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Integrated Control of Eurasian Water Milfoil, *Myriophyllum spicatum*, by a Fungal Pathogen and a Herbicide

K. K. SORSA, E. V. NORDHEIM AND J. H. ANDREWS[1]

ABSTRACT

Factorial experiments were conducted on rooted Eurasian water milfoil grown in a biphasic culture system to evaluate the impact of the fungal pathogen *Colletotrichum gloeosporioides*, the herbicide Endothall at 0.65, 1.29 and 2.58 ppm and high or low phosphorus conditions on growth. Growth, measured as post-treatment biomass increase, declined significantly ($p<0.01$) from the untreated control levels under all treatment conditions. Growth decline of 42-90% (shoot) or 41-88% (shoot and root biomass) was observed as herbicide concentration was increased (from 0.65-2.58 ppm Endothall). Fungal inoculation alone depressed biomass increment values to 90% (for both shoots alone and shoots plus roots). Complementary growth assessments, based on incremental biomass and total biomass increase from the start of the experiments, confirmed the effect of the herbicides ($p<0.001$) or pathogen alone ($p<0.01$) or combined ($p<0.05$). Low phosphorus conditions further reduced biomass for all treatments, but the data were otherwise consistent with that for the high phosphorus regimen. The results suggest that pathogens even if marginally effective alone, could accentuate the impact of chemical control and offer promise for integrated control based on biological, chemical, and physiological components.

Key words: Biocontrol, *Colletotrichum gloeosporioides*, Endothall, milfoil growth.

INTRODUCTION

Eurasian water milfoil (*Myriophyllum spicatum* L.) (hereafter called "milfoil") ranks among the most aggressive nuisance aquatic weeds in Norh America (Grace and Wetzel 1978). The plant is adaptable, grows rapidly, and reproduces prolifically thereby displacing native vegetation. Since being introduced in the early 1800's (Reed 1977), milfoil continues to expand its geographic range. As a submersed weed, it possesses most, if not all, the detractions of the emersed group and additionally it impedes water flow more severely and is less amenable to control.

The traditional control methods for milfoil are mechanical harvesting or use of herbicides (Dunst and Nichols 1979). The former is costly (Koegel and Livermore 1979); the latter is controversial because of the real or perceived threat to human health and the environment. Since our studies (Andrews and Hecht, 1981; Andrews et al. 1982) indicate that milfoil is susceptible to fungal infections, there is the prospect for integrated control using a fungal pathogen and modified herbicide program, to-

[1]Postdoctoral Research Associate, Department of Soil Science, University of Wisconsin-Madison, Madison, WI 53706; Associate Professor, Department of Forestry and Statistics, University of Wisconsin-Madison, Madison, WI 53706: Professor, Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706.

gether with manipulation of stress factors (e.g. nutrient levels). We report here the first evidence that *Colletotrichum gloeosporioides* may have a potential role in an integrated strategy for milfoil control.

MATERIALS AND METHODS

The aquarium culture system used in this study has been previously described in length by Hoffmann et al. (1984). Briefly, it was comprised of an aerated, biphasic system with artificial sediment representative of calcareous lake sediments in the Madison area and an overlying diluted nutrient medium modified after Gerloff (1975). The 21-L aquaria were partitioned such that each half contained four plants. Two to three aquaria halves served as replicates. Statistical testing proved this procedure justifiable. The prevailing aquarium conditions were: 25 C (± 1 C), photoperiod 15 h light: 9 h dark, and a light intensity of 250 $\mu E \cdot m^{-2} \cdot sec^{-1}$. Two levels of phosphorus, high [sediment loaded with 0.2 mM $(NH_4)_2 PO_4$] and low (sediment loaded with 0.4 mM NH_4Cl, no added P.), were used.

Colletotrichum gloeosporioides was isolated from a milfoil stem lesion on a plant growing in small pond at the University of Wisconsin Arboretum. The fungus was purified by conventional single-spore methods (Tuite 1969) and preserved on silica gel (Trollope 1975).

