July 1, 1993

LEGISLATIVE COMMISSION ON MINNESOTA RESOURCES (LCMR) WORK PROGRAM 1992-93

Final Status Report - Summary-Research

I. **Biological Control of Pests** - Agriculture 2.

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A. M.L. Ch 254 Sec 14 Minnesota Resources — Total biennial appropriation $650,000
subd. 6 (a) Agriculture-Biological Control of Pests — Balance $5,007

This appropriation is from the Minnesota environment and natural resources trust fund to the commissioner of agriculture to collect and identify potential biological control agents, and to develop and test biological control agents for a variety of pests. A grant request to supplement this appropriation must be submitted to the U.S. Department of Agriculture and the results reported to the Legislative Commission on Minnesota Resources.

B. Not applicable.

C. Not applicable.

II. Narrative

A. Ground and surface waters of Minnesota continue to be threatened by contamination with residues of pesticides used in agricultural and urban development.

B. Renewed efforts in the development of pesticide alternatives are underway nationwide. Of particular utility is **biological control** of which the best example is the use of natural enemies to reduce pest populations.

C. The Minnesota Department of Agriculture and the University of Minnesota are currently in the fourth year of LCMR funded projects to develop biological controls for several Minnesota pests. These include leafy spurge, Canada thistle, European corn borer, corn rootworm, forest defoliators, filth flies, and grasshoppers, among others. This is a comprehensive interdisciplinary approach to a major problem threatening Minnesota's natural resources. It involves 18 scientists from three departments at the University of Minnesota as well as scientists from the Minnesota Department of Agriculture.

Project 1 Title: **Develo[illegible]nt of Biological Control for Leafy Spurge a[illegible] Canada Thistle in Minnesota; and Smother Plant System for Control of Weeds in Corn.**

III. Objectives

A. **Biological control of leafy spurge in Minnesota**
Dr. David Ragsdale

A.1. Narrative: Leafy spurge *(Euphorbia esula)* is a weed native to Europe. In North America it invades non-cropland, displacing native forage. Leafy spurge is not a problem on land that is regularly cultivated. Grazing animals avoid spurge infested areas because the milky latex is irritating to mucous membranes. Controlling spurge with herbicides is costly (ca. $100/ac) and is largely a short term strategy (Harris et al. 1985). Several insects have been screened and approved for release by the USDA. Any exotic insect approved for release in the U.S. has been thoroughly screened by USDA personnel and an independent technical advisory group (TAG) (Klingman and Coulson 1983). An insect's feeding preference and host range is determined and no insect approved for release in the U.S. will feed on any cultivated crop or endangered plant species (Pemberton 1985). Through this proposal, we are participating in a multistate leafy spurge biological control program in cooperation with the USDA Biological Control of Weeds Laboratory in Bozeman, Montana and local APHIS personnel. During 1989-90 we established one insect, a flea beetle, *Aphthona nigriscutus* in Becker, Washington, Hennepin and Carver counties. We released another flea beetle species, *A. cyparissiae*, in 1989, but have yet to recover this species. *A. nigriscutus* has been recovered from all 1989 release sites (6) in Minnesota. *A. nigriscutus* has been able to control spurge in Manitoba, Canada and is now established in several northern states (Harris, personal commun.). It is the immature form of this beetle that is damaging and adults in Minnesota are only present from mid-June to early August, depending on location. As a part of the multistate program we are characterizing each release site and will deposit our site characterization data with the USDA lab in Montana. Analysis of these data will enable us to predict success of future release sites and will allow us to fully exploit the biological control potential of this flea beetle. Understanding these following site characteristics is essential to properly plan an expanded release program of this insect in Minnesota.

