I. **Project Title:** BIOLOGICAL CONTROL OF ECOLOGICALLY HARMFUL EXOTIC SPECIES: EURASIAN WATERMILFOIL

Program Manager: Luke Skinner
Agency Affiliation: Minnesota Department of Natural Resources
Division of Fish and Wildlife
Address: Ecological Services Section
Box 25, 500 Lafayette Road
St. Paul, MN 55155-4025
Phone: (612) 297-3763

A. Appropriation: $160,000.00
Balance: $ 0

Laws of Minnesota 1992, Chp. , Sec. , Subd. . This appropriation is from the Minnesota future resources fund to the commissioner of natural resources for a research program leading to biological control of Eurasian watermilfoil. It is available upon final enactment and is otherwise subject to the provisions of Laws 1991, chapter 254, article 1, section 14. Subd. 9(b)

B. n\a

C. n\a

II. **NARRATIVE:** The submersed aquatic plant, Eurasian watermilfoil (*Myriophyllum spicatum*), has recently invaded Minnesota and is now established in 47 lakes and 2 rivers. The largest infestations are located in the Twin Cities metropolitan area, including Lake Minnetonka. The present control strategy for Eurasian watermilfoil is chemical, with some mechanical control.

The purpose of this biological control project is to initiate a research program leading to the long term control of Eurasian watermilfoil. Research will be carried out to assess the potential of native insects and fungi, leading to integrated control strategies for controlling Eurasian watermilfoil. Principal cooperators under this program are the Department of Natural Resources, the University of Minnesota, and the EcoScience Corporation.

III. **OBJECTIVES:**

A. **TITLE: The potential for biological control of Eurasian watermilfoil with native and naturalized invertebrates.**

A1. **NARRATIVE:** Native aquatic insects known to feed on Eurasian watermilfoil have been identified in the Canadian Province of Ontario and the State of Wisconsin, both of which border Minnesota. Surveys will be conducted to verify the presence of these insects of Minnesota, and laboratory studies will be carried out to determine their feeding preference.

A2. **PROCEDURES:**

a. **Extensive survey of 40-50 lakes (sites).** Sampling will occur in late summer 1992 and include metro area lakes, as well as outstate lakes. Results will be used in a correlational analysis of the following factors:
1. Presence of potential biocontrol insects.
2. Macrophyte community structure and relative density.
3. Qualitative assessment of plant damage.
4. Location, physical, and chemical characteristics.

b. **Intensive study of 5-10 selected lakes with and without potential biocontrol invertebrates.** These sites will be sampled 3 times in the summer and fall of 1992, and once in late spring or early summer of 1993. Results will provide a more detailed and quantitative assessment of the potential of biocontrol herbivores for milfoil control. The following measurements will be made:
1. Quantitative estimates of invertebrate herbivore densities.
2. Quantitative assessment of plant damage.
3. Quantitative estimates of plant density and community structure.
4. Location, physical, and chemical characteristics.

c. **Laboratory feeding and preference studies of potential biocontrol invertebrates.** These studies will provide information on plant preference and damage potential of the invertebrates.

A3. **BUDGET:**

a. Amount budgeted: $55,000.00
b. Balance: $ 0

A4. **TIMELINE:**

	7/92	1/93	6/93
Site selection	****	***	
Intensive field sampling	**********		
Extensive field sampling	*****		
Field consumption/preference	********		
Lab consumption/preference	****************		
Identify/enumerate samples	*********************		
Physical/chemical analysis		******	
Analyze results	******************	*****	
Preliminary reports		*****	
Final report			*****

A5. **STATUS:** In total, 30 sites were examined in 25 lakes reported to contain EWM; three lakes, Island Lake, Bryant Lake, and Lake Wabasso were found to have very little or no EWM and were thus not suitable for sampling. Control efforts in several other lakes precluded their use. Twenty-seven sites at twenty-one lakes in Minnesota and Wisconsin were thus surveyed for presence of control agents, plant stand and environmental characteristics, and EWM density and damage. Milfoil has been in two of the Wisconsin sites (Fish Lake and Devil's Lake) for over 12 years and these were used as examples of well established or possibly declining (Devil's Lake) sites. Eight of the Minnesota sites were selected as intensive sites. These sites were sampled several times to identify changes over time in such parameters as biomass and community structure of aquatic macrophytes and their associated invertebrate fauna, herbivore damage to the plant community and physical and chemical characteristics of the water and sediments. These sites are being resampled in June 1993; these data and a more detailed presentation of our results will be provided in a report to the Minnesota Department of Natural Resources this summer.

There was a range of milfoil densities at the sites sampled. Late summer milfoil biomass (wet weight) ranged from 23 g/m^2 to over 2000 g/m^2 and total plant biomass ranged from 400 to over 4500 g/m^2. Five sites had low densities of EWM, five sites had high densities and in the other sites, densities were moderate, or patchy and limited to relatively small areas. Sites in Lake Minnetonka and Fish Lake, Wisconsin, had the highest densities of EWM, all being over 1100 g/m^2. Stem densities averaged from 100/m^2 to over 1300/m^2. These densities are similar to others reported in the literature and with the densest sites in Wisconsin. Over half of the sites had five or more species of macrophyte in addition to EWM, however, almost one fourth of the sites only had two species other than EWM. *Ceratophyllum demersum* (coontail) and *Myriophyllum sibiricum* (northern watermilfoil) were most commonly associated with EWM and in five instances were at equal to or higher densities than EWM.

The maximum depth of milfoil was related to light levels, with greatest depths being attained in clearer lakes with deep secchi depths and low chlorophyll levels. In 1992, maximum depths ranged from 1 to 6.6 m, however, in most sites, milfoil was not found deeper than 3 m. The unusually wet and cold summer may have limited milfoil growth and densities. Milfoil densities were related to sediment ammonium (nutrient) content with the highest density stands occurring in high ammonium sediments; milfoil density was positively related to secchi depth and sediment ammonium. There was also a weak negative relationship between milfoil density and coarser (sandy) sediment as has been observed in other studies. These results suggest that, as elsewhere, improved water clarity may enhance milfoil growth, but, reduced sediment nutrients may limit milfoil growth. Somewhat surprisingly, total plant biomass was negatively related to secchi depth which contrasts with the positive relation of milfoil density ' secchi depth.

Plant damage by invertebrates ranged from an average of 4% to 19% of total leaf area lost, with the highest percent damaged noted in Devil's Lake and two sites in Lake Minnetonka. Plant damage increased with depth, and was positively related to snail densities. These damage estimates are similar to the few other reports for invertebrate damage. Stem damage was also extensive at some sites and generally associated with stem boring chironomids or weevils. We did not obtain enough sites with high enough densities of specialist herbivores (i.e., weevils, caterpillars or the milfoil midge) to detect any relation between plant damage and their density.

Invertebrate densities in milfoil beds ranged from 60 to 9000/m^2. Amphipods, midges, snails and caddisflies were most common. The total abundance of invertebrates per m^2 of substrate increased with total plant biomass, however, invertebrate abundance was negatively related to milfoil density. These results suggest that while high plant abundance is good for invertebrates high abundance of milfoil is not. This may either be a direct effect of EWM or the fact that diversity of plant abundance is lower at high milfoil densities

Herbivores with some potential to control EWM were found at 2 Wisconsin sites and at 6 Minnesota sites. We have professionally verified identifications of three potential control agents in Minnesota; the weevil, *Euhrychiopsis lecontei*, in Lake Auburn, Otter Lake and Gideon's and Smith's Bays of Lake Minnetonka(C. O'Brien); the caterpillar *Acentria ephemerella* (= *A. nivea*) in Lakes Auburn and Virginia (S. Passoa); the caddisfly *Nectopsyche albida* at Shady Island in Lake Minnetonka (R. Holzenthal). We suspect we have the midge, *Cricotopus myriophylli* in one lake and another caterpillar *Parapoynx* in another, but have not yet had our identifications of these verified. Snails were abundant at many sites, but are not likely to be good control agents.

