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2,4-D and *Mycoleptodiscus terrestris* for Control of Eurasian Watermilfoil

LINDA S. NELSON AND J. F. SHEARER[1]

ABSTRACT

Growth chamber studies were conducted to evaluate the impact of an indigenous fungal pathogen, *Mycoleptodiscus terrestris* (Gerd.) Ostazeski, and the herbicide 2,4-D applied alone and in combination with one another, on the growth of a nuisance submersed plant, Eurasian watermilfoil (*Myriophyllum spicatum* L.). Treatments included 0.25, 0.50, and 1.00 mg L^{-1} 2,4-D; 0.08, 0.16, and 0.32 ml L^{-1} *M. terrestris*; combinations of both agents at all rates (applied simultaneously); and untreated controls. Six weeks after application, all treatments except the lowest rate of *M. terrestris*, had significantly reduced shoot biomass compared with untreated controls. Herbicide and pathogen combinations provided better control of Eurasian watermilfoil than either agent used alone. Based on the Colby statistic, interactions between the two agents were either synergistic or additive. Rates as low as 0.25 mg L^{-1} 2,4-D combined with 0.16 ml L^{-1} *M. terrestris* reduced shoot biomass more than 90%. To achieve similar results with herbicide alone required 2,4-D rates of 1.00 mg L^{-1}. The highest rate of *M. terrestris* applied alone reduced plant biomass by only 79%. Combined treatments effectively suppressed perennial rootstock which would reduce the potential for weed re-establishment. When 2,4-D and *M. terrestris* were applied as one treatment, a 24-hr contact time was sufficient to effectively control Eurasian watermilfoil. The ability to achieve weed control while minimizing herbicide rate and contact time requirements would improve management in systems with flowing water and where chemical impacts on sensitive species are of concern. These data support the potential for effective integrated weed management strategies using biological and chemical agents in aquatic environments.

Key words: Aquatic plant management, biocontrol, fungal pathogen, herbicide, integrated weed management, invasive plant, *Myriophyllum spicatum*, (2,4-dichlorophenoxy)acetic acid.

INTRODUCTION

Eurasian watermilfoil (*Myriophyllum spicatum* L.) is a submersed, aquatic perennial native to Europe, Asia, and northern Africa (Couch and Nelson 1985). Although the exact time, location and method of introduction into the U.S. are unknown and often disputed, Couch and Nelson (1985) contend that Eurasian watermilfoil was first collected from the District of Columbia in October, 1942. Since then, this highly invasive weed has spread to 45 states and 3 Canadian provinces (Mullin et al. 2000). Once established, excessive growth of this plant can hinder recreational activities (boating, fishing, swimming), impede navigation, and clog water intakes used for industrial and power generation.

A survey of state natural resource agencies conducted by Bartodziej and Ludlow (1997) revealed that Eurasian watermilfoil is the most widely managed aquatic weed in the U.S. According to Mullin et al. (2000), millions of dollars are spent annually to control Eurasian watermilfoil in northern states. A recent economic valuation study of the Truckee Riv-

[1]Plant Physiologist and Plant Pathologist, U.S. Army Engineer Research and Development Center, Waterways Experiment Station, 3909 Halls Ferry Road, Vicksburg, MS 39180-6199. Corresponding author's e-mail: Linda.S.Nelson@erdc.usace. army.mil. Received for publication January 25, 2005, and in revised form March 7, 2005.

er watershed (western NV and northeastern CA) showed that a 1% decrease in recreation activities as a result of nuisance Eurasian watermilfoil infestations, corresponded to a minimum loss of $500,000 in recreation revenue per year (Eiswerth et al. 2000).

In addition to impacts on human activities and the subsequent monetary consequences, high densities of Eurasian watermilfoil can negatively affect aquatic ecosystems by reducing native plant species richness and abundance. Vegetation surveys conducted over a three-year period in Lake George, NY, showed that expanding Eurasian watermilfoil populations significantly suppressed the native plant community (Madsen et al. 1991). Total number of native species found in Eurasian watermilfoil beds decreased linearly over time from 20 species in 1987 to 9 species in 1989 (Madsen et al. 1991). Correspondingly, Getsinger et al. (1997) recorded a significant increase in native plant populations following herbicide treatments to remove dense Eurasian watermilfoil stands from the Pend Oreille River, WA. Excessive plant growths can also degrade fish habitat and influence predator-prey interactions (Petr 2000, Maceina et al. 1991, Newroth 1985, Nichols and Shaw 1986, Borawa et al. 1979).

