 C, 19.2 at 20 C, 15.8 at 25 C, 15 at 30 C, and 5.8 at 35 C. Two weeks later, water temperature range between 15 to 35 C had little or no effect on the levels of shoot damage caused by *F. culmorum* or *H. pakistanae* alone. However, water temperature had an effect on the damage caused by the *H. pakistanae* plus *F. culmorum* treatment at both HP1 and HP2 levels of herbivory (HP1 and HP2) (Table 3). The *H. pakistanae* plus *F. culmorum* treatment combinations caused the most severe damage at 20 and 25 C (Table 3, Figure 3). When data from all treatments at a given temperature were pooled and analyzed, the temperature range between 20 to 30 C provided the most conducive environment for *F. culmorum* and *H. pakistanae* as effective biocontrol agents of hydrilla (Table 4).

DISCUSSION

The results presented herein confirm the findings from a previous study by Shabana et al. (2003) in which *F. culmorum*, among the microorganisms tested, produced the highest lev-

el of damage to hydrilla in laboratory tests. Unlike an isolate of *F. culmorum* isolated in the Netherlands that was reported by Charudattan and McKinney (1978) as a potential biocontrol agent for hydrilla, the *F. culmorum* isolate used in the current study is indigenous to Florida. Thus, the use of an indigenous fungus as a biological control agent should be more acceptable to the public and regulatory agencies than a nonindigenous organism such as the isolate from the Netherlands. However, the host range of the Florida isolate should be determined before it can be presented as a safe biocontrol agent.

The subject of biocontrol of hydrilla with the leaf-mining fly, *H. pakistanae*, has been extensively reviewed by Buckingham et al. (1989), Buckingham and Okrah (1993), Wheeler and Center (1996), Center et al. (1997), and Wheeler and Center (2001). Though *H. pakistanae* is widely established in Florida after its initial release in 1987 (Buckingham 1988, Center et al. 1997), little or no effect on hydrilla stand density has been achieved (Forno and Julien 2000, Wheeler and
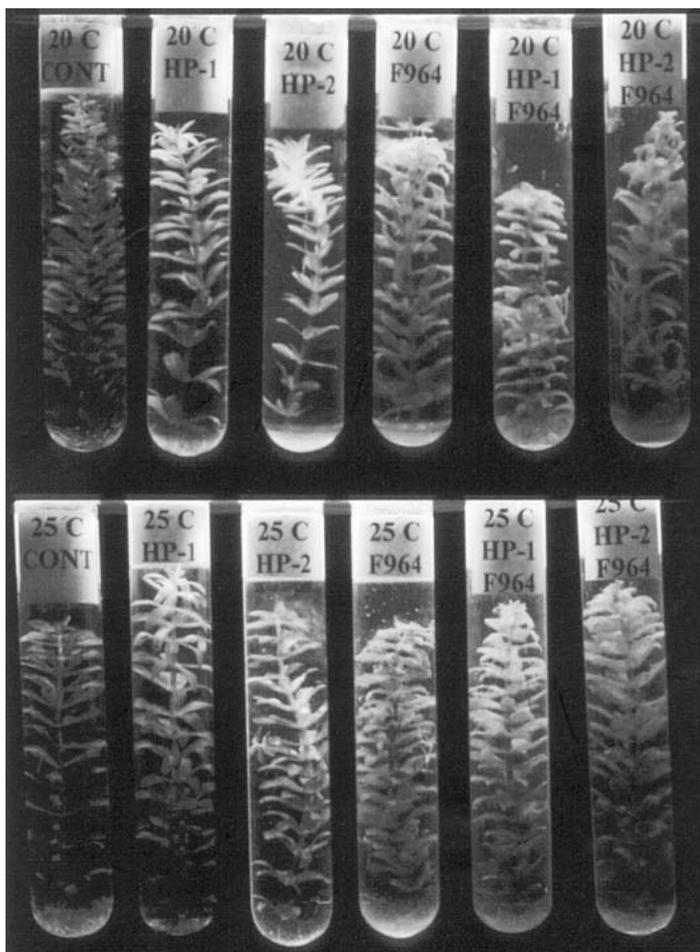
Figure 3. Effects of water temperatures 20 C (top) and 25 C (bottom) on the level of disease damage on hydrilla shoots caused by *Hydrellia pakistanae* and *Fusarium culmorum*. Cont., no insect or fungus; HP-1, the lower level of *H. pakistanae* damage (created when *H. pakistanae* eggs were placed on leaves 11 days prior to fungal inoculation); HP-2, the higher level of *H. pakistanae* damage (created when *H. pakistanae* eggs were placed on leaves 28 days prior to fungal inoculation); and HP+F964 combinations, HP1 plus *F. culmorum* and HP2 plus *F. culmorum*.

