Date of Report: 29 December 1995
LCMR Research Work Program 1993 - Summary

I. Project Title: BIOLOGICAL CONTROL OF EURASIAN WATERMILFOIL AND PURPLE LOOSESTRIFE

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A. Legal Citation M.L. 93 Chpt. 172, Sec. 14, Subd. 12(L)

Total Biennial LCMR Budget: $400,000
Balance: $0

This appropriation is from the trust fund to the commissioner of natural resources to research biological control for purple loosestrife and Eurasian watermilfoil. The purple loosestrife research must be done in cooperation with the commissioner of agriculture. $100,000 is for the propagation, release, and evaluation of insects for purple loosestrife control; $50,000 is for the development of mycoherbicides to control purple loosestrife; $200,000 is for evaluation of biocontrol agents for Eurasian watermilfoil fungi and insects; and $50,000 is to research the biology of Eurasian watermilfoil. The $250,000 for Eurasian watermilfoil must be matched by $200,000 of nonstate funds.

The project is extended to December 31, 1995; on that date the appropriations cancel and no further payment is authorized, Minnesota Laws 1995, Chap. 220, Sec. 19, Subd. 19.

B. LMIC Compatible Language: Not applicable.

C. Status of Match Requirement: On March 7, 1994, the DNR and the Department of the Army completed a cooperative agreement that commits the Corps of Engineers Aquatic Plant Control Research Program to conduct $200,000 worth of research as a match of 'in-kind' services. On 5 April 1994, the Minnesota Lakes Association (MLA) contributed $7,967 to the DNR to support evaluation of biocontrol agents for Eurasian watermilfoil. In 1995, the $7,967 received from the MLA was allocated to sampling to be done during spring before the beginning of the biennium of Fiscal Years 1996 and 1997.

II. Project Summary:

The overall goal of this project is to provide long-term control of Purple loosestrife (*Lythrum salicaria*) and Eurasian watermilfoil (*Myriophyllum spicatum*) utilizing biological control methods. Purple loosestrife and Eurasian watermilfoil (EWM) are exotic (non-native) plants that have caused negative impacts to Minnesota's natural ecosystems. The loss of plant diversity, wildlife habitat, and recreational opportunities is the basis for the need to control these plant species. Currently, the best available method for controlling these two exotic plants is the use of herbicides. Unless loosestrife or EWM infestations are small, chemical control methods are limiting and typically short term in nature.

Biological controls strategy for exotic species, such as these, is to reunite the pest species with its natural enemies. The natural enemies are specific to the pest and will live and reproduce on the pest plant. Typically, natural enemies are insects. They can feed on the roots, stems, leaves and flowers or any combination thereof. Once the insects become established in the environment, they can provide long-term control usually dramatically reducing pest populations, but not eliminating them completely. If purple loosestrife and EWM can be kept in check and not dominate in their respective environments, then they can be considered effectively controlled. An effective biological control approach will provide long-term control of exotic pests and will reduce our dependence on herbicides for controlling the exotic species.

III. Statement of Objectives:

A. Propagation, release and evaluation of three insect species for biological control of purple loosestrife.

B. Development of mycoherbicides (fungi) to control purple loosestrife.

C. Research biology of Eurasian watermilfoil.

D. Evaluation of biological control agents for Eurasian watermilfoil: fungi and insects.

IV. Research Objectives

A. Title of Objective: Propagation, release and evaluation of three insects species for biological control of purple loosestrife.

A.1. Activity: Propagation, release and evaluation of three insect species for control of purple loosestrife.

A.1.a. Context within the project: Three European insects, one weevil (*Hylobius transversovittatus*) and two beetles (*Galerucella spp.*), have been identified as promising candidate biological control agents for introduction into the U.S. (Batra et al. 1986) and have received federal and state approval for release in the United States and Minnesota as potential natural enemies of purple loosestrife (*Lythrum salicaria* L.). Biological control offers the most suitable and environmentally safe technique to manage loosestrife long term, especially in nature reserves (Thompson et al. 1987). The idea is to increase stress on purple loosestrife by introducing predators that eat the plant. The two beetles in particular can cause high plant mortality, reduce shoot growth, suppress flowering and reduce seed output. All three species have been released in cages at one large stand of purple loosestrife near St. Paul, Minnesota during the late summer of 1992. This site will serve as a breeding colony and insects will ultimately be redistributed to additional sites.

At this time, however, it is unknown whether the insects will become established and thrive in Minnesota.

Two major activities will be conducted to evaluate these insects as biological control agents. The first activity will be to determine if the beetles have established self-reproducing populations under Minnesota climatic conditions. In the event that the initial released beetles did not establish, additional beetles will be requested from other release sites in the United States or Canada. The second activity will determine ways to increase insect population more efficiently.

A.1.b. Methods: For the insects to establish in Minnesota, it will be necessary for them to overwinter successfully, and to lay a sufficient number of eggs so that large numbers of adults can mature. We will conduct overwintering studies to determine factors that affect overwintering ability, such as temperature, snow cover or duration of cold temperatures. Loosestrife in the cages will be sampled during the summer to determine the population density of insects.

Overwintering adult beetles will be subjected to various soil moisture regimes to determine its effect on mortality. Overwintering success will be confirmed by field observations in the spring. The beetles lay their eggs on loosestrife leaves. Egg densities will be estimated frequently and their fate will be monitored, which will provide us with some indications of the factors that might limit these populations.

Conditions will be modified to within test areas to determine how selected

environmental conditions may affect beetle populations. The beetles appear to prefer to feed on young developing leaves of loosestrife. They can be tested by artificially increasing the density of young leaves in a cage by periodic clipping of some of the loosestrife in a cage to stimulate new growth. Natural enemies, such as other insects and spiders are quite abundant on loosestrife during mid-summer, and are expected to eat immature leaf beetles. Natural enemies will be removed in some of the cages to evaluate their effect on beetle population growth as compared to the untreated cages.

Finally, if beetles establish and thrive, experiments will be conducted to determine how far beetles disperse in order to develop predictions about how many beetles to release at a site and in what manner they should be released. If beetles are found dispersed too widely, they may have difficulty locating mates and larger quantities may need to be released or dispersal may need to be inhibited.

A.1.c. Materials: Field and lab materials necessary to accomplish this objective include plastic pots, field cages, permanent station markers, insect nets, data forms, clipping shears, insect aspirators, small insect cages, insecticides, shovels, sprayer, hygrothermograph, soil/surface moisture meter.

A.1.d. Budget: $100,000 **Balance:** $0

A.1.e. Timeline:

	7/93	1/94	6/94	1/95	6/95	11/95
Propagation experiments	**********	**********	**********			
Establishment exper.	**********	**********	**********			
Evaluate methods			**********	**********	**********	**********
Evaluate impacts			**********	**********	**********	**********

A. **Status:** Insects released during the summer of 1992, survived the winter and produced offspring. This is a positive step forward knowing the insects can survive winter in Minnesota. Unfortunately, most of the insects died when the Mississippi River flooded and covered the research site at Pigs Eye Lake in 1993.