Fungal inocula, consisting of mycelium and spores, were produced by growth for 7 days on potato dextrose agar (PDA) plates followed by spore harvest and resuspension in 850 mL Richard's solution in 2,000 mL Erlenmeyer flasks (Daniel et al. 1973). The flasks were incubated on a shaker operating at 100 rpm at 25-28 C for 48 hours. Spores were concentrated by centrifugation (6,000 rpm, 15 min), washed with distilled water and aliquots of uniform suspension adjusted to 8×10^5 conidia $\cdot mL^{-1}$ for all experiments. Although higher spore concentrations have greater impact on milfoil (Andrews et al. unpublished), for this work lower densities were used to increase resolution between different treatments. Freshly harvested fungal suspensions were injected into aquaria from sterile syringes. To provide uniform distribution aquarium aeration rate was increased for 2 minutes after inoculation.

Initially, three herbicides were screened: 2,4-D (2,4-dichlorophenoxy acetic acid; Albaugh Chem. Corp., Ankeny, IA); Endothall (dipotassium salt of 7 oxabicyclo [2,2,1]heptane-2,3-dicarboxylic acid; Pennwalt Corp., Philadelphia, PA) and Diquat® (6.7-dihydrodipyrido[1,2-α:2′,1′-c] pyrazidiinium dibromide; Chevron Corp., San Francisco, CA). These pilot trials were conducted in jars containing milfoil shoots subjected to fungal conidia and dosages of the herbicides down to levels about one-tenth of the manufacturers' recommendations for milfoil control. Based on preliminary experiments, the Endothall-fungus permutation appeared to have the most potenial for synergism and, accordingly is the subject of this paper. Stock herbicide concentrations were prepared with distilled, deionized water and filter-sterilized before use.

Factorial experiments were conducted to assess the effect of pathogen, herbicide, and phosphorus stress. Pathogen was considered at two levels—present or absent and herbicide at four levels—0, 0.65, 1.29, and 2.58 ppm. The factorial was repeated at two phosphorus levels—high and low. Two or three of the four aquaria halves (=8 or 12 plants) were used for final harvest data for each combination of factors; however, due to the lethality on milfoil no treatments at 2.58 ppm Endothall and low phosphorus were tested. The experiments were replicated at 0 and 0.65 ppm Endothall; they yielded results similar to the first experiments. Four types of evaluation were conducted at harvest: (1) The state of all plants was characterized visually. Symptoms were estimated by the percentage of color change (browning and blackening of tissue). Additionally, plants were analyzed for (2) biomass, (3) tissue phosphorus (APHA 1980), and (4) pigment contents (Wetzel 1965). Biomass data proved to be the best indicator and are reported here. Plant biomass was determined by drying plants at 70 C for 48 hr to constant weight.

The impact of various treatment combinations was estimated both as total biomass (final harvest day biomass) and as biomass increment after the treatment date. These biomass values represent an average response of eight to twelve plants per treatment. For these experiments, one aquarium with two halves, each half with 4 plants, was harvested on the treatment day to provide initial weights. Unless indicated otherwise, symptoms were recorded on all plants; following this, two of the 4 plants in each half aquarium were subjected to dry weight and tissue phosphorus analyses, the other two were used for pigment analysis.

Aquaria were treated on the 12th day after planting and harvested on the 25th day. This growth period was chosen because previous work with the system (Hoffmann et al. 1984) showed that exponential growth (and hence our sensitivity to detect impact) ended at about this time. Response was evaluated both as shoot biomass (a measure of immediate control) and also with contributions from both shoot and root. The contribution of roots was included because root biomass is a measure of growth potential over extended periods since roots can maintain their vigor and are a means of survival, even under conditions of disease and herbicide toxicity, while the shoot biomass has degraded.

Analyses of variance (ANOVA) (Snedecor and Cochran 1980, SAS [Statistical Analysis System], SAS Institute Gary, NC) were calculated from mean values from aquaria half-tanks as the experimental unit, after logarithmic transformation of the data. We report here partial sum of square values (type III sum of squares which do not add up to the model SS). We analyzed the data 1) by comparing all treatments together and 2) breaking down and comparing controls (=0 ppm herbicide and no pathogen) against each treatment group. We report here only statistical comparisons for biomass increment data on selected combinations to demonstrate the consistent trends in all break down comparisons. The total (final harvest day) biomass ANOVA data are essentially the same.