Center 2001). One approach to improve the efficacy of insect natural enemies may be to incorporate one or more microbial pathogens into the system. Based on the results presented here, it may be possible to achieve a higher level

TABLE 4. EFFECTS OF TEMPERATURE TREATMENT ON OVERALL PERFORMANCE OF *FUSARIUM CULMORUM* AND/OR *HYDRELLIA PAKISTANAE*.

Temperature (°C)	Average damage (%)
15	18.47 c[a]
20	45.56 a
25	40.97 a
30	35.07 ab
35	27.36 bc

[a]Values represent the means of six replicates within a given temperature. Values followed by the same letter(s) are not significantly different according to Tukey's Studentized Range test (P < 0.05).

of hydrilla control through integration of insects and pathogens. It appears that leaf mining by *H. pakistanae* not only reduces the photosynthetic capacity of the plant but also facilitates fungal infection of leaves and stems and onset of disease-induced stress. *H. pakistanae* can also aid the spread of the pathogen by passively transporting the pathogens' propagules. Data presented in Tables 1-3 show that the level of shoot damage caused by the synergistic effect of insect-pathogen combination was 1.6 times and 2.8 to 3.0 times more than the fungus alone or insect alone, respectively.

Higher levels of hydrilla control were achieved when the fungal pathogen was applied to hydrilla after inducing a higher level (25%) of insect damage. This suggests that scheduling of the application of the microbial pathogen may be crucial in enhancing synergistic effects on hydrilla control.

Previous studies have shown that the surface-water temperature is a critical to the development of *H. pakistanae* on hydrilla (Cuda and Fox 1997, Wheeler and Center 2001). In our study, water temperature had insignificant effect on fungus but had a significant effect on insect's ability to damage hydrilla. Also, the surface-water temperature influenced the level of damage caused by the insect plus fungus combination. Maximum damage was obtained with the combination HP2 plus *F. culmorum* at 20 and 25 C compared to 15 or 30 and 35 C. Lower or higher water temperatures presumably may have negative effects on the development and hydrilla shoot damaging activity of both insect and pathogen. The optimum temperature range at which maximum damage occurred in our study is comparable to the temperature of the surface water in Florida waters and therefore it should be possible to duplicate the hydrilla control efficacy of the *F. culmorum*-insect combination.

This is the first report of an attempt of evaluating a pathogenic fungus and an insect for use in integrated biocontrol of hydrilla. However, further studies are needed to assess the efficacy of the *F. culmorum* plus *H. pakistanae* combination for integrating in hydrilla control in natural lakes.

ACKNOWLEDGMENTS

LITERATURE CITED

Baloch, G. M. and Sana-Ullah. 1974. Insects and other organisms associated with *Hydrilla verticillata* (L. f.) L. C. (Hydrocharitaceae) in Pakistan, p. 61-66. *In:* A. J. Wapshere (ed.). Proc. III Int. Symp. Biol. Control Weeds, September 1973, Montpellier, France. Commonw. Inst. Biol. Control, Silwood Park, UK.

Baloch, G. M., Sana-Ullah and M. A. Ghani. 1980. Some promising insects for the biocontrol of *Hydrilla verticillata* in Pakistan. Trop. Pest Manage. 26:194-200.

Blackburn, R. D., L. W. Weldon and R. R. Yeo. 1969. Identification and distribution of certain similar-appearing submersed aquatic weeds in Florida. Hyacinth Control J. 8:17-21.

Bowes, G., T. K. Van, L. A. Garrard and W. T. Haller. 1977. Adaptation to low light levels of hydrilla. J. Aquat. Plant Manage. 15:32-35.

Buckingham, G. R. 1988. Reunion in Florida-hydrilla, a weevil, and a fly. Aquatics 10:19-25.

Buckingham, G. R. and E. A. Okrah. 1993. Biological and Host Range Studies with Two Species of *Hydrellia* (Diptera: Ephydridae) That Feed on Hydrilla. Technical Report A-93-7, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS, USA.

Buckingham, G. R., E. A. Okrah and M. C. Thomas. 1989. Laboratory host range tests with *Hydrellia pakistanae* (Diptera; Ephydridae), an agent for biocontrol of *Hydrilla verticillata* (Hydrocharitaceae). Environ. Entomol. 18:164-171.

Caesar, A. J. 2000. Insect - pathogen synergisms are the foundation of weed biocontrol, p. 793-798. *In:* N. R. Spencer (ed.). Proc. 10th Int. Symp. Biol. Control Weeds, July 4-14, 1999, Montana State University, Bozeman, Montana, USA.

Center, T. D., M. J. Grodowitz, A. F. Cofrancesco, G. Jubinsky, E. Snoddy and J. E. Freedman. 1997. Establishment of *Hydrellia pakistanae* (Diptera: Ephydridae) for the biological control of the submersed aquatic plant *Hydrilla verticillata* (Hydrocharitaceae) in the southeastern United States. Biol. Control 8:65-73.

