Past and Current Research on Diseases of Eurasian Watermilfoil (Myriophyllum spicatum L.)[1, 2]

H. F. HAYSLIP AND F. W. ZETTLER

Graduate Assistant and Associate Professor, Department of Plant Pathology, University of Florida, Gainesville, Florida, 32601.

INTRODUCTION

Eurasian watermilfoil *(Myriophyllum spicatum* L.) was first observed in Florida in the mid 1960's (6). Since then it has become a severe problem in several areas of Florida including the Crystal River, Deer Point Lake, and Lake Seminole. Other areas of the United States such as Currituck Sound in North Carolina (9) and the Tennessee Valley[3] have similar problems with Eurasian watermilfoil.

In recent years, biocontrol agents have been given increasing consideration as a means of controlling this weed. Phytophagous insects have been reported on Eurasian watermilfoil (1, 14) but none are currently being used as biocontrols. Other phytophagous animals such as fish, manatees, and snails have also been investigated (5) but their value as biocontrols of Eurasian watermilfoil remains to be determined. Unfortunately, relatively little attention has been given to pathogens of this plant despite the obvious potentials of viruses, fungi, bacteria, and nematodes as biocontrols (20, 21). Although the nematode *Ditylenchus dipsachi tobaensis* was collected from *Myriophyllum verticillatum* L., its potential as a biocontrol of Eurasian watermilfoil has not been considered (11).

A virus was incriminated as being responsible for the Northeast disease which decimated the Eurasian watermilfoil populations in the Chesapeake Bay from 1965 to 1969 but this hypothesis was never proved, and attempts to infect plants with tobacco ringspot, alfalfa mosaic, potato X, potato Y, and tobacco mosaic viruses were not successful (3). In 1968 Lammers (13) obtained 15 bacterial isolates *(Bacillus* sp., *Erwinia* sp., *Xanthomonas* sp., *Aerobacter* sp., and several Corynebacteriaceae[4]) from plants infected with the Northeast disease. He reported that several induced symptoms in inoculated plants of Eurasian watermilfoil, but whether these bacteria are responsible for the Northeast disease remains to be determined. Another abnormal condition of Eurasian watermilfoil was noted in the Chesapeake Bay during the 1960's and was referred to as the Lake Venice disease (3). Little was done to determine its cause, however. As the decline of the Eurasian watermilfoil population continued in the Chesapeake Bay, most plants developed symptoms of both the Lake Venice and Northeast diseases (3). These symptoms have not been seen elsewhere on Eurasian watermilfoil and attempts to establish the Northeast disease in Florida's Crystal River were apparently unsuccessful.

A program to locate organisms deleterious to Eurasian watermilfoil was recently initiated at the Institute for Plant Protection in Belgrade, Yugoslavia. Plant pathologists there isolated many fungal species, some of which induced symptoms on Eurasian watermilfoil plants. These fungi were: *Sclerotium hydrophyllum, Dactylella microaquatica, Flagellospora stricta, Fusarium roseum, Fusarium solani, Fusarium oxysporium,* and *Fusarium moniliforme* (14).

A search for phytopathogens as biocontrols of waterweeds, including Eurasian watermilfoil, was initiated at the University of Florida in 1970. Although several diseases of aquatic plants have been reported by workers on this project (7, 10, 16, 17, 18), none have been reported on Eurasian watermilfoil. This paper reports the progress of this program with respect to Eurasian watermilfoil.

METHODS AND MATERIALS

In October 1971, we obtained the bacteria isolated by Lammers (13) from plants with symptoms of the Northeast disease. The following procedure was established for treating the cultures: (1) Bacterial cells of each isolate were streaked onto nutrient agar to ascertain viability and purity. (2) The tobacco hypersensitivity test as described by Klement et al. (12), was conducted. This test indicates pathogenicity of certain types of bacteria to plants. Bacterial cells were transferred to nutrient broth tubes and shaken for 24 hr. If, at the end of 24 hr the broth was not turbid, the culture remained on the shaker for an additional 24 hr. The suspension was centrifuged at about 1000 g for 10 minutes and the pellet resuspended in buffered saline. The inoculum was standardized spectrophotometrically to 10^6 bacterial cells/ml. Leaves of tobacco plants were watersoaked by subcuticular injections of bacterial suspensions with a 26-gauge hypodermic syringe. Leaves were injected with saline as controls. Treated plants were examined for necrotic lesions indicative of pathogenicity. (3) Each isolate was tested for its ability to produce soft rot symptoms in potatoes (15). Thin-skinned potatoes were washed with a solution of commercial sodium hypochlorite and peeled with a knife flamed in alcohol. Slices of potato were placed in petri plates that contained filter paper dampened with water. Bacterial cells were streaked on the middle of each potato

[1]Based upon the M.S. thesis research of the senior author.