Leaf-eating beetles were released in 8 sites in Minnesota during the 1993 field season. Insects were received from Europe and cleared quarantine before being shipped to Minnesota. The releases were made within 48 hours of receiving the insects. Plant species diversity and density were measured at each location to establish a baseline from which to assess impact of the insects on purple loosestrife. All sites will be sampled in

the spring of 1994 for evidence of over-wintering survival, egg laying and larval feeding.

Plans for 1994 include field releases of insects across the state. Leaf-eating beetles will be released as far north as International falls to test insect survival in different climates. Root boring weevils and more leaf-eating beetles will be received from Cornell University in June 1994, for lab and field rearing. The root boring weevil is much slower growing than the leaf-eating beetles and will take longer to establish in Minnesota. However, the weevils are predicted to play an important role in controlling purple loosestrife. Potential success of biological control efforts are enhanced when a number of insects species are used.

1993 Leaf-eating beetle releases survived the winter at all field sites and had one or more life stages of beetles present. No sites had a large enough insect population to warrant estimation of insect density. Defoliation was minimal as beetle populations were too small to have any appreciable impact on purple loosestrife in 1994.

Laboratory colonies of the leaf-eating beetles are maintained at the University of Minnesota. More than 5,000 insects were reared from June through December 1994. All but 500 were either released in the field or used in conjunction with a defoliation/root depletion experiment. These remaining insects were fed foliage under short day length for two weeks and placed at (4°C) until November. Two hundred loosestrife root stocks were dug and held in cold storage to be used as food for insect colonies during the winter. Currently several colonies have been started with the goal of producing at least 5,000 insects for distribution in May 1995.

Leaf-eating beetles were released in 13 new sites encompassing 7 counties in 1994. This included Anoka, Crow Wing, Dakota, Hennepin, Rice, St. Louis and Washington counties.

At two sites, the beetles reproduced at a very high rate increasing their populations significantly in the first year.

Flower-feeding weevils, *Nanophyes marmoratus*, were released for the first time in Minnesota in 1994. A total of 1000 weevils were divided up and released at 5 sites in the metro area. Their first test will be to survive the winter in Minnesota.

A small number of Root-boring weevils were received from Cornell University to be

used as brood stock. The root weevils were kept in the lab and as eggs were produced, they were transferred to the purple loosestrife plants in the field. More than 1,000 root weevil eggs were collected and transferred into the field.

The project was expanded in January 1995 to include mass rearing of the two leaf-beetles. From March 31 to June 30, a total of 54,291 insects were reared and released into wetlands of 17 counties (Houston, Winona, Goodhue, Rice, Dakota, Ramsey, Hennepin, Washington, Crow Wing, St. Louis, Stevens, Carlton, Hubbard, Cass, Mahnomen, Wadena and Becker).

Insects released in 1994 established very well at three sites. The leaf-beetle populations on these three sites have increased significantly and have spread to many of the loosestrife plants in the immediate area. At Circle Lake (Rice County) release site, eggs and larvae were found some 200 meters from the release point in June of 1995. Moreover, nearly every loosestrife stem within a 25 meter radius of the release site showed intense adult and larval feeding.

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Leaf beetles releases continued through July 12, 1995. Total number of leaf beetles released in 1995 was 101,203 at over 45 sites statewide (Ragsdale, 1995). This is up sharply from the 5,850 beetles released in 1994. Rearing techniques were greatly modified to efficiently mass rear the insects for release in Minnesota (Ragsdale, 1995). The leaf-beetles show much potential for being successful bio-control agents in Minnesota.

Root-boring weevils have been found at all sites in 1995 where eggs were inoculated into loosestrife plants in 1994. This research on the root-boring weevil was carried out at the University of MN by Dr. David Andow. His final report was returned for revisions and will be completed soon.

A study to characterize the genetic diversity of loosestrife in Minnesota began in 1991. This research was continued in this project. This study was started because one important aspect to the success of a biological control program is the extent of genetic diversity. Biological control agents may not be adapted to the divers range of genotypes within a target species and therefore may not be successful (Sheppard, 1992). This study found that cultivars are genetically distinguishable from weedy populations (Darmo et al., 1991). However, many cultivars of putative *Lythrum salicaria* origin were not distinguishable from cultivars of putative *L. virgatum*, indicating that nursery records may be suspect

and/or the taxonomy of these two species need to be reviewed. In follow up studies, it was found that the cultivars are showed moderate to high identity with weedy populations (Becker et al., 1995).

Problems:

B. Title of Objective: Development of mycoherbicides to control purple loosestrife.

B.1. Activity: Development of mycoherbicides to control purple loosestrife.

B.1.a. Context within the project: Work on this project has been ongoing for approximately one year. During this time, 2,000 fungal cultures have been isolated from spots on purple loosestrife plants from 23 locations around the state of Minnesota. The predominant fungi isolated are *Alternaria* sp., *Sclerotinia* sp., and *Fusarium* sp. Because of the amount of damage caused to the leaves by natural infection of these fungi, there is an excellent possibility that one or more of the isolated fungi will prove to be a suitable mycoherbicide. The proposed work will continue on isolation of fungi from purple loosestrife plants and testing for pathogenicity.

B.1.b. Methods: Fungi are isolated from leaf spots caused by natural infection. Isolated fungi will be tested in the lab for its ability to kill purple loosestrife.

Once identified, suitable candidate mycoherbicides will be field tested in natural stands of purple loosestrife. Another question that must be addressed is the proper time to apply the mycoherbicide. Application should be at a stage in the plant's growth so that adequate foliage is involved for infection; approximately 15-20 cm in height. This hypothesis will be tested on other wetland plants likely to be found in the vicinity of the purple loosestrife plants to insure they are not harmed.

Similar work developing mycoherbicides for other weeds has taken several years. It is anticipated development of a mycoherbicide for purple loosestrife may take 5 to 10 years to complete. The results in the last year have been extremely positive and it is expected that a candidate mycoherbicide will be identified in 1993.

B.1.c. Materials: Agar solutions, chemicals, petri dishes, lab glassware, plastic pots, microscope and other lab equipment.

B.1.d. Budget: $50,000 **Balance:** $0

B.1.e. Timeline:

	7/93	1/94	6/94	1/95	6/95	11/95
Isolate fungi	**********	**********	**********			
Evaluate effectiveness			**********	**********	**********	

B. Status: Thirty-six species of fungi have been isolated on purple loosestrife plants sampled throughout Minnesota. To date, two fungi species show promise as potential control agents. *Botrytis cinerea* and *Alternaria alternata* both caused damage when applied to young loosestrife plants. Future work is needed to find a suitable way to apply the fungi to loosestrife plants, determine how much fungi is needed to effectively control loosestrife, test other fungi for pathogenicity, and start an interaction study between the fungi and insects in the spring of 1994.