RESULTS

Fungal suspensions introduced into the aquaria caused transient turbidity that dissipated after a few hours when

the spores had settled. *Colletotrichum* induced brown to black localized stem and leaf lesions 5 days after inoculation. Tissue blackening was observed occasionally on the upper portions of roots, and infected plants were very fragile and broke apart easily upon harvest.

Mild impact of Endothall was evident a day after treatment and became more intensive with time. The herbicide which acts as a contact herbicide, induced tissue browning starting from leaf tips and advancing over the entire plant to varying degrees depending on herbicide concentration. It also caused deformation of the growing points.

The results obtained for high phosphorus treatment (Figure 1) are similar to those for low P treatment as will be shown in Figure 3. (Shoots only data are shown in Figures 1 and 3). Endothall caused a decline in total biomass equalling that in post-treatment biomass increment. This reduction from untreated (no herbicide, no fungus) control plants progressed wih herbicide concentration to the same degree whether expressed as total biomass or shoot biomass [average decline of 42-90% (shoot biomass) and 41-88% (shoot and root biomass) with 0.65-2.58 ppm Endothall]. Fungal application further depressed biomass increment values to 90% (shoot only or shoot and root). Similar decline patterns were observed among total milfoil biomass (final harvest day biomass) levels. Endothall application at 0.65-2.58 ppm resulted in average decline of 31-70% and 39-71% (shoot biomass and shoot + root biomass, respectively) without fungus and in 70-78% decline with fungus. The additive effect of the fungus on biomass reduction is evident in the biomass increment vs. ppm Endothall treatment graphs at all but the highest herbicide concentration, where it was masked by the toxicity of the chemical alone. Figure 2 shows the equivalent slopes of the comparisons with and without fungus and the lower y intercept with fungal treatment. In addition to deformed growth tips, Endothall application at 0.65, 1.29 and 2.58 ppm resulted in about 20, 40 and 80% shoot browning, respectively. These levels increased to about 70, 80 and 95% when *Colletotrichum* was also present and the plants disintegrated at the highest Endothall concentration.

The favorable effect of treatments consisting of fungus and herbicide for integrated control of milfoil growth was

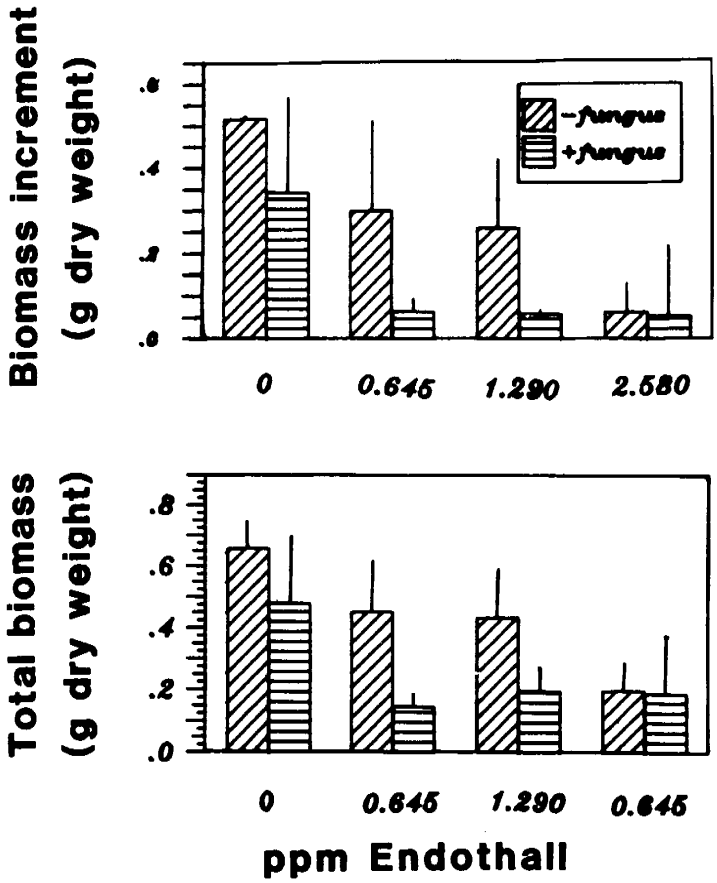

Figure 1. Incremental growth (top) and final biomass (bottom) of milfoil expressed as shoot only, under high phosphorus conditions following treatment with or without Endothall at 3 concentrations and the fungus *Colletotrichum gloeosporioides*. The error bars represent 95% confidence intervals for the data.