A.2. Procedures: Site characterization includes noting: vegetation type, dominate tree, shrub and herb species, altitude, slope, aspect, micro-, meso- and macro-scale relief, shade, moisture regime, soil surface cover, soil texture, land use, and previous herbicide use at site (if any). By taking twenty 0.1 m^2 biomass samples at each location we will determine the following: percent cover and biomass of the target weed, grasses, forbs, and woody perennials and weed canopy height. When monitoring the sites we will record temperature, weather, insect numbers per 20 sweeps, area of spread (m^2), distance of spread from the release point, direction of spread, and direction in relation to topography. These data will be sent to the USDA lab in Bozeman and a common base for the five states and four provinces participating in the program will be assembled. We currently have ten sites where flea beetles have been released (six in 1989 and four in 1990). We will continuously redistribute flea beetles as the established populations in Minnesota build up to sufficient numbers to allow some insects to be removed. We also will receive additional shipments of these and other insects as they become available from the USDA facility in Montana. Our goals are to double the number of release sites of *A. nigriscutus* and to release other insects or other biocontrol agents as they become available from USDA.

To this end, up to 25 percent of the budget will be used to support the CIBC research station at Delemont, Switzerland where insects and other biocontrol agents are first screened (Schroeder 1989).

A.3. Budget

	LCMR Funds	Matching Funds
a. Amount Budgeted:	$40,000	-0-
b. Balance:	$-0-	-0-

A.4. Timeline for Products/Tasks

	July 91	Jan 92	June 92	Jan 93	June 93
Field studies	—		———	———	
Data Analyses		———		———	
Reports		—	—	—	—

A.5. Status: Final status report June 30, 1993. This year marks the first recovery of an overwintering population of *Aphthona cyparissiae*, the first release of a new species, *A. flava*, and the first redistribution from a Minnesota field insectary of *A. nigriscutus*. Soil samples were taken from all Minnesota sites and are currently being processed in Montana. Once optimum site characteristics are determined for these species, the success of any further redistribution should be greatly improved. Additionally, a degree-day emergence model is being developed to better predict beetle population dynamics.

We have established 10 overwintering populations of *Aphthona nigriscutis* in Minnesota We currently have 17 release sites in Minnesota consisting of three species of flea beetle, *Aphthona nigriscutus*, *A. cyparissiae*, and *A. flava*. Both *A. nigriscutus* and *A. cyparissiae* populations have been established in Minnesota for at least two years. One of these populations was a redistribution from a Minnesota insectary, the first in Minnesota. In addition, 1993 marks the second redistribution to a new site from a Minnesota insectary. When beetles become established, adapted to local conditions, and generate large population levels, they can be distributed to nearby areas where leafy spurge is a problem. We also have two overwintering populations of *A. cyparissiae*, again the first in Minnesota. This year marks the first recovery of a third species of flea beetle in Minnesota, *A. flava*, released in 1992. Again, site information for each species is being collected and analyzed in order to determine optimum conditions for a potential new release site.

Results from the analysis on the national data base of field site characteristics are inconclusive. No one character or suite of characters can accurately predict success of beetle establishment. The only significant factor was the size of the initial population with positive linear correlation of percent spurge control with the number of beetles released. However, some general recommendations (using unpublished data) concerning selection of a release site include: at least an acre of contiguous leafy spurge; spurge densities should be moderate, e.g., you should be able to see individual stems from a distance, and, on closer inspection, you should be able to see bare ground between stems; flowering stem heights should be two feet or less; sites should be well drained, but not excessively well drained (loam or sandy-loam); sites should be exposed to full sun and have little or no shade; and finally all cultural treatments (mowing, grazing, herbicides, and insecticides) should be avoided in or near the release site. Areas to avoid include: very sparse spurge stands (stems less than 12" tall and separated by 5" or more) or extremely dense stands (stems more than 3 ft. tall and ground surface totally obscured by spurge).

Site characterization continues in all established sites and in all new sites. Data on the soil profiles from Minnesota sites is inconclusive as a method to evaluate effectiveness of a potential release site. Weather computers have been placed in two sites in order to further refine an emergence model in order to predict key population levels of these beetles. In the future, data will continue to be collected on site characteristics, insect population levels, and leafy spurge densities in the release sites. When the degree-day emergence model is refined, it will be a valuable tool to predict optimum sampling times in order to continue redistribution of this biological control agent throughout the State of Minnesota.