The caddisfly *Nectopsyche* was relatively dense (210/m^2) at the Shady Island site in Lake Minnetonka; this site also had one of the highest levels of leaf damage (mean of 19% with a high of 32%) and most of the leaf damage was consistent with feeding by the caddisfly. We suspect that the caddisfly will eat other plants as readily as milfoil and thus it may not be a good control agent, however, feeding choice and preference experiments with the caddisfly need to be done this summer to determine its range of feeding.

The weevils collected in Lake Auburn were patchily distributed and only locally abundant. Some areas had extensive damage and other areas appeared to harbor no weevils. Weevils seemed similarly distributed at the other sites in Minnesota; damage was evident at some sites but very few weevils were located. Conversely, at Devil's and Fish Lakes, Wisconsin, weevil densities were higher; weevils have been documented to occur in these Wisconsin lakes since the 1980s. We estimated weevil densities at 6/m^2 at Devil's Lake and 2/m^2 at Fish Lake, h ver, we think that a ance behavior may have resulted in underestimates of we densities.

The weevil is thought to be native to North America and probably Minnesota. Its original host plant is unknown, but thought to be northern watermilfoil. We suspect that for some reason the weevil does not normally attain high population levels on northern watermilfoil but can attain high populations on EWM, which our lab studies have shown it to prefer. The relatively short time that EWM has been in Minnesota may not have allowed weevils to yet reach high densities. Fish and Devil's Lake in Wisconsin have had dense milfoil for over 12 years, which would permit time for development of weevil populations. Plant damage was extensive in these lakes and there is some suggestion that increased densities of weevils may be reducing milfoil biomass; milfoil has been reported to have declined Devil's Lake and appears to be declining in Fish Lake. We did note that lakes with weevils had significantly higher clarity (secchi depths) than lakes without weevils, however, this may be interrelated with higher densities of milfoil being more likely to harbor weevils.

Laboratory and greenhouse cultures of Eurasian and northern watermilfoil were developed, along with other native species. A reproducing stock of the weevil was established and at least 3 laboratory generations (eggs to adults) were produced between September and February. However, due to nutrient depletion of sediments by the milfoil in mid-winter and underestimation of the extent of milfoil damage by weevils we were not able to provide enough healthy milfoil to maintain the weevil colony after March. A new colony of weevils and larger stock of weevil-free and nutrient replenished milfoil is being established this spring.

Milfoil consumption rates were estimated for the caddisfly, *Nectopsyche*, at 6 mg per larvae per day and they can significantly reduce milfoil growth. These results are consistent with our field observations at Shady Island. However, we suspect that they will also feed on other plants. The caddisflies pupated before multiple choice experiments could be conducted. These studies will be pursued this summer.

Multiple choice experiments with the weevil indicate that it strongly prefers Eurasian watermilfoil over other aquatic plants. In multiple choice oviposition tests, 7 of 12 female weevils oviposited on Eurasian watermilfoil in the first round; mean time to oviposition was 1 day. The 5 other weevils did not oviposit on any plants. The average weevil spent about 60% of its time on Eurasian watermilfoil and less than 5% of its time on any other taxa. In the second round, Eurasian watermilfoil was not included and weevils were presented with six taxa. None of the weevils oviposited within 5 days and most of their time was spent on no plants or northern watermilfoil. Although no oviposition was noted on northern watermilfoil, we have observed oviposition on northern watermilfoil several times in other settings. It does appear, from limited experimentation, that most oviposition is oriented toward Eurasian watermilfoil. These experiments will be replicated several more times this summer. In addition, we will attempt to grow

weevils on northern watermilfoil, which is presumably the original host, but does not seem to be currently preferred.

This work has shown that herbivores with the potential to control Eurasian watermilfoil occur in Minnesota. These herbivores are either native or naturalized, have not had undesirable effects on native vegetation and are capable of surviving the Minnesota climate. Given its apparent specificity and potential for damage to milfoil, we believe the weevil, *Euhrychiopsis lecontei* is the best candidate for control, however, the potential of the other invertebrates needs to be explored further. It is unclear what is controlling and limiting the distribution of the weevil and other control agents. This will need to be determined before they can be successfully used in a control program. We suspect that actions to enhance the populations of one control agent may also enhance populations of other control agents. Further testing to ensure host specificity, especially of organisms raised on plants other than Eurasian watermilfoil is needed to ensure that enhancing control agent populations or introducing them to new waters will not harm native plants. More information on the mechanisms of plant damage by these herbivores and their effects on plant populations in the field and laboratory is also needed. This information, along with and understanding of what is currently limiting their populations and distribution will be needed for effective biological control. We intend to investigate these topics during the upcoming biennium.

A6. BENEFITS: The ability of native or naturalized insects capable of biological control of Eurasian watermilfoil will be determined in this objective. The information from all three of the above components will be used to make recommendations for integrated control strategies. This could ultimately minimize the impacts of current control methods on aquatic ecosystems.

B. TITLE: Isolation of fungi native to Minnesota for control of Eurasian watermilfoil.

B1. NARRATIVE: Mycoleptodiscus terrestris (M.t.) is a naturally occurring fungus which has the ability to colonize and degrade Eurasian watermilfoil. In aquatic environments, M.t. is host specific, colonizing and causing disease in Eurasian watermilfoil while not affecting native plant species. Strains of M.t. will be selected which are found in close association with Eurasian watermilfoil from different geographic areas in Minnesota.

B2. PROCEDURES:

a. **Selection of strains of M.t. with greater virulence against Minnesota Eurasian watermilfoil populations at temperatures typical of northern latitudes.** Samples will be collected from all lakes known to have Eurasian watermilfoil populations in Minnesota. Strain isolates will be cultured, formulated, and evaluated at 59°F and 77°F for the following:

1. Disease incidence.
2. Rate of disease development.
3. Disease severity.

b. **Determination of the susceptibility of different geographic populations of Eurasian watermilfoil to M.t.** Different strains of Eurasian watermilfoil may have differential susceptibility of attack by M.t., necessitating the development of selected M.t. strains for specific regions of Minnesota. Samples of Eurasian watermilfoil from different geographic regions in Minnesota will be cultured in the laboratory. Bioassays using differing Eurasian watermilfoil and M.t. strains will be conducted and assessed under the same criteria as in 2(a), including percent reduction in biomass.

c. **Selection of M.t. strains with enhanced growth and accelerated disease progression at 59°F.** Isolates of M.t. found in other regions of the U.S. have been found to have accelerated growth, and induce disease progression and severity of infection faster at 77°F. In the same way, isolates of M.t. from different Eurasian watermilfoil populations will be screened and evaluated for these same traits in water temperatures more common to Minnesota (i.e. 59°F).