The fungi will be tested on purple loosestrife in the field for the first time in May 1994. The fungi will be applied to test plots near Morris, Minnesota by itself and in combination with the leaf eating-beetles. It is thought that the insects will cause lesions on loosestrife plants allowing the fungi easier access into the plant.

Testing of pathogens in the field could not be accomplished until a suitable carrier was developed to apply the pathogen effectively to loosestrife plants. A variety of carriers were developed and fungal pathogens were incorporated into the carriers to test effectiveness. This development process took more time than anticipated. Two carriers were tested on a limited basis in 1994 at one site in Morris, MN. Testing will resume on a larger scale at more than one site in the state in the spring of 1995.

The Mycoherbicides will also be tested in conjunction with insects as an integrated approach. It was hoped that this portion of the research would begin in 1994. However, a suitable carrier was not ready when the insects were available for field releases.

A new fungus, *Colletotrichum truncatum*, has been found to have mycoherbicidal activity. This and other fungi are being evaluated further as potential mycoherbicides.

Two field test sites were established to test the mycoherbicide by itself and in conjunction with leaf-beetles (Stevens and Ramsey Counties). Insect and mycoherbicide were applied to the test plots in early June. This will be followed this summer and through 1997.

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Much work apparently remains to be completed in apply fungi to plants in the field. Although a very limited test in 1994 appeared to be successful, the 1995 results on a larger scale have been disappointing. Suspected reasons why the fungi might not have worked include: 1) Fungi applied too late in growing season. 2) Fungi must be applied more than once. 3) Fungal spore concentration might be too low. 4) Weather was extremely hot and dry following application (Nyvall, 1995). This research is continuing though the next biennium.

Problems:

C. **Title of Objective**: Research Biology of Eurasian Watermilfoil

C.1 Activity: Investigation into natural declines and environmental effects on growth of Eurasian watermilfoil.

C.1.a. Context within the project: Information on what EWM needs to survive is required for any type of control program. Although there is a great deal of information on these topics, this information is lacking in relation to its use in coordinated biological control programs. The State of Vermont is the only other state agency that is conducting similar work on environmental monitoring and biological control.

Major declines in populations of Eurasian watermilfoil have been reported and evaluated (Carpenter 1980). Declines could be caused by nutrient depletion, shading, self- shading, attack by parasites or pathogens, long-term effects of harvesting and/or herbicides, accumulation of a toxin, or insect damage, but none of the declines were explained. In many locations EWM populations increased to a high level of dominance, maintained dominance for a few years, and then declined. To date, declines have occurred in Chesapeake Bay area, in Wisconsin lakes near Madison area lakes, and in several southern Ontario lakes. In these locations, the period of peak EWM abundance ranged from approximately 5 to 10 years (Carpenter 1980). When the EWM declined, the rate and amount of decline varied from lake to lake.

C.1.b. Methods: EWM populations will be studied primarily in the field. Accumulation and allocation of nutrients, carbohydrate status, and growth rate will be monitored under varying environmental conditions.

C.1.c. Materials: Standard laboratory assay equipment will be used for environmental monitoring of field collected plant samples. Field sampling will require a boat, outboard motor, trailer, and plant grapple hook.

C.1.d. Budget: State funds: $9,000 Corps of Eng.: $60,000
Balance: State funds: $0 Corps of Engineers: $50,000??

C.1.e. Timeline:

	7/93	1/94	6/94	1/95	6/95	11/95
Laboratory assays	**********	**********	**********			
Field collections	*****		*****			
Field assays	*****		*****			
Evaluations			**********	**********	**********	

C.2 Activity: Genetic variation of Eurasian watermilfoil populations in Minnesota.

C.2.a. Context within the project: Previous research on genetic variability of EWM populations in Minnesota has been conducted on several metropolitan area lakes. Two separate types of Eurasian watermilfoil types have been identified, one found predominantly in Hennepin County, the other found primarily in Carver County. In each of these counties, only one type was found in each lake, implying that the spread of EWM within a lake is by cloning, or asexual reproduction.

It is well known that different pest types, whether plant or animal, react differently to chemical and biological control techniques. This activity is aimed at determining the total number of types of EWM present in Minnesota, their location geographically, and whether or not multiple types are present in the same lake. Control techniques can then be prescribed on a lake by lake basis, or a regional basis. In addition, samples of Eurasian watermilfoil collected outside Minnesota will be analyzed to determine whether additional genotypes of this exotic plant might be expected to be introduced to the state and to more completely assess the range of genetic variability in this species.

C.2.b. Methods: To estimate the number of different types of Eurasian watermilfoil in Minnesota lakes, a maximum of 15 plants will be samples from each of the infestations reported. Newly reported populations will be sampled as they are reported in 1993-94. In addition, the Army Corps of Engineers and other

cooperators will be asked to collect samples of at least ten plants from each of four lakes in other regions of the country in which *M. spicatum* has been present for at least fifteen years to determine whether multiple genotypes exist within lakes. The Army Corps of Engineers and other cooperators also will be asked to collect samples of at least five plants from at least ten states in other regions of the country to estimate levels of genetic variability in *M. spicatum* in North America.

To determine whether multiple variations of EWM exist within lakes, and to estimate the variability within Minnesota, DNA fingerprinting will be used.

C.2.c. Materials: Standard aquatic plant sampling equipment will be used for field work, as described in Activity C.1. DNA fingerprinting will be done by extraction of DNA from plants and use of randomly amplified polymorphic DNA markers (RAPD).

C.2.d. Budget: $28,000 **Balance:** $0

C.2.e. Timeline:	7/93	1/94	7/94	1/95	7/95	11/95
Design culture system	****					
Build culture system		**********				
Laboratory assays	**********	**********	**********			
Field collections	*****		*****			
Field assays	*****		*****			
Evaluations				**********	**********	

C.3 Activity: Methods of reliably distinguishing native watermilfoils and Eurasian watermilfoil.

C.3.a. Context within the project: Eurasian watermilfoil was first identified in Minnesota in 1987. There are, however, several other species of the genus *Myriophyllum*, the watermilfoils, which are native to the state. Taxonomic differences between the native species, and the exotic species, EWM, are very subtle. Even experts in the field have difficulty in distinguishing the various species of watermilfoils. Certainty in identifying EWM is critical to eradication of EWM and preservation of native watermilfoils.

To verify identification of Eurasian watermilfoil, the Department of Natural

Resources typically forwards plant material to specialists at Oral Roberts University in Oklahoma. Identification is performed solely on the basis of meristic characters. In these cases, there is considerable room for error, and results are often not repeatable between plant specialists.