Charudattan, R. and D. E. McKinney. 1978. A Dutch isolate of *Fusarium roseum* 'Culmorum' may control *Hydrilla verticillata* in Florida, p. 219-224. *In:* Anonymous (ed.). Proc. EWRS 5th Symp. on Aquatic Weeds. P.O. Box 14, Wageningen, The Netherlands.

Charudattan, R. 1986. Integrated control of waterhyacinth (*Eichhornia crassipes*) with a pathogen, insect, and herbicides. Weed Sci. 34:26-30.

Charudattan, R. 1990. Biological control by means of fungi, p. 186-201. *In:* K. J. Murphy and A. Pieterse (eds.). Aquatic Weeds. Oxford Univ. Press, Oxford.

Charudattan, R. 2001. Are we on top of aquatic weeds? Weed problems, control options, and challenges, p. 43-68. *In:* C. R. Riches (ed.). 2001 BCPC Symposium Proceedings No. 77: The World's Worst Weeds. The British Crop Protection Council, Farnham, Surrey, U.K.

Charudattan, R., B. D. Perkins and R. C. Littell. 1978. Effects of fungi and bacteria on the decline of arthropod-damaged waterhyacinth (*Eichhornia crassipes*) in Florida. Weed Sci. 26:101-107.

Cuda, J. P. and A. M. Fox. 1997. Evaluation of biocontrol insects on hydrilla. USDA/ARS-IFAS/University of Florida Cooperative Agreement Final Report. Center for Aquatic and Invasive Plants, Gainesville.

Deonier, D. L. 1978. New species of *Hydrellia* reared from aquatic macrophyte in Pakistan (Diptera: Ephydridae). Entomol. Scan. 9:188-197.

Deonier, D. L. 1993. A critical taxonomic analysis of the *Hydrellia pakistanae* species group (Diptera: Ephydridae). Insecta Mundi 7:133-158.

Forno, I. W. and M. H. Julien. 2000. Success in biological control of aquatic weeds by arthropods, p. 159-187. *In:* G. Gurr and S. Wratten (eds.). Measuring Success in Biological Control. Kluwer Academic Publishers, Boston.

Hoagland, D. R. and D. I. Arnon. 1950. The water-culture method for growing plants without soil. Calif. Agric. Exp. Stn. Circular 347 (Revised).

Langeland, K. A. 1996. *Hydrilla verticillata* (L.F.) Royle (Hydrocharitaceae), "The Perfect Aquatic Weed." Castanea 61:293-304.

Langeland, K. A. and K. C. Burks. 1998. Identification and biology of non-native plants in Florida's natural areas. University of Florida, Gainesville, FL.

Nachtigal, G. de F. and R. A. Pitelli. 1999. *Fusarium* sp. as a potential biocontrol agent for *Egeria densa* and *E. najas*, p. 68. *In:* N. Spencer and R. Nowierski (eds.). Program and Abstracts, X Int. Symp. Biol. Contr. Weeds. USDA-ARS and Montana State Univ., Bozeman.

Netherland, M. D. and J. F. Shearer. 1996. Integrated Use of Fluridone and A Fungal Pathogen for Control of Hydrilla. J. Aquat. Plant Manage. 34:4-8.

Pieterse, A. H. 1977. Biological control of aquatic weeds: perspectives for the tropics. Aquat. Bot. 3:133-141.

Pieterse, A. H. 1981. *Hydrilla verticillata*—A review. Abstr. Trop. Agric. 7:9-34.

SAS Institute. 2000. SAS Statistical Software Release 8.1, Cary, NC, USA.

Schardt, J. D. 1997. Maintenance control (hydrilla), p. 229-244. *In:* Simberloff, D., D. C. Schmitz, and T. C. Brown (eds.). Strangers in Paradise: Impact and Management of Nonindigenous Species in Florida. Island Press, Washington, D.C.

Shabana, Y. M. and R. Charudattan. 1996. Microorganisms associated with hydrilla in ponds and lakes in North Florida. J. Aquat. Plant Manage. 34:60-68.

Shabana, Y. M., J. P. Cuda and R. Charudattan. 2003. Evaluation of pathogens as potential biocontrol agents of hydrilla. J. Phytopathology 151: in press.

Shearer, J. F. 1998. Biological Control of Hydrilla Using an Endemic Fungal Pathogen. J. Aquat. Plant Manage. 36:54-46.

Verma, U. and R. Charudattan. 1993. Host range of *Mycoleptodiscus terrestris*, a microbial herbicide candidate for Eurasian watermilfoil, *Myriophyllum spicatum*. Biol. Control 2:271-280.

Wheeler, G. S. and T. D. Center. 1996. The influence of hydrilla leaf quality on larval growth and development of the biological control agent *Hydrellia pakistanae* (Diptera: Ephydridae). Biol. Control 7:1-9.

Wheeler, G. S. and T. D. Center. 2001. Impact of the biological control agent *Hydrellia pakistanae* (Diptera: Ephydridae) on the submersed aquatic weed *Hydrilla verticillata* (Hydrocharitaceae). Biol. Control 21:168-181.