B3. BUDGET:

a. Amount budgeted: $54,000.00
b. Balance: $ 0

EcoScience Corporation (ECS) agrees to reimburse the Minnesota Future Resources Fund all support paid to ECS for up to the full level of support paid to ECS under this research and development grant. Such reimbursement will be made by ECS upon first sale of any milfoil control product in Minnesota, or from the first sale of any milfoil control product anywhere based on material derived from this program. The mechanism for the reimbursement would be either through cost reduction for finished goods or payment by ECS of up to thirty percent (30%) of its profits generated from sale of a milfoil control product in Minnesota. Such repayment will continue until the full amount of support received by ECS has been repaid.

B4. TIMELINE:

	7/92 1/93 6/93
M.t. strain isolation	***************************************
M.t/EWM laboratory bioassays	***************************************
Temperature studies	***************************************

B5. STATUS: Milfoil was collected from 16 lakes throughout Minnesota and microbial isolations were made from approximately 100 plants per lake. Five lakes (Auburn, Bavaria, Minnewashta, Pierson, and Zumbra) were sampled at monthly intervals. *Mycoleptodiscus terrestris*, M.t., isolations appeared greater in July and September than in June. The overall frequency of M.t. isolation from all lakes varied from 0% in Lake Waverly and Otter to 100% in Lake Bavaria. From these plants, 310 isolates were obtained of which 288 proved amenable to standard culture and preservation techniques.

The isolates were evaluated for virulence against Eurasian watermilfoil. Variation in virulence was identified using Quantitative Virulence Assay (QVA) as a method of assesment. Isolates with rates of disease progression faster and slower than the current strain have been identified. The relative rates of disease progression were consistent over four assays, including one blind assay. Additional methods of assessment are now underway, including one meter column assays to determine the nature and extent of disease development on larger milfiol plants.

Potential variation in temperature response has been identified. Isolates that exhibit faster disease progression dynamics at both 25 and 15 degree celcius relative to the reference strain have been isolated from Minnesota milfoil. Qualitative differences among isolates in rate of lesion development or severity of disease were not apparent at 25 or 15 degrees celcius.

Milfoil plants from several geographic locations in Minnesota as well as from other states were tested for susceptibility to M.t.. No populations of milfoil were identified as being resistant to M.t.. Disease symptoms and rates of disease progression for Minnesota milfoil populations were within the range observed in milfoil populations from around the United States.

B6. BENEFITS: A strain of M.t. with enhanced virulence towards northern populations of Eurasian watermilfoil and an expanded temperature range more typical of Minnesota lakes will be obtained. Possible differences in milfoil susceptibility will be investigated, and selected M.t. strains that will provide consistent control of Eurasian watermilfoil in northern latitudes will be studied.

C. TITLE: Comparative effectiveness of M.t. strains for milfoil control in field trials.

C1. NARRATIVE: As part of the EcoScience Experimental Use Permit field program for 1993, the three most effective strains of M.t. identified in laboratory screens will be evaluated under Minnesota field conditions in spring and summer of 1993.

C2. PROCEDURES: Treatments will be applied to milfoil stands with full canopies when water temperatures are optimum for growth of the three stra[illegible] Prior to treatm[illegible], two isolation tubes will be placed in each tank. One wi[illegible] covered during treatment and will serve as a control.

Treatments will be replicated three times using a randomized complete block design.

Percent cover will be estimated prior to and 6 weeks after application of materials to determine canopy reduction. Total above ground biomass in isolation tubes will be harvested at 6 weeks post treatment. Water quality will be monitored weekly throughout the study. Plants, sediments, and water column will be sampled for the presence of M.t. at 6 weeks post treatment. Percentage data will be transformed accordingly, subjected to analysis of variance, and where applicable, means will be separated (P=0.05).

C3. **BUDGET:**

a. Amount budgeted: $51,000.00
b. Balance: $ 0

The same grant reimbursement language used in Objective B applies.

C4. **TIMELINE:**

	7/92	1/93	6/93
M.t. field trials			****

C5. **STATUS:** The research leading up to the implementation of this objective has shown that although we have identified isolates that show potential against milfoil, more research is needed before field applications can be made. Smaller more controlled tests (possibly outdoor enclosure work) need to be implemented. This portion is being worked on. Secondly, due to a vacancy, the project's Program Manager has changed during the past year. The new manager has concerns regarding project direction, implementation and reporting. These concerns are being addressed but not in time for a more complete report on this objective.

C6. **BENEFITS:** Enhanced activity of the biological control product.

IV. **EVALUATION:** The main objectives of this project are to collect and establish baseline information on the potential of using biological agents for the control of Eurasian watermilfoil in Minnesota. This study will be the first research project of its kind since Eurasian watermilfoil was first identified in Minnesota in 1987. The duration of this project will last approximately 14 months. Accordingly, its length and scope will allow for only preliminary observations and conclusions. The findings reported from this work will act as precursors to future biological control research projects for the 1994-1995 biennium.

V. CONTEXT: RELATED CURRENT AND PREVIOUS WORK

A. The current work will be of short duration and will have a much narrower focus than if the project had been funded and approved for the entire biennium. Since there has been no previous work done in this area this will be the first biocontrol research program of its kind in Minnesota for Eurasian watermilfoil.

B. The results of this study will supplement existing biological control information obtained from studies conducted in other areas of the U.S. and Canada. This information will be used by the Minnesota DNR in the development of integrated control strategies.

C. This program was originally developed by the Freshwater Foundation with the assistance of the DNR, effective July 1, 1991. To implement this project, the LCMR had approved $100,000.00, to be matched by $200,000.00 by the Freshwater Foundation.

The Freshwater Foundation could not raise the match, and in December 1991 declined further participation in the biological control research program. The DNR volunteered to continue with development and implementation of the project, conducted an additional peer review process on existing proposals, and submitted a revised proposal at roughly half ($160,000.00) the original funding level of $300,000.00.

LCMR has not previously funded research programs for Eurasian watermilfoil. A proposal for furthering biological control research on Eurasian watermilfoil was developed by the DNR and is under consideration for funding during the 1994-1995 biennium.

D. Water Recreation Account, Ecological Services, Eurasian Watermilfoil FY 90-92, $125,262.00 each fiscal year. These funds have been allocated to develop programs for public education and awareness, law enforcement, survey and monitoring, and control and eradication of milfoil.

E. Not yet available.

VI. QUALIFICATIONS:

1. **Program Manager**
Luke Skinner
Minnesota DNR
Ecological Services Section
Box 25, 500 Lafayette Road
St. Paul, MN 55155-4025
(612) 297-3763

EDUCATION:
Bachelor of Science Degree 1987. University of Minnesota
Major: Biology
Minor: Chemistry

POSITIONS:
Coordinator, Purple Loosestrife Program, Minnesota Department of Natural Resources, St. Paul; March 1990 - Present.

Purple Loosestrife Project Coordinator, Hennepin Parks, Hennepin County, MN; May 1988 - December 1989.

Senior Laboratory Technician, Center for Water and the Environment, Natural Resources Research Institute, University of Minnesota, Duluth; June 1986 - September 1986 and June 1987 - November 1987.

Research Assistant, Department of Biology, University of Minnesota, Duluth; September 1985 - March 1986.

Assisted in research on the diet of Lake Superior trout and salmon.

SELECTED PUBLICATIONS:
Skinner, L.S., W.J. Rendall and E.L. Fuge. In Press. Minnesota's Purple Loosestrife Program: History, Findings and Management Recommendations. Special Publication 145, Minnesota Department of Natural Resources, 32 pages.