The objective of this activity is to develop genetic markers which managers can use as a tool to confirm, before expensive control efforts are undertaken, that samples from a suspected infestation are indeed Eurasian watermilfoil, and not a native watermilfoil.

C.3.b. Methods: Plants will be collected from various regions of the state where representative members of the genus *Myriophyllum* are located. The Army Corps of Engineers will also be asked to collect samples for this effort.

Results of DNA fingerprinting will be compared to measurements of morphological characteristics of plant samples to determine the utility of these two approaches to identification of milfoil species. The product will be a reliable technique that can be used to verify the identification of a milfoil specimen.

C.3.c. Materials: Standard aquatic plant sampling equipment will be used for field work, as described in Activity C.1. DNA fingerprinting will be done by extraction of DNA from plants and use of randomly amplified polymorphic DNA markers (RAPD). Selected morphological features will be characterized for plant specimens and voucher specimens will be deposited at the University of Minnesota herbarium.

C.3.d. Budget: $10,000 **Balance:** $0

C.3.e. Timeline:	7/93	1/94	7/94	1/95	7/95	11/95
Design culture system	****					
Build culture system		**********				
Electrophoresis assays	**********	**********	**********			
Field collections	****		****			
Product				**********	**********	

C.4 Activity: Viability of Eurasian watermilfoil seeds.

C.4.a. Context within the project: It has been assumed by managers that the

primary means by which Eurasian watermilfoil is spread is by vegetative fragmentation. Preliminary results of work carried out on eight Minnesota lakes by researchers at the University of Minnesota support this hypothesis. Questions still remain, however, as to whether seed germination may occur in lakes having different soil characteristics. Management strategies would change if seed are found to be viable.

C.4.b. Methods: Samples of EWM will be collected from all known infested waterbodies. Under laboratory conditions, researchers will attempt to germinate seeds produced by EWM.

C.4.c. Materials: EWM plants will be cultured using the plant culture system described in Activity C.1.

C.4.d. Budget: $3,000 **Balance:** $0

C.4.e. Timeline:	7/93	1/94	7/94	1/95	7/95	11/95
Design culture system	****					
Build culture system		**********				
Laboratory assays	**********	**********	**********			
Field collections	*****		*****			
Report					**********	

C. **Status:** Funding was not made available for this research in 1993 because the final form of the matching, non-state funds for this project was not confirmed by the provider, the Army Corps of Engineers (ACE), until March, 1994. In April 1994, a contract was fully executed by the Department of Natural Resources (DNR) and the University of Minnesota for this research. The ACE will provide the match for this project by conducting research which is considered 'in-kind services.' Minnesota's efforts to evaluate the potential for biological control of Eurasian watermilfoil will benefit greatly from the involvement of researchers from the ACE. The ACE has been involved in the study of the ecology and management of Eurasian watermilfoil and other aquatic plants for well over 25 years. They have on their staff some of the most respected scientific authorities in the field of ecology of aquatic plants. The insights and capabilities of ACE researchers will enhance the Minnesota research. The initial step taken by the ACE was to review proposals submitted to the DNR by researchers at the University of Minnesota for LCMR research on milfoil to identify specific projects that the ACE should undertake as their part of this overall research

effort. The status of work on activities listed under Objective C is described below.

Activity C.1. Investigation into natural declines and environmental effects on the growth of Eurasian watermilfoil.

University of Minnesota

The purpose of this research is to investigate relationships between among abundance of milfoil and, abundance of other plants, and selected environmental factors. Results of these investigations will be used to evaluate changes that may be associated with declines in the abundance of milfoil, if they occur. Initial analyses of occurrences of different plant species failed to identify any clusters of lakes with milfoil. Subsequent analyses of similarity indices identified two clusters or groups of lakes with milfoil. The first group or community has a relatively high level of abundance of Eurasian watermilfoil, average secchi depth of 2.4 m, and 5.4 mg m^{-3} chlorophyll a; characteristic plant taxa are *Chara, Najas, Potamogeton richardsonii*, and *Bidens beckii* (Newman et al. 1994; Holmberg 1994). The second community has a relatively low level of abundance of milfoil, average secchi depth of 1.3 m, and 20.4 mg m^{-3} chlorophyll a; characteristic plant taxa are *Potamogeton crispus*, and *Elodea canadensis*. No large or widespread declines in milfoil were observed in the study lakes during 1992 and 1993. Future analyses will include information on concentrations of nutrients and carbohydrates in plants. It is hoped that these efforts will improve our understanding of relationships between environmental variables and abundance of Eurasian watermilfoil.

Research in this area is being conducted by two researchers at the University of Minnesota. The contract with the University of Minnesota under which this research is being done will be amended to extend the due date for the final report from 15 June 1995 to 1 November 1995. This extension will enable the DNR to thoroughly review reports on research done under this activity and request revisions, if necessary, from the researchers. The results of this research will be reported to the LCMR in the work program to be submitted on 31 December 1995.

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This effort did not detect any dramatic and sustained natural declines in the four populations of Eurasian watermilfoil under study before the end of June, 1995 when this effort was concluded (Perry and Penner 1995, Newman and Ragsdale 1995; copies attached). Continuing research which is supported by funding provided through the DNR after appropriation by the Minnesota Legislature as recommended by the LCMR (M.L. 1995, ch. 220, sec. 19, subd. 13 (a)) indicates that one of these populations might be

declining (see Newman et al 1995).

These investigators found various correlations among measures of milfoil abundance, milfoil condition, and environmental variables (Perry and Penner 1995). Generally, these findings were consistent with results of previous, published studies. It is expected that the results of these efforts will be useful in the future interpretation of long-term studies causes of of declines in Eurasian watermilfoil.

Army Corps of Engineers

The ACE plans to spend approximately $60,000 on research in this area. This effort will be directed by Dr. John Barko of the Waterways Experiment Station. This research will include studies designed to generate predictions of the possible spread of milfoil in Minnesota based on the environmental characterization of lakes. The first phase of work to be done by the ACE in this area is a feasibility study. This will begin with development of a list of factors likely to influence the distribution of milfoil. The ACE will then evaluate the availability of information on these factors for lakes in Minnesota and in other northen states or Canadian provinces. In Minnesota, this information is likely to be available from the DNR and Pollution Control Agency. Completion of the feasibility study in early 1995 will enable the ACE to decide whether to proceed with the second phase of this effort, predicting the susceptibility of different classes of Minnesota lakes to dominance by milfoil. These predictions will be made by examining information on relationships between abundance of milfoil and environmental conditions in lake in other northern states and perhaps Canadian provinces where this exotic plant has been present for a number of years. In addition, this characterization of lake environments will likely help predict where potential biological control agents may live and how effective they will be in controlling milfoil. This information also will be useful to understanding where and why future declines of milfoil occur, assuming that they will occur in Minnesota.

