1995 Project Abstract

31 December 1997

Program Manager: Chip Welling
Organization: Minnesota Department of Natural Resources
Mail Address: Box 25, 500 Lafayette Road, St. Paul, MN 55155-4025
Phone: (612) 297-8021
E-mail: chip.welling@dnr.state.mn.us

Legal Citation: **M.L. 95, Ch. 220, Sec. 19, Subd. 13(a)**
Appropriation amount: **$300,000**

Objectives: A. Evaluate insects or pathogens or both as biological control agents for Eurasian watermilfoil.

B. Evaluate insects and fungi as biological control agents for purple loosestrife.

Results of Project:

Evaluation of potential biological control agents for Eurasian watermilfoil, *Myriophyllum spicatum*, by researchers at the University of Minnesota is primarily focused on a weevil, *Euhrychiopsis lecontei*, which is a native insect. Researchers sampled nine sites known to have weevils and documented apparent declines of varying degrees in milfoil in six sites. It is believed that three of these declines may be associated with weevils. Increases in abundance of native plants following declines in milfoil appear to be important in preventing the exotic from returning to high levels of abundance.

Release of weevils into study plots in lakes neither produced high densities of weevils nor did it reduce the density of milfoil. Researchers completed additional studies of factors that may limit populations of weevils, and hence limit their potential to control milfoil. Future research (M.L. 1997 Chapter 216, Sec. 15, Subd. 20(b)) will 1.) attempt to reduce milfoil in lakes by introduction of weevils to plots as was done under controlled conditions in tanks, and 2.) attempt to document relationships between declines in milfoil and weevils or other potential biological control agents by continued surveys of Minnesota lakes.

Implementation of biological control of purple loosestrife, *Lythrum salicaria*, was expanded significantly through rearing and distribution efforts at the University of Minnesota. New rearing protocols were successfully tested and implemented during this biennium. In 1996, 168,000 leaf-eating beetles, *Galerucella* spp., were reared and released in 34 loosestrife infestations statewide. In 1997, over 800,000 leaf-eating beetles were reared and released in 150 sites statewide. Research was conducted to evaluate rearing success, establishment of field released insects and impact these insects have on purple loosestrife.

Use and Dissemination of Results

The DNR views the research on the potential for biological control of Eurasian watermilfoil as basic research. Consequently, the primary means for dissemination of results are publications in peer-reviewed scientific journals and presentations at conferences. The researchers at the University of Minnesota have published five papers, given numerous presentations at conferences, and created a web page [http://www.fw.umn.edu/research/milfoil/milfoilbc.html] where information on the potential for biocontrol of milfoil is presented. An extension publication on rearing and releasing *Galerucella* for management of purple loosestrife is under production at the University of Minnesota. This publication, with its companion slide set, will teach resource managers how to raise and release leaf-eating beetles for the control of loosestrife. It should be ready for dissemination in January, 1998.

Date of Report:30 December 1997

LCMR 1995 Work Program Update

I. Project Title and Project Number: BIOLOGICAL CONTROL OF EURASIAN WATERMILFOIL AND PURPLE LOOSESTRIFE - CONTINUATION

Program Manager: Chip Welling
Agency Affiliation: Minnesota Department of Natural Resources
Division of Fish and Wildlife
Mail Address: Ecological Services Section
Box 25, 500 Lafayette Road
St. Paul, MN 55155-4025
Phone: (612) 297-8021

A. Legal Citation: M.L. 95, Ch. 220, Sec. 19, Subd. 13(a)
Total biennial LCMR budget: $300,000
Balance: 0

Appropriation Language: This appropriation is from the trust fund to the commissioner of natural resources for research to develop biological controls for Eurasian watermilfoil and purple loosestrife.

B. LMIC Compatible Data Language: N/A

C. Status of Match Requirement: N/A

II. Project Summary: This project will continue the development of biological controls for these two exotic plants. In the case of Eurasian watermilfoil (milfoil), the project will further the evaluation of the potential for native or naturalized insects or pathogens to control milfoil. If one (or more) organism(s) is (are) found to be able to control milfoil, then we will begin research on implementation of the approach to control. In the case of purple loosestrife, this project will complete the initial implementation using four Eurasian insects known to contribute to the control of this plant. In addition, this project will further the evaluation of the potential for native or naturalized fungi to control loosestrife. Development of biological controls will enhance stewardship of Minnesota's resources by minimizing the negative effects of these exotic plants and our need to use herbicides.

III. Six Month Work Program Update Summary:

Evaluation of potential biological control agents for Eurasian watermilfoil by researchers at the University of Minnesota is primarily focused on a weevil (*Euhrychiopsis lecontei*), which is a native insect. Researchers sampled nine sites known to have weevils. In two of four sites intensively followed for four years and in one site followed for two years, significant and persistent declines in milfoil were documented. Two of these declines appear to be associated with weevils. In one of these cases, researchers found an

aquatic moth (*Acentria epehemerella*), another potential control agent, at densities that were high for Minnesota, but less than those reported from New York lakes where the moth is believed to have contributed to declines in milfoil (Johnson 1995 a and 1995b). The third decline was tentatively attributed to winter-kill.

In three sites that were less intensively followed for three years, researchers also documented declines in milfoil. Nevertheless, the association of weevils with these declines is unclear. In a ninth site sampled by the University of Minnesota during two years, researchers from the Wisconsin Department of Natural Resources detected a decline in which weevils are implicated (Lillie 1996, Lillie and Helsel 1997). The decline was followed by return to pre-decline levels over a seven year period (R. Lillie, pers. comm.).