Recently, scientists have verified the existence of hybrid plant populations between the non-indigenous Eurasian watermilfoil (*M. spicatum*) and the indigenous *M. sibiricum* V. Kamarov in several Minnesota and Wisconsin lakes (Moody and Les 2002). These hybrids were noticeably aggressive and had formed dense, monotypic stands. The potential implications of hybrid populations on the effectiveness of current management strategies is of great concern. Moody and Les (2002) reported that there is correlative evidence suggesting the hybrid genotype may influence the efficacy of the watermilfoil weevil (*Euhrychiopsis lecontei* Dietz) as a biocontrol agent. The differential response to chemical management strategies among hybrid populations is unknown however, there have been reports of decreased sensitivity of some Eurasian watermilfoil populations in Minnesota when using recommended application rates of the herbicide 2,4-D ((2,4-dichlorophenoxy)acetic acid) (J. Skogerboe, pers. comm.[2]).

Strategies currently used to manage Eurasian watermilfoil infestations include herbicides (Green and Westerdahl 1990, Netherland et al. 1991, Netherland and Getsinger 1995, Getsinger et al. 1997, Parsons et al. 2001), mechanical harvesters (Painter 1988, Crowell et al. 1994, Unmuth et al. 1998), water level manipulation (i.e., drawdowns) (Stanley et al. 1976, Bates et al. 1985), placement of bottom fabrics (Helsel et al. 1996), and insect biocontrol agents (Johnson and Blossey 2002, Newman et al. 2001). Fungal pathogens have been identified as potential mycoherbicides for control of submersed aquatic plants (Sorsa et al. 1988, Shearer 1996) but to date, are not commercially available. According to Smith and Barko (1990) and Madsen and Smith (1997), control efforts in use today are largely directed towards "maintenance," since eradication of this weed is improbable, given its ability to readily reproduce via vegetative fragmentation.

Of the control technologies listed above, herbicides provide the quickest and most effective results (Ross and Lembi 1985). However, over-reliance on chemical methods may lead to weed resistance to herbicides, weed population shifts, and can result in off-target movement of herbicides and subsequent impacts to non-target organisms. The development of herbicide-resistant hydrilla (*Hydrilla verticillata* (L.f.) Royle) (Michel et al. 2004) and the discovery of hybridity between native and invasive *Myriophyllum* species (Moody and Les 2002) are recent examples of the challenges currently encountered by many aquatic plant managers. The ability of plant communities to shift in response to control practices suggests the need to develop more diverse weed management strategies for future use.

The practice of integrating various weed management methods may provide an alternative strategy for submersed plant control. Recent laboratory and outdoor mesocosm studies have shown the potential for integrating a native fungal pathogen, *Mycoleptodiscus terrestris* (Gerd.) Ostazeski, with sublethal doses of herbicide for control of nuisance submersed plants such as Eurasian watermilfoil and hydrilla (Shearer and Nelson 2002, Nelson et al. 1998, Netherland and Shearer 1996). In these studies, combined treatments of herbicide and pathogen resulted in better weed control compared to either agent used independently. Additional treatment benefits included reduced chemical input into the environment, longer-term weed control, and increased selectivity as a result of lower herbicide use rates. Future development and implementation of integrated weed management programs will be important in the overall effort to minimize the impacts incurred by repeated use of a single management strategy.

The objective of this research was to evaluate the effectiveness of combining 2,4-D with the fungal pathogen, *M. terrestris*, as an integrated control strategy against Eurasian watermilfoil.

MATERIALS AND METHODS

Studies were conducted at the U.S. Army Engineer Research and Development Center (USAERDC), Vicksburg, MS, in a walk-in growth chamber equipped with 55-L aquariums (0.75 m tall by 0.80 m^2). Conditions in the growth chamber were maintained at 22 ± 1 C with a light intensity of 580 ± 50 µmol $m^2 s^{-1}$ and a 14:10-hr light-dark photoperiod.

