Date of Report: 31 December 1999
Date of Next Status Report:
Date of Work Program Approval: 23 June 1997
Project Completion Date: 31 December 1999

LCMR Work Program 1997

I. PROJECT TITLE: Biological control of Eurasian watermilfoil and purple loosestrife - *Continuation* (Project P 2)

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Total Biennial Project Budget:

LCMR:	$150,000
LCMR Amount Spent:	$ 150,000
$Match:	(see section VII on cooperation)
$Total	$150,000
=LCMR Balance:	$ 0

A. Legal Citation: Minnesota Laws 1997, Chapter 216, Section 15, Subdivision 20(b). Appropriation Language: "This appropriation is from the trust fund to the commissioner of natural resources for the third biennium of a five-biennium project to develop biological controls for Eurasian watermilfoil and purple loosestrife. This appropriation is available until June 30, 2000, at which time the project must be completed and final products delivered, unless an earlier date is specified in the work program."

B. Status of Match Requirement: Not Applicable.

II. PROJECT SUMMARY AND RESULTS:

The purpose of this comprehensive project is to develop and implement biological controls for Eurasian watermilfoil, *Myriophyllum spicatum*, and purple loosestrife, *Lythrum salicaria*, two exotic aquatic plants that are degrading Minnesota's aquatic resources statewide.

A. Eurasian watermilfoil

Dr. Newman and colleagues have documented one decline that is clearly attributable to weevil stem mining, and have evidence that weevil damage, at least in shallower sites of Lake Auburn and Smiths

Bay, have reduced milfoil abundance. The Eurasian watermilfoil decline at Cenaiko Lake has persisted; an increase in milfoil in early summer 1998 was met with high weevil populations and a subsequent decrease of milfoil, and that decline has persisted through the summer of 1999. It is not clear what permits development of such high weevil populations in Cenaiko Lake, however, low predation by sunfish appears to be a factor.

The longer and slower decline of Eurasian watermilfoil leveled at Smith's Bay; at the shallower sites milfoil remains suppressed and native plants have developed extensively. At deeper sites, with little evidence of weevil damage, Eurasian watermilfoil remains quite dense, but well beneath the surface. In Cedar Lake, the improved water clarity and very low weevil densities resulted in a continued increase in Eurasian watermilfoil that persisted through the summer. Milfoil is slowly recovering at Otter Lake, but still remains well below historic highs; the slow increase may be due to a combination of plant competition and herbivore pressure.

The response of Lake Auburn remains puzzling. The early season decline of milfoil in 1998 was associated with relatively low weevil densities but much apparent damage (personal observation). However, for some reason the weevil population crashed and the poor light probably prevented regrowth of milfoil and other plants. It remains to be seen if milfoil will remain suppressed in Lake Auburn, or if the milfoil will return in force with low weevils densities, as suggested by the low overwinter densities. Continuation of their plant removal and competition experiments should shed light on the role of plant competition in suppressing milfoil.

Two conditions are needed for successful biological control of weeds: adequate agent densities and a negative response of the target to the control agent (Newman et al. 1998). The potential importance of plant community response to stress imposed on Eurasian watermilfoil was addressed above. It is also clear that at many of their sites weevil populations have not built to adequate densities; Cenaiko Lake provides a clear example of the potential for high weevil populations and subsequent effects on milfoil. Given the potential for population increase in the summer, and the lack of a strong correlation between in-lake and onshore densities, it does not appear that overwinter populations are the main limiting factor at least at Lake Auburn and Smith's Bay where detectible populations have been found in early summer each year. Fish exclusion experiments suggest that fish predation could be one important factor and that milfoil genotype and sediment may also affect weevil performance.

It is clear that milfoil suppression can be obtained given adequate densities of weevils throughout the summer, and perhaps positive plant community response. On-going focused research should shed additional light on the factors that regulate weevil populations and their effects on plant communities. Once these factors have clearly been identified, management strategies, such as piscivore enhancement or water clarity improvements, can be tested to determine their feasibility for enhancing biological control of Eurasian watermilfoil.