A.6. Benefits: Biological control agents will be established with greater probability of success once site specific characteristics are identified. A larger land area could be under biological control reducing reliance on herbicides on lands with marginal returns (roadside rights-of-way, non-cropland, and unimproved pasture). New agents will stabilize the influence of biocontrol and further reduce spurge populations. Development of ties with European and Canadian biological control facilities will accelerate the introduction and establishment of new agents.

B. Biological control of Canada thistle in Minnesota

Dr. Donald Wyse

B.1. Narrative: Efforts to control Canada thistle (*Cirsium arvense*) in both crop and non-cropland areas with herbicides have been costly, ineffective, and environmentally questionable. The Canada thistle population has continued to increase in Minnesota despite attempts at control with herbicides.

Minnesota's Canada thistle population has been suppressed in recent years by an "apical chlorosis" disease of unknown etiology. The symptoms include distortion, dwarfing, yellowing and bleaching of the upper leaves, stems and flowers. Established thistles may be killed when severely affected, and even mildly symptomatic plants are often prevented from flowering or producing viable seed. These effects suggest that the causal agent of the yellows condition might be developed as a biological control for Canada thistle. The objectives of this study are to identify the organisms infecting Canada thistle in Minnesota and to explore the potential for development of these organisms as biological control agents.

B.1.a. Objectives:

1. To determine the etiology of the unknown apical chlorosis disease of Canada thistle and isolate the causal agent.
2. To develop a practical inoculation method for infection of Canada thistle in the field.
3. To evaluate the potential of the apical chlorosis as a biological control agent.

B.2. Procedures: Research on the isolation, identification and production of the causal bacterial agent will continue. Over the next six months, we believe that the causal agent will be isolated from Canada thistle and identified. Once techniques for culture and storage of the organism has been developed, research will be initiated to determine the feasibility of developing it as a biological control agent for Canada thistle. Suspensions of bacterial cells will be sprayed on Canada thistle plants to investigate the potential for direct infection of Canada thistle leaves. The use of surfactants for enhancing foliar infection will also be investigated. Other factors that may enhance expression of the "apical chlorosis" disease in Canada thistle will be studied.

Procedures Objective 1: **Etiology of apical chlorosis**
Progress has been made in the isolation and identification of the "thistle apical chlorosis" causal organism.

Yellows diseases are often caused by mycoplasmalike organisms (MLOs), viruses, or fastidious xylem-limited prokaryotes. MLOs have been reported in Canada thistle (7), but these were not shown to produce yellows or other symptoms. Extensive light and electron microscopy of affected thistles from Minnesota have not detected any organisms which might cause the observed symptoms. Leaf dips of affected thistle sap viewed by transmission electron microscopy (TEM) detected no virus particles or MLOs. Stem sections viewed by scanning electron microscopy (SEM) revealed only a small population of prokaryotes in xylem and phloem. We conclude that thistle apical chlorosis is not caused by any MLO, virus or xylem-limited prokaryote.

An apical chlorosis disease caused by the bacterium *Pseudomonas syringae* pv. *tagetis* has been reported in certain members of the compositae (4). The bacterium produces tagetitoxin, which specifically inhibits RNA polymerase III and causes severe chlorosis by preventing plastid biogenesis (1, 3). The yellows symptoms observed in Canada thistle strongly resemble those produced in known hosts of *P. syringae* pv. *tagetis* when they are naturally or artificially infected with the bacterium.

Needle inoculation of thistles with stock cultures of *P. syringae* pv. *tagetis* produced characteristic apical chlorosis symptoms in five days. No isolates of *P. syringae* pv. *tagetis* have yet been identified from Canada thistle, but evidence that it is the causal agent of thistle apical yellows is accumulating.