Eurasian watermilfoil was collected from ponds located at the Lewisville Aquatic Ecosystem Research Facility, Lewisville, TX. Plants from this site were previously evaluated for endophytic *M. terrestris* infection and were found to be free of this pathogen. Four apical stem cuttings of Eurasian watermilfoil (approximately 15 cm in length) were planted 5-cm deep into sediment-filled, glass beakers (300 ml). After planting, a thin layer of silica sand was added to the sediment surface to prevent sediment and nutrient dispersion into the water column. The sediment was collected from Brown's Lake, Vicksburg, MS, and amended with ammonium chloride at a rate of 200 mg NH_4Cl L^{-1} of sediment. Nine beakers of plants were placed in each aquarium which were pre-filled with 52 L of Smart and Barko (1984) culture solution. Air was gently bubbled in each aquarium to provide circulation of the culture solution. Twice weekly, one half the volume of culture solution was replaced in each aquarium to minimize nuisance algal growth. After each culture solution exchange, the insecticide malathion (O,O-dimethyl phosporodithioate

[2]J. Skogerboe, USAERDC, Eau Galle Aquatic Ecology Laboratory, P.O. Box 237, Spring Valley, WI 54767.

of diethyl mercaptosuccinate) was applied at a rate of 100 µl ferti•lome® Mal-A-Cide formulation[3] (Voluntary Purchasing Groups, Inc., Bonham, TX) to each aquaria to control moth larvae (namely *Parapoynx* spp.) that feed on aquatic plants. Plants were established under these conditions for 21 days prior to treatment. Malathion application was suspended one week prior to application of experimental treatments.

The *M. terrestris* isolate used for inoculum was obtained from Eurasian watermilfoil collected in Alabama (Shearer 2001). Stock cultures of *M. terrestris* were stored as 1-mm agar plugs in 10% glycerol in a cryofreezer (Revco, Asheville, NC). The plugs were plated onto potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI) and allowed to grow at room temperature for 1 week prior to initiation of liquid fermentation processes.

Inoculum was produced in 250-ml baffled, Erlenmeyer flasks containing a 100-ml volume of sterile medium composed of a basal salts solution amended with a carbon source (glucose) and either corn steep liquor powder or cottonseed meal as a nitrogen source (Solulys, Rochette Industries, France or Pharmamedia Traders, Memphis, TN, respectively). The defined basal salts solution used in all liquid cultures contained per liter of deionized water: KH_2PO_4, 4 g; $CaCl_2 \cdot 2H_2O$, 0.80 g; $MgSO_4 \cdot 7H_2O$, 0.60 g; $FeSO_4 \cdot 7H_2O$, 0.10 g; $CoCl_2 \cdot 6H_2O$, 37 mg; $MnSO_4 \cdot H_2O$, 16 mg; $ZnSO_4 \cdot 7H_2O$, 14 mg; thiamine, riboflavin, pantothenate, niacin, pyridozamine, thiotic acid, 500 mg each; and folic acid, biotin, vitamin B_{12}, 50 µg each. Cultures were incubated at room temperature and 300 rpm on a rotary shaker (Innova 4000, New Brunswick Scientific, Edison, NJ).

Mycoloeptodiscus terrestris inoculum was produced in a two-step process. The fungus was first grown in a preculture broth to initiate hyphal growth. The broth consisted of the basal salts solution amended with corn steep liquor powder (1.5%) and glucose (1%). Glucose stock solutions (20% wt/vol; Difco Laboratories, Detroit, MI) were autoclaved separately. The flasks were inoculated with one PDA plate culture of *M. terrestris* chopped into 1-mm pieces. The precultures were incubated for 4 days at which time abundant short hyphal fragments of *M. terrestris* were present in the medium.

The final inoculation medium was prepared by supplementing the basal salts solution with glucose (6%) and cottonseed meal (4.5%) as the carbon and nitrogen sources, respectively. A 10-ml aliquot of the preculture fermentation slurry was added to each flask. Following a 4-day incubation as described above, a fungal matrix developed in the flasks that was a combination of microsclerotia initials, microsclerotia, and melanized hyphae. The fungal matrix used in the application was rated at 5×10^6 colony forming units (cfu) ml^{-1}. Flasks were hand-shaken frequently to inhibit mycelial growth on the flask wall.

