Date of Report: June 30, 1995

LCMR Research Work Program 1994-95, Final Status Report

I. Project Title: Integrated Control of Purple Loosestrife.

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A. Legal Citation: M.L.93 Chpt. 172, Art. 1, Sect. 14, Subd. 12(n).

Total Biennial LCMR Budget: $90,000
Balance: $ -0-

Subd. 12(n). This appropriation is from the Future Resources Fund to the commissioner of agriculture in cooperation with the commissioner of natural resources to accelerate evaluation of integrated biological control agents for purple loosestrife infestations in Houston, Hennepin, Wabasha, and Goodhue counties.

B. LMIC Compatible Data Language: Not applicable.

C. Status of Match Request: Not applicable.

II. Project Summary: Control of purple loosestrife needs to be accelerated in southeast Minnesota. Various chemical and cultural control methods to date have been ineffective and costly. Development of a natural control program against purple loosestrife is a viable alternative. Based on considerable background work already done in Europe we propose four study sites of varying landscapes and levels of infestation in Minnesota to investigate the impact of integrating combinations of natural control agents. The two species of insects will be evaluated on the basis of single/multiple species introductions; similarly fungal pathogens introduced as single/multiple applications; and finally combinations of insects and fungal pathogens. Geographic separation of study sites will provide useful information in determining the best combination of ecological factors for successful introduction and establishment of biological control agents against purple loosestrife.

This is a cooperative project involving County Agriculture personnel, U of M researchers, MDA and DNR.

Abstract - June 30, 1995

This research assessed the impact of *Galerucella* feeding on purple loosestrife in Minnesota wetlands. Supportive research was conducted to characterize storage carbohydrates in roots and crowns of purple loosestrife as well as to monitor the impact of stressors such as *Galerucella* feeding on carbohydrate reserves. The outcomes of this project are:

- *Galerucella pusilla* and *G. calmariensis* did successfully overwinter, but are not yet present at high enough populations to monitor impact.
- Storage carbohydrate (CHO) was successfully characterized qualitatively and quantitatively.
- Purple loosestrife crowns were collected from the field and a methodology developed for seasonal carbohydrate characterization. Samples will be assayed during the next LCMR biennium on DNR funding.
- Preliminary studies showed that clipping, but not insect stressors, did significantly lower purple loosestrife storage carbohydrate levels in crown and root tissue. Long-term impacts will be clarified in the next LCMR biennium.

The fungi, *Alternaria alternata* and *Botrytis cinerea* appear to be the most promising candidates as mycoherbicides. A liquid carrier has been developed to apply fungi in the laboratory. Field tests are underway and the results will be summarized in the next LCMR biennium.

III. Statement of Objectives:

A. Investigate impact of insect introductions and establishment against purple loosestrife (*Lythrum salicaria* L.)

B. Augment and enhance native fungal pathogens for control of purple loosestrife.

IV. Research Objectives:

A. Title of Objective: Investigate impact of insect introductions and establishment against purple loosestrife (*Lythrum salicaria* L.)

A.1. Activity: Investigate impact of ecological variables on the successful establishment of insect introductions against purple loosestrife. This study will concentrate on 4 sites selected from watersheds located in Houston, Hennepin, Wabasha, and Goodhue counties.

A.1.a. Context within Project: Three natural enemies of purple loosestrife have been introduced from Europe during 1991 and 1992 into Pennsylvania, New York and Maryland, and in 1992 into Virginia, Minnesota, Oregon and Guelph, Canada. These phytophagous agents are: a weevil, *Hylobius tranversovittatus* and two leaf beetles, *Galerucella calmariensis* and *G. pusilla*. All three beetles are host specific to purple loosestrife. Introductions in New York have shown establishment and look promising in other states.

A.1.b. Methods: Selection of sampling sites: The 4 sites will be located in watersheds. Baseline data including site history will be collected prior to insect introductions. The density of populations of *L. salicaria* will be estimated as (1) percent cover (purple loosestrife and associated vegetation), (2) number of *L. salicaria* plants within random quadrats, and (3) number of stems per plant within each quadrat. Other site selection criteria will include marsh or wetland with little or no submersion of roots, with dense to newly established populations. Release sites must be free from chemical or cultural control and major environmental changes due to human or agricultural activity . Sites must have easy access and neighboring stands of purple loosestrife to permit spread of the beetles out of the release site upon establishment (Hight & Drea, 1991). Abiotic data will include soil type, topography and site orientation. The study sites will be monitored periodically from May through October.

Data analyses: Comparisons will be made within and between release sites to determine which combinations of biocontrol agents are most effective in controlling purple loosestrife. Comparisons will be made to determine effects of climate on the agents. There will be evaluations of plant density and spread of the control agents. A cost/benefit analysis will be made. Published base temperatures for development will be tested against heat unit accumulations in detecting first and peak occurrence of the beetles' feeding damage and densities of attack.

A.1.c. Materials: The beetles will be obtained for release through the USDA-ARS Insect Biocontrol Laboratory in Maryland, other materials include field cages, rental vehicles, two incubators and detailed county maps in addition to existing laboratory facilities.

A.1.d. Biennial Budget: $70,000 **Balance:** $ -0-

A.1.e. Timeline:

	6/93	1/94	6/94	1/95	6/95
Select sites in watersheds	-------				
Field releases; lab studies	-------		-------		----
Analyses	-------		-----		----
Reports		----	----	----	----

A.1.f. Status: Final status report, June 30, 1995. ROGER BECKER, DAVID RAGSDALE, and ELIZABETH KATOVICH

***Galerucella* Release Site Selection and Monitoring.** Subsequent to the release of *Galerucella* beetles at the four sites targeted for extensive monitoring in 1993, observations during the summer of 1994 and 1995 revealed that the beetles overwintered successfully at each site, but beetle populations were not yet established at high enough populations to provide measurable feeding damage, or to assess the impact of *Galerucella* feeding on non-host plant biomass or population. Successful biological control measures such as this will take several years, possibly five to ten years to effectively control purple loosestrife at the release sites. We will continue to monitor and maintain these release sites during the next LCMR biennium (funding through the Minnesota Department of Natural Resources). Results of the initial plant counts and plant biomass in 0.25 m^2 quadrants adjacent to insect release sites are presented by location in Tables 1, 2, 3 and 4. Plant species present at the four release sites are listed in Table 5. Monitoring of the four release sites will be continued and if beetle feeding is significant, the change in plant species number and biomass will be determined.

Table 1. Initial Year Baseline Plant Counts and Plant Biomass. Ramsey County - White Bear Lake, July 1993.

Quadrant	Total Cover	Purple loosestrife		Sedge[3]		Broadleaved arrowhead[4]		Jewelweed[5]		Reed canarygrass[6]		Broadleaved cattail[7]	
		Shoots[1]	Biomass[2]	Shoots	Biomass	Shoots	Biomass	Shoots	Biomass	Shoots	Biomass	Shoots	Biomass
	%	(no.)	(g. dry wt.)	(no.)	(g. dry wt.)	(no.)	(g. dry wt.)	(no.)	(g. dry wt.)	(no.)	(g. dry wt.)	(no.)	(g. dry wt.)
Northeast 1	100	15	- - -	0	- - -	0	- - -	1	- - -	24	- - -	0	- - -
Northeast 2	100	0	71.5	27	42.1	0	0	4	0	39	21.5	0	0
Northwest 1	100	17	- - -	9	- - -	0	- - -	2	- - -	17	- - -	0	- - -
Northwest 2	100	9	78.0	0	0	0	0	0	0	0	0	2	12.5
Southeast 1	100	14	- - -	30	- - -	0	- - -	2	- - -	7	- - -	0	- - -
Southeast 2	100	8	94.4	70	13.9	0	0	0	1.1	11	25.6	0	0
Southwest 1	100	12	- - -	16	- - -	0	- - -	0	- - -	2	- - -	0	- - -
Southwest 2	100	15	25.4	0	0	2	0.02	0	0	5	35.0	1	5.2

1. Number of shoots per 0.25 m^2
2. g dry weight per 0.25 m^2
3. *Carex* spp.
4. *Sagittaria latifolia*
5. *Impatiens capensis*
6. *Phalaris arundinacea*
7. *Typha latifolia*

Additional species present: Common bugleweed (*Lycopus amerianus*), Beggartick (*Bidens* spp.), Dock (*Rumex* spp.), Fern (*Osmunda* spp.)

Table 2. Initial Year Baseline Plant Counts and Plant Biomass. Goodhue County, July 1993.

Quadrant	Total cover %	Purple loosestrife		Sedge[3]		Broadleaved arrowhead[4]		Jewelweed[5]		Broadleaved cattail[6]		Rush[7]	
		Shoots[1] (no.)	Biomass[2] (g. dry wt.)	Shoots (no.)	Biomass (g. dry wt.)	Shoots (no.)	Biomass (g. dry wt.)	Shoots (no.)	Biomass (g. dry wt.)	Shoots (no.)	Biomass (g. dry wt.)	Shoots (no.)	Biomass (g. dry wt.)
Northeast 1	85	16	- - -	6	- - -	0	- - -	22	- - -	1	- - -	0	- - -
Northeast 2	100	24	225.5	0	0	0	0	25	0	0	0	0	0
Northwest 1	100	45	- - -	45	- - -	3	- - -	6	- - -	0	- - -	0	- - -
Northwest 2	90	109	51.1	109	51.4	2	3.6	16	0.1	0	0	0	0
Southeast 1	90	2	- - -	75	- - -	7	- - -	2	- - -	2	- - -	49	0
Southeast 2	100	6	107.4	123	80.2	2	1.82	7	0	0	0	0	0
Southwest 1	98	5	- - -	75	- - -	3	- - -	3	- - -	0	- - -	2	- - -
Southwest 2	100	100	112.8	60	30.4	5	0.9	1	0	2	8.7	4	4.2

1. Number of shoots per 0.25 m^2
2. q dry weight per 0.25 m^2
3. *Carex* spp.
4. *Sagittaria latifolia*
5. *Impatiens capensis*
6. *Typha latifolia*
7. *Scirpus* spp.

Additional species present: Fern (*Osmunda* spp.), Beggartick (*Bidens* spp.), Box elder (*Acer negundo*), Catchweed bedstraw (*Galium aparine*).

Table 3. Initial Year Baseline Plant Counts and Plant Biomass. Winona County, July 1993.

Quadrant	Purple loosestrife		
	Total Cover %	Shoots[1] (no.)	Biomass[2] (g. dry weight)
Northeast 1	100	14	186.9
Northeast 2	100	5	- - -
Northwest 1	100	21	457.8
Northwest 2	95	8	- - -
Southeast 1	100	46	331.3
Southeast 2	100	9	- - -
Southwest 1	50	6	118.5
Southwest 2	90	9	- - -

1. Number of shoots per 0.25 m^2
2. g dry weight per 0.25 m^2

Additional species present: Rush (*Scirpus* spp.)

Table 4. Initial Year Baseline Plant Counts and Plant Biomass. Houston County, July 1993.

Quadrant	Total Cover	Purple loosestrife		Sedge[3]		Narrow-leaved cattail[4]		Rush[5]		Dock[6]		Reed canarygrass[7]	
		Shoots[1]	Biomass[2]	Shoots	Biomass	Shoots	Biomass	Shoots	Biomass	Shoots	Biomass	Shoots	Biomass
	%	(no.)	(g. dry wt.)	(no.)	(g. dry wt.)	(no.)	(g. dry wt.)	(no.)	(g. dry wt.)	(no.)	(g. dry wt.)	(no.)	(g. dry wt.)
Northeast 1	40	11	106.6	6	0.3	0	0	0	0	2	5.5	0	0
Northeast 2	40	12	- - -	5	0	5	- - -	0	- - -	0	- - -	0	- - -
Northwest 1	90	29	129.7	1	0	0	0	0	0	1	0	5	0.4
Northwest 2	95	23	- - -	0	- - -	5	- - -	11	- - -	0	- - -	0	- - -
Southeast 1	55	11	61.8	1	0	1	0.03	0	0	1	- - -	6	1.0
Southeast 2	65	10	- - -	0	- - -	2	- - -	0	- - -	0	0	0	- - -
Southwest 1	50	9	118.4	0	0	0	0	0	0	0	0	0	0
Southwest 2	90	6	- - -	0	- - -	0	- - -	0	- - -	0	- - -	0	- - -

1. Number of shoots per 0.25 m^2
2. g dry weight per 0.25 m^2
3. *Carex* spp.
4. *Typha angustifolia*
5. *Scirpus* spp.
6. *Rumex* sp.
7. *Phalaris arundinacea*

Additional species present: Beggartick (*Bidens* spp.), Catchweed bedstraw (*Galium aparine*)

Table 5. Species List for Galerucella Release Sites - 1994

1. Houston County Site.

Common Name	Genus/Species
1. Purple loosestrife	*Lythrum salicaria*
2. Jewelweed	*Impatiens capensis*
3. Marsh milkweed	*Asclepias incarnata*
4. Tear thumb	*Polygonum sagittatum*
5. Red-osier dogweed	*Cornus stolonifera*
6. Broad-leaved arrowhead	*Sagittaria latifolia*
7. Joe-pye weed	*Eupatorium maculatum*
8. Clearweed	*Pilea pumila*
9. Catchweed bedstraw	*Galium aparine*
10. Water dock	*Rumex orbiculatus*
11. Willow	*Salix* spp.
12. Beggartick	*Bidens* spp.
13. Skullcap	*Scutellaria lateriflora*
14. White heath aster	*Aster pilosus*
15. Lessor duckweed	*Lemna minor*
16. Broad-leaved cattail	*Typha latifolia*
17. Violet	*Viola* spp.
18. Common dandelion	*Taraxacum officinale*
19. Reed canarygrass	*Phalaris arundinacea*
20. Sedge	*Carex* spp.
21. Smartweed	*Polygonum punctatum*
22. Water hemlock	*Cicuta bulbifera*
23. Sweet flag	*Acorus calamus*
24. Wild tobacco	*Lobelia inflata*

2. Goodhue County Site.

Common Name	Genus/Species
1. Purple loosestrife	*Lythrum salicaria*
2. Narrow-leaved cattail	*Typha angustifolia*
3. Broad-leaved cattail	*Typha latifolia*
4. Wild mint	*Mentha arvensis*
5. Common bugleweed	*Lycopus americanus*
6. Jewelweed	*Impatiens capensis*
7. Clearweed	*Pilea pumila*
8. Joe-pye weed	*Eupatorium maculatum*
9. Broad-leaved arrowhead	*Sagittaria latifolia*
10. Violet	*Viola* spp.
11. Red-osier dogweed	*Cornus stolonifera*
12. Water dock	*Rumex orbiculatus*
13. Skullcap	*Scutellaria lateriflora*

3. White Bear Lake Site, Ramsey County.

Common Name	Genus/Species
1. Purple loosestrife	*Lythrum salicaria*
2. Hummock sedge	*Carex stricta*
3. Joe-pye weed	*Eupatorium maculatum*
4. Clearweed	*Pilea pumila*
5. Tear thumb smartweed	*Polygonum sagittatum*
6. Common bugleweed	*Lycopus americanus*
7. Water dock	*Rumex örbiculatus*
8. Broad-leaved cattail	*Typha latifolia*
9. Reed canarygrass	*Phalaris arundinacea*
10. Sedge	*Carex comosa*
11. Nimblewill	*Muhlenbergia* spp.
12. Southern tickseed sunflower	*Bidens coronata*

4. Winona County Site.

Common Name	Genus/Species
1. Purple loosestrife	*Lythrum salicaria*

Impact of Clipping and *Galerucella* Feeding on Carbohydrate Levels in Roots and Crowns of Purple Loosestrife. Starch was determined to be the major storage carbohydrate in purple loosestrife roots and crowns. Samples collected in 1994 have been analyzed for starch. Results indicate no differences in the level of starch between root and crown tissue, therefore starch levels in both tissue types are combined for presentation. Starch levels were highest in control plants. Levels of starch in the screened controls, and treatments where insects were placed in screened cages were significantly lower than the non-screened controls but did not differ from each other. Shoot clipping treatments at two and four

weekly intervals resulted in lower levels of starch than other treatments but did not differ from each other. Starch level in crown and root tissue as a result of *Galerucella* feeding did not differ from the caged control treatment. However, beetle feeding on caged plants did not completely defoliate the plants. The maximum amount of defoliation attained was approximately 25 percent of total leaf area. Since the initiation of the study in the summer of 1994, rearing and feeding behavior of *Galerucella* are better understood and these treatments are being repeated in 1995 to test the effect of significantly higher levels of adult and larval beetle defoliation on sugar and starch levels.

All plant crowns in duplicate treatments set aside to study second year effects of stressors failed to produce any significant regrowth of purple loosestrife in 1995 after overwintering regardless of clipping or feeding stressor treatments. Only one nonvigorous shoot had emerged on one control replicate. This indicates severe winter damage of loosestrife crowns in this study. Similar damage was widespread in alfalfa, a cultivated perennial with a large crown, in this geographic region as well in the winter of 1994-95 indicating climatic events were primarily responsible for this phenomena, not clipping or feeding stressors to purple loosestrife. The study will be repeated to further clarify these issues. The seasonal fluctuation of carbohydrates in loosestrife crowns will be analyzed following completion of the 1995 collections.

Field Releases: The project goals were expanded in the winter of 1994 to include a mass rearing component focusing on producing the two leaf beetles, *Gallerucella pusilla* and *G. calmariensis*. From 31 March to 30 June a total of 58,001 insects were reared and released into wetlands of 17 counties (including: Houston, Winona, Goodhue, Rice, Dakota, Ramsey, Hennepin, Washington, Crow Wing, St. Louis, and Stevens). Additional insects will be released the first 2 weeks in July 1995. In addition to the field releases, a total of 2,557 insects were made available to three other investigators to augment their work with purple loosestrife biological control (Bob Nyvall-Plant Pathology, Grand Rapids Experiment Station, David Andow-Entomology, St. Paul, and Roger Becker-Agronomy and Plant Genetics, St. Paul). Approximately 5 percent of the insects produced were used by these scientists.

Most field sites where leaf beetles were released in 1993 and 1994 were visited at least once in the summer of 1995. Some sites where *Gallerucella* spp. were released in prior years had one or more stages of beetles present (adults, eggs, or larvae). No insects were found at White Bear Lake (Washington), French Park (Hennepin), Eagle Lake (Hennepin), Black Dog (Dakota) Bay Lake (Crow Wing), Rush Lake (Crow Wing), Long Lake (Crow Wing), and Frontenac (Goodhue) release sites. Most of these sites had little evidence of feeding in 1994 so it is not surprising that insects could not be found in 1995. At the remaining sites only a few insects were found. The exception was Circle Lake (Rice) where a large reproducing population of *G. calmariensis* was found.

A decision was made to augment each of the 1993 and 1994 release sites in 1995 with lab reared insects. By June 1995 each of these sites (designated # 2 in the attached table) were augmented with 525 to 4,275 laboratory reared insects. Each site was revisited within 7 days of release to remove cages. All insects were found feeding and eggs were found at most sites.

At the Circle Lake site (Rice), where insects were first released in July 1994, a reproducing population of *G. calmariensis* was found. Eggs and larvae were found some 200 meters from the original release site on 16 June 1995. Moreover, nearly every stem within a 25 meter radius of the release site showed intense adult and larval feeding. One unique feature of this site is that the loosestrife is found in a drier habitat. We suspect that beetles are able to establish a reproducing population faster on a dry site and thus we are focusing our release efforts in 1995 on drier habitats to foster successful establishment.

Laboratory Production. Laboratory colonies of *Gallerucella pusilla* and *G. calmariensis* were maintained throughout the spring and early summer. A total of 124 colonies were started from 31 March through 22 May 1995. To date a total of 58,001 insects have been reared and released from these colonies in 1995. It is anticipated that another 25 to 30 thousand insects will emerge for release during July 1995. Because we can successfully rear both leaf beetles, we will no longer import *Gallerucella* spp. from Europe which cost as much as $2.00 per insect.

On average the *Gallerucella pusilla* colonies produced 408 insects per plant while the *G. calmariensis* colonies produced nearly twice as many adults per plant at 798. An experiment was designed to determine if 10, 20 or 40 adults placed on a single plant ca. 0.7 m tall would differ in production. No significant difference was found in production from these colonies for either species. This would suggest that even with 10 adults, resources are limiting production of adults.

Detailed analysis of egg production and survival to adult was conducted on several single plant colonies. Both species laid approximately the same number of eggs, 15,713 and 14,859 for *G. pusilla* and *G. calmariensis*, respectively. The average number of eggs laid per mass was also similar with *G. pusilla* laying 5.3 eggs per mass and *G. calmariensis* laying 6.6 eggs per mass. Each female laid on average 10 eggs per day for 39 days. *Gallerucella calmariensis* showed a definite preference for the top of the plant with two-thirds of its eggs being laid in the top 1/3 of the plant. *Gallerucella pusilla* showed no preference for distribution of eggs within the plant. Both species laid 60 percent of their eggs on the stems and 40 percent of their eggs on leaves. From these data there does not appear to be any substantial difference which could account for *G. calmariensis* colonies from being 50 percent larger.

Table 1. Releases made during 1995 (through 30 June 1995) from laboratory reared *Gallerucella pusilla* and *G. calmariensis*.

Date Released	*G. pusilla* Released	*G. calmariensis* Released	Total No. Released	County	Location
30 May 1995	170	355	525	Rice	Circle Lake #2
30 May 1995	352	651	1,003	Rice	Milton's Pasture
1 June 1995	1,439	1,370	2,809	Goodhue	Frontenac #2
2 June 1995			697	Ramsey	Ivan Savoy's experiments
2 June 1995	540	0	540	Stevens	Morris, mycoherbicide study w/ B. Nyvall
6 June 1995	734	394	1,128	Winona	Lake Winona, #2
8 June 1995	1,029	0	1,029	Dakota	Dodge Nature Center, Delaware St.
8 June 1995	0	3,082	3,082	Dakota	Dodge Nature Center, Cheyenne St.
20 June 1995	0	960	960	Ramsey	J. Katovich experiments
21 June 1995	803	1,222	2,025	Ramsey	White Bear Lake, #2
21 June 1995	937	1,306	2,243	Hennepin	Clifford French Park, #2
21 June 1995	0	1,263	1,263	Dakota	Black Dog Preserve, #2
23 June 1995	0	360	360	Ramsey	St. Paul, mycoherbicide study
27 June 1995	5,264	11,373	16,637	Crow Wing	#2 release at Rush, Bay & Long L; release at 5 additional sites (Donna)
29 June 1995	962	9,413	10,375	St. Louis	#2 release near Hibbing; release at 2 additional sites (L. Skinner)
29 June 1995	181	3,984	4,165	Houston	#2 release near Reno; (D. Sreenivasam)
29 June 1995	0	3,000	3,000	Houston	Brownsville, Gretta Lockhart owner
29 June 1995	1,357	1,790	3,147	Winona	#3 release at Winona, same as 6 June site.
TOTALS	13,768	43,536	58,001		

B. Title of Objective: Augment and enhance native fungal pathogens for control of purple loosestrife.

B.1. Activity: Application and evaluation of fungal pathogen isolates taken from native purple loosestrife.

B.1.a. Context within the project: Since the summer of 1991, efforts have been underway to develop a mycoherbicide that would serve as a biocontrol agent of purple loosestrife. Fungi that cause leafspots or other diseases are first isolated from purple loosestrife and identified. Approximately 3,000 fungal cultures have been isolated from plants at 16 sites throughout Minnesota. To date, five previously unreported fungi (*Alternaria* sp., *Colletotrichum* sp., *Curvularia* sp., *Fusarium* sp., and *Sclerotinia* sp.) have been consistently isolated from purple loosestrife. Additionally, one previously reported fungus, *Septoria* sp. has also been isolated. Fungi from 4 of the 5 genera have previously been reported as potential mycoherbicides to other weeds.

B.1.b. Methods: Suitable candidate fungal pathogens (mycoherbicides) will be field tested in natural stands of purple loosestrife located at the 4 sites. The exact field plot configuration will depend upon the size and shape of the natural purple loosestrife stand at each test site. Each culture will be considered a treatment and ideally will be applied in a suitable carrier consisting of an energy source and material to prevent desiccation of spores. Timing of application involves the maximum susceptibility of purple loosestrife plants. This is unknown at this time but likely young plants are the most susceptible.

Evaluation of field testing. Parameters to be evaluated are plant height and weight, number of plants within a plot, size and number of lesions, and ability of plants to overwinter (measured by biomass).

B.1.c. Materials: Centrifuge, shakers, sprayers, a field vehicle rental and collecting tools in addition to existing laboratory facilities.

B.1.d. Biennial Budget: $20,000 **Balance:** $ -0-

B.1.e. Timeline:

	7/93	1/94	6/94	1/95	6/95
Plot layout	-----	---------			
Lab testing	-----	---------	----		---
Field testing			------		---
Analyses		------	-------	----	
Reports	----	---	------		---

B.1.f. Status: Final status report, June 30, 1995. Robert Nyvall.
Results. Isolation of fungi. In a 3-year study, fungi were isolated from diseased purple loosestrife plants at 16 sites throughout Minnesota. A total of 5,265 fungal isolates were obtained. Nineteen species in 26 genera were identified of which 18 species in 24 genera had not been reported from purple loosestrife in the United States.

Inoculation of purple loosestrife in growth chambers: One isolate each of *Alternaria alternata, Botrytis cinerea , Colletotrichum truncatum*, and *Septoria lythrina* have been identified as potential mycoherbicides on the basis of their consistent pathogenicity to purple loosestrife. These four fungi had the highest disease ratings of all fungi tested and on the basis of this work were chosen for further testing in the field. However pathogenicity testing will also continue with other fungi.

Geographical origin of fungi: Origin of fungi in relation to pathogenicity was determined. Isolates of *A. alternata* and *B. cinerea* were selected from three different locations and their pathogenicity determined. There was no difference among origins with *A. alternata* but isolates of *B. cinerea* from kabetogama and Tamarack were more pathogenic than an isolate from Pigs Eye.

Effect of plant age to inoculation in the growth chamber: There was no effect of age of purple loosestrife plants at inoculation on disease ratings. However, 6-wk-old plants are utilized instead of 4-wk-old plants because they are easier to rate for disease severity.

Effectiveness of carrier: Digs was the most effective carrier compared to tween 20 and Invert , in which to incorporate fungi as measured by disease severity. DIGS was easier to apply than Invert and accentuated pathogenicity better than either Invert or tween 20.

Maintenance of fungus cultures: Maintaining pathogenicity of cultures in storage may be a problem with some fungi. The culture of *B. cinerea* most pathogenic to purple loosestrife lost its pathogenicity in culture. However, a pathogenic culture was regained by reisolating from leaf spots on inoculated leaves. A suitable method to maintain pathogenicity in culture is being formulated.

Field testing: The *Botrytis*/pesta combination appeared promising when tested on purple loosestrife plants in the field in 1994. The results of the 1995 field tests are unknown at this time; however, preliminary observations of the fungus only inoculations did not appear effective, but the fungus/insect combination appeared more effective than either biological agent alone.

Discussion: Much progress has been made in developing a mycoherbicide to control purple loosestrife in the last four years. Several fungi have been identified as being pathogenic to purple loosestrife and progress has been made on developing a carrier in which to incorporate the fungi and apply to plants. We have a clear understanding on laboratory procedures and protocol on testing pathogenicity. A major effort was made in growing purple loosestrife from seed and raising it to a suitable size before inoculation. The fungi *A. alternata* and *B. cinerea* appear to be the most promising candidates as mycoherbicides; however others are still being tested. *Colletotrichum truncatum* has also been pathogenic in growth chamber tests; however pathogenicity by this fungus has been less reliable than with the other two fungi. Additionally, it is better understood that origin of fungal isolation is not a factor in pathogenicity but length of time of storage is a factor in pathogenicity, at least with *B. cinerea*.

We have developed a liquid carrier that has proven superior to carriers used by other researchers to apply fungi in the laboratory. However much work remains before a suitable carrier can be used in the field in which to apply fungi.

Much work apparently remains to be done in application of fungi to plants in the field. Although a very limited test last year appeared successful, this years results on a larger scale have been disappointing. However, preliminary observations of fungi in combination with insects appears promising at this time in the growing season. Suspected reasons why the fungi have not been pathogenic in the field this year are as follows: 1) Fungi applied too late in the growing season. 2) Mycoherbicides must be applied more than once. 3) Fungal spore concentration must be increased.

4) Weather was extremely hot (32-36 C) and dry following inoculation. Using this information, field tests will be modified next year to answer the preceding parameters.

In conclusion, it is my opinion that it is possible to develop a mycoherbicide to control or partially control purple loosestrife. The knowledge to accomplish this has been partially developed in the laboratory. It is now necessary to modify laboratory techniques and apply this knowledge to field situations. Our future work will focus on the following: 1) Determining an effective carrier in which to apply fungal spores to purple loosestrife plants, particularly in the field. 2) Testing of carrier/fungi combinations in field tests. 3) Testing of fungi in combination with insects in field tests. This work will be in cooperation with Dr. Dave Ragsdale, U. of Minnesota. 4) Obtaining and evaluating fungus isolates for pathogenicity to purple loosestrife.

Publications.

Nyvall, R. F. 1995. Fungi associated with purple loosestrife (*Lythrum salicaria*) in Minnesota. Mycologia 87: (In Press).

Nyvall, R. F. and Hu, A. 1993. *Botrytis cinerea* and *Alternaria alternata* as potential mycoherbicides of purple loosestrife. (Abstr.) Phytopathology 83:1366.

Nyvall, R. F. and Hu, A. 1995. Efforts to develop a mycoherbicide to control purple loosestrife in the United States. (Abstr.) XIII International Plant Protection Congress.

Nyvall, R. F. and Hu, A. 1995. Laboratory evaluation of fungi as potential mycoherbicides to control purple loosestrife. (To be submitted to Biological Control).

V. Evaluation: The project can be evaluated by its ability to: (1) adequately assess the effectiveness and practicality of the biological control agents (2) identify areas where further research and field experimentation is needed and (3) transfer technology and educate managers and measure success based on adoption of the new technology.

In the long-term, the project should be evaluated by its ability to: (1) successfully utilize knowledge of the relationships between biological control agents and their ecological dynamics; (2) to develop and implement sound management practices to maintain and improve status of our biocontrol fauna and flora; and (3) compare and analyze the budgets of farmers who adopted biological pest control with those who use chemical-based control.

VI. Context within field: To date, work on biological control agents in Minnesota, has largely focused on isolation, identification and rearing of several biocontrol organisms, their biology, distribution, virulence and pest impact levels. Some investigations have extended to imported biological control agents introduced in previous years, their establishment and current levels of impact. Our recent work has repeatedly demonstrated that behavior patterns, spatial dynamics, cultural practices, selective control of weeds with bioherbicides and rearing conditions affecting quality are important in the maximization of benefits of biological control.

DNR's project manager of purple loosestrife has been consulted. MDA's part of the project will focus on expanding the biological control effort to 4 watershed sites in 4 counties (Hennepin, Goodhue, Wabasha and Houston). This will allow for as many different ecological conditions as possible, thereby increasing the chances for establishment. Once established future redistribution of biocontrol agents is a logical next step.

Cooperation with county agriculture personnel is an integral part of this project. Local involvement is considered key to successful future implementation. The cooperation between DNR and MDA will help strengthen relationships with the clientele. Cooperators on the project come from varied academic and professional backgrounds. Integration of all of the above will provide a basis to continue our biological control efforts state-wide as well as nation-wide.

VII. Benefits: Protection of Minnesota's watersheds is important to the birds and wildlife dependent upon the food and shelter that watersheds provide. Livestock also benefit from accessible water sources. The control of purple loosestrife and its spread will insure the quality of watersheds. Biological control agents of weeds have shown to be effective for long term control of weeds. Cost per acre for mechanical and chemical means of control will be considerably reduced by establishment and spread of biological control agents. Consequences of short term funding for control will be lessened by reducing dependence on herbicides and mechanical methods. Future augmentative releases of biological control agents will be considerably less expensive and time consuming. Private property will benefit esthetically by retaining native vegetation without burned or mowed shorelines. Field insectaries will be established and used to further distribute the biocontrol agents to new sites.

VIII. Dissemination: Results can be displayed to the public through posters, brochures, fact sheets, and media coverage. Evaluations will also be summarized at regional or other special meetings. A scientific note will be published in an appropriate biological control journal, as well as in regional and local newsletters.

IX. Time: Two years of funding is requested for the proposed project.

X. Cooperation:

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XI. Reporting Requirements: Semiannual status reports will be submitted no later than Jan. 1, 1994, July 1, 1994, Jan. 1, 1995, and a final status report by June 30, 1995.