[2]Florida Agricultural Experiment Stations Journal Series No. 4512.

[3]Smith, G. E. 1970. Resume of studies and control of Eurasian watermilfoil *(Myriophyllum spicatum* L.) in Tennessee Valley from 1960 to 1969. Paper given before Meeting of the Industry/Government Task Force on Eutrophication. Mimeo. 9 p.

[4]Roger, M. L. 1970. TVA memorandum "A study of potential pathogens for Eurasian watermilfoil" to C. Wayne Holley dated April 10, 1970.

slice with a transfer "L" stylet. Noninoculated slices served as controls. Plates were incubated at 28 C and observed daily for 3 days. (4) Each isolate was tested for pathogenicity on Eurasian watermilfoil. Bacterial suspensions were standardized to concentrations of 10^8 or 10^7 cells/ml. Sprigs of Eurasian watermilfoil were placed in these suspensions in 100 ml test tubes capped with aluminum foil. The tubes were kept at 22-25 C under banks of fluorescent lights on a 12-hr cycle. Noninoculated plants served as controls. Sprigs were observed daily for symptoms which could be attributed to the bacteria.

Fungi from Florida were obtained from the Florida Type Culture Collection of the Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville. These were: *Fusarium roseum, Fusarium solani, Fusarium oxysporium,* and *Fusarium moniliforme* originally from *Ficus elastica, Platanus occidentalis, Lycopersicon esculentum,* and *Dracaena marginata,* respectively. These fungi were tested as pathogens of Eurasian watermilfoil and parrotfeather *(Myriophyllum brasilinese* Camb.). Parrotfeather, a species with emergent foliage, was used to evaluate effects of these fungal isolates on plant tissue formed above the water.

Single sprigs of Eurasian watermilfoil, approximately 12 cm long and containing a growing point, were taken from rooted stock and placed in 100 ml test tubes that contained aged tap water. Plugs approximately 1 cm square were cut from plates containing potato-dextrose agar (PDA) on which the fungus had been growing for 4 days. One plug was placed on the petiole of a leaf about 6 cm from the top of the sprig.

Eurasian watermilfoil was also grown in 1 gallon jars that contained fine grade quartz sand and water from nearby Big Hatchet Creek. After the plants rooted, the water was removed and the plants trellised on 2.5 cm mesh poultry wire. These plants were placed in a mist chamber and agar plugs containing inoculum placed in the leaf axils.

Apical sprigs were taken from stock plantings of parrotfeather. These were placed into plastic lined clay pots that contained potting soil and 2.5 cm of water. These plants were grown in a greenhouse for 4 days prior to inoculation to allow roots to form. As described above, agar plugs were placed in the leaf axils. Treated plants were covered with plastic bags. Sterile agar plugs were placed on plants as controls.

Fungi reported to be pathogens on other plant species were tested on Eurasian watermilfoil (14). Species of Phycomycetes were tested for pathogenicity to watermilfoil because many produce zoospores which are motile in water. The following species were tested: *Pythium aphanidermatum, P. carolinianum, P. helicoides, P. irregulare, P. polytylum, P. ultimum, P. vexans, P. scleroteichum, P. splendens, P. debaryanum, P. oligandrum, Phytophthora cinnamomi, P. citricola, P. drechsleri, P. erythroseptica, P. hibernalis, P. palmivora,* and *P. parasitica.* These fungi were grown on lima bean agar for 5 days. The growth plates were flooded with distilled water and examined for zoospores after 24 hr. The agar and water solution that contained each fungus was then comminuted with a Waring blender. This zoospore and mycelial suspension was used as inoculum and was added to tubes that contained distilled water and Eurasian watermilfoil sprigs.

Ridings and Zettler (16) reported a lethal fungal pathogen of *Echinodorus brevipedicellatus* (O. Kuntze) Buchenau, a submergent aquatic ornamental. This fungus, identified as an isolate of *Aphanomyces euteiches,* was tested as a pathogen of Eurasian watermilfoil in this study. Growing, rooted plants as well as unrooted sprigs of Eurasian watermilfoil planted adjacent to rooted plants of *E. brevipedicellatus* contained in 1 gal jars were treated with zoospore and mycelial suspensions of the fungus.