For the herbicide, a concentrated stock solution was prepared by dissolving the aqueous formulation DMA4™ IVM (Dow AgroSciences, Indianapolis, IN) into glass-distilled water. The DMA4 IVM formulation is approved for aquatic sites and contains 46.3% of the active ingredient, 2,4-D, as a dimethylamine salt. The stock solution was mixed using a stir plate and magnetic stir bar and was prepared approximately 0.5 hr prior to treatment.

Both the fungal inoculum and the herbicide stock solution were dispensed to the water surface in each aquarium using pipettes. Treatments included 0.25, 0.50, and 1.00 mg 2,4-D L^{-1}, 0.08, 0.16, and 0.32 ml L^{-1} of *M. terrestris*, combined treatments of each herbicide rate with each *M. terrestris* rate, and untreated controls. For the combined treatments, the herbicide and fungal inoculum were applied simultaneously. Following a 24-hr exposure to treatment, each aquarium was emptied and refilled with fresh water three times to remove treatment residues (namely herbicide residues). After rinsing, the twice weekly solution exchange as described above was continued for the duration of the experiment.

The experiment was maintained for 6 weeks following treatment application. At the end of the study, shoot and root biomass were collected, dried to a constant weight, and dry weights recorded. Visual assessments of plant health, herbicide injury and development of pathogen infection were recorded weekly.

Treatments were randomly assigned to aquariums and replicated three times. The experiment was conducted twice. Data were subjected to analysis of variance procedures using SAS[4]. To meet the assumptions for normality and equality of variance, data were transformed using log(x+1) (Snedecor and Cochran 1980). Since there were no significant differences between experimental trials, the data were combined and are presented as means over two trials. When significant treatment effects were found, means were separated using Fisher's protected Least Significant Difference (LSD) test at the 0.05 level of significance. For simplicity and clarity of presentation, non-transformed data are presented with statistical interpretations based upon transformed data.

The Colby test for interactions was performed on shoot biomass data (using percent-of-control values) to determine the nature of the interaction (synergistic, antagonistic, or additive) of combined treatments (Colby 1967). The expected responses were calculated according to the following equation:

Expected Response = control by herbicide applied alone + control by *M. terrestris* applied alone – [(control by herbicide applied alone × control by *M. terrestris* applied alone)/100].

Expected and observed responses were compared with Fisher's Protected LSD. An observed response was determined synergistic when it was greater than the expected response by at least the LSD value. An observed response lower than the expected response by the LSD, was determined to be antagonistic. If the difference between the two values was not significant, then the combination was considered additive.

RESULTS AND DISCUSSION

Treatment effects on Eurasian watermilfoil shoot and root biomass 6 weeks after application are presented in Table 1. Compared to untreated plants, a 24-hr exposure to all rates

[3]Citation of trade names does not constitute an official endorsement or approval of the use of such commercial products.

[4]Statistical Analysis System, SAS Institute, Inc., Cary, NC.

Table 1. Effects of a 24-hr exposure to 2,4-D, *M. terrestris*, and combinations of 2,4-D and *M. terrestris* on Eurasian watermilfoil shoot and root dry weight (DW) biomass. Data were collected 6 weeks after treatment application.

Treatment	Shoot biomass (g DW)	Root biomass (g DW)
Untreated Control	11.20 a	4.53 a
2,4-D alone		
0.25 mg L^{-1}	6.50 bc	3.03 ab
0.50 mg L^{-1}	6.09 bc	2.32 abc
1.00 mg L^{-1}	1.33 de	0.35 e
M. terrestris alone		
0.08 ml L^{-1}	9.65 ab	5.15 a
0.16 ml L^{-1}	4.78 c	2.72 ab
0.32 ml L^{-1}	2.34 d	1.15 cd
2,4-D + *M. terrestris*		
0.25 mg L^{-1} + 0.08 ml L^{-1}	2.68 d*	2.50 bc
0.25 mg L^{-1} + 0.16 ml L^{-1}	1.00 ef*	0.98 de
0.25 mg L^{-1} + 0.32 ml L^{-1}	0.48 ef*	0.46 de
0.50 mg L^{-1} + 0.08 ml L^{-1}	0.55 ef*	0.41 de
0.50 mg L^{-1} + 0.16 ml L^{-1}	0.60 ef*	0.64 de
0.50 mg L^{-1} + 0.32 ml L^{-1}	0.46 ef*	0.68 de
1.00 mg L^{-1} + 0.08 ml L^{-1}	0.57 ef*	0.38 de
1.00 mg L^{-1} + 0.16 ml L^{-1}	0.08 f*	0.48 de
1.00 mg L^{-1} + 0.32 ml L^{-1}	0.15 f+	0.52 de
LSD (0.05)	1.60	1.57

Within each column, values followed by a different letter are significantly different according to Fisher's protected LSD test at $p \leq 0.05$; n = 6.
An asterisk (*) denotes synergism and a plus sign (+) indicates an additive response based on the Colby test for interactions.

of 2,4-D applied alone significantly reduced shoot biomass however, a dose of 1.00 mg 2,4-D L^{-1} was required to eliminate >85% of shoots, which in terms of management, can be considered a successful field application. Root biomass was reduced by 92% with the highest 2,4-D rate, thus greatly diminishing the potential for recovery from perennial rootstock. Lower doses of 2,4-D (0.25 and 0.50 mg L^{-1}) reduced shoot biomass by only 42 to 46% and had no significant impact on roots. Typical 2,4-D symptoms (leaf cupping and curling, stem epinasty, leaf chlorosis) were observed on all herbicide-treated plants as early as 1 day after treatment (DAT). Stem necrosis occurred as early as 14 DAT on plants exposed to 1.00 mg 2,4-D L^{-1}. As expected, severity of 2,4-D injury was more pronounced on plants exposed to higher chemical rates. Green and Westerdahl (1990) reported similar biomass reductions (40%) following a 24-hr exposure of 0.50 mg L^{-1} 2,4-D on Eurasian watermilfoil. These researchers also observed severe injury (88%) to plants exposed to 1.00 mg L^{-1} 2,4-D, however total plant biomass collected 4 weeks after treatment (WAT) was reduced only by 60% whereas an 88% reduction was recorded 6 WAT in this study. Our results also compare favorably with studies by Netherland (1991) that showed a 75% reduction in Eurasian watermilfoil biomass following a 24-hr exposure to 1.00 mg L^{-1} 2,4-D.

For *M. terrestris* applied alone, shoot biomass decreased with increasing rate of application. A similar rate response with *M. terrestris* has been reported (Shearer and Nelson 2002, Netherland and Shearer 1996). In this study, rates of 0.16 and 0.32 ml L^{-1} significantly reduced shoots by 57 and 79% compared with untreated plants, whereas the 0.08 ml L^{-1} rate had no effect on shoot biomass. Only the highest rate of *M. terrestris* applied alone significantly affected roots, reducing root growth by 75% compared to untreated plants. There were no statistical differences in shoot biomass between the high rate of *M. terrestris* (0.32 ml L^{-1}) and the high rate of 2,4-D (1.00 mg L^{-1}).

Combined treatments of 2,4-D and *M. terrestris* reduced shoot biomass of Eurasian watermilfoil better than either agent applied alone. With exception of 0.25 mg L^{-1} 2,4-D + 0.08 ml L^{-1} *M. terrestris*, all integrated treatments were statistically similar and reduced shoot biomass more than 90%. Results were similar on roots with biomass reductions averaging 87% compared to untreated plants. Although less effective than higher rate combinations, 0.25 mg L^{-1} 2,4-D + 0.08 ml L^{-1} *M. terrestris* inhibited shoot and root biomass by 76 and 45% respectively, and was still more efficacious than either agent applied alone at these rates.

Results of the Colby test indicated that interactions between 2,4-D and *M. terrestris* were either synergistic or additive. Combined treatments showed synergistic interactions at all rates except 1.00 mg L^{-1} 2,4-D + 0.32 ml L^{-1} *M. terrestris*, which showed an additive interaction. In no instance did the combinations of these two agents show antagonism.