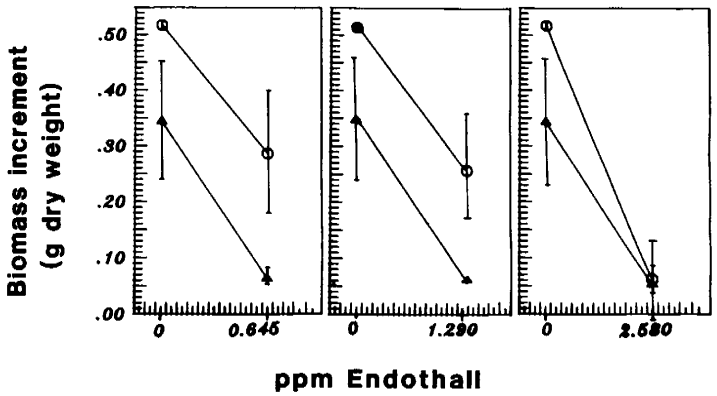

Figure. 2. Effects of fungus (Δ) on reduction in incremental growth of milfoil shoot biomass following treatment by Endothall at 3 concentrations (with 95% confidence limits). Impact of fungus is additive except at 2.58 ppm Endothall where effect is masked by herbicide.

TABLE 1. ANALYSIS OF VARIANCE OF BIOMASS INCREMENT (SHOOTS ONLY) OF MILFOIL AS INFLUENCED BY ENDOTHALL AND *C. GLOEOSPORIOIDES* AT HIGH LEVELS OF PHOSPHORUS. TREATMENT COMPARISONS: 0 PPM, 0.65, 1.29 AND 2.58 PPM ENDOTHALL, NO FUNGUS AND *C. GLOEOSPORIOIDES* AT 8 X 10^5 SPORES ML^{-1} (I.E. 8 TREATMENTS). A) INCLUDING ALL VARIABLES. BREAKDOWN OF VARIANCE DATA. B) 1.29 PPM ENDOTHALL AND *C. GLOEOSPORIOIDES* AND NO HERBICIDE, NO FUNGUS.

Dependent variable	Source of variation	dF	Sum of squares	F value
Biomass increment	treatment	7	2.112	9.58***
	fungus (F)	1	0.498	15.82**
	herbicide (H)	3	1.269	13.43***
a)	F*H	3	0.460	4.87*
	error	11	0.346	
	total	18	2.458	
Biomass increment	treatment	3	1.037	14.52**
	fungus	1	0.433	18.20**
	herbicide	1	0.663	27.87**
b)	F*H	1	0.118	4.96
	error	6	0.143	
	total	9	1.180	

*significant at least at 5% level
**significant at least at 1% level
***significant at least at 0.1% level

supported by the statistical significance of the treatment comparisons. The ANOVA (Table 1) illustrated that total milfoil biomass and biomass increment were significantly related to fungal treatment ($p<0.01$) and the effect of herbicides ($p<0.001$); their combined effect was still significant ($p<0.05$), but not the major contributor. The breakdown analyses showed that the contribution of the fungus was, however, masked at the highest Endothall level. Similar levels of statistical significance were observed irrespective of whether total biomass or biomass increment data were analyzed. Endothall treatment at 0.65 ppm level was an exception; the significance level of fungal treatment changed from $p<0.01$ to $p<0.05$ and herbicide treatment from $p<0.001$ to $p<0.01$ for biomass increment data. This was probably due to increased variability of the values resulting from the subtraction procedure. When treatment day values are subtracted from final harvest day values to obtain biomass increment data, there is a risk that the variability among different plants may reduce the statistical difference between their means, increasing replication requirements for demonstrating the response.