Increases in abundance of native plants following declines in milfoil appear to be important in preventing the exotic from returning to high levels of abundance. Responses of natives to declines in milfoil appear to be affected by clarity of water and concentration of nutrients in sediments.

Release of weevils by researchers into study plots neither produced high densities of weevils nor did it reduce the density of milfoil. Factors affecting populations of weevils during summer appear to be more important than those affecting over-winter survival of weevils in determining densities of weevils observed during summer, which in turn appears to be related to effects on milfoil plants. Researchers also completed studies of over-wintering in weevils, dispersal of adult weevils, development of weevils in relation to temperature, predation on weevils by fish, and genetic variation in weevils in relation to host plant.

Minnesota researchers conducting the weevil studies are making good progress on publication of results in peer-reviewed journals. Four papers were published the past two years (Newman et al. 1996, Solarz and Newman 1996, Sutter and Newman 1997, Newman et. al. 1997).

Future research (M.L. 1997 Chapter 216, Sec. 15, Subd. 20(b)) will address two key objectives: 1.) attempt to reduce milfoil biomass in lakes by introduction of weevils to plots as was done under controlled conditions in tanks (Newman et al. 1996), and 2.) attempt to document declines in milfoil and relationships between these declines and weevils or other potential biological control agents by continued surveys of lakes.

Implementation of biological control of purple loosestrife was expanded significantly through rearing and distribution efforts at the University of Minnesota. New rearing protocols were successfully tested and implemented during this biennium. In 1996, 168,000 leaf-eating beetles, *Galerucella* spp., were reared and released in 34 loosestrife infestations statewide. In 1997, over 800,000 leaf-eating beetles were reared and released in 150 sites statewide. Research was conducted to evaluate rearing success, establishment of field released insects and impact these insects have on purple loosestrife.

Leaf-eating beetle releases established at approximately 80% of the release sites. Certain sites showed extensive populations of leaf-eating beetles, two to three years after release. These sites were studied for what effect the leaf-beetles have on carbohydrate stores in the loosestrife root-crowns and on seed production. Storage carbohydrates were reduced after one to two years of *Galerucella* leaf feeding. What impact this has on loosestrife mortality is not known. *Galerucella* feeding on loosestrife shoot tips appears to reduce the number of seed capsules produced on a loosestrife plant.

Development of a mycoherbicide to control loosestrife continued during this biennium. Several fungi have been identified as being pathogenic to purple loosestrife and progress has been made to develop a carrier in which to incorporate the fungi and apply to plants. Field tests of fungal pathogen on loosestrife, however, have not been successful. At best, the fungi may prove to work best in conjunction with insect releases. There are no successful mycoherbicides at this time.

IV. Statement of Objectives:

Objective A. Evaluation of insects or pathogens or both as biological control agents for Eurasian watermilfoil.

This project will further the evaluation of the potential for native or naturalized aquatic insects or pathogens to control Eurasian watermilfoil. This project will enable us to determine which potential biological control agents merit further evaluation and which agents do not merit further evaluation.

Objective B. Evaluation of insects and fungi as biological control agents for purple loosestrife.

This project will further the evaluation of the potential for establishment in Minnesota of insects known to damage purple loosestrife. This project also will continue evaluation of fungi to control purple loosestrife.

Timeline for Completion of Objectives:

	7/95	1/96	6/96	1/97	6/97
Objective A. Evaluation of insects as biological control agents for Eurasian watermilfoil.					*****
Objective B. Evaluation of insects and fungi as biological control agents for purple loosestrife.					*****

V. Objectives/Outcome:

A. Title of Objective/Outcome: Evaluation of insects as biological control agents for Eurasian watermilfoil.

A.1. Activity: Research to evaluate the potential of native or naturalized aquatic insects to control Eurasian watermilfoil in Minnesota.

A.1.a. Context within the project: The aim of the research is to (1) monitor by survey a set of previously sampled milfoil populations with the potential to decline, (2) determine by intensive sampling the factors that may be limiting control agent densities in the field and whether chronic effects such as disruption of milfoil nutrient stores are responsible for declines of milfoil associated with herbivores, (3) determine the immediate and over winter effects of summer-long augmentations of weevils, and (4) conduct additional studies of factors that may limit potential control agents.

A.1.b. Methods:

A.1**(-1).b** Attempt to detect declines in milfoil by survey of previously sampled sites in MN & WI: We will select a subset of approximately 4 lakes or distinct bays to survey for the presence of control agents and density of milfoil. These sites will be selected from those that we surveyed in 1992 or 1993 (Newman et al. 1993: Work done with state funds appropriated by the Minnesota legislature as recommended by the LCMR (M.L. 1992, Ch. 513, Art. 2, Sec. 9)), so that we have at least one year of prior data. Four additional lake sites will be more intensively sampled as permanent transect sites (see objective 2), but the below mentioned GPS (global positioning system) surveys will be conducted at these sites as well, resulting in approximately 8 sites with broad scale coverage data.

Each site will be sampled once in mid-summer 1995 and once in 1996. At each site, the extent of the surfaced (matted) milfoil and the plant limit depth will be mapped with a GPS unit. This will provide information of the extent of milfoil coverage. Standard limnological measures such as Secchi depth, conductivity and light and temperature profiles will be measured. At the same locations from which we gathered data in 1992 and 1993 (Newman et al. 1993), we will collect samples of plants along three transects running from shore. Plants will either be collected from 0.1 m^2 quadrats with SCUBA (preferred approach), from the surface with a defined area rake (Crowell et al. 1994), or with the grapple hook

modification of Jessen and Lound (1962; see Newman et al., 1993 for discussion of this approach). Samples collected will be scanned for the presence of control agent, visually assigned a damage rating (0-5), and rinsed of invertebrates. Plants will be sorted by taxa, weighed, dried, reweighed, and ash-free-dry-mass (AFDM). Collected invertebrates will be preserved for later analysis if a decline is noted at that site. Because the aim of sampling these sites is to obtain a fairly rapid assessment of potential declines, we will not conduct a detailed analysis of invertebrates unless it is noted at that site, or if a decline is noted in the following year. Sediment and plant carbohydrate samples will only be collected if a decline is noted. If a decline appears, or appears imminent, we will intensify our sampling of that site to provide explanatory power to interpret the cause of the decline.

(2) Attempt to assess factors that limit potential control agents, especially *Euhrychiposis*, by intensive sampling at four sites in Minnesota: To assess limiting factors and to monitor for potential declines, we plan to continue an intensive sampling regime at the four Minnesota sites, Lake Auburn, Cedar Lake, Otter Lake and Smith's Bay of Lake Minnetonka and to collaborate with the Wisconsin DNR at the Fish Lake site. To reduce overall effort and processing time, we will sample each site twice in 1995 (mid July and late summer) and three times in 1996 (late spring, mid July and late summer). We aim to collect samples in spring 1995, based on supplemental funding to our current project (M.L. 1993, Ch. 172, Sec. 14, Subd. 12(1)). At each site, total macrophyte and milfoil biomass (wet, dry and AFDM), stem density and herbivore density (larvae, pupae and adults) will be determined for 0.1 m^2 samples collected via SCUBA along 5 semipermanent transects (see Newman et al. 1994). Damage will be scored using the visual inspection approach outlined above, rather than the more extensive quantitative approach we are currently using. A subsample of additional plants, roots and shoots, (3 samples from each of three transects) will be collected for carbohydrate analyses (Raguse and Smith 1965, Smith 1969, Newman et al. *in prep*). Standard limnological variables (Secchi depth, light and temperature profile, conductivity) will be measured during each sampling period.

Recording thermistors will be placed and maintained at two sites. Fall and spring shoreline samples (0.1-0.25 m^2) will be taken adjacent to the transects and at other sites along the lake to determine shoreline densities and overwinter survival. Samples of weevils will be periodically dissected to confirm our previous observations on reproductive tissue, flight muscle, and fat body condition, as well as the presence of parasites. These data will allow us to 1) determine seasonal

and yearly changes in milfoil and herbivore densities, 2) determine if declines occur, 3) determine what factors are associated with declines, 4) determine when and where weevil population bottlenecks occur and 5) develop and calibrate a crude population model for weevils based on degree days, overwinter populations and milfoil density.

We will also collaborate with the Wisconsin DNR at their Fish Lake site (R.A. Lillie, WI DNR, pers. comm.). We will collect a set of underwater transect samples in the south bed once each summer to quantify milfoil and herbivore densities in a manner comparable to our 1992 and 1993 samples. The WI DNR will be collecting and analyzing additional milfoil and invertebrate samples throughout the lake several times during the summer. However, during our sampling in mid summer we will also collect sediment for ammonium, nitrogen and bulk density analysis and milfoil shoots and roots for carbohydrate analysis. We will also have the WI DNR collect an additional set of sediment and plant shoot and root samples later in the season each year. We will conduct fall and spring shoreline sampling to determine overwinter populations and overwinter success. Although the Wisconsin DNR will be doing most of the in-lake work assessing changes in milfoil and weevil densities, they do not have the funds or mandate to collect the additional information on sediments, plant carbohydrates or shoreline weevil densities (Lillie, pers. comm.). These data will be essential to interpret any decline that may persist in Fish Lake.

Addition of Cenaiko Lake to four sites subject to intensive sampling.

$5,800

While searching for a better site to conduct manipulations of weevil densities we found a high population of the weevil (*Euhrychiopsis lecontei*) in an extensive bed of Eurasian watermilfoil at Cenaiko Lake. The plants were heavily damaged and preliminary estimates indicate that this is the highest density of weevils we have yet seen in the upper midwest. The high density of weevils was surprising because the lake has a relatively recent milfoil infestation (1992), is human-made and has no connections to other water bodies, and has no woody vegetation or leaf litter (thought to be important for weevil overwintering) within 50-100m of the lake shore. It does have a steeply sloping grassy-dike shoreline. This site gives us an excellent opportunity to follow a weevil population, that may be high enough to cause a direct decline, before the decline occurs, and to contrast this site with our other permanent transect sites that have much lower weevil densities. We therefore mapped the lake and the milfoil bed and took 25 samples along four transects in mid-July 1996. These samples have been

processed and we plan to collect and process another set of samples in September 1996. This site will be added to our set of regular sample sites and we will do limited monitoring of overwintering populations at this site to determine if overwinter success is better at this site or if the weevils are colonizing from overwinter sites distant from shore.

Increase in the number of samples that are processed in the lab. $7,000

At each of our permanent transect sites we typically collect 25-30 samples (depending upon site). Typically, we are able to only process (sort, weigh and count weevils) 10-15 samples per site due to time and personnel constraints. With changes occurring in plant community structure and patchy weevil distribution, higher sample sizes improve our ability to interpret the data. For our June 1996 sampling, we processed 20-30 samples at each site. We will continue to process at least 20 samples per site from our August and September collections to provide quality data on plants and control agents.

Sampling during June 1997. $8,000

For the 1995-1997 biennium we proposed sampling only in summer 1995 and 1996. We propose to collect a set of samples at our permanent transect sites and Cenaiko lake in June 1997. This set of samples will include the full complement of biomass and weevil samples (20-30 per site), a subsample of plants for carbohydrates, sediment collection and water quality data. These data will be important to determining if milfoil populations have changed substantially from fall 1996 (as they did in one lake in spring 1996), continuing our assessment of spring weevil populations and provide a baseline for additional sampling that may be continued in 1997 after June. These funds will cover sampling, initial equipment preparation and June vehicle and boat charges.

(3) Augmentation of weevil populations: We propose to evaluate field releases or augmentation at 2 sites. Because large scale enclosures present numerous problems and at least during the summer the weevils appear to have limited dispersal and mobility, we propose to introduce known numbers of weevils to replicate plots within each site. The two sites will be chosen based on security, access, occurrence of high milfoil densities, low or absent weevil densities and background data. Currently, we are considering Niccum's Pond as one site (permission obtained from Bill Niccum) and either Cedar, Auburn or Otter Lake as the other site. Otter Lake is probably the best site, however, we suspect the milfoil there may be different from the other sites and may not be as susceptible to weevils.

At each site, we will locate 10-16 plots 4 m in diameter (12.6 m^2). Plots will be marked with a center buoy and 4 peripheral buoys (2 m from center) delineating a cross through the plots. Plots will be >30 m apart. One half of the plots will be randomly assigned as controls and one half as treatments (introduction or augmentation). Prior to weevil introduction to the treatment plots, all plots will be sampled (0.1 m^2 quadrat samples) for milfoil biomass and the occurrence of control agents, as well as plant root and shoot carbohydrates and sediment characteristics. Sampling will be initiated along one of the crosses, 10 m from the center buoy, with samples collected at 10, 6, 3 and 1 m from the center buoy. This will result in 4 subsamples (two within the introduction area and two outside) for each of the 5-8 replicates of weevil level. One hundred and twenty-five weevils will then be introduced evenly within the 12.6 m^2 area of the treatment plots, which should result in an initial density of 10/m^2. This density is relatively low, however, in our tank experiments it was more than adequate to result in densities >400/m^2 within one month. We will attempt to stock only adult weevils - weevils may be collected from fall to spring litter samples, lake collections, or if needed from reared weevils developed in outdoor tanks. Weevils collected from fall to spring litter samples can be maintained for >5 months at 4°C (Ragsdale, pers. obs.). We will need 1200-2000 weevils per summer to introduce at these densities.

Once each week the previously sampled transect will be surveyed by snorkeling for quick counts of weevils, eggs, damage and height of milfoil. Approximately three weeks after introduction, one of the remaining four cross transects will be sampled for biomass, weevil density, plant condition (root and shoot biomass, carbohydrates and if needed nitrogen) and sediment characteristics, and a third cross transect will be sampled approximately six weeks after introduction. Provided funding is in place soon enough in 1995 and we can thus collect adequate numbers of weevils, we will conduct the experiment in 1996 and re-augment the treatment plots in 1997. Although we do not expect that weevil densities to carry over to our treatment plots than the controls and we will be able to evaluate both one season effects and the response to two seasons of elevated summer densities of weevils. The experimental design will permit analysis with standard ANOVA techniques (Wilkinson 1989).

(4) Conduct additional studies of factors that may limit potential control agents. **Continuing studies of weevil performance on and preference for the native milfoils vs the exotic Eurasian watermilfoil.** $2,200

Graduate student Susan Solarz has continued her research on the host range expansion of the weevil from the native northern watermilfoil to the exotic Eurasian watermilfoil. She is performing a genetic study to examine if differences in weevil performance on and preference for the native vs the exotic milfoils is genetic, behavioral or due to phenotypic plasticity. She is supported by my Agricultural Experiment Station project, and received additional supply monies from the Community Genetics Program, and help from a DNR Youth in Natural Resources intern. An undergraduate assistant has been also needed for the experiment to collect weevils, help maintain plant populations and assist with keeping track of the hundreds of individual weevils that are being followed in the experiment.

Development of weevils in relation to temperature. $500

A summer life sciences student initiated an experiment to determine the developmental rates of E. lecontei at 5 different temperatures (15, 19, 23, 27 and 31 degrees C). These data will be important to predict weevil development in the field and the number of generations that can occur in a summer. The results will be linked to our continuous temperature monitoring initiated this spring to help determine what might be limiting weevil populations. The student was supported with MNAES funds, CNR funds and NSF Aquatic and Environmental Science funds (ca $3,400). All of the eggs have been laid and hatched, and most weevils have completed development at the two highest temperatures, however, development of weevils in the remaining chambers must be monitored for another 3-4 weeks. This monitoring will cost approximately $480.

A.1.c. Materials: Field grids, thermistors and sampling gear need to be repaired and built. SCUBA equipment maintenance and supplies, and boat fuel, maintenance storage and miscellaneous supplies are also requested (boat supplied with 1992 LCMR funding (M.L. 1992, Ch. 513, Art. 2, Sec. 9) will be used for this project). Chemicals and laboratory supplies are needed for plant carbohydrate analysis and limnological analysis.

One computer is requested (Mac 6100 with DOS 486 card; 16 mb Ram, 350 mb hard drive). Our current computers are unable to efficiently handle our data base; computations requiring an hour using current computers could be done in 5-10 min. using the requested new computer. Analysis of GPS data requires upgrade DOS capabilities. The proposed computer will be used, along with existing

computers in my lab, to process, store and analyze data on Eurasian watermilfoil and its relation with potential biological control agents such as E. lecontei. The computer will continue to be used for these purposes after the expiration of the project to ensure scientific publication of the results and to continue research on the milfoil/control agent relationship and biological control of Eurasian watermilfoil. Thus, even if additional funding from LCMR or the MN DNR is not forthcoming for continuation of this work, I will be pursuing other funding sources and anticipate that this topic will be a major focus of our work for the next 5 years, which is the maximum reasonable useful life of such a computer. I do not anticipate significant changes in use of this computer through that time frame.

In April, 1996 a spectrophotometer used in analysis carbohydrate content of samples began to malfunction and the researchers at the University of Minnesota requested additional funds to purchase a replacement. They plan to buy a reconditioned Beckman Model 35 spectrophotometer for $1500, an amount much less than the $4000 to $5000 which would be required to purchase a new instrument. The reconditioned spectrophotometer will be used to process samples collected for this project.

A.1.d. Budget:

Total biennial LCMR budget:	$150,000
Amount allocated to current research:	$150,000
(Addition to capital budget equipment):	($1,500)
Amount not yet allocated:	$0
Match:	None required
Balance:	$25,400

A.1.e. Timeline: Project will be completed by 31 December 1997.

	7/95	1/96	6/96	1/97	6/97
PRODUCT #1		Report			
PRODUCT #2			Report		
PRODUCT #3				Report	
PRODUCT #4					Report

PRODUCT #1: (Deliverable 1). Completion of 1995 field sampling and preliminary analysis of collected samples and completed experiments.
Due Date: 15 November 1995

Deliverable: Fall shoreline and in lake sampling will be collected and a preliminary analysis of summer 1995 samples and results will be presented in a progress report. These results will be preliminary and not all samples will be processed. Results will include observed milfoil coverage at the survey sites, initial milfoil densities, sediment characteristics and plant physiological status at the transect sites and preliminary observations from the augmentation sites.

PRODUCT #2: (Deliverable 2). Report of results from 1995.
Due Date: 15 April 1996

Deliverable: Sample processing and analysis of 1995 samples will be completed and the results will be summarized in a multi-page progress report. Results from all data collected will be reported and interpreted, including observations of milfoil coverage and occurrence of declines at the survey sites, milfoil and weevil densities with associated plant and sediment status at the transect sites and the results of the augmentation experiments.

PRODUCT #3: (Deliverable 3). Completion of 1996 field sampling and preliminary analysis of collected samples and completed experiments.
Due Date: 15 November 1996

Deliverable: Fall shoreline and in lake sampling will be completed and a preliminary analysis of summer 1996 samples will be presented in a progress report. These results will be preliminary and not all samples will be processed. Results will include observed milfoil coverage at the survey sites, initial milfoil densities, sediment characteristics and plant physiological status at the transect sites and preliminary observations from the augmentation sites.

PRODUCT #4: (Deliverable 4). Report of results from 1996.
Due Date: 15 April 1997

Deliverable: Processing and analysis of samples collected during 1996, including samples from Cenaiko Lake and processing for which additional funding was provided, will be completed. In addition, studies of performance and preference of weevils in relation to genetics and studies of development of weevils in relation to temperature will be completed. The Beckman Model 34 spectrophotometer, which is necessary for analysis of carbohydrates in various samples, will have been purchased. The results will be summarized in a multi-page progress report. Results from all data collected will be reported and interpreted, including observations of milfoil coverage, occurrence of declines at the survey sites, milfoil and weevil densities, sediment

characteristics and plant physiological status at the transect sites and preliminary observations from the augmentation sites. We will begin to analyze the entire data set collected, including previously collected data, to identify declines, factors limiting or facilitating control, and the results of the augmentation releases. These analyses and interpretations will be preliminary and may be revised in a subsequent report.

PRODUCT #5 (Deliverable 5.) Final Report.
Due Date: 15 November 1997

Deliverable: A final report on the field analyses, including results of sampling done during June 1997, will be completed and included in a report summarizing the results of all objectives. An introductory overview of the purpose, scope and literature related to the project will be presented. Individual chapters containing the results of each objective will follow, and a synthetic analysis and interpretation of the combined results will be presented. Recommendations for the likelihood of continued success with invertebrate biocontrol agents will be presented along with a suggested course of action. This will be the final report.

A.1.f. Work Program Update: 30 December 1997

See Six Month Work Program Update Summary at the beginning of this work program or Newman et al (1997b).

B. Title of Objective/Outcome: Evaluation of insects and fungi as biological control agents for purple loosestrife.

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

B.1.a. Context within the project: Four European insects, one root-boring weevil (*Hylobius transversovittatus*), two leaf beetles (*Galerucella spp.*) and one flower-feeding weevil (*Nanophyes marmoratus*), 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. Research on these insects began in Minnesota in 1992 with insects released at one release site.

B.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 experiments in the field to test the insects' ability to become established in different habitat types. We will also evaluate the effects of these different habitat types on the insects reproduction, dispersal and effect on purple loosestrife.

Conditions will be modified within test areas to determine how selected environmental conditions may affect beetle and weevil 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.

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 second component of this research is to develop field release strategies for all four species to enhance establishment success. Factors such as insect release densities, mixture of insect species and phenology of plant at time of release will be studied to develop a protocol for achieving optimal establishment of insect populations in the field.

Before the first two components of this research can start, large numbers of insects must be reared in the lab to accommodate needs for field releases and lab projects. Rearing is underway and will be continued year-round to supply enough insects for the duration of this project.

A third component of this study will be to measure impact of previous insect releases in 1992,1993 and 1994. Effects of insect defoliation will be measured by quantifying reduced shoot growth, suppression of flowering, reduction in seed

output, and plant mortality.

The fourth component will be to evaluate a combined insect and fungal pathogen interaction as part of a integrated strategy.

A standard monitoring and evaluation protocol has been written and is being used by the researchers to ensure the data collected from all research projects is compatible (Ivan Savov, University of MN, In Press). The field monitoring protocol for insects and vegetation is now being adopted by many other states as the standard for evaluating change in the field. The Minnesota Department of Natural Resources has established a statewide database for all purple loosestrife insect releases that have occurred since insects were first released in 1992. This database will act as a clearinghouse for all information related purple loosestrife biological control activities in Minnesota.

B.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.

B.1.d. Budget: $120,000
Balance: $0

B.1.e. Timeline: Project will be completed by June 30, 1997.

	7/95	1/96	6/96	1/97	6/97
Insect Propagation	**********	**********	**********	**********	**********
Establishment exper.	**********	**********	**********	**********	**********
Test Release Strategies		******		**********	
Evaluate impacts	**********	**********	**********	**********	**********
Test Fungi/Insects	******		******		

B.1.f. Work Program Update: 30 December 1997

From July 1, 1997 to December 30, 1997, time was spent writing final report. Please refer to attached Final research reports by Dr. D. Ragsdale and A. Loos; Dr. D.A. Andow; and Dr. R. Becker and Dr. E. Stamm-Katovich.

B.2.Activity: Development of mycoherbicides to control purple loosestrife.

B.2.a. Context within the project: Work on this project has been ongoing for approximately three years. During this time, over 5,000 fungal cultures have been isolated from spots on purple loosestrife plants from 16 locations around the state of Minnesota. To date, two fungi, *Alternaria alternata* and *Botrytis cineria*, have been discovered that offer possibilities as potential mycoherbicides. Because of the amount of damage caused to the leaves by natural infection of these fungi, there is an excellent possibility one of these two fungi will prove to be a suitable mycoherbicide. The proposed work will continue isolation of fungi from purple loosestrife plants and testing for pathogenicity.

B.2.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. When the mycoherbicides are ready to be tested in the field, the same monitor and evaluation protocol will be used as in purple loosestrife insect releases. 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 soon be identified.

B.2.c. Materials: Agar solutions, chemicals, petri dishes, lab glassware, plastic pots, microscope and other lab equipment.

B.2.d. Budget: $30,000
Balance: $0

B.2.e. Timeline: Project will be completed by June 30, 1997.

	7/95	**1/96**	**6/96**	**1/97**	**6/97**
Develop carrier	**********				
Test Fungi in field	******		*****		
Test Fungi/Insects Comb.	******		*****		
Evaluate effectiveness		**********	**********	**********	**********

B.2.f. Work Program Update: 30 December 1997

From July 1, 1997 to December 30, 1997, time was spent writing final report.

Please refer to attached Final research report by Dr. R.Nyvall.

VI. Evaluation: In the case of milfoil, this project will be successful if it enables us to determine which potential biological control agents merit further evaluation and which agents do not merit further evaluation. This project will further the evaluation of the potential for establishment in Minnesota of insects known to damage purple loosestrife. This project also will continue evaluation of fungi to control purple loosestrife. The benefit of this effort is that it will indicate whether or not additional State funds should be spent on research in this area. If this research leads to successful control of milfoil and loosestrife, then the project will benefit the State because it should reduce the need to use herbicides in aquatic environments and also reduce the demand for staff time to monitor control with herbicides.

VII. Context within the field: Efforts to identify a biological control agent for Eurasian watermilfoil (milfoil) were initiated over 20 years ago. Unfortunately, no successful 'classical' organism has yet been identified. The existence of a native or naturalized organism that damages this exotic is suspected because declines of milfoil that have occurred in North America (see review by Smith and Barko 1990). Nevertheless, no native or naturalized agent has been found to reliably and consistently control milfoil.

Efforts to identify biological control agents of milfoil in Eurasia are being continued by the Army Corps of Engineers, the U.S. Department of Agriculture, and their cooperators. The purpose of this project is to continue efforts to evaluate native or naturalized organisms as potential biological control agents for Eurasian watermilfoil.

Native or naturalized organisms that have potential to control milfoil include three species of insects. Circumstantial evidence suggested that a decline in milfoil in lakes of southern Ontario was due to grazing by insects, in particular the moth *Acentria nivea* (Painter and McCabe 1988). Other research suggested that this insect and a weevil, *Eurhychiopsis lecontei*, caused a decline in milfoil in Vermont (Creed and Sheldon 1992). Lastly, a midge, *Cricotopus myriophylli*, has been shown to damage milfoil and may have contributed to declines observed in British Columbia (MacRae et al. 1990). All three of these insects have been found in Minnesota (Newman and ~~Perry~~ Maher ~~1994~~ 1995). Recent experiments have demonstrated the potential of *E. lecontei* alone or *E. lecontei* and *A. nivea* together to damage milfoil in small tanks with volumes of less than 400 liter (Creed, Sheldon, and Cheek 1992; Creed and Sheldon 1993; Newman ~~and Ragsdale~~ et al ~~1994~~ 1996). Native or naturalized pathogens that have potential to control milfoil include the

fungus *Mycoleptodiscus terrestris* (Shearer 1994, 1996) and other organisms. Surveys of pathogens on milfoil in the upper midwest are continuing.

The studies cited above have shown potential of certain organisms to damage milfoil under controlled conditions in small volumes of water, but it has been very difficult to produce similar damage in field environments, i.e., stands of milfoil growing in lakes. Though declines have been observed in field environments, we lack strong evidence that a particular organism caused such a decline. The purpose of the proposed project is to attempt to obtain such evidence. If declines are observed, efforts should be made to elucidate environmental conditions or factors that either promote or prevent declines in order to further our ability to determine the potential of an organism(s) to control milfoil.

VIII. Budget context:

In 1991, the Minnesota legislature followed recommendations from the LCMR and appropriated $100,000 from the Minnesota Environment and Natural Resources Trust Fund for research leading to biological control of Eurasian watermilfoil (M.L. 1991, Ch. 254, Art. 1, Sec. 14, Subd. 9(b)). This appropriation was contingent on a match of $200,000 from the Freshwater Foundation, which notified the DNR in December that they would not provide these matching funds. In 1992, the Minnesota legislature appropriated $160,000 from the Minnesota Future Resources Fund for this research (M.L. 1992, Ch. 513, Art. 2, Sec. 9).

In 1993, the Minnesota legislature followed recommendations from the LCMR and appropriated $250,000 from the Minnesota Environment and Natural Resources Trust Fund for continuing research leading to biological control of Eurasian watermilfoil (M.L. 1993, Ch. 172, Sec. 14, Subd. 12(l)). This appropriation was contingent on a match of $200,000 of non-state funds. The Minnesota Lakes Association attempted to raise these funds but was only able to generate donations totaling $7,967. The Army Corps of Engineers subsequently agreed to meet this requirement of a match by providing 'in-kind' services in the form of research to be conducted by their Aquatic Plant Control Research Program.

In 1993, the Minnesota legislature followed recommendations from the LCMR and appropriated $150,000 from the Minnesota Environment and Natural Resources Trust Fund for continuing research leading to biological control of purple loosestrife (M.L. 1993, Ch. 172, Sec. 14, Subd. 12(l)).

In August, 1994, the LCMR recommended that the DNR support continued research on the potential for biological control of purple loosestrife and Eurasian watermilfoil with funds other than those appropriated by the legislature as recommended by the LCMR. The

LCMR suggested that the DNR consider supporting this research with funds raised for management of exotic species by the surcharge on watercraft licenses. The DNR plans to follow this recommendation and not apply to the LCMR for funds this research after the FY 96-97 biennium.

IX. Dissemination: It is expected that the results of this project will be published in peer-reviewed scientific journals and also in special publications and newsletters. Results also 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 use the results of this project. An extension publication on rearing and releasing Galerucella is under production at the University of Minneosta. This publication, with its companion slide set, will teach resource managers how to raise and release leaf-eating beetles for the control of purple loosestrife. It should be ready for dissemination in January 1998. The University of Minnesota has created a web page [http://www.fw.umn.edu/research/milfoil/milfoilbc.html] where information on the potential for biocontrol of milfoil is presented.

X. Time: Development of biological control of undesirable plants typically requires more than ten years' effort. For example, biological control of alligatorweed, an exotic aquatic plant of the southern states, required over twelve years of work to develop and implement (Spencer and Coulson 1976). The duration of the current effort to evaluate the potential for biological control of Eurasian watermilfoil already has exceeded two years (see section on Budget Context) and likely will continue beyond 1997.

XI. Cooperation:

Objective A research is to be done by contracts with the cooperators listed below. These cooperators are the authors of the proposal submitted to the DNR by the University of Minnesota and selected for funding on 21 April 1995. This is the first-ranked proposal submitted in response to the Request for Proposals, which was published in the State Register on 30 January 1995, and is the only one that we will be able to fund at this time. No written time commitments have been received from these cooperators because the DNR has not yet established a contract with the University of Minnesota for this research.

1. Dr. Raymond M. Newman
   Department of Fisheries and Wildlife
   University of Minnesota

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

3. Dr. David D. Biesboer
   Department of Plant Biology
   University of Minnesota

Objective B research projects are 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 (Activity 1). 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 B.

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 (Activity 1). Dr. Ragsdale will contribute 15% of the time needed to complete Objective B.

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 (Activity 2). 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 Activity 2.

XII. **Reporting Requirements:** Semiannual six-month work program update reports will be submitted not later than January 1, 1996, July 1, 1996, January 1, 1997, and a final six-month work program update and final report by June 30, 1997.

Literature Cited

APHA. 1989. Standard methods for the examination of water and wastewater. 17th

edition APHA-AWA-WPCF, Washington, DC.

Batra, S.W.T., D. Schroeder, P.E. Boldt, and W. Mendl. 1986. Insects associated with purple loosestrife, *Lythrum salicaria* L., in Europe. Proceedings of the Entomological Society of Washington 88:748-759.

Creed, R. P., and S.P. Sheldon. 1992. The effect of the weevil, *Euhrychiopsis lecontei*, on Eurasian Watermilfoil: results form Brownington Pond and Norton Brook Pond. Proceedings, 27th Annual Meeting, Aquatic Plant Control Research Program. Miscellaneous Paper A-93-2, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Creed, R.P., S.P. Sheldon, and D.M. Creek. 1992. The effect of herbivore feeding on the buoyancy of Eurasian watermilfoil. Journal of Aquatic Plant Management 30:75-76.

Creed, R. P., and S.P. Sheldon. 1993. The effect of feeding by a North American weevil, Euhrychiopsis lecontei, on Eurasian Watermilfoil (*Myriophyllum spicatum*). Aquatic Botany 45:245-256.

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

Jessen, R. and R. Lound. 1962. An evaluation of a survey technique for submerged aquatic plants. Minnesota Department of Conservation, Division of Game and Fish, Section of Research and Planning, Fish and Wildlife Surveys Unit, [Current address: Minnesota Department of Natural Resources, 500 Lafayette Rd., St. Paul, MN 55155].

Johnson, R. L. 1995a. Monitoring aquatic vegetation in Tompkins County: Project completion report SFY 1993-1994. Section of Ecology and Systematics, Cornell University, Ithaca, NY.

Johnson, R. L. 1995b. Monitoring aquatic vegetation in Seneca County: Project completion report. Section of Ecology and Systematics, Cornell University, Ithaca, NY.

Lillie, R.A. 1996. A milfoil decline associated with aquatic weevils in a southern Wisconsin lake. (Abstract) 16th Annual Symposium of the North American Lake Management Society, 13-16 November in Minneapolis, MN.

Lillie, R.A., and D. Helsel. 1997. A native weevil attacks Eurasian watermilfoil. Research Management Findings Number 40, Bureau of Research, Wisconsin Department of Natural Resources, P.O. Box 7921, Madison, WI 53707.

Macrae, I. V., N. N. Winchester, and R. A. Ring. 1990. Feeding Activity and Host Preference of the Milfoil Midge, *Cricotopus myriophylli* Oliver (Diptera: Chironomidae). Journal of Aquatic Plant Management 28: 89-92.

Newman, R.M. and L.M. Maher. 1995. New records and distribution of aquatic insect herbivores of watermilfoils (Haloragaceae: *Myriophyllum* spp.) in Minnesota. Entomological News 106: 6-12.

Newman, R.M., K.L. Holmberg, D.D. Biesboer and B.G. Penner. 1996. Effects of a potential biocontrol agent, *Euhrychiopsis lecontei*, on Eurasian watermilfoil in experimental tanks. Aquatic Botany 53:131-150.

Newman, R.M., and J.A. Perry. 1994. The potential for biological control agents for Eurasian watermilfoil in Minnesota with native and naturalized insects. Unpublished

revision of a final report by the University of Minnesota submitted to the Department of Natural Resources in June.

Newman, R.M., J.A. Perry, K.L. Holmberg, L. Mizner-Maher and S.L. Solarz. 1993. The potential for biological control agents for Eurasian watermilfoil with native and naturalized invertebrates. Completion Report to the Minnesota Department of Natural Resources, Ecological Services.

Newman, R.M., and D.W. Ragsdale. 1994. Evaluation of insects as biological control agents for Eurasian watermilfoil. Unpublished report by the University of Minnesota submitted to the Department of Natural Resources on 15 September.

Newman, R.M., M.E. Borman, and S.W. Castro. 1997a. Developmental performance of the weevil *Euhrychiopsis lecontei* on native and exotic watermilfoil host plants. *Journal of the North American Benthological Society* 16(3):627-634.

Newman, R.M., D.D. Biesboer, and D.W. Ragsdale. 1997b. Can Eurasian watermilfoil be managed in Minnesota by biological control native and naturalized insects? Final report submitted as deliverable 5. Unpublished report dated 15 November and submitted to the Minnesota Department of Natural Resources, Section of Ecological Services, 500 Lafayette Rd., St. Paul, MN 55155 by the Department of Fisheries and Wildlife, University of Minnesota, St. Paul, MN 55108.

Painter, D.S., and K.J. McCabe. 1988. Investigation into the disappearance of Eurasian watermilfoil from the Kawartha Lakes. Journal of Aquatic Plant Management 26:3-12.

Raguse, C.A., and D. Smith. 1965. Carbohydrate content in alfalfa herbage as influenced by methods of drying. Journal of Agricultural and Food Chemistry 13:306-309.

Shearer, J.F. 1994. A historical perspective of biological control of submersed macrophytes *Myriophyllum spicatum* and *Hydrilla verticillata* using plant pathogens. Proceedings, 28th Annual Meeting, Aquatic Plant Control Research Program. Miscellaneous Paper A-94-2, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Shearer, J.F. 1996. Microorganisms collected from *Myriophyllum spicatum* L. in the upper Midwest and their evaluation as potential biocontrol agents for milfoil management. Draft report submitted on 4 November to the Minnesota Department of Natural Resources, Section of Ecological Services, 500 Lafayette Rd., St. Paul, MN 55155 by. The U.S. Army Engineer Waterways Experiment Station, CEWES-ER-A, Aquatic Plant Control Research Program, 3909 Halls Ferry Rd., Vicksburg, MS 39180.

Smith, D. 1969. Removing and analyzing total nonstructural carbohydrates from plant tissue. Wisconsin Agricultural Experiment Station Research Report 41. pp. 1-13.

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

Solarz, S.L., and R.M. Newman. 1996. Oviposition specificity and behavior of the watermilfoil specialist, *Euhrychiopsis lecontei*. Oecologia 106:337-344.

Spencer, N.R., and J.R. Coulson. 1976. The biological control of alligatorweed, *Alternathera philoxeroides*, in the United State of America. Aquatic Botany 2:177-190.

Sutter, T.J. and R.M. Newman. 1997. Is predation by sunfish (*Lepomis* spp.) an important source of mortality for the Eurasian watermilfoil biocontrol agent

Euhrychiopsis lecontei? Journal of Freshwater Ecology 12(2):225-234.

Thompson, D.Q., R.L. Stuckey and E.B. Thompson. 1987. Spread, Impact and control of purple loosestrife, *Lythrum salicaria*, in North American Wetlands. U.S. Fish and Wildlife Service, *Fish and Wildlife Research* 2. 55 pp.

Wilkinson, L. 1989. SYSTAT: the system for statistics. SYSTAT Inc., Evanston, IL.

XIII. Qualifications: See resumes attached to workprogram of 19 June 1995.