Overall the data demonstrated that under these experimental conditions, combining both agents as a simultaneous treatment, significantly improved control of Eurasian watermilfoil. Rates as low as 0.25 mg L^{-1} 2,4-D combined with 0.16 ml L^{-1} *M. terrestris* reduced plant growth by more than 90%. A 2,4-D rate four times as high was required to produce the same effect if the herbicide was used alone. Lowering herbicide use rates reduces the risk of chemical injury to sensitive non-target vegetation, reduces cost of application, and may minimize impacts from label-imposed use restrictions. Low doses of both agents applied alone were insufficient to eliminate perennial rootstock and as a result, plants maintained some regenerative capacity which contributed to final shoot biomass. However, combining low doses of both herbicide and pathogen had significant impacts on root biomass (>85% reduction), thus greatly minimizing the potential for plant re-establishment following treatment.

In addition to lower herbicide rates, the data also showed that >90% Eurasian watermilfoil control could be achieved with a minimal 24-hr contact time when combining either 0.25 mg L^{-1} 2,4-D with 0.16 ml L^{-1} *M. terrestris* or 0.50 mg L^{-1} 2,4-D with 0.08 ml L^{-1} *M. terrestris*. Concentration-exposure time studies by Green and Westerdahl (1990) and Netherland (1991) showed that achieving this level of plant control required 2,4-D rates of 2.00 or 1.00 mg L^{-1} at contact times of 36 and 48 hrs, respectively. Reducing the contact time requirements by combining 2,4-D with *M. terrestris* should improve plant control where contact time is influenced by water exchange.

Results of these and previous studies (Netherland and Shearer 1996, Nelson et al. 1998, Shearer and Nelson 2002) indicate that *M. terrestris* is highly compatible with a variety of herbicides with different modes of action (i.e., 2,4-D, endothall (7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid), and

fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1*H*)-pyridinone). All of these studies confirmed that by integrating *M. terrestris* with herbicides, chemical use rates could be significantly reduced and impacts to non-target vegetation minimized. In addition, isolates of *M. terrestris* have shown a high degree of specificity for the target plants Eurasian watermilfoil and hydrilla. Host specificity tests on 46 species of aquatic, wetland, and crop plants demonstrated that only duck lettuce (*Ottelia alismoides* (L.) Pers.) was susceptible to *M. terrestris* at rates sufficient to control target plants (Joye and Cofrancesco 1991). (Note: *M. terrestris* was mis-identified in these early studies as *Macrophomina phaseolina* (Tassi) Goid.).) These data support the potential of integrated weed management as an effective, reduced-risk alternative for nuisance submersed plant control. Cooperative research between the U.S. Department of Agriculture, Agricultural Research Service, National Center for Agricultural Utilization Research (USDA-ARS-NCAUR) and the US-AERDC, is currently underway to develop *M. terrestris* as a marketable bioherbicide formulation for submersed aquatic weed control (Shearer and Jackson 2003).

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LITERATURE CITED

Bates, A. L., E. R. Burns and D. H. Webb. 1985. Eurasian watermilfoil (*Myriophyllum spicatum* L.) in the Tennessee Valley: an update on biology and control, pp. 104-115. *In*: L. W. J. Anderson (ed.), Proceedings of the First International Symposium on watermilfoil (*Myriophyllum spicatum*) and related Haloragaceae species. Aquat. Plant Manage. Soc., Washington, DC.

Bartodziej, W. and J. Ludlow. 1997. Aquatic vegetation monitoring by natural resource agencies in the United States. J. of Lake and Res. Manage. 13(2):109-117.

Borawa, J. C., J. H. Kerby, M. T. Huisch and A. W. Mullis. 1979. Currituck Sound fish populations before and after infestation by Eurasian watermilfoil. Proc. Ann. Conf. S.E. Assoc. Fish and Wildlife Agencies. 32:520-528.

Colby, S. R. 1967. Calculating synergistic and antagonistic responses of herbicide combinations. Weeds. 15:20-22.

Couch, R. and E. Nelson. 1985. *Myriophyllum spicatum* in North America, pp. 8-18. *In*: L. W. J. Anderson (ed.), Proceedings of the First International Symposium on watermilfoil (*Myriophyllum spicatum*) and related Haloragaceae species. Aquat. Plant Manage. Soc., Washington, DC.

Crowell, W., N. Troelstrup, Jr., L. Queen and J. Perry. 1994. Effects of harvesting on plant communities dominated by Eurasian watermilfoil in Lake Minnetonka, MN. J. of Aquat. Plant Manage. 32:56-60.

Eiswerth, M. E., S. G. Donaldson and W. S. Johnson. 2000. Potential environmental impacts and economic damages of Eurasian watermilfoil (*Myriophyllum spicatum*) in western Nevada and northeastern California. Weed Tech. 14:511-518.

Green, W. R. and H. E. Westerdahl. 1990. Response of Eurasian watermilfoil to 2,4-D concentrations and exposures times. J. Aquat. Plant Manage. 28:27-32.

Getsinger, K. D., E. G. Turner, J. D. Madsen and M. D. Netherland. 1997. Restoring native vegetation in a Eurasian water milfoil-dominated plant community using the herbicide triclopyr. Regul. Rivers: Res. Mgmt. 13:357-375.

Helsel, D. R., D. T. Gerber and S. Engel. 1996. Comparing spring treatments of 2,4-D with bottom fabrics to control a new infestation of Eurasian watermilfoil. J. Aquat. Plant Manage. 34:68-71.

Joye, G. F. and A. F. Cofrancesco. 1991. Studies on the use of fungal pathogens for control of *Hydrilla verticillata* (L.f.) Royle. Technical Report A-91-4. U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS. 26 pp.

Johnson, R. L. and B. Blossey. 2002. Eurasian watermilfoil, pp. 79-90. *In:* R. Van Driesche, B. Blossey, M. Hoddle, S. Lyon and R. Reardon (eds.), Biological Control of Invasive Plants in the Eastern United States. U.S. Department of Agriculture, Forest Service Pub. FHTET-2002-04.

Maceina, M. J., P. W. Bettoli, W. G. Klussmann, R. K. Betsill and R. L. Noble. 1991. Effect of aquatic macrophyte removal on recruitment and growth of black crappies and white crappies in Lake Conroe, Texas. N. Amer. J. Fish. Manage. 11:556-563.

Madsen, J. D. and D. H. Smith. 1997. Vegetative spread of Eurasian watermilfoil colonies. J. Aquat. Plant Manage. 35:63-68.

Madsen, J. D., J. W. Sutherland, J. A. Bloomfield, L. W. Eichler and C. W. Boylen. 1991. The decline of native vegetation under dense Eurasian watermilfoil canopies. J. Aquat. Plant Manage. 29:94-99.

Michel, A., B. E. Scheffler, R. S. Arias, S. O. Duke, M. D. Netherland and F. E. Dayan. 2004. Somatic mutation-mediated evolution of herbicide resistance in the non-indigenous invasive plant hydrilla (*Hydrilla verticillata*). Molec. Ecol. 13:3229-3237.

Moody, M. L. and D. H. Les. 2002. Evidence of hybridity in invasive watermilfoil (*Myriophyllum*) populations. PNAS 99(2):14867-14871.

Mullin, B. H., L. W. J. Anderson, J. M. DiTomaso, R. E. Eplee and K. D. Getsinger. 2000. Invasive plant species. Council for Agricultural Science and Technology (CAST), Issue Paper No. 13. 18 pp.

Nelson, L. S., J. F. Shearer and M. D. Netherland. 1998. Mesocosm evaluation of integrated fluridone-fungal pathogen treatments on four submersed plants. J. Aquat. Plant Manage. 36:73-77.

Nelson, L. S. and J. F. Shearer. 2002. Response of Eurasian watermilfoil to integrated fluridone-fungal pathogen treatment. APCRP Technical Note, TN APCRP-IC-03. U.S. Army Engineer Research and Development Center, Vicksburg, MS. 7 pp.

Netherland, M. D. 1991. Herbicide concentration/exposure time relationships for Eurasian watermilfoil and hydrilla. Proceedings, 24th Annual Meeting, Aquatic Plant Control Research Program. Miscellaneous Paper A-91-3. U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS. pp. 205-209.