Skinner, L.S., T.P. Hollenhorst and C.J. Evenson. 1989. Control of Purple Loosestrife (Lythrum Salicaria L.) with Triclopyr Amine. Research Report, North Central Weed Science Society. Volume 46, pp. 104-106.

Skinner, L.S. and T.P. Hollenhorst. 1989. Incidence, Spread and Control Studies of Purple Loosestrife (Lythrum Salicaria L.) in Hennepin Parks. Special Publication published by Suburban Hennepin Regional Park District, MN. 75 pages.

Skinner, L.S. 1988. Purple Loosestrife (Lythrum Salicaria L.) Control Studies in Hennepin Parks, MN. 43rd Annual North Central Weed Control Conference Proceedings, Weed Science Society of America, Volume 43, p. 69.

2A. Cooperators/other investigators: Objective A.
Raymond M. Newman
Fisheries and Wildlife
University of Minnesota
St. Paul, MN 55108
(612) 625-5704

EDUCATION:
Ph.D. (Fisheries) 1985, University of Minnesota, St. Paul, MN
M.S. (Fisheries) 1982, University of Minnesota, St. Paul, MN
B.S. (Biology) 1978, Slippery Rock University, Slippery Rock, PA

POSITIONS:
Assistant Professor, Fisheries (1988-present), University of Minnesota; Investigator (Summer 1987, 1988), University of Michigan Biological Station; Postdoctoral fellow, Renewable Natural Resources (1986-1988), University of Connecticut; Research Specialist, Forest Resources (1985-1986), University of Minnesota.

SELECTED PUBLICATIONS:
Newman, R.M. 1991. Herbivory and detrivory on freshwater macrophytes by invertebrates: a review. J. No. Am. Benthol. Soc. 10:89-114.

Newman, R.M. 1990. Effects of shredding amphipod density on watercress *Nastutium officinale* breakdown. Holarctic Ecology 13:293-299.

Newman, R.M., W.C. Kerfoot, and Z. Hanscom III. 1990. Watercress and amphipods: potential chemical defense in a spring-stream macrophyte. J. Chem. Ecol. 16:245-259.

Newman, R.M. and J.A. Perry. 1989. The combined effects of chlorine and ammonia on litter breakdown in outdoor experimental streams. Hydrobiologia 184:69-78.

2.B. Cooperators/other investigators: Objectives B and C.
James P. Stack
EcoScience Laboratories, Inc.
One Innovation Drive
Worchester, MA 01605
(508) 754-0300

EDUCATION:
Ph.D. (Plant Pathology) 1984. Cornell University.
M.S. (Plant Pathology) 1978. University of Massachusetts.
B.S. (Plant Pathology) 1976. University of Massachusetts.

POSITIONS:
Manager of Plant Studies (1989-present), EcoScience Laboratories; Assistant Professor, Plant Pathology & Microbiology (1986-1989), Texas A&M University; Postdoctoral Research Associate, Plant Pathology & Microbiology (1983-1986), Texas A&M University· Graduate Research Assistant, Plant Pathology (1978-1983) [illegible]nell University

SELECTED PUBLICATION:

Stack, J.P., and C.M. Kenerley. 1989. Pathogenesis of *Phymatotrichum omnivorum* sclerotia by *Gliocadium roseum, G. catenulatum*, and *G. virens*: Infection and latent periods. Phytopathology (in preparation).

Stack, J.P., and Y.H. Park. 1989. Gliotoxic synthesis by *Gliocladium virens* as a function of carbon and nitrogen nutrition. Phytopathology (in preparation).

Stack, J.P., C.M. Kenerley, and R.E. Petit. 1988. Application of biological control agents. In: Biocontrol of Plant Diseases, K.G. Mukerji and K.L. Garg, eds. CRC Press, Boca Raton, FL.

Martyn, R.D., and J.P. Stack. 1988. Biological control of soil-borne plant pathogens with antagonistic fungi. In: Laboratory Exercises in Plant Pathology, A.B.A.M. Baudoin, ed. APS Press, St. Paul, MN.

VII. REPORTING REQUIREMENTS: Semi-annual reports will be submitted not later than July 1, 1992, January 1, 1993, and a final status report by June 30, 1993.

Title:	Biological Control of Ecologically Harmful Exotic Species: Eurasian Watermilfoil
Program Manager:	Luke Skinner
Organization:	Minnesota Department of Natural Resources
Legal Citation:	M.L. 1992, Chap. 513, Art. 2
Appropriation Amount:	$160,000

Statement of Objectives: To develop long-term biological controls techniques for controlling Eurasian Watermilfoil utilizing native insects and fungal pathogens. The current program focuses in on identifying naturally occurring control organisms and selecting those which inflict the most damage for additional research and development.

Overall Project Results: Three native aquatic insects known to feed on Eurasian watermilfoil have been found in six sites in Minnesota through an extensive survey of 25 Minnesota lakes. The three insects identified are a weevil, *Euhrychiopsis lecontei*; a caterpillar, *Acentria ephemerella*; and a caddisfly, *Nectopsyche albida.* It is the weevil which shows the most promise as an agent for controlling Eurasian watermilfoil. Multiple choice experiments(insects are put into cages with Eurasian watermilfoil and other aquatic plants) with the weevil indicate that it strongly prefers Eurasian watermilfoil. This portion of the research is in its early stages and is continuing through 1995.

Mycoleptodiscus terrestris (M.t.) is a naturally occurring fungus in Minnesota lakes which has the ability to colonize and cause damage Eurasian watermilfoil. 16 Minnesota lakes were sampled for M.t.. 288 different isolates were collected and tested for virulence towards Eurasian watermilfoil. The three isolates which display the greatest virulence will be mass cultured and then tested in field plots for effectiveness. This research is ongoing and will continue through 1995.

Project Results Use and Dissemination: As information becomes available from this research, it will be published in peer reviewed scientific journals and in special publications through the Department of Natural Resources. This information will be shared with other researchers from other agencies (e.g. Sallie Sheldon, University of Middlebury, Vermont) working on the same problem. Maintaining close collaboration is important to insure that the most promising biological control options are pursued. Since this research is ongoing, no recommendations for biological control are presently warranted.

I. **Project Title:** BIOLOGICAL CONTROL OF ECOLOGICALLY HARMFUL EXOTIC SPECIES: EURASIAN WATERMILFOIL

Program Manager: Luke Skinner
Agency Affiliation: Minnesota Department of Natural Resources
Division of Fish and Wildlife
Address: Ecological Services Section
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St. Paul, MN 55155-4025
Phone: (612) 297-3763

A. Appropriation: $160,000.00
Balance: $ 0

Laws of Minnesota 1992, Chp. , Sec. , Subd. . This appropriation is from the Minnesota future resources fund to the commissioner of natural resources for a research program leading to biological control of Eurasian watermilfoil. It is available upon final enactment and is otherwise subject to the provisions of Laws 1991, chapter 254, article 1, section 14. SubD 9-B

B. n\a

C. n\a

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The purpose of this biological control project is to initiate a research program leading to the long term control of Eurasian watermilfoil. Research will be carried out to assess the potential of native insects and fungi, leading to integrated control strategies for controlling Eurasian watermilfoil. Principal cooperators under this program are the Department of Natural Resources, the University of Minnesota, and the EcoScience Corporation.