A fungus was reported by Freeman and Zettler (10) as pathogenic on waterhyacinth *Eichhornia crassipes* (Mart.) Solms. This organism, *Rhizoctonia solani,* was originally found on anchoring waterhyacinth, *Eichhornia azurea* (Schwartz) Kunth., in Panama and induces a blight on waterhyacinth. Tests were undertaken to determine its effects on Eurasian watermilfoil and parrotfeather. Using the agar-plug technique, watermilfoil and parrotfeather plants were treated with this fungus. In addition, parrotfeather was also treated by planting sprigs in pots that contained soil in which fungal mycelium and sclerotia had been incorporated. These plants were grown in a greehouse and observed daily for foliar symptoms. Plants were uprooted at 1, 2, and 3 weeks for observation of roots. These were compared with noninoculated plants. Waterhyacinth plants were treated as controls to verify the pathogenicity of the isolate used.

RESULTS

All bacterial isolates from plant tissue that displayed symptoms of Northeast disease grew when streaked onto nutrient agar and appeared not to be contaminated. No lesions were observed on any of the tobacco leaves injected with the bacterial suspensions or with the saline solution. Similarly, no isolate consistently caused rotting on the tested potato slices, and after three weeks, no differences were noted between inoculated and control plants of Eurasian watermilfoil.

No evidence of infection was observed on any of the plants inoculated with the *Fusarium, Phytophthora* or *Pythium* species from Florida and, after 4 weeks, inoculated plants did not differ in appearance from noninoculated ones used as controls.

Similarly, *A. euteiches* did not infect Eurasian watermilfoil. Within 10 days, however, leaves of *E. brevipedicellatus* used as controls became necrotic at the base. Over a period of 4 weeks, the leaves were almost totally skeletonized and many plants had died. The Eurasian watermilfoil plants growing in the same containers showed no symptoms at the end of 4 weeks.

Variable results were obtained from the *Rhizoctonia* trials. In all experiments, necrosis occurred but degree of infection and time required to induce symptoms varied. With agar plug treatments of parrotfeather, mycelium grew out from the plug within 48 hr and extended over the plant surface. The leaflets covered with mycelium began to turn brown. For several days the necrosis spread in both directions along the stem. In most cases the

emersed portion of the plant toppled over within 5 days. However, in no case did infection result in death of the plant, and healthy side shoots emerged from the lower stems. No symptoms were observed on plants growing in soil into which mycelia and sclerotia had been incorporated. Leaves of waterhyacinth plants used as controls became necrotic within 48 hr, however.

DISCUSSION

Our data do not support the hypothesis that a phytopathogen was responsible for the Northeast and Lake Venice diseases of Eurasian watermilfoil. Thus, the precise cause of Northeast disease remains unsolved. Lammers' results (13), which suggested that bacteria would infect watermilfoil, were not reproduced in this study even when concentrations of bacteria far surpassing those found under natural conditions were used to treat Eurasian watermilfoil.

It is possible that the demise of the Eurasian watermilfoil populations of the Chesapeake Bay was due to abiotic causes. Workers such as Steenis[5] have suggested that pollution, siltation, autotoxins, or a combination of some factors may have been involved. Whatever the cause, it has been effective, and interest in this condition has waned since the plant is no longer a problem in the Chesapeake Bay (4).

The preliminary report from Yugoslavia of fungi pathogenic to Eurasian watermilfoil seems to be the most promising work to date. Inoculations with Florida isolates of some of the fungi described in Yugoslavia did not result in infection, however, although different isolates of fungi can vary greatly in pathogenicity. It is interesting to note that some of their fungi such as *Dactylella microaquatica* and *Flagellospora stricta* are organisms commonly found in the United States as saprophytes on decaying submerged vegetation (2, 8). This work should be continued after cultures of the isolates from Yugoslavia are obtained before conclusions can be drawn.

The waterhyacinth isolate of *Rhizoctonia* caused symptoms in Eurasian watermilfoil and parrotfeather but the infections by this fungus were limited. *Rhizoctonia* infection did not kill Eurasian watermilfoil or parrotfeather plants and it is doubtful that this isolate could be an effective biocontrol of these species.

Although only limited success was achieved with phytopathogens in this study, this research should be continued. About 40 species of *Myriophyllum* occur throughout the world (19). Because of the possibility that Eurasian watermilfoil was introduced into areas of the U. S. without its natural enemies, other areas of the world should be surveyed for pathogens attacking *Myriophyllum* species. Eurasian watermilfoil is native to Europe and Asia and in these areas it presumably has attained a balance with its environment in large part due to natural control. The large number of fungi isolated from Eurasian watermilfoil in Yugoslavia by Lekic (14) and the phytophagous insects found on this plant in Yugoslavia and Pakistan (1) is evidence of this. It is highly unlikely that Eurasian watermilfoil has no viruses, fungi, nematodes, or bacteria as natural enemies and the paucity of reports of Eurasian watermilfoil diseases is probably due to insufficient research.

[5]Steenis, H. H. 1970. Eurasian watermilfoil and associated submerged species in the Chesapeake Bay area. Paper prepared for the Weed Sci. Soc. Meeting in Montreal, Feb. 3-5.

ACKNOWLEDGEMENTS

This research was funded by U. S. Army Corps of Engineers Contract No. DACW 73-71-C0002, and supported by the Florida Department of Natural Resources Contract No. 3 and by the United States Department of the Interior, Office of Water Resources Research, as authorized under the Water Resources Research Act of 1964. Appreciation is extended to the Tennessee Valley Authority for making available Dr. Lammers' bacterial isolates for our study.

LITERATURE CITED

1. Baloch, G. M., A. G. Kahn, and M. A. Ghani. 1972. Phenology, biology, and host-specificity of some stenophagous insects attacking *Myriophyllum* spp. in Pakistan. Hyacinth Contr. J. 10:13-16.
2. Barnett, H. L. and B. B. Hunter. 1972. Illustrated Genera of Imperfect Fungi, 3rd ed. Burgess Publishing Company, Minneapolis. 241 p.
3. Bayley, S. E. M. 1970. The ecology and disease of eurasian watermilfoil (*Myriophylum spicatum* L.) in the Chesapeake Bay, Ph.D. Dissertation, School of Hygiene and Public Health of the Johns Hopkins University. 190 p.
4. Bayley, S. E. M., H. Rabin, and C. H. Southwick. 1968. Recent decline in the distribution and abundance of eurasian milfoil in Chesapeake Bay. Chesapeake Sci. 9:173-181.
5. Blackburn, R. D., D. L. Sutton, and T. Taylor. 1971. Biological control of aquatic weeds. Irrigation Drainage Div. Proc. Am. Soc. Civil Eng. 97:421-432.
6. Blackburn, R. D. and L. W. Weldon. 1967. Eurasian watermilfoil—Florida's new underwater menace. Hyacinth Contr. J. 6:15-18.
7. Charudattan, R. 1973. Pathogenicity of fungi and bacteria from India to hydrilla and waterhyacinth. Hyacinth Contr. J. 11:44-48.
8. Conway, K. 1969. Some aquatic hyphomycetes of Florida. Florida Acad. Sci. 32:210-220.
9. Crowell, T. E., J. H. Steenis, and J. L. Sinock. 1967. Recent observations on eurasian watermilfoil in Currituck Sound, N. C. and other coastal southeastern states. Proc. Southern Weed Conf. 20:348-352.
10. Freeman, T. E. and F. W. Zettler. 1971. Rhizoctonia blight of waterhyacinth. Phytopathology 61:892. (Abstr.)
11. Goodey, J. B., M. T. Franklin, and D. J. Hooper. 1965. Goodey's the nematode parasites of plants catalogued under their hosts. Commonwealth Agr. Bur. Fordham Royal, Bucks, England. 214 p.
12. Klement, Z., G. L. Farkas, and L. Lovrekovich. 1964. Hypersensitive reaction induced by phytopathogenic bacteria in the tobacco leaf. Phytopathology 54:474-477.
13. Lammers, W. 1968. Report on the isolation and characterization of microorganisms from *Myriophyllum spicatum*. TVA mimeo report of June 20.
14. Lekic, M. 1970. Establishing of insect species, causes of disease and other parasitic organisms which reduce the numerical strength of the populations of *Myriophyllum spicatum*. Annual Progress Report for research supported by PL-480 Grant E30-ENT-10.
15. Lelliott, R. A., E. Billing, and A. C. Hayward. 1966. A determinative scheme for the fluorescent plant pathogenic Pseudomonads. J. Appl. Bacteriol. 29:470-489.
16. Ridings, W. H. and F. W. Zettler. 1972. *Aphanomyces* blight of amazon sword plants. Phytopathology 62:806 (Abstr.)
17. Rintz, R. and T. E. Freeman. 1972. *Fusarium roseum* pathogenic to water hyacinth in Florida. Phytopathology 62:806. (Abstr.)
18. Rintz, R. E. 1973. A zonal leaf spot of wathchyacinth caused by *Cephalosporium zonatum*. Hyacinth Contr. J. 11:41-44.
19. Stodola, Jiri. 1967. Encyclopedia of Water Plants. T. F. H. Publications, Inc. New Jersey. 368 p.
20. Wilson, C. L. 1969. Use of plant pathogens in weed control. Annu. Rev. Phytopathol. 7:411-434.
21. Zettler, F. W. and T. E. Freeman. 1972. Plant pathogens as biocontrols of aquatic weeds. Annu. Rev. Phytopathol. 10:455-470.