Netherland, M. D. and K. D. Getsinger. 1995. Laboratory evaluation of threshold fluridone concentrations under static conditions for controlling hydrilla and Eurasian watermilfoil. J. Aquat. Plant Manage. 33:33-36.

Netherland, M. D., W. R. Green and K. D. Getsinger. 1991. Endothall concentration and exposure time relationships for the control of Eurasian watermilfoil and hydrilla. J. Aquat. Plant Manage. 29:61-67.

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.

Newman, R. M., D. W. Ragsdale, A Milles and C. Oien. 2001. Overwinter habitat and the relationship of overwinter to in-lake densities of the milfoil weevil, *Euhrychiopsis lecontei*, a Eurasian watermilfoil biological control agent. J. Aquat. Plant Manage. 39:63-67.

Newroth, P. R. 1985. A review of Eurasian water milfoil impacts and management in British Columbia, pp. 139-153. *In:* L. W. J. Anderson (ed.), Proceedings of the First International Symposium on watermilfoil (*Myriophyllum spicatum*) and related Haloragaceae species. Aquat. Plant Manage. Soc., Washington, DC.

Nichols, S. A. and B. H. Shaw. 1986. Ecological life histories of the three aquatic nuisance plants, *Myriophyllum spicatum*, *Potamogeton crispus*, and *Elodea canadensis*. Hydrobiologia 131:3-21.

Painter, D. S. 1988. Long-term effects of mechanical harvesting on Eurasian watermilfoil. J. Aquat. Plant Manage. 26:25-29.

Parsons, J. K., K. S. Hamel, J. D. Madsen and K. D. Getsinger. 2001. The use of 2,4-D for selective control of an early infestation of Eurasian watermilfoil in Loon Lake, Washington. J. Aquat. Plant Manage. 39:117-125.

Petr, T. 2000. Interactions between fish and aquatic macrophytes in inland waters. A review. FAO Fisheries Technical Paper. No. 396. Rome, FAO. 185 pp.

Ross, M. A. and C. A. Lembi. 1985. Applied weed science. Macmillan Publishing Company, New York, NY. 340 pp.

Shearer, J. 1996. Potential of a pathogen, *Mycoleptodiscus terrestris*, as a biocontrol agent for the management of *Myriophyllum spicatum* in Lake Guntersville Reservoir. Technical Report A-96-4. U.S. Army Corps of Engineers, Waterways Experiment Station, Vicksburg, MS. 28 pp.

Shearer, J. F. 2001. Recovery of endophytic fungi from *Myriophyllum spicatum*. APCRP Technical Note, TN APCRP-BC-03. U.S. Army Engineer Research and Development Center, Vicksburg, MS. 11 pp.

Shearer, J. F. and M. A. Jackson. 2003. Mycoherbicidal compositions and methods of preparing and using the same. United States Patent Office, No. 6,569,807.

Shearer, J. F. and L. S. Nelson. 2002. Integrated use of endothall and a fungal pathogen for management of the submersed aquatic macrophyte *Hydrilla verticillata*. Weed Tech. 16:224-230.

Smart, R. M. and J. W. Barko. 1984. Culture methodology for experimental investigations involving rooted submersed aquatic plants. Miscellaneous Paper A-84-6. U.S. Army Corps of Engineers, Waterways Experiment Station, Vicksburg, MS. 20 pp.

Smith, C. S. and J. W. Barko. 1990. Ecology of Eurasian watermilfoil. J. Aquatic Plant Manage. 28:55-64.

Snedecor, W. G. and W. G. Cochran. 1980. Statistical methods. 7th Edition. The Iowa State Press, Ames, IA. 507 pp.

Sorsa, K. K., E. V. Nordheim and J. H. Andrews. 1988. Integrated control of Eurasian water milfoil, *Myriophyllum spicatum*, by a fungal pathogen and a herbicide. J. Aquat. Plant Manage. 26:12-17.

Stanley, R. A., E. Shackelford, D. Wade and C. Warren. 1976. Effect of season and water depth on Eurasian watermilfoil. J. Aquat. Plant Manage. 14:32-36.

Unmuth, J. M., D. J. Sloey and R. A. Lillie. 1998. An evaluation of close-cut mechanical harvesting of Eurasian watermilfoil. J. Aquat. Plant Manage. 36:93-100.