III. OBJECTIVES:

A. TITLE: The potential for biological control of Eurasian watermilfoil with native and naturalized invertebrates.

A1. NARRATIVE: Native aquatic insects known to feed on Eurasian watermilfoil have been identified in the Canadian Province of Ontario and the State of Wisconsin, both of which border Minnesota. Surveys will be conducted to verify the presence of these insects of Minnesota, and laboratory studies will be carried out to determine their feeding preference.

A2. PROCEDURES:

a. **Extensive survey of 40-50 lakes (sites).** Sampling will occur in late summer 1992 and include metro area lakes, as well as outstate lakes. Results will be used in a correlational analysis of the following factors:
   1. Presence of potential biocontrol insects.
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   3. Qualitative assessment of plant damage.
   4. Location, physical, and chemical characteristics.

b. **Intensive study of 5-10 selected lakes with and without potential biocontrol invertebrates.** These sites will be sampled 3 times in the summer and fall of 1992, and once in late spring or early summer of 1993. Results will provide a more detailed and quantitative assessment of the potential of biocontrol herbivores for milfoil control. The following measurements will be made:
   1. Quantitative estimates of invertebrate herbivore densities.
   2. Quantitative assessment of plant damage.
   3. Quantitative estimates of plant density and community structure.
   4. Location, physical, and chemical characteristics.

c. **Laboratory feeding and preference studies of potential biocontrol invertebrates.** These studies will provide information on plant preference and damage potential of the invertebrates.

A3. BUDGET:

a. Amount budgeted: $55,000.00
b. Balance: $ 0

A4. TIMELINE:

	7/92	1/93	6/93
Site selection	****	***	
Intensive field sampling	**********		
Extensive field sampling	*****		
Field consumption/preference	********		
Lab consumption/preference	*****************		
Identify/enumerate samples	**********************		
Physical/chemical analysis		******	
Analyze results	******************	*****	
Preliminary reports		*****	
Final report			*****

A5. STATUS:

In total, 30 sites were examined in 25 lakes reported to contain EWM; three lakes, Island Lake, Bryant Lake, and Lake Wabasso were found to have very little or no EWM and were thus not suitable for sampling. Control efforts in several other lakes precluded their use. Twenty-seven sites at twenty-one lakes in Minnesota and Wisconsin were thus surveyed for presence of control agents, plant stand and environmental characteristics, and EWM density and damage. Eight of the Minnesota sites were selected as intensive sites. These intensive sites were sampled several times to identify changes over time in such parameters as biomass and community structure of aquatic macrophytes and their associated invertebrate fauna, herbivore damage to the plant community and physical and chemical characteristics of the water and sediments. These sites are being resampled in June 1993; these data and a more detailed presentation of our results will be provided in a report to the Minnesota Department of Natural Resources this summer.

The 19 extensive sites were sampled once in August or September 1992. These sites were used to broaden our coverage of potential areas containing milfoil herbivores and permitted a correlational analysis of factors associated with herbivore occurrence. Sites selected bounded the range of established (prior to 1992) populations of milfoil in Minnesota; the sites ranged from Otter and Vadnais Lakes (Ramsey Co.) in the east, to Clearwater and Waverley Lakes (Wright Co.) in the west, several sites in the Mississippi River in the south, and 17 sites in Hennepin or Carver Co. The extensive sites also included two lakes near Madison, Wisconsin (Devil's and Fish Lakes), which have had Eurasian watermilfoil for over 12 years and also had populations of potential control agents. These two sites were used as examples of well established or possibly declining (Devil's Lake) milfoil populations.

The eight intensive sites were sampled several times to identify changes over time in such parameters as biomass and community structure of aquatic macrophytes and their associated invertebrate fauna, herbivore damage to the plant community and physical and chemical characteristics of the water and sediments. Two rounds of sampling, July-August and September, were completed at these sites in 1992, with the exception of Lakes Minnewashta, Cedar and Vadnais, which were not sampled the second time, due to weather constraints before milfoil dieback. All intensive sites are all being resampled in June 1993.

At each intensive site a milfoil bed was identified for sampling. Water quality parameters were measured in the open water at the edge of the bed. These parameters included temperature and light (PAR) profiles (0.5 m), secchi depth, turbidity, chlorophyll, conductivity (μS/cm), total alkalinity and pH. Three transects, perpendicular to shore, were set up with 5-6 sampling stations per transect. The aim of the transect sampling stations w[illegible] cover a depth range

from the inner to outer margins of the bed or 2-5m depth. Usually, the stations were 30m apart, however, in several instances with narrow beds, stations were placed 15m apart. At each station, plant community structure and plant density was sampled with standardized grapple hook samples (4 throws per station; Jessen and Lound 1962). Plant height, plant disk depth (depth at which a secchi disk disappears in the plant bed; Crowell 1992), and bottom depth were recorded at each station.

Quantitative samples of plant material were collected to assess plant damage and invertebrate occurrence along 2 of the transects at each site. These samples were collected using SCUBA and consisted of all the vegetation included in a 0.1m^2 quadrat. The quadrats were located at each station and half-way between each station along the transect (i.e., 9 quadrats at 15 or 7 m intervals) so that changes in herbivory along a depth gradient could be identified. Samples were refrigerated at 4°C, rinsed to remove invertebrates and then plants and invertebrates were preserved in a 10% formalin solution for future examination. Plant and invertebrate biomass was quantified for each sample along with an estimate of plant damage (% leaf area lost; Mizner et al. 1993).

In addition, at each intensive site, replicate sediment cores were taken at two depths (shallow and deep; see biomass samples below) within each bed, oxygen concentration at the sediment-water interface was taken at two depths and seven quantitative plant biomass/invertebrate density samples were collected. The quantitative plant biomass samples (0.2 m^2 area) were obtained by a diver operated "super-sampler" (Creed and Sheldon 1992), a poly bag that is placed downward over the plants, which are then clipped at the bottom, and sealed into the sampler with a mesh lid. Three samples were collected in shallow water (1-2 m), and two samples at each depth were collected in medium (2-3 m) and deep (3-5 m) water. Plant biomass samples consist of all the plant and animal material in the column of water sampled. The plant material and invertebrates were returned to the laboratory intact, where they were washed throughly with water to remove attached invertebrates, which were collected in a 150μm sieve. The plants were separated into species and frozen; they were later dried, weighed, and ashed to obtain ash-free-dry-weights (AFDW). Voucher specimens of plants were collected when field identification was questionable. Sediment cores were placed on ice and returned to the laboratory. Within 24 hours, the pore water was extracted from the top 15 cm of the cores by centrifugation. The water was acidified to pH < 2 and the remaining core was frozen. Within a week after extraction, the pore waters were analyzed for ammonium concentration by the selective electrode method (APHA 1989). The frozen sediments were later thawed and analyzed for % organic matter, texture and grain size.

Similar, but abbreviated, methods were used at each extensive site. At each extensi[illegible] site, an EWM bed was identified and water quality paran[illegible]s were measured at the edge of the bed as was described for the intensive sites. Sediment cores were collected at 2 depths. Plant community structure, bed characteristics and plant damage samples were obtained from two transects with 3 sampling stations placed at shallow (1m), moderate (2m) and deep (3-4m) depths, using the same methods at each sampling station that were described for the intensive sites. No biomass samples were collected at extensive sites.