Decreased phosphorus resulted in a general decline of milfoil biomass (Figure 3). The treatment trends were otherwise consistent with those observed in the presence of high phosphorus. Endothall application resulted in the average of 39.8 and 65.4 (shoot only) and 39.4 and 59.9 (shoot and root) percent depression in the incremental growh due to 0.65 and 1.29 ppm Endothall. When combined with the fungus, Endothall induced an additional decline of 11.2 and 24.3 (shoot) and 17.1 and 29.1 (shoot and root) percent. Corresponding depression values for the total milfoil biomass at the conclusion of the experiment are 23.8 and 40.9 percent (shoot only) and 25.3 and 45.5 percent (shoot and root) due to 0.65 and 1.29 ppm Endothall, respectively. The fungus depressed biomass further up to 49 percent (shoot only) and 61 percent (shoot and root), respectively. Similar to the previous experiment, Endothall treatment at 0.65 and 1.29 ppm respectively, resulted in about 20 and 40 percent shoot deterioration; the fungus, as before, further enhanced this effect.

Statistical analysis of Endothall and fungus treatment combinations revealed that total biomass and biomass increment were influenced by both the fungus and herbicide ($p<0.05$-0.001). Further breakdown of the analyses of total biomass data also points out the influence of the fungus alone ($p<0.05$) and the herbicide ($p<0.001$) as well as their interactive effect ($p<0.01$) lending support for an integrated control strategy. At 1.29 ppm Endothall level, the influence of *Colletotrichum* and its interaction with the herbicide on milfoil's biomass increment were more notable ($p<0.01$). On the other hand, at 0.65 ppm Endothall level a lesser significance of the herbicide ($p<0.05$) and no statistical significance of the fungus were observed among he biomass increment values, probably due to the variability resulting from subtraction of treatment day values. We present here ANOVA results for biomass increment data (Table 2), where partial sum of square values are reported.

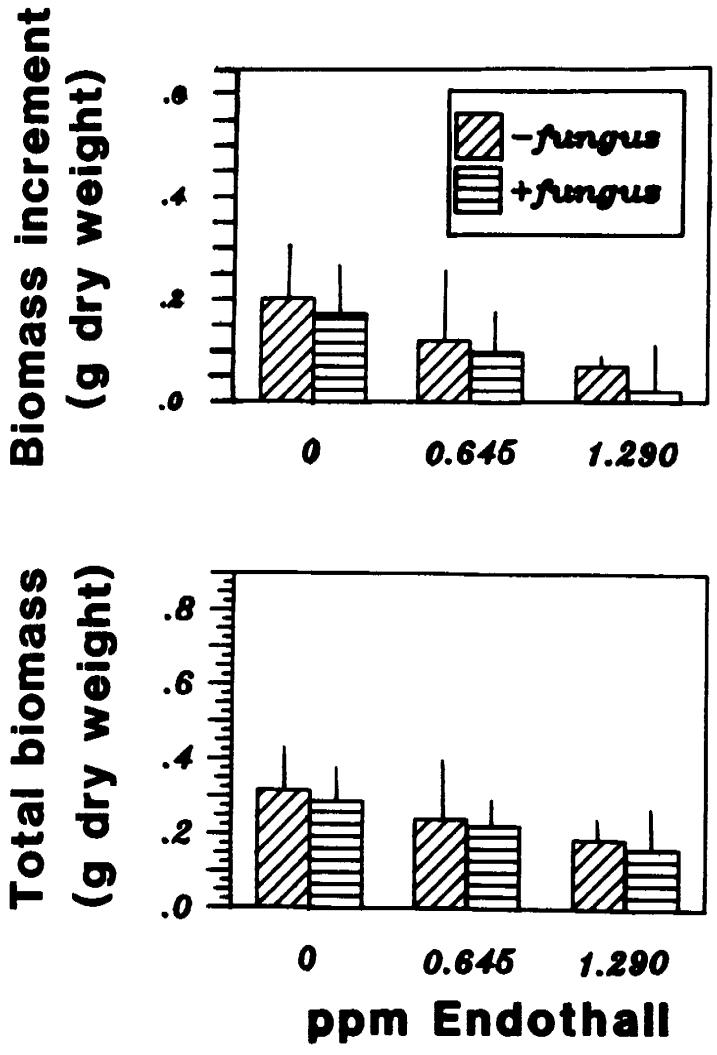

Figure 3. Biomass increment and final biomass of milfoil (shoots only), grown under low phosphorus conditions, as influenced by various Endothall levels and the fungus *C. gloeosporioides*. 95% confidence intervals shown.

DISCUSSION

Of the various treatments investigated to reduce milfoil, the best permutation from an integrated standpoint appears to be a low level (0.65-1.29 ppm) of the herbicide Endothall in conjunction with application of the fungus *Colletotrichum* to the plants. This conclusion is valid regardless whether impact is measured on shoots alone or both shoots and roots, and also whether the assessment is based on biomass change at the end of the experiment or only incremental growth following treatment. These results await confirmation from field trials, where it might be expected that plants would need to be considerably more stressed by each of the factors for sufficient impact to be realized.

The idea of treating herbicide-stressed plants with pathogens was promped by numerous observations from agriculture that a side-effect of herbicidal treatment is increased disease in the non-target crop (e.g. Katan and Eshel 1973). In our system, without any additional stress, levels of herbicides well below those recommended commercially resulted in death or major destruction of milfoil.

TABLE 2. ANALYSIS OF VARIANCE OF MILFOIL BIOMASS INCREMENT (SHOOTS ONLY) AS INFLUENCED BY ENDOTHALL AND *C. GLOEOSPORIOIDES* AT LOW LEVELS OF PHOSPHORUS, A) ALL TREATMENT COMPARISONS (0 PPM, 0.65 AND 1.29PPM ENDOTHALL, NO FUNGUS AND *C. GLOEOSPORIOIDES* AT 8 X 10^5 SPORES ML^{-1} (I.E. 6 TREATMENTS). BREAKDOWN OF VARIANCE DATA B) 1.29 PPM ENDOTHALL AND *C. GLOEOSPORIOIDES* AND NO HERBICIDE, NO FUNGUS.

Dependent variable		Source of variation	dF	Sum of squares	F value
Biomass increment	a)	treatment	5	2.248	15.22***
		fungus (F)	1	0.394	13.33**
		herbicide (H)	2	1.800	30.45***
		F*H	2	0.446	7.54**
		error	11	0.325	
		total	16	2.573	
Biomass increment	b)	treatment	3	2.220	41.61***
		fungus	1	0.493	27.73**
		herbicide	1	1.758	98.88***
		F*H	1	0.343	19.28**
		error	7	0.124	
		total	10	2.344	

*significant at least at 5% level
**significant at least at 1% level
***significant at least at 0.1% level

This is not surprising, since the application conditions and subsequent fate of the chemical as influenced by dilution, inactivation, and degradation would be quite different in nature and in the laboratory. The pathogen, although somewhat effective, acted mainly to enhance effectiveness of the treatments. There is considerable evidence that nutrients, including phosphorus, have influenced disease severity in terrestrial plants (e.g. Last 1962, Huber 1980). We do not know how nutrient status affects disease severity in this system. One might expect, however, that nutrient-deficient plants when exposed to environmental stress factors, including disease, have less chance of prevailing than their well nourished counterparts. For example, vigorous plants with a sufficient nutrient supply could outgrow the infection. Even though the area of infected tissue may be large, the ability of healthy shoots and roots to support and increase the biomass is probably greater than among poorly nourished plants.

We conducted experiments during the exponential growth phase and did not investigate how culture aging influences treatment results. This was done to achive high sensitivity to detect impact. It is probable, however, that declining growth rate and onsetting nutrient depletion in the closed system at a later stage would make the culture at least, if not more, susceptible to the integrated treatment.

Our results suggest that pathogens indigenous to a natural system may be manipulated to be components of an integrated control program. If not markedly effective alone against vigorous plants, they may play a role in situations where plants are nutrient-stressed or by enabling applications of herbicides at concentrations that otherwise would be ineffective. Although bodies of water where aquatic weeds are a problem are typically eutrophic, nutrient stress and other predisposing factors (e.g. temperature fluctuations; shading) can occur. Milfoil beds in portions of Lake Wingra, Wisconsin, have, for example, been progressing toward phosphorus-limited conditions (Adams and Prentki 1982) because milfoil has been metabolizing sediment reserves of phosphorus at 170 percent of the renewal rate over the 21-year lifetime of that community (Prentki 1979). Thus, our work on a biologically based, integrated strategy, while awaiting field testing, holds out promise that a significant reduction of nuisance aquatic weeds to acceptable economic and social levels, can be achieved by a more environmentally sound management program. These tactics are based on the assumption that depression of one predominant weed would ultimately result in increased species diversity and improvement of water quality.

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Selective Patterns of Herbicide Application for Improved Biological Control of Waterhyacinth

KIM H. HAAG[1], MARGARET S. GLENN[2], AND JACQUELINE C. JORDAN[3]

ABSTRACT

The effects of two different herbicide application patterns on waterhyacinth regrowth and waterhyacinth weevil population dynamics were studied in 7 ponds in Alachua Co. In Treatment 1, after half of the weed mat was sprayed, the waterhyacinths were left with a short boundary area along which daughter plants could form and colonize open water. In these ponds (# 1, 4, 5) a reduced plant expansion rate fostered the success of the biocontrol agents (*Neochetina* sp.) and the resulting heavy insect feeding damage caused a total decline of the weed populations. In Treatment 2, after half of the weed mat was sprayed, the waterhyacinths were left with a long boundary area along which daughter plants could form. In these ponds (# 2, 3, 6, 7) the plant population rapidly expanded to fill available open water. Plant growth rate surpassed the weevil population rate of increase, and insect feeding damage was not sufficient to control the weed mats.

Key words: Eichhornia crassipes, biocontrol, *Neochetina,* integrated pest management.

INTRODUCTION

There is growing evidence that the acreage of waterhyacinth (*Eichhornia crassipes*) [Mart.] Solms) has diminished in Florida and elsewhere as a result of damage by the released biological control agents *Neochetina eichhorniae* Warner and *N. bruchi* Hustache (Center 1982, DeLoach and Cordo 1983, Theriot 1982, Wright 1980). However, waterhyacinth continues to be a problem at some intensively used sites and in these situations herbicides are used to manage the weed. Contact with herbicide does not kill *Neochetina* weevils (Haag 1986a), but rapid loss of habitat following herbicidal control may reduce weevil populations. Previous research efforts have focused on limiting the extent of herbicide application, thereby conserving portions of a waterhyacinth mat as a reservoir for waterhyacinth weevil populations. Results have shown that weevils will disperse from sprayed plants to adjacent unsprayed waterhyacinths if they are available (Haag 1986b). This study was designed to examine the effects of various herbicide spray patterns on waterhyacinth regrowth and subsequent population dynamics of the biological control insects involved.

MATERIALS AND METHODS

Seven ponds on the University of Florida campus were chosen as experimental sites. The shallow, clay-lined ponds (mean depth 1.5 m) are rectangular (30 m x 150 m) and receive pump-circulated water from Bivan's Arm Lake. At the beginning of the study (August 1984) approximately 60% of the water surface in Pond 1 was covered with waterhyacinth. Waterhyacinth weevils were present on these plants at a density of approximately 0.5 weevils per plant. Small populations of waterhyacinth (less than 20% total pond surface area) were present in Ponds 2, 4 and 5. No waterhyacinths were present in Ponds 3, 6 and 7. In March 1985 waterhyacinth plants were collected from southeastern Alachua Co. and transported to the experimental site. A quantity of plants was added to each of ponds 2, 4 and 5 to increase the total plant surface area coverage to approximately 20%. Plants were added to Ponds 3, 6 and 7 to provide approximately 20% total surface area coverage. Waterhyacinth weevils were present on these introduced plants at an average density of 0.5 weevils per plant.

Plants were allowed to colonize the ponds for several months in order to obtain complete surface coverage. Three separate analyses of water quality between August 1984 and April 1985 indicated that nutrient levels were relatively low (total N: $\bar{x} = 1.3$ ppm; total P: $\bar{x} = 0.9$ ppm). Consequently inorganic fertilizer (10N-10P-10K) was added to each pond at a rate of 85.25 kg/ha (75 lb/acre) in late May, 1985. In a further attempt to promote water surface coverage by the plant, foliar fertilizer (Sunniland™,

[1]Postdoctoral research associate, Dept. of Entomology and Nematology, Univ. of Florida, Gainesville, FL 32611.
[2]Biologist, Agronomy Department, University of Florida.
[3]Lab Technician, Department of Entomology and Nematology, Univ. of Florida.