December, 1995

The U.S. Army Corps of Engineers (USACE) decided to proceed with the second phase of this effort, predicting the susceptibility of different classes of Minnesota lakes to dominance by Eurasian watermilfoil. In 1995, this effort was directed by Drs. John Barko and John Madsen of the Waterways Experiment Station (WES). These predictions will be based upon correlations between levels of dominance by miloil in lakes of north-temperate North America and environmental conditions. Information on Wisconsin lakes was obtained from both existing databases and surveys of 12 lakes conducted by USACE WES personnel specifically for this project. This information will be used for the initial assessment which will be described in a report to be completed by 29 February 1996. In

addition, data from north-temperate lakes in other states, including Minnesota, and Canadian provinces have been requested. This information will form the basis for a final assessment which will be described in a report to be completed by 30 April 1996 using additional funding from the USACE. This project wi;; aid in planning allocation of resources to management on those lakes most likely to be infested with milfoil and experience problems caused by this exotic plant.

Activity C.2. Genetic variation of Eurasian watermilfoil.

In the contract between DNR and the University, the amount of State funds allocated to this activity was increased from the $15,000 originally proposed to $28,000. This increase in funding was made in response to very favorable reviews of this research that were received from the ACE researchers. During the spring of 1994, considerable efforts were made to refine techniques involving the use of Randomly Amplified Polymorphic DNA markers (RAPD) to analyze the genetic 'fingerprints' of milfoil specimens (first part of this study C.2.1). In addition, arrangements have been made to obtain specimens of milfoil from researchers and natural resource agencies outside Minnesota.

Research in this area is being conducted by a researcher at the University of Minnesota. The contract with the University of Minnesota under which this research is being done will be amended to extend the due date for the final report from June 15, 1995 to November 1, 1995. This extension will enable the DNR to thoroughly review reports on research done under this activity and request revisions, if necessary, from the researchers. The results of this research will be reported to the LCMR in the work program to be submitted on December 31, 1995.

Genetic variation in Eurasian watermilfoil was examined using RAPD for samples collected from 27 lakes and one river in Minnesota as well as two lakes outside the state (Furnier et al. 1994). These efforts identified 11 different genotypes, a number significantly greater than the two genotypes identified previously by the use of isozymes (Furnier and Mustaphi 1992). In 11 samples collected from White Bear Lake, nine different genotypes have tentatively been identified (Furnier et al. 1994).

This level of variation may be higher than that in most lakes with milfoil. For example, only one genotype was found in 17 of 20 Minnesota lakes from which two samples were collected. These results are generally consistent with the belief that most reproduction in Eurasian watermilfoil is clonal or asexual, though they do suggest that some sexual

reproduction may be occurring in Minnesota lakes.

December, 1995

The use of randomly amplified polymorphic DNA (RAPD) markers to analyze genetic 'finger-prints' of Eurasian watermilfoil (Furnier et al. 1994, Furnier et al. 1995; copies attached) indicted a much higher level of genetic variation in Eurasian watermilfoil than was expected based on previous studies of isozymes (Furnier and Mustaphi 1992). Observed variation may be attributable to either sexual reproduction in North American lakes or introduction of multiple genotypes into these lakes. Overall, these results indicate that clonal reproduction is very important in Eurasian watermilfoil. Future efforts to evaluate the efficacy of control agents should include a number of different milfoil genotypes.

In the second part of this study (C.2.2), variation within individual lakes was examined using ten to 11 samples collected from each of four lakes, two in Minnesota, one in California, and one in Washington (Furnier et al. 1994). Genetic variation was observed among samples of milfoil collected from each lake, but one genotype was usually predominant. In the third part of this study (C.2.3), variation among individual lakes in Minnesota was examined using samples collected from 27 lakes and one river. Analyses of these samples identified 11 distinct genotypes. Among the lake sampled, one milfoil genotype predominated, occurring in 60% of the plants assayed.

In the fourth part of this study (C.2.4), variation among North American populations of milfoil was examined using 149 plants collected from approximately 80 lakes and rivers (Furnier et al. 1995). These bodies of water were located in Alabama, Arizona, California, Michigan, Texas, Vermont, Washington, and Wisconsin, though most of them were in Minnesota.. Analyses of these 149 samples identified 85 distinct genotypes. These results indicate more genetic variation in milfoil than was previously found by this lab using techniques to evaluate isozymes as opposed to RAPD (Furnier and Mustaphi 1992). These results are generally consistent with the belief that most reproduction in Eurasian watermilfoil is clonal or asexual, though they do suggest that some sexual reproduction may be occurring in Minnesota lakes.

Activity C.3. Methods of distinguishing among milfoil species.

In the contract between DNR and the University, the amount of State funds allocated to this activity was decreased from the $15,000 originally proposed to $10,000. This decrease was suggested by Dr. Glenn Furnier, the researcher responsible for this work. Specimens of whorled watermilfoil, *Myriophyllum verticillatum*, were analyzed in the

laboratory during 1993 and found to be distinguishable from Eurasian watermilfoil, M. *spicatum*. During the spring of 1994, arrangements have been made to obtain specimens of milfoil from researchers and natural resource agencies outside Minnesota. No written report on this activity is due until 15 June 1995.

Research in this area is being conducted by a researcher at the University of Minnesota. The contract with the University of Minnesota under which this research is being done will be amended to extend the due date for the final report from June 15, 1995 to November 1, 1995. This extension will enable the DNR to thoroughly review this report and request revisions, if necessary, from the researcher. The results of this research will be reported to the LCMR in the work program to be submitted on December 31, 1995.

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Results of this effort indicated that randomly amplified polymorphic DNA (RAPD) markers can be used to distinguish between Eurasian and northern watermilfoil (Furnier et al. 1995; copy attached). Genetic variation among North American populations of Eurasian, northern, and whorled watermilfoil was examined using 219 plants collected from 84 lakes and rivers. These bodies of water were located in Alabama, Arizona, California, Michigan, Texas, Vermont, Washington, and Wisconsin, though most of them were in Minnesota. Analyses of 149 samples of Eurasian watermilfoil and 65 samples of northern watermilfoil identified several RAPD fragments that would be useful in distinguishing between these two species. Unfortunately, the number of samples of whorled watermilfoil collected was too small to allow a meaningful analysis of genetic variation in this species. Analyses of the 65 samples of northern watermilfoil identified 58 distinct genotypes, a higher percentage (89%) than that observed in Eurasian watermilfoil (57% - see C.2).

Activity C.4. Viability of Eurasian watermilfoil seeds.

In the contract between DNR and the University, the amount of State funds allocated to this activity was decreased from the $5,000 originally proposed to $3,000. This decrease was suggested by Dr. David Biesboer, the researcher responsible for this work. This change is consistent with reviews received from the ACE which recommended that this effort be de-emphasized or redirected.

Sampling of inflorescences of Eurasian watermilfoil in one Minnesota lake indicated seed set of up to 1,400,000 seeds per hectare per year (see Biesboer 1995a). No germination was observed in 100 seeds in petri dishes in the lab. In addition, no seedlings emerged from 600 seeds sewn in soil in aquaria filled with water. A germination rate of 1.5% was

observed in 270 seeds placed in nylon mesh bags placed in three Minnesota lakes during fall, 1993 and examined in May, 1994. These results indicate that Eurasian watermilfoil in Minnesota can produce significant numbers of seeds, but the rate of germination in these seeds is low. These results are generally consistent with those from studies conducted elsewhere in North America.

Research in this area is being conducted by a researcher at the University of Minnesota. The contract with the University of Minnesota under which this research is being done will be amended to extend the due date for the final report from June 15, 1995 to November 1, 1995. This extension will enable the DNR to thoroughly review this report and request revisions, if necessary, from the researcher. The results of this research will be reported to the LCMR in the work program to be submitted on December 31, 1995.

December, 1995

Results of this effort (Biesboer 1995a; copy attached) are consistent with previous research which has shown limited germination in seeds of Eurasian watermilfoil but little if any survival of seedlings, much less establishment of adult plants. Biesboer (1995a) also descried the extent of seedling development following germination, which did not proceed beyond "... a slight elongation of the plumule."

D. Title of Objective: Evaluation of biological control agencies for Eurasian watermilfoil: fungi and insects.

D.1. Activity: Survey of Eurasian watermilfoil in Minnesota and the upper midwest for pathogens.

D.1.a. Context within the project: Beginning in 1988, a program was initiated to isolate a fungus as a biological control for Eurasian watermilfoil. As a result of the screening program for microorganisms, a fungus, *Mycoleptodiscus terrestris* (M.t.), was isolated. When introduced to milfoil in the laboratory, it resulted in decomposition of the plants.

M.t. was field tested at high concentrations in a small lake in Massachusetts with a heavy infestation of EWM and the EWM was killed within 4 to 6 weeks showing the potential M.t. might have in the future.

Unfortunately, EcoScience Corporation has been unsuccessful when it attempted to

control milfoil in field environments with Mt. Consequently, the purpose of this activity is to survey Minnesota and the upper midwest for pathogens of Eurasian watermilfoil.

D.1.b. Methods: Plants, sediments, and the water column will be sampled as necessary to determine whether fungi or other pathogens of milfoil are present.

This hypothesis will be tested on selected study sites on two lakes supporting dense populations of EWM. On each of these two lakes, both contact and systemic herbicides will be tested.

Army Corps of Engineers staff will collect samples of Eurasian watermilfoil to be analyzed for presence of potential pathogens. Efforts will be made to have field personnel of the COE and other agencies, other institutions, or individuals report suspected occurrences of disease in milfoil. If merited, such reports will be followed by collection of plant materials from populations thought to be diseased.

D.1.c. Materials: Samples collected by this survey will be analyzed by the ACE.

D.1.d. Budget: Corps of Engineers: $65,000
Balance: Corps of Engineers: $40,000

D.1.e. Timeline:	7/93	1/94	7/94	1/95	7/95	11/95
Laboratory assays	**********					
Apply M.t.	******	*****				
Evaluate M.t.	******	*******	****			
Field surveys	******	*******	****			
Lab strain selection	**********					
Report				**********		

D.2. Activity: Evaluation of insects as biological control agent for Eurasian watermilfoil.

D.2.a. Context within the project: This research has been initiated to investigate the potential for biological control of EWM with native or naturalized insects.

Investigators have identified various native or naturalized aquatic insects that feed on

EWM. These invertebrates include a caterpillar in Ontario, a weevil in Vermont (*Euhrychiopis lecontei*, Creed and Sheldon 1991), and a midge in British Columbia (*Cricotopus myriophylli*, MacRae et al. 1990). All three species have been associated with milfoil declines. Furthermore, two of these species have been confirmed to occur in the neighboring state of Wisconsin, but no research on their control potential has been conducted there. The presence of these insects in Minnesota would ensure that they can survive our environment and reduce concerns associated with the introduction of another exotic. Laboratory studies will be conducted to determine feeding preference and consumption rates for all three insects, which will further determine their suitability as control agents.

Research on the biological control potential of insects was conducted by the University of Minnesota as recommended by LCMR during the 1992-1993 biennium. Researchers confirmed one species on aquatic invertebrate, the weevil, *Euhrychiopsis lecontei*, in one Minnesota lake, and found evidence of its presence in three other lakes. There is further evidence that the midge *Cricotopus myriophylli* may occur in one other lake.

The purpose of research to be conducted by the University of Minnesota is to: 1) Determine the oviposition preferences of these aquatic insects under laboratory conditions; 2) Evaluate the effects of weevils on EWM in confined conditions in large tanks (> 0.4 m^3); 3) Evaluate environmental conditions and the impacts of these conditions on insect populations and potential control success, and 4) Investigate Herbivore/fungal interactions which may lead to increased control success.

D.2.b. Methods: Cultures of the weevil, EWM, and various species of native aquatic plants are currently being raised in a greenhouse at the University of Minnesota. These cultures of aquatic plants and invertebrates will serve as the basis for further assessment of seasonal feeding patterns and plant selectivity. Plant damage will be assessed according to a classification system developed by University of Minnesota researchers.

Throughout the course of the study, a breeding population of each of the aquatic insects known to feed on EWM will be established and maintained in the laboratory. These populations will be supplemented by field collections. Studies to determine if environmental conditions impact insect population and control success will be made and to evaluate interaction between insects and fungi.

D.2.c. Materials: Laboratory and greenhouse cultures of EWM and northern watermilfoil, along with other native species of aquatic plants, will be maintained from previous research efforts. Laboratory cultures of potential control agents will likewise be raised in similar facilities.

D.2.d. Budget: State funds: $205,967 Corps of Engineers: $65,000
Balance: State funds: $0 Corps of Engineers: $40,000

D.2.e. Timeline:	7/93	1/94	7/94	1/95	7/95	11/95
Invertebrate surveys	****	****				
Plant preference	**********	**********	**********			
Propagate invertebrates	**********	**********	**********			
Introduce invertebrates			***	***		
Evaluate Invertebrates		****		***		
Report					**********	**********

D. Status: Funding was not made available in 1993 because the final form of the matching, non-state funds for this project was not confirmed by the provider, the Army Corps of Engineers, until March, 1994. In April 1994, a contract was fully executed by the Department of Natural Resources (DNR) and the University of Minnesota for this research. The Army Corps of Engineers (ACE) will provide the match for this project by conducting research which is considered 'in-kind services.' Minnesota's efforts to evaluate the potential for biological control of Eurasian watermilfoil will benefit greatly from the involvement of researchers from the ACE. The ACE has been involved in the study of the ecology and management of Eurasian watermilfoil and other aquatic plants for well over 25 years. They have on their staff some of the most respected scientific authorities in the field of biological control of aquatic plants. The insights and capabilities of ACE researchers will enhance the Minnesota research. The initial step taken by the ACE was to review proposals submitted to the DNR by researchers at the University of Minnesota for LCMR research on milfoil to identify specific projects that the ACE should undertake as their part of this overall research effort. These projects are described below.

Activity D.1. Research on the potential of the fungus Mycoleptodiscus terrestris *to control Eurasian watermilfoil.*

Funding of research on *Mycoleptodiscus terrestris* (Mt) by EcoScience Corporation was canceled. This action was taken because EcoScience Corporation has been unsuccessful when it attempted to control milfoil in field environments with Mt in studies funded by the legislature as recommended by the LCMR (M.L. 1992, Ch. 513,

Art. 2, Sec. 9). Similar problems with Mt have been encountered by researchers of the ACE who were working independently on the same organism. Although the outlook for Mt is not promising, the search for pathogens of milfoil has not ceased. The search continues because disease has been observed to affect milfoil plants in field environments and is suspected to have contributed to past declines observed in milfoil in North America. The ACE plans to spend approximately $65,000 on survey in Minnesota and the upper midwest for pathogens of Eurasian watermilfoil. The ACE has developed a study to be done by Dr. Judy Shearer at the Waterways Experiment Station with the following objectives:

1) Isolate fungal pathogens causing infection in and damage to *M. spicatum* in the field
2) Investigate biological and ecological characteristics of the pathogens infecting *M. spicatum* in the upper midwest
3) Conduct preliminary screens of pathenogenicity and host-specificity in fungal isolates

Initial isolations of fungi have been completed and more results will become available later.

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A report on this research is expected in 1996.

Activity D.2. Evaluation of insects as biological control agents for Eurasian watermilfoil.

In the contract between DNR and the University of Minnesota, the amount of State funds allocated to this activity was $200,000. The researchers at the University have made interesting progress on understanding the life history of the weevil *Euhrychiopsis lecontei* in Minnesota by discovering over-wintering weevils in litter and soil along the shore of a lake. This new information is critical to the evaluation of the potential of this insect to control milfoil because it ultimately should enable researchers to predict how many weevils will survive Minnesota winters and be available to feed on milfoil in spring.

University of Minnesota

Reports on research done by the University of Minnesota are listed under the four objectives included in the last paragraph of section D.2.a of this workplan. The contract with the University of Minnesota under which this research is being done will be amended to extend the due date for the final report from June 15, 1995 to November 1, 1995. This extension will enable the DNR to thoroughly review this report and

request revisions, if necessary, from the researcher. The results of this research will be reported to the LCMR in the work program to be submitted on December 31, 1995.

Activity D.2.1. Study of oviposition preferences of weevils under laboratory conditions.

The purpose of this reasearch was to examine the potential oviposition by weevils on Eurasian and northern watermilfoils as well as six other species of submersed aquatic plants used in this effort. Only three females oviposited on a plant other than Eurasian or northern watermilfoil. Weevils reared on Eurasian watermilfoil oviposited more frequently on this species than on northern watermilfoil. Weevils reared on northern watermilfoil were more likely than those reared on Eurasian to shift hosts, i.e., to oviposit on Eurasian watermilfoil. Analysis of time budgets showed that female weevils generally spent the most time on Eurasian watermilfoil.

December, 1995

The results of this research indicate that the weevil *Euhrychiopsis lecontei* is a specialist on watermilfoils and prefers Eurasian watermilfoil over northern watermilfoil (Newman et al 1995; copy attached). Very little oviposition on 'non-watermilfoils' was observed. This result is consistent with that reported by Sheldon and Creed (1995) who also found 1) that the weevils do more damage to Eurasain watermilfoil than to northern watermilfoil and 2) found little feeding on, or damage to, nine 'non-watermilfoil' plant species. The results described above were included in a thesis written by Solarz (1995) and in a manuscript by Solarz and Newman (in press) which has been accepted for publication by *Oecologia*, an international peer-reviewed journal.

Activity D.2.2. Study of the effects of weevils on Eurasian watermilfoil in large tanks.

These studies were done in July and August, 1994. In these experiments, weevils reached densities of up to 300 per square meter. Biomass of milfoil above ground was reduced by up to 60% four weeks after introduction of weevils into tanks; biomass of milfoil below ground was reduced by up to 45%. Biomass of milfoil that was detached and either floating or sunken increased with numbers of weevils introduced into tanks, suggesting that the insects damaged plants rather than consumed them. In addition, the concentrations of sugars and total nonstructural carbohydrates in both roots and shoots were reduced by weevils. This result suggests that weevils may have potential to reduce survival over winter of Eurasian watermilfoil and perhaps lead to reduced growth during the following spring.

December, 1995

Results of this experiment conducted under controlled conditions identified that the weevil *Euhrychiopis lecontei* may have potential to control Eurasian watermilfoil. These results have been reported in a manuscript which has been accepted for publication in *Aquatic Botany*, an international peer-reviewed journal (Newman et al., In press). These results are consistent with those of Sheldon and Creed (1995) who reported that this weevil may be a suitable biological control agent for Eurasian watermilfoil.

Activity D.2.3. Study of Eurasian watermilfoil and weevils in four Minnesota lakes.

Abundance of Eurasian watermilfoil and weevils was monitored along semi-permanent transects established in four Minnesota lakes during 1993 and 1994. Weevils and damage to milfoil plants were found in these lakes. Nevertheless, these efforts have not yet detected any strong patterns or changes in the abundance of milfoil that are related to the abundance of weevils. The maximum density of weevils observed in one of these lakes was 55 per square meter; average densities for three of the four lakes in May was 13 weevils per square meter. These values are much lower than the level of approximately 300 weevils per square meter shown to affect milfoil in tanks (see *Activity D.2.2*). Efforts are being made to determine what factors might be limiting densities of weevils in Minnesota lakes.

December, 1995

Monitoring of Eurasian watermilfoil, weevils, and environmental conditions in the four study lakes was continued during June, 1995 by allocating to this effort an additional $7,967 which was received from the Minnesota Lakes Association as part of the matching funds required for this project. This effort did not detect any dramatic and sustained natural declines in the four populations of milfoil under study before the end of June, 1995 when this effort was concluded (Perry and Penner 1995, Newman and Ragsdale 1995; copies attached). Continuing research which is supported by funding provided through the DNR after appropriation by the Minnesota Legislature as recommended by the LCMR (M.L. 1995, ch. 220, sec. 19, subd. 13 (a)) indicates indicates that one of these populations might be declining (Newman et al 1995).

Activity D.2.4. Investigate interactions between herbivores and fungi which may increase control.

Samples of damaged and undamaged Eurasian watermilfoil were collected by the University of Minnesota and sent to Dr. Judy Shearer at the Waterways Experiment Station. Dr. Shearer isolated seven pathenogenic fungi from this material Of these, *Mycoleptodiscus terrestris* was most common. In addition, samples were collected from weevils dissected by the University of Minnesota and examined by Dr Shearer for microbes that might be pathenogenic to milfoil. Preliminary results do not indicate that weevils are vectors for pathogens of milfoil.

December, 1995

This effort indicates that it is unlikely that weevils are vectors for organisms that are pathogenic to Eurasian watermilfoil.

Army Corps of Engineers

The COE plans to spend approximately $65,000 on evaluation of insects as biological control agents for Eurasian watermilfoil. The COE has developed a study to be done by other plant species was examined. Over 150 female weevils, some of which were reared on Eurasian watermilfoil and some of which were reared on northern watermilfoil were

Dr. Sallie Sheldon at Middlebury College with the following objectives:

1) Quantify the impacts of weevils on native watermilfoils in feeding experiments conducted in the lab

2) Examine the impact of weevil larvae on some native plants with stems similar to

those of Eurasian watermilfoil
3) Determine temperature maxima for eggs, larvae, and adults
4) Determine life history stage for release
5) Follow the fate of plants damaged by weevils
A progress report on this project should be available early in 1995.

December, 1995

Results of this effort indicate that the weevil *Euhrychiopsis lecontei* had no significant negative effect on five native milfoil species under laboratory conditions (Sheldon 1995). Under field conditions, the weevil altered the growth of northern watermilfoil but did not have a significant negative effect on this native plant. An aditonal report is expected in 1996.

V. Evaluation: For the FY94-95 biennium the program can be evaluated by its ability to: 1) determine if establishment and propagation is possible for three insects species that show promise as potential biocontrol agents for purple loosestrife (objective A.); 2) determine the potential for developing a mycoherbicide for purple loosestrife control by isolating and testing native fungi (Objective B); 3) better understand the biology, physiology (crown formation and crown bud development) and ecology of EWM and integrate this knowledge with known and experimental methods of control (Objective C); 4) evaluate the potential use of insects and fungi (mycoherbicides) as biological control of EWM (Objective D).

In the long-term, the project should be evaluated by its ability to successfully utilize knowledge gained to control harmful exotic plant species to protect our native ecosystems.

VI. Context within the fields: To date, work on purple loosestrife and Eurasian watermilfoil control in Minnesota has been focused on the use of cultural, mechanical and chemical control methods. These three methods can provide short-term control, but show little potential to eradicate these exotic plants. Pesticides, in general, have a negative connotation with many people. The continuing proliferation of pesticide use, especially in aquatic systems has the potential to impact the environment. Pesticides used for aquatic weed control tend to only provide one to two years of control (reduction in pest population to an acceptable level). Research continues on developing better herbicides and application methodologies.

Biological controls, especially insects, have the potential to reduce pest populations to an acceptable level and keep the populations down over long periods of time. The U. S. Department of Agriculture is coordinating research on insects as a biological control for purple loosestrife. Five states, including Minnesota are working with the USDA to continue this research at the state level. Minnesota is leading the nation in research on purple loosestrife.

Biological control research on Eurasian watermilfoil (EWM) in Minnesota began in 1992 and is being carried out by two different research organizations. Much of the research is being funded by the DNR through previous funding from the Minnesota state legislature as recommended by the LCMR. Both insects and fungi are being researched as possible controls for EWM. Insect research is on going in Minnesota as well as British Columbia and Vermont. A mycoherbicides (the use of fungi) was being researched by the Ecoscience

Corporation with funding from the state of Minnesota. The U.S. Corp. of Engineers has initiated cooperative research on EWM with our researchers to facilitate the research here in Minnesota.

VII. Benefits: Long-term control of purple loosestrife and Eurasian watermilfoil would protect Minnesota's vast aquatic diversity of plants and animals. Protecting native plants will ensure high diversity of animals which rely on these plants for habitat such as food, shelter and nesting sites. Recreational opportunities will be enhanced with the control of these exotic pests. The use of biological controls lessen the burden of controlling these two species with pesticides. Biological controls are a more natural, less damaging way of controlling pest species.

VIII. Dissemination: Results from this project will be presented at national, regional and state scientific meetings to peers in the field, as well as to resource managers and planners who will be users of the information and results. Following presentations of results at such meetings, they will be published as special publications, newsletters, other various forms and in peer-reviewed scientific journals. When management approaches using biocontrol are developed, they will be targeted at resource managers.

IX. Time: Biological control research requires an extensive commitment. The intent of this project is to develop control methods that will last as long as the problem exists. This development process can take up to 10 years at the same funding level. Funding beyond the FY94-95 biennium will continue to be requested from the LCMR.

X. Cooperation:

This research project is carried out through contracts with the cooperators listed below. Contracts have not been established with the cooperators, therefore no written time commitments have been received from them. Listed below are estimates of time commitments from the cooperators for the objective of which they will be involved in.

1. Dr. David Andow
   Department of Entomology
   University of Minnesota

   A researcher and entomologist, Dr. Andow will implement field studies of insects for purple loosestrife control (Objective A). Dr. Andow will contribute 20% of the time needed to complete Objective A, and a graduate student will contribute 50% of the time to complete Objective A.

2. Dr. David Ragsdale
   Department of Entomology
   University of Minnesota

   A biological control researcher and entomologist, Dr. Ragsdale will work with Dr. Andow to implement studies of insect biological control of purple loosestrife (Objective A). Dr. Ragsdale will contribute 15% of the time needed to complete Objective A.

3. Dr. Robert Nyvall
   North Central Experiment Station
   University of Minnesota

   A mycologist, Dr. Nyvall's primary role will be to study fungi as a possible biological control of purple loosestrife (Objective B). Dr. Nyvall will contribute 75% of the time needed to complete Objective B. A research assistant will contribute 25% of the time needed to complete Objective B.

4. Dr. Ray Newman
   Department of Fisheries
   University of Minnesota

   An aquatic ecologist, Dr. Newman's primary role will be to study native insects for Eurasian watermilfoil control (Objective C and D). Dr. Newman will contribute 15% of the time needed to complete Objective C and D. Other UM faculty and graduate students specializing in a variety of activities listed will contribute 85% of time needed to complete these two objectives.

5. U.S. Army Corps of Engineers
   Mr. Edward McNally
   St. Paul District

   Mr. McNally is the coordinator of the Corps of Engineers' Aquatic Plant Control Program in the St. Paul District. He will coordinate research efforts to be undertaken by staff of the Waterways Experiment Station as cooperative work initiated as 'in-kind' match services.

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