- Dr. Newman and colleagues noted declines in Eurasian watermilfoil biomass at Cenaiko Lake and Lake Auburn in 1997-1998. Milfoil increased in Otter Lake but the native plant community remains healthy and milfoil is still ≤ 20% of total plant biomass. In Smith's Bay,

milfoil remained suppressed at the shallower sites with high non-milfoil biomass and much evidence of weevil damage, but increased at the deeper sites that show little evidence of weevil damage. Milfoil biomass at Cenaiko Lake increased relative to 1997, but by the end of the summer had declined to <7% of plant biomass. Native plants continued to increase at Cenaiko. A weevil survey indicated very high densities of weevils in September 1998

- Milfoil biomass in Lake Auburn declined over the winter of 1997-1998 and continued to decline during the summer of 1998; weevil damage appeared high in late spring, but by early summer the weevil population had disappeared. Milfoil biomass remained high in Cedar Lake during 1998, likely due to increased water clarity that persisted through the summer. Coontail makes up a large percentage of the non-milfoil plant biomass at both of these lakes and the lack of development of a rooted native plant community may reduce competitive stress on Eurasian watermilfoil.

- Milfoil biomass increased or remained the same at two of our three survey sites: Grays Bay, and Lake-of-the-Isles. Visual coverage at our survey sites ranged from 4 to 59%, with small increases at two lakes and small decreases at two others. A major decline occurred in 1998 at Piersons Lake which had about half the milfoil density that was found in 1997.

- Shoreline weevil densities at Smith's Bay and Lake Auburn were low in fall 1998, being similar to their previous fall low in 1994, however, overwinter mortality was quite low at Smith's Bay and was low at both lakes during the recent mild winters of 1997-1999. Shoreline densities and overwinter survival may be controlled by regional climatic factors.

- A fish exclusion experiment suggested that fish predation may limit weevil populations and that introduced weevils protected from fish predation may reduce milfoil. These effects were weak and more experimentation is required.

- Weevil performance (developmental rate, size and survival) was influenced by rearing plant and rearing plant sediment. Sediment effects appear larger than plant genotype effects, and these results may explain differential performance of weevils in different lakes. Performance was poorest on Otter plants grown in Otter sediment. However, overall plant size may have influenced these results and it is not clear that internal plant quality affected survival and development.

- Weevil temperature-development models appear useful for predicting trends and matching field observations. A combination of modeling, observation and experiment may help explain which stages are critical or what factors may be limiting populations.

- Based on their laboratory results and life history information, weevil populations should reach much higher densities than they have seen at most sites except Cenaiko. Further work is needed to identify what is limiting weevil populations.

B. Purple loosestrife

***Hylobius* Rearing**

The lab rearing of Hylobius on artificial diet was unsuccessful. Very few adult weevils were produced. Effort to determine why the larvae did not develop was investigated. To date, it is unknown why the rearing efforts failed. Closer collaboration with Cornell University, where rearing has been successful, will commence during the next biennium. We expect to solve the problem and move forward with rearing the weevils. Please see attached report by Ragsdale and Milles.

Effect of *Galerucella calmariensis* and *G. pusilla* leaf defoliation on carbohydrate levels in roots and crowns of purple loosestrife.

The results of this work, as reported in previous LCMR reports, has been accepted for publication to Weed Science (draft copy attached with last report). All details in attached final report from researchers Becker, Katovich and Ragsdale.

Impact of *Galerucella* spp. on seed production in purple loosestrife.

Results of this work, as reported in previous LCMR reports is currently in preparation for publication. In general, even moderate *Galerucella* spp. shoot tip feeding resulted in a 72% reduction in seed capsules present on damaged shoot tips. All details in attached final report from researchers Becker, Katovich and Ragsdale.

Impact of *Nanophyes marmoratus* on seed production in purple loosestrife.

We conclude that the population densities of *N. marmoratus* has not yet reached the level to consistently result in measurable reduction of seed capsule production. All details in attached final report from researchers Becker, Katovich and Ragsdale.

IV. OUTLINE OF RESULTS OF THE PROJECT

Detailed descriptions of the background for each objective listed below, as well as proposed methods to accomplish these objectives, are provided in two detailed proposals written by the researchers who will do this work. The proposals are attached as addenda 1 and 2 to the workprogram.

A. Eurasian watermilfoil

Result A-1. Identify factors that limit populations of potential biological control agents, particularly the weevil, *Euhrychiopsis lecontei,* and their effectiveness at reducing the abundance of Eurasian watermilfoil by continued long-term sampling in five intensive study sites in different Minnesota lakes.

Budget: LCMR: $42,500 Balance: LCMR: $0
Other: $42,500 Other: $0
Completion Date: December 31, 1999

Result A-2. Attempt to detect additional lake-wide declines of Eurasian watermilfoil that may be related to the presence of potential biological control agents, and identify environmental variables associated with any identified declines by short-term sampling in approximately five (5) whole lakes or bays in Minnesota.

Budget: LCMR: $10,000 Balance: LCMR: $0
Other: $10,000 Other: $0
Completion Date: December 31, 1999

Result A-3. Introduce weevils, *Euhrychiopsis lecontei*, at replicated sites to determine effects of artificial augmentation on the density and condition of Eurasian watermilfoil and other macrophytes during a single growing season. This effort will include evaluation of the possible effects of fish predation on weevil density.

Budget: LCMR: $10,000 Balance: LCMR: $0
Other: $10,000
Other: $0
Completion Date: December 31, 1999

Result A-4. Determine the competitive interactions between the native northern watermilfoil and the exotic Eurasian watermilfoil and whether development of the weevil *Euhrychiopsis lecontei* varies on Eurasian watermilfoil from two different populations.

Budget: LCMR: $7,500 Balance: LCMR: $0
Other: $7,500 Other: $0
Completion Date: December 31, 1999

Result A-5. Continue development of a mechanistic model of declines in populations of Eurasian watermilfoil. Development of this model will be based on comparison of control agent densities and limiting factors, site characteristics, and carbohydrate status in field environments with results predicted from laboratory and simulation studies.

Budget: LCMR: $5,000 Balance: LCMR: $0
Other: $5,000 Other: $0
Completion Date: December 31, 1999

B. Purple loosestrife

Result B-1. Rearing and release of the flower feeding weevil, *Nanophyes marmoratus* and root boring weevil, *Hylobius transersovittatus* . Both of these insects current populations are very low in Minnesota. *Both Nanophyes marmoratus* and *Hylobius transersovittatus* will reared for research efforts and field releases. Insects will not become available for rearing and release until June 1998, pushing our completion date to June 1999. Hylobius will be obtained from Cornell University as part of a cooperative effort.

Budget: LCMR: $30,000 Balance: LCMR: $0
Other: $30,000 Other: $0
Completion Date: June 30, 1999

Result B-2. Study impact of previously field released *G. calmariensis, G. pusilla* and *Nanophyes marmoratus* on *Lythrum* and wetland communities. The impacts of *G. calmariensis* and *G. pusilla* on individual purple loosestrife plants and plant community interactions will be studied at four locations where *G. calmariensis* spp. have been established successfully and have populations at levels that should begin to alter plant community dynamics. This objective will include a study of the impact of *Galerucella calmariensis* feeding on root and crown carbohydrate and the effect on *Lythrum* survivability. An ongoing study on the impact of *Galerucella* on purple loosestrife crown carbohydrate levels and the role and dynamics of carbohydrate depletion on survivability of purple loosestrife will be completed to build on three previous years of research. The impact on seed production by these three insect will also be studied. A small portion of the study effort will focus on further development of the plant pathogen, *Microsphaeropsis* spp., as a biological control agent aided by *Galerucella* wounding, which may increase infection and improve management of purple loosestrife populations.

Budget: LCMR: $30,000 Balance: LCMR: $0
Other: $30,000 Other: $0
Completion Date: December 31, 1999

Result B-3. Study impact of *Hylobius transversovittatus* on *Lythrum*. Work will be conducted to further characterize the extent of damage *Hylobius* can cause to purple loosestrife root-crowns.

Budget: LCMR: $15,000 Balance: LCMR: $0
Other: $15,000 Other: $0
Completion Date: December 31, 1999

V. 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.

VI. CONTEXT

A. Significance: Eurasian watermilfoil is a significant problem in Minnesota because it can produce dense mats at the water's surface. Mats of milfoil can severely limit water recreation and also reduce the biodiversity of aquatic ecosystems.

Efforts to identify a biological control agent in Eurasia for Eurasian watermilfoil (milfoil) were initiated over 20 years ago (For reviews, see Buckingham 1994, Sheldon and Creed 1995:1128). Observations such as this: "*Myriophyllum spicatum* constitutes part of the background or natural flora throughout most of Europe and rarely reaches weed status" by Harvey and Evans (1997:2) suggest that potential biological control agents may exist in Europe. Unfortunately, no successful 'classical' biological control organism in Eurasia has yet been identified.

The existence in North America of a native or naturalized organism that damages Eurasian watermilfoil is suspected because drastic declines of milfoil have occurred here (see review by Smith and Barko 1990). Nevertheless, no native or naturalized agent has yet been demonstrated to reliably and consistently control milfoil in lakes.

The Minnesota Legislature has directed the DNR to initiate research on biological control of milfoil (M.S. 84D.02, subdivision 2(3)). The purpose of this project is to continue efforts to evaluate native or naturalized organisms, primarily insects, 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, *Euhrychiopsis lecontei*, caused a decline in milfoil in Vermont (Creed and Sheldon 1995). 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 Maher 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 et al.1992; Creed and Sheldon 1993; Newman at al. 1996). Recent research indicated that weevils are not likely to damage native plants other than milfoil species (Sheldon and Creed 1995, Solarz and Newman 1996). In addition, weevils appear to be likely to do more damage Eurasian watermilfoil than to northern watermilfoil.

Though these studies have shown the potential of weevils, and to a lesser extent other organisms, to damage milfoil under controlled conditions in small volumes of water, it has been very difficult to produce similar damage in field environments, i.e., stands of milfoil growing in lakes. One factor that might limit the abundance of weevils, and hence their ability to reduce milfoil, in lakes is predation by fish (Sutter and Newman 1997).

Though declines have been observed in field environments, we lack strong evidence that a particular organism caused such declines. Examination of milfoil plants collected from Minnesota lakes has documented damage, presumably by insects, to the plants (e.g., Newman et al 1997). Based on available research, densities of weevils in Minnesota lakes appear to be lower than densities observed under controlled experimental conditions where milfoil was severely damaged by weevils (Newman et al. 1996).

The purpose of the current project is to attempt to obtain strong evidence that a particular organism(s), most likely an insect, can cause declines in milfoil in Minnesota lakes. 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.

Researchers will also watch for possible effects of pathogens on milfoil. Efforts to identify pathogens in Eurasia that might act as biological control agents of milfoil are being continued by the Army Corps of Engineers and their cooperators (e.g., Shearer 1997a, Harvey and Evans 1997). Efforts to identify native or naturalized pathogens in North America that might act as biological control agents of milfoil are being continued by the Army Corps of Engineers and their cooperators (e.g., Shearer 1996, Shearer 1997b). Shearer (1996) attempted to isolate pathogenic fungi from milfoil collected in Minnesota and other northern states. Endemic organisms isolated from milfoil during this study did not appear to have potential to control Eurasian watermilfoil. This lack of potential as biocontrol agents was evidenced by the low degrees of virulence demonstrated by the isolated organisms.

Research efforts suggest that biological control of purple loosestrife is very feasible. Extensive research conducted on loosestrife in Europe has demonstrated that the plant is successfully controlled by insect herbivores. Research completed in the United States has

demonstrated that these European insects pose no known threat to native plants. Four European insects, one root-feeding weevil, one flower-feeding weevil, and two leaf-eating beetles, have been identified as promising candidate biological control agents for introduction into the U.S. and have received federal and state approval for release in the United States and Minnesota as potential natural enemies of purple loosestrife.

Biological control offers the most suitable and environmentally safe technique to manage loosestrife long term, especially in nature reserves. Many times a combination of insects is more effective than one species by itself. The idea is to increase stress on purple loosestrife by introducing predators that feed on leaves, flowers and roots of the plant. The two beetles in particular can cause high plant mortality, reduce shoot growth, suppress flowering and reduce seed output. Testing combinations of these insects will be an important part of the research. All four species have been released in stands of purple loosestrife in Minnesota. Currently 1,000,000 leaf-eating beetles have been released on 200 sites statewide. All four insect species have survived the winter in Minnesota and are reproducing. This is a big step forward towards finding a successful biological control.

Literature cited

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B. Time: Development of biological controls for milfoil in Minnesota has been underway for four years and may well require six or more years' of additional effort. Development of biological controls for loosestrife in Minnesota began six years ago. Achieving successful control may well require 10 or more years of effort. The project proposed for the 1997 Biennium should be extended to 31 December 1999 in order to allow researchers to work in the field during the whole of the summer of that year.

C. Budget Context: Information to describe the project context and budget history is presented as follows: 1) funding history which summarizes expenditures for the previous three biennia; 2) proposed and Anticipated Expenditures for the FY98-99 and FY00-01 biennia; and 3.) Detailed budget.

1. Funding History

	July 91-June 93	July 93-June (Dec)95	July 95-June (Dec)97
	Prior Expenditures	Prior Expenditures	Prior Expenditures
LCMR	$ 160,000	$ 400,000	$ 300,000
Other State	-	$ -	-
Non State Match	$ -	$ -	
In-Kind	-	$ 200,000	$ -
Total	$ 160,000	$ 400,000	$ 300,000

2. Proposed and Anticipated Expenditures

	July 97-June(Dec) 99	July 99-June (Dec)01
	Proposed Expenditures	Future Expenditures
LCMR	$ 150,000	$ 150,000
Other State	$ 150,000	$ 150,000
Non State Match	$ -	$ -
In-Kind	$ -	$ -
Total $	300,000	$ 300,000

3. Detailed Budget: This work will be done by the University of Minnesota under contract to the DNR.

A. Eurasian watermilfoil - Budget

1. Salaries and Wages 97-98		98-99	Total	
Academic Staff				
(Newman, 1mo each summer)		4,401	4,622	9,023
Graduate Assistants				
Undergraduate Assistants		16,000	18,000	34,000
Technical (1 @ 100%, 1 @15%)		29,000	30,200	59,200
Total Salaries and wages		49,401	52,822	102,223
2 Fringe		10,410	10,852	21,262
(Acad 27% ; Civil Service 30.6% 95-6; 29.1% 96-7)				
3. Travel	6,250 7,250	13,500		
4. Purchased chemical analyses		1,000	1,000	2,000
5. Supplies (field/lab), phone, mail, equipment <$500		6,500	4,500	11,000
Subtotal	73,561	76,424	149,985	
Indirect Costs	0	0	0	
TOTAL	73,561 76,424	149,985		

B. Purple loosestrife

1. Salaries and fringe		
Technicians		136,500
Undergraduates		2,000
2. Supplies	4,000	
3. Travel	2,500	
4. Vehicle lease	5,000	
Total	150,000	

VII. **Cooperation**: The DNR's Exotic Species Program will apply $150,000 from the Water Recreation Account, designated as 'other' in this work program, towards this project over a two year period. This support in conjunction with funding that we hope the legislature will appropriate at the recommendation of the LCMR will provide $300,000 for this research. This project will be directed by Charles (Chip) Welling with assistance from Luke Skinner, both of the DNR.

A. Eurasian watermilfoil
Cooperators at the University of Minnesota include: Drs. Raymond Newman, David Ragsdale, and David Biesboer.

Cooperator	Dollars received	Percent time spent on project
R. Newman	$150,000	20%

B. Purple loosestrife
Cooperators at the University of Minnesota include: Drs. Roger Becker, David Ragsdale, and Elizabeth Stamm Katovich. Technical expertise on loosestrife will be provided by Dr. Bernd Blossey of Cornell University, and Dr. Dharma Sreenivasam, Minnesota Department of Agriculture Technical expertise on milfoil will be provided by the Army Corps of Engineers.

Cooperators	Dollars received	Percent time spent on project
R. Becker and D. Ragsdale*	$135,000	15% each
B. Blossey**	$15,000	20%

*Includes DNR Funding contribution
**Dr. Blossey's funding is from the DNR contribution not LCMR $$

VIII. **Location**: Big Woods, St. Croix Moraines & Outwash Plains, Anoka Sand Plain, Mille Lacs Uplands, Pine Moraines & Outwash Plains

IX. **Reporting Requirements**: Periodic workprogram progress reports will be submitted not later than 15 November 1999. A final workprogram report and associated products will be submitted by December 31, 1999. A list of deliverables is presented below:

Deliverable A-1. Completion of 1997 field sampling initiated after June, 1997 and preliminary analysis of collected samples and completed experiments.
Due Date: 1 December 1997 $36,000

Content: In-lake sampling and fall shoreline sampling initiated after June, 1997 will be completed. Preliminary analyses of summer 1997 samples will be presented in a status report. These results will be preliminary and not all samples will be processed. Results will include observed milfoil coverage at approximately five (5) extensive survey sites, initial milfoil densities, sediment characteristics and plant physiological status at the transect sites and preliminary observations from the augmentation/fish exclosure sites. Progress, problems and qualitative observations will be reported for these efforts.

Deliverable A-2. Report of results from 1997.
Due Date: 15 May 1998 $28,000

Content: Sample processing and analysis of 1997 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 weevils or declines at approximately five (5) extensive survey sites, milfoil and weevil densities with associated plant and sediment status at the transect sites and the results of the augmentation/fish exclosure experiments.

Deliverable A-3. Completion of 1998 field sampling and competition experiments, and preliminary analysis of collected samples and completed experiments.
Due Date: 1 December 1998 $46,000

Content: A one to two page status report on progress during the 1996<u>8</u> field season will be presented. We will have completed in-lake and fall field sampling at our survey sites and our semi-permanent transect sites as well as our milfoil competition-weevil effect experiment. Progress, problems and qualitative observations will be reported for these efforts.

Deliverable A-4. Report of results from 1998.
Due Date: 15 May 1999 $30,500

Content: Processing and analysis of samples collected and experiments conducted during 1998 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, occurrence of declines at approximately five (5) extensive survey sites, milfoil and weevil densities, sediment characteristics and plant physiological status at the transect sites and preliminary observations from the competition-herbivory experiments. We will begin to analyzed the entire data set collected, including previously collected data, to identify declines, factors limiting or facilitating control, and the results of the augmentation releases. We will also explore the reparameterization of the INSECT and MILFO models for use with our system.

Deliverable A-5. Final report.
Due Date: 1 December 1999 $9,500

Content: Field analyses will be completed and included in a report summarizing the results of all objectives. The field results will be integrated with the lab and experimental results, and if feasible, compared to simulation predictions. These results will be presented in a synthetic report which will also attempt to predict when and where successful control might be expected and what circumstances are needed for control. This will be the final report.

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Deliverable B-1. Progress report.
Due Date: 31 December 1997 $30,000
Content:
Distribute Eggs of *Hylobius transversovittattus* in selected wetlands
Monitor impact of leaf-beetles, *Galerucella* spp., from selected release sites
Complete harvesting of carbohydrate depletion study
Complete harvesting of seeds from plants impacted by seed-feeding weevil and leaf-beetles
Monitor impact of root-boring weevil from selected sites
Collect and store root crowns for maintenance of *Galerucella* spp. Colonies
Develop educational materials for use by educators, extension personnel and resource managers to teach varoius audiences about purple loosestrife biological control programs

Deliverable B-2. Progress report.
Due Date: 30 June 1998 $35,000
Content:
Assist in collecting and distributing *Galerucella* spp. to cooperators for rearing insects during the summer
Evaluate the establishment and survival of *Hylobius transversovittatus* eggs released in wetlands, fall

1997
Initiate rearing of root-boring weevil, *Hylobius transversovittattus*
Develop methods to rear seed-feeding weevil, *Nanophyes marmoratus*
Complete analysis of carbohydrate samples for carbohydrate depletion study
Seed germination from plants impacted by seed-feeding weevil and leaf-beetles

Deliverable B-3. Progress report.
Due Date: 31 December 1998 $40,000
Content:
Rearing of *Hylobius transversovittatus* on a synthetic diet initiated if diet is available from Cornell Univerity
Continue monitoring of leaf-beetles and root-boring weevils on selected sites
Continue study of impact of seed-feeding weevils and leaf-beetles on germination of seeds
Monitor establishment of insects at selected release sites

Deliverable B-4. Progress Report.
Due Date: 30 June 1999 $30,000
Contents:
Evaluate field establishment of lab and field reared insects
Release lab-reared Hylobius transversovittatus if successful diet developed

Deliverable B-4. Final report.
Due Date: 31 December 1999 $15,000

X. Research Projects: Refer to the attached abstracts from the two proposals that were attached to the previous work program as addenda. If you would like to receive additional copies of the complete proposals, please contact Skinner.