Results:

There was a range of milfoil densities at the sites sampled. Late summer milfoil biomass ranged from 23 g wet/m^2 in Waverly Lake to over 2000 g wet/m^2 in Smiths Bay (2 to 207 g/m^2 AFDW) and total plant biomass ranged from 400 g wet/m^2 in Lake Minnewashta to over 4500 g wet/m^2 in Waverly Lake (33 to 380 g/m^2 AFDW). Five sites had low densities of EWM (density rating $\leq$ 1.5), five sites had high densities (rating $\geq$ 4.0) and in the other sites, densities were moderate, or patchy and limited to relatively small areas. Sites in Lake Minnetonka and Fish Lake, Wisconsin, had the highest biomass of EWM, all being over 1100 g wet/m^2 (typically > 150 g/m^2 AFDW). Milfoil stem densities averaged from 100/m^2 in Lake Mallalieu, WI, to over 1300/m^2 in Fish Lake; the range for Minnesota sites was 120 to 900/m^2. These biomasses and densities are similar to others reported in the literature and the high values in Minnesota are comparable to high values for other infestations (Smith and Barko 1990, Lillie 1990, Lillie and Budd 1992). Over half of the sites had five or more species of macrophyte in addition to EWM, however, almost one fourth of the sites only had two species other than EWM. *Ceratophyllum demersum* (coontail) and *Myriophyllum sibiricum* (northern watermilfoil) were most commonly associated with EWM and in five instances were at equal to or higher densities than EWM. Other plants commonly found were several *Potamogeton* species such as *P. pectinatus* and *P. richardsoni,* and *Chara.*

The maximum depth of milfoil was related to light levels, with greatest depths being attained in clearer lakes with deep secchi depths and low chlorophyll levels. Secchi depths and chlorophyll levels ranged from 0.4 m and 22 mg/m^3 respectively to 3.6 m and 1 mg/m^3. In 1992, maximum EWM depths ranged from 1 to 6.6 m, however, in most sites, milfoil was not found deeper than 3 m. The unusually wet and cold summer may have limited milfoil growth and densities. Milfoil densities were also related to sediment ammonium (nutrient) content with the highest density stands occurring in high ammonium sediments ($\geq$ 8 mg/L in sediment pore water). Sediment ammonium levels ranged from 1-11 mg/L with a mean of 4 mg/L. Sediment organic matter ranged from 6- 24% with a mean of 9% and percent sand ranged from 0.3 to 7%. Multiple regression showed that milfoil density was positively related to secchi depth and sediment ammonium. There was also a weak negative relationship between milfoil density and coarser (sandy) sediment as has been observed in other studies (Smith and Barko 1990, Crowell 1992). These

results suggest that, as elsewhere, improved water clarity may enhance milfoil growth, but, reduced sediment nutrients may limit milfoil growth (Smith and Barko 1990). Somewhat surprisingly, total plant biomass was negatively related to secchi depth which contrasts with the positive relation of milfoil density and secchi depth.

Plant damage by invertebrates ranged from an average of 4% to 19% of total leaf area lost, with the highest percent damaged noted in Devil's Lake (16%) and Shady Island (19%) and Gray's Bay (15%) in Lake Minnetonka. Plant damage increased with depth, and was positively related to snail densities. These damage estimates are similar to the few other reports for invertebrate damage (e.g., Jacobsen and Sand-Jensen 1992). Stem damage was also extensive at some sites and generally associated with stem boring chironomids or weevils. We did not obtain enough sites with high enough densities of specialist herbivores (i.e., weevils, caterpillars or the milfoil midge) to detect any relation between plant damage and their density.

Invertebrate densities in milfoil beds ranged from 60 to 9000/m^2. Amphipods, midges, snails and caddisflies were most common, with high densities of 3600, 1400, 1400, and 280/m^2, respectively. The total abundance of invertebrates per m^2 of substrate increased significantly with total plant biomass, however, invertebrate abundance was negatively related to milfoil density. These results suggest that while high plant abundance is good for invertebrates high abundance of milfoil is not. This may either be a direct effect of EWM or the fact that diversity of plant abundance is lower at high milfoil densities. Results from others studies have produced conflicting results relative to the effects on milfoil on invertebrate densities (e.g., Chilton 1990, Smith and Barko 1990).

Herbivores with some potential to control EWM were found at 2 Wisconsin sites and at 6 Minnesota sites. We have professionally verified identifications of three potential control agents in Minnesota; the weevil, *Euhrychiopsis lecontei*, in Lake Auburn, Otter Lake and Gideon's and Smith's Bays of Lake Minnetonka (C. O'Brien); the caterpillar *Acentria ephemerella* (= *A. nivea*) in Lakes Auburn and Virginia (S. Passoa); and the caddisfly *Nectopsyche albida* at Shady Island in Lake Minnetonka (R. Holzenthal). We suspect we have the midge, *Cricotopus myriophylli* in one lake and another caterpillar *Parapoynx* in another, but have not yet had our identifications of these verified. Snails were abundant at many sites, but are not likely to be good control agents.

The caddisfly *Nectopsyche* was relatively dense (210/m^2) at the Shady Island site in Lake Minnetonka; this site also had one of the highest levels of leaf damage (mean of 19% with a high of 32%) and most of the leaf damage was consistent with feeding by the caddisfly. We suspect that the caddisfly will eat other plants as readily as milfoil and thus it may not be a good control agent, however, feeding choice and preference experiments with the caddisfly need to be done this summer to determine its range of feeding.

The weevils collected in Lake Auburn were patchily distributed and only locally abundant. Some areas had extensive damage and other areas appeared to harbor no weevils. Weevils seemed similarly distributed at the other sites in Minnesota; damage was evident at some sites but very few weevils were located. Conversely, at Devil's and Fish Lakes, Wisconsin, weevil densities were higher; weevils have been documented to occur in these Wisconsin lakes since the 1980s (R.A. Lillie, WI DNR, personal communication). We estimated weevil densities at $6/m^2$ at Devil's Lake and $2/m^2$ at Fish Lake, however, we think that avoidance behavior may have resulted in underestimates of weevil densities.

The weevil is thought to be native to North America (e.g. O'Brien and Wibmer 1982) and probably Minnesota. Its original host plant is unknown, but thought to be northern watermilfoil (Creed and Sheldon 1993). We suspect that for some reason the weevil does not normally attain high population levels on northern watermilfoil but can attain high populations on EWM, which our lab studies have shown it to prefer. The relatively short time that EWM has been in Minnesota may not have allowed weevils to yet reach high densities. Fish and Devil's Lakes in Wisconsin have had dense milfoil for over 12 years, which would permit time for development of weevil populations. Plant damage was extensive in these lakes and there is some suggestion that increased densities of weevils may be reducing milfoil biomass; milfoil has been reported to have declined Devil's Lake (Smith and Barko 1990) and appears to be declining in Fish Lake (R.A. Lillie, personal communication). We did note that lakes with weevils had significantly higher clarity (secchi depths) than lakes without weevils, however, this may be interrelated with higher densities of milfoil being more likely to harbor weevils.

Laboratory and greenhouse cultures of Eurasian and northern watermilfoil were developed, along with other native species. A reproducing stock of the weevil was established and at least 3 laboratory generations (eggs to adults) were produced between September and February. However, due to nutrient depletion of sediments by the milfoil in mid-winter and underestimation of the extent of milfoil damage by weevils we were not able to provide enough healthy milfoil to maintain the weevil colony after March 1993. A new colony of weevils and larger stock of weevil-free and nutrient replenished milfoil has been established this spring.

Milfoil consumption rates were estimated for the caddisfly, *Nectopsyche*, at 6 mg per larvae per day. The caddisflies were able to significantly reduce milfoil growth at high densities in the laboratory. These results are consistent with our field observations at Shady Island. However, we suspect that they will also feed on other plants. The caddisflies pupated before multiple choice experiments could be conducted. These studies will be pursued this summer.

Multiple choice experiments with the weevil indicated that it strongly prefers Eurasian watermilfoil over other aquatic plants. The plants tested were Eurasian watermilfoil, northern milfoil (*Myriophyllum sibiricum*), *Elodea canadensis*, *Potamogeton zosteriformis*, *P.pectinatus*, *Megalodonta beckii*, and *Ceratophyllum demersum*. In multiple choice oviposition tests, 7 of 12 female weevils oviposited on Eurasian watermilfoil; mean time to oviposition was 1 day. The 5 other weevils did not oviposit on any plants. The average weevil spent about 60% of its time on Eurasian watermilfoil and less than 5% of its time on any other taxa; four to 5% of the time weevils were found on either northern watermilfoil, coontail or *Potamogeton pectinatus*, and less than 1% of the time on *Elodea* or *Megalodonta*. In a second round, Eurasian watermilfoil was not included and weevils were presented with six taxa. None of the weevils oviposited within 5 days and most of their time was spent on no plants (50%) or northern watermilfoil (34%). Although no oviposition was noted on northern watermilfoil, we have observed oviposition on northern watermilfoil several times in other settings. It does appear, from limited experimentation, that most oviposition is oriented toward Eurasian watermilfoil. These experiments will be replicated several more times this summer. In addition, we will attempt to grow weevils on northern watermilfoil, which is presumably the original host, but does not seem to be currently preferred.

This work has shown that herbivores with the potential to control Eurasian watermilfoil occur in Minnesota. These herbivores are either native or naturalized, have not had undesirable effects on native vegetation and are capable of surviving the Minnesota climate. Given its apparent specificity and potential for damage to milfoil (e.g., Creed et al. 1992, Creed and Sheldon 1993), we believe the weevil, *Euhrychiopsis lecontei* is the best candidate for control, however, the potential of the other invertebrates needs to be explored further. It is unclear what is controlling and limiting the distribution of the weevil and other control agents in Minnesota. This will need to be determined before they can be successfully used in a control program. We suspect that actions to enhance the populations of one control agent may also enhance populations of other control agents. Further testing to ensure host specificity, especially of organisms raised on plants other than Eurasian watermilfoil is needed to ensure that enhancing control agent populations or introducing them to new waters will not harm native plants. More information on the mechanisms of plant damage by these herbivores and their effects on plant populations in the field and laboratory is also needed. This information, along with an understanding of what is currently limiting their populations and distribution will be needed for effective biological control. We intend to investigate these topics during the upcoming biennium.

A6. BENEFITS: The ability of native or naturalized insects capable of biological control of Eurasian watermilfoil will be determined in this objective. The information from all three of the above components will be used t~ make recoı ndations for integrated control strategies. This could ult. ;ely minimize the impacts of current control methods on aquatic ecosystems.

B. TITLE: Isolation of fungi native to Minnesota for control of Eurasian watermilfoil.

B1. NARRATIVE: Mycoleptodiscus terrestris (M.t.) is a naturally occurring fungus which has the ability to colonize and degrade Eurasian watermilfoil. In aquatic environments, M.t. is host specific, colonizing and causing disease in Eurasian watermilfoil while not affecting native plant species. Strains of M.t. will be selected which are found in close association with Eurasian watermilfoil from different geographic areas in Minnesota.

B2. PROCEDURES:

a. **Selection of strains of M.t. with greater virulence against Minnesota Eurasian watermilfoil populations at temperatures typical of northern latitudes.** Samples will be collected from all lakes known to have Eurasian watermilfoil populations in Minnesota. Strain isolates will be cultured, formulated, and evaluated at 59°F and 77°F for the following:
   1. Disease incidence.
   2. Rate of disease development.
   3. Disease severity.

b. **Determination of the susceptibility of different geographic populations of Eurasian watermilfoil to M.t.** Different strains of Eurasian watermilfoil may have differential susceptibility of attack by M.t., necessitating the development of selected M.t. strains for specific regions of Minnesota. Samples of Eurasian watermilfoil from different geographic regions in Minnesota will be cultured in the laboratory. Bioassays using differing Eurasian watermilfoil and M.t. strains will be conducted and assessed under the same criteria as in 2(a), including percent reduction in biomass.

c. **Selection of M.t. strains with enhanced growth and accelerated disease progression at 59°F.** Isolates of M.t. found in other regions of the U.S. have been found to have accelerated growth, and induce disease progression and severity of infection faster at 77°F. In the same way, isolates of M.t. from different Eurasian watermilfoil populations will be screened and evaluated for these same traits in water temperatures more common to Minnesota (i.e. 59°F).

B3. BUDGET:

a. Amount budgeted: $54,000.00
b. Balance: $ 0

EcoScience Corporation (ECS) agrees to reimburse the Minnesota Future Resources Fund all support paid to ECS for up to the full level of support paid to

ECS under this research and development grant. Such reimbursement will be made by ECS upon first sale of any milfoil control product in Minnesota, or from the first sale of any milfoil control product anywhere based on material derived from this program. The mechanism for the reimbursement would be either through cost reduction for finished goods or payment by ECS of up to thirty percent (30%) of its profits generated from sale of a milfoil control product in Minnesota. Such repayment will continue until the full amount of support received by ECS has been repaid.

B4. TIMELINE:

	7/92 1/93 6/93
M.t. strain isolation	***************************************
M.t/EWM laboratory bioassays	***************************************
Temperature studies	***************************************

B5. STATUS: Milfoil was collected from 16 lakes throughout Minnesota and microbial isolations were made from approximately 100 plants per lake. Five lakes (Auburn, Bavaria, Minnewashta, Pierson, and Zumbra) were sampled at monthly intervals. *Mycoleptodiscus terrestris*, M.t., isolations appeared greater in July and September than in June. The overall frequency of M.t. isolation from all lakes varied from 0% in Lake Waverly and Otter to 100% in Lake Bavaria. From these plants, 310 isolates were obtained of which 288 proved amenable to standard culture and preservation techniques.

The isolates were evaluated for virulence against Eurasian watermilfoil. Variation in virulence was identified using Quantitative Virulence Assay (QVA) as a method of assesment. Isolates with rates of disease progression faster and slower than the current strain have been identified. The relative rates of disease progression were consistent over four assays, including one blind assay. Additional methods of assessment are now underway, including one meter column assays to determine the nature and extent of disease development on larger milfiol plants.

Potential variation in temperature response has been identified. Isolates that exhibit faster disease progression dynamics at both 25 and 15 degree celcius relative to the reference strain have been isolated from Minnesota milfoil. Qualitative differences among isolates in rate of lesion development or severity of disease were not apparent at 25 or 15 degrees celcius.

Milfoil plants from several geographic locations in Minnesota as well as from other states were tested for susceptibility to M.t.. No populations of milfoil were identified as being resistant to M.t.. Disease symptoms and rates of disease progression for Minnesota milfoil populations were within the range observed in milfoil populations from around the United States.

B6. BENEFITS: A strain of M.t. with enhanced virulence towards northern populations of Eurasian watermilfoil and an expanded temperature range more typical of Minnesota lakes will be obtained. Possible differences in milfoil susceptibility will be investigated, and selected M.t. strains that will provide consistent control of Eurasian watermilfoil in northern latitudes will be studied.

C. TITLE: Comparative effectiveness of M.t. strains for milfoil control in field trials.

C1. NARRATIVE: As part of the EcoScience Experimental Use Permit field program for 1993, the three most effective strains of M.t. identified in laboratory screens will be evaluated under Minnesota field conditions in spring and summer of 1993.

C2. PROCEDURES: Treatments will be applied to milfoil stands with full canopies when water temperatures are optimum for growth of the three strains. Prior to treatment, two isolation tubes will be placed in each tank. One will be covered during treatment and will serve as a control.

Treatments will be replicated three times using a randomized complete block design.

Percent cover will be estimated prior to and 6 weeks after application of materials to determine canopy reduction. Total above ground biomass in isolation tubes will be harvested at 6 weeks post treatment. Water quality will be monitored weekly throughout the study. Plants, sediments, and water column will be sampled for the presence of M.t. at 6 weeks post treatment. Percentage data will be transformed accordingly, subjected to analysis of variance, and where applicable, means will be separated (P=0.05).

C3. BUDGET:

a. Amount budgeted: $51,000.00
b. Balance: $ 0

The same grant reimbursement language used in Objective B applies.

C4. TIMELINE:

	7/92	1/93	6/93
M.t. field trials			****

C5. STATUS: The research leading up to the implementation of this objective has shown that although we have identified isolates that show potential against milfoil, more research is needed before field applications can be made. Smaller more controlled tests (possibly outdoor enclosure work) need to be implemented. This portion is being worked on. Secondly, due to a vacancv, the project's Program Manager has changed during the past year. The new aager has concerns

regarding project direction, implementation and reporting. These concerns are being addressed but not in time for a more complete report on this objective.

C6. BENEFITS: Enhanced activity of the biological control product.

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O'Brien, C. W., and G. J. Wibmer. 1982. Annotated checklist of the weevils (Curculionidae *sensu lato*) of North America, Central America, and the West Indies (Coleoptera: Curculionidae). Memoirs of the American Entomological Institute 34: 1-.

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IV. EVALUATION: The main objectives of this project are to collect and establish baseline information on the potential of using biological agents for the control of Eurasian watermilfoil in Minnesota. This study will be the first research project of its kind since Eurasian watermilfoil was first identified in Minnesota in 1987. The duration of this project will last approximately 14 months. Accordingly, its length and scope will allow for only preliminary observations and conclusions. The findings reported from this work will act as precursors to future biological control research projects for the 1994-1995 biennium.

V. CONTEXT: RELATED CURRENT AND PREVIOUS WORK

A. The current work will be of short duration and will have a much narrower focus than if the project had been funded and approved for the entire biennium. Since there has been no previous work done in this area this will be the first biocontrol research program of its kind in Minnesota for Eurasian watermilfoil.

B. The results of this study will supplement existing biological control information obtained from studies conducted in other areas of the U.S. and Canada. This information will be used by the Minnesota DNR in the development of integrated control strategies.

C. This program was originally developed by the Freshwater Foundation with the assistance of the DNR, effective July 1, 1991. To implement this project, the LCMR had approved $100,000.00, to be matched by $200,000.00 by the Freshwater Foundation.

The Freshwater Foundation could not raise the match, and in December 1991 declined further participation in the biological control research program. The DNR volunteered to continue with development and implementation of the project, conducted an additional peer review process on existing proposals, and submitted a revised proposal at roughly half ($160,000.00) the original funding level of $300,000.00.

LCMR has not previously funded research programs for Eurasian watermilfoil.

A proposal for furthering biological control research on Eurasian watermilfoil was developed by the DNR and is under consideration for funding during the 1994-1995 biennium.

D. Water Recreation Account, Ecological Services, Eurasian Watermilfoil FY 90-92, $125,262.00 each fiscal year. These funds have been allocated to develop programs for public education and awareness, law enforcement, survey and monitoring, and control and eradication of milfoil.

E. Not yet available.

VI.. QUALIFICATIONS:

1. **Program Manager**

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EDUCATION:
Bachelor of Science Degree 1987. University of Minnesota
Major: Biology
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POSITIONS:
Coordinator, Purple Loosestrife Program, Minnesota Department of Natural Resources, St. Paul; March 1990 - Present.

Purple Loosestrife Project Coordinator, Hennepin Parks, Hennepin County, MN; May 1988 - December 1989.

Senior Laboratory Technician, Center for Water and the Environment, Natural Resources Research Institute, University of Minnesota, Duluth; June 1986 - September 1986 and June 1987 - November 1987.

Research Assistant, Department of Biology, University of Minnesota, Duluth; September 1985 - March 1986.

Assisted in research on the diet of Lake Superior trout and salmon.

SELECTED PUBLICATIONS:
Skinner, L.S., W.J. Rendall and E.L. Fuge. In Press. Minnesota's Purple Loosestrife Program: History, Findings and Management Recommendations. Special Publication 145, Minnesota Department of Natural Resources, 32 pages.

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2A. Cooperators/other investigators: Objective A.

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M.S. (Fisheries) 1982, University of Minnesota, St. Paul, MN
B.S. (Biology) 1978, Slippery Rock University, Slippery Rock, PA

POSITIONS:
Assistant Professor, Fisheries (1988-present), University of Minnesota; Investigator (Summer 1987, 1988), University of Michigan Biological Station; Postdoctoral fellow, Renewable Natural Resources (1986-1988), University of Connecticut; Research Specialist, Forest Resources (1985-1986), University of Minnesota.

SELECTED PUBLICATIONS:
Newman, R.M. 1991. Herbivory and detrivory on freshwater macrophytes by invertebrates: a review. J. No. Am. Benthol. Soc. 10:89-114.

Newman, R.M. 1990. Effects of shredding amphipod density on watercress *Nastutium officinale* breakdown. Holarctic Ecology 13:293-299.

Newman, R.M., W.C. Kerfoot, and Z. Hanscom III. 1990. Watercress and amphipods: potential chemical defense in a spring-stream macrophyte. J. Chem. Ecol. 16:245-259.

Newman, R.M. and J.A. Perry. 1989. The combined effects of chlorine and ammonia on litter breakdown in outdoor experimental streams. Hydrobiologia 184:69-78.

2.B. Cooperators/other investigators: Objectives B and C.

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SELECTED PUBLICATION:
Stack, J.P., and C.M. Kenerley. 1989. Pathogenesis of *Phymatotrichum omnivorum* sclerotia by *Gliocadium roseum, G. catenulatum*, and *G. virens*: Infection and latent periods. Phytopathology (in preparation).

Stack, J.P., and Y.H. Park. 1989. Gliotoxic synthesis by *Gliocladium virens* as a function of carbon and nitrogen nutrition. Phytopathology (in preparation).

Stack, J.P., C.M. Kenerley, and R.E. Petit. 1988. Application of biological control agents. In: Biocontrol of Plant Diseases, K.G. Mukerji and K.L. Garg, eds. CRC Press, Boca Raton, FL.

Martyn, R.D., and J.P. Stack. 1988. Biological control of soil-borne plant pathogens with antagonistic fungi. In: Laboratory Exercises in Plant Pathology, A.B.A.M. Baudoin, ed. APS Press, St. Paul, MN.

VII. REPORTING REQUIREMENTS: Semi-annual reports will be submitted not later than July 1, 1992, January 1, 1993, and a final status report by June 30, 1993.