Attempts to isolate the bacterium from symptomatic thistle will continue. Numerous bacterial cultures have been isolated from leaves, roots and stems of affected thistles, and these are currently being screened with a sunflower bioassay. Promising isolates are identified to species or pathovar using the Biolog Microplate System (2), gas chromatography of cellular fatty acids (5, 9), and standard bacteriological methods (6). Efforts to isolate the bacterium have been hampered by the presence of saprophytic organisms and possibly by bactericidal substances in the host cytoplasm, which are released during the culture process. Efforts to overcome these problems will center on development of selective culture media and extraction techniques which minimize cell damage or neutralize toxic cytoplasmic substances.

Procedures Objective 2: **Inoculation method for infection of Canada Thistle**
The usefulness of bacterial pathogens as biological control agents has been limited in the past by the necessity of wounding the plant to initiate infection. Needle inoculation of thistles with *P. syringae* pv. *tagetis* resulted in typical apical chlorosis symptoms, but spray applications with aqueous bacterial suspensions failed to produce infection. Also, thistles became increasingly resistant with age, and needle inoculations were only effective in young plants.

A new inoculation method has been developed which dramatically improves infection of Canada thistle by *P. syringae* pv. *tagetis*. Low pressure spray application of bacterial suspension in phosphate buffer with an organosilicon surfactant (8) has reliably produced apical chlorosis symptoms of greater severity than those produced by needle inoculation. Further, the spray applications have produced infection in thistles at all growth stages. Unlike inoculation techniques requiring physical injury to the target plant, this spray application method could easily be adapted for field application.

Procedures Objective 3: **Potential of apical chlorosis as a biological control agent**
Factors that may increase infection or disease severity will be studied further to develop the most efficacious formulation. Optimal surfactant and bacterial cell concentrations will be determined by visual evaluation of plants inoculated with various concentrations of surfactant and bacterial cells.

The impact of natural and artifical bacterial infection on thistles will be measured by comparison of fresh weight and carbohydrate content of roots and shoots from healthy and infected plants. Root carbohydrate concentrations of naturally infected, artifically infected and healthy field plants will be compared. The effect of artificial infection on fresh weight and carbohydrate content of whole plants will be determined in greenhouse studies.

B.3. Budget

a. Amount Budgeted:	$20,000
b. Balance:	$ -0-

B.4. Timeline for Products/Tasks

	July 91	Jan 92	June 92	Jan 93	June 93
Detail design	———				
Fieldwork	———	———	———		
Laboratory research	———	———	———		
Data analysis & evaluation		———	———	———	
Reports	—	—	—	—	—

B.5. Status: Final status report June 30, 1993. Minnesota's Canada thistle population was suppressed in recent years by an apical chlorosis disease of unknown etiology. The symptoms included distortion, dwarfing, yellowing and bleaching of the upper leaves, stems and flowers. Established thistles were killed if severely infected, and even mildly symptomatic plants were often prevented from flowering or producing viable seed. We chose to investigate the potential of this disease as a biological weed control because of its apparent virulence on Canada thistle and because of increasing regulatory costs, environmental impacts, public concern, and

government restrictions associated with synthetic herbicides. We isolated the causal organism from symptomatic Canada thistles and identified it as the phytopathogenic bacterium *Pseudomonas syringae* pv. *tagetis*. Spray application of our *P. syringae* pv. *tagetis* isolate as a buffered bacterial suspension (5×10^8 cells/ml) with an organosilicone surfactant caused apical chlorosis and significant damage to Canada thistle, common ragweed, giant ragweed, horseweed, Jerusalem artichoke and several other weed species, many of which are not reported hosts of the bacterium. Infection was consistent under extremes of temperature and humidity, and disease was more severe than in natural infections. In replicated field experiments, five applications of the bioherbicide severely injured or eradicated Canada thistle in soybean. In field experiments where fewer than five applications were made, significant current-season suppression of Canada thistle occurred, and spring regrowth was greatly diminished or nonexistent. Five biweekly applications to corn and soybeans caused no detectable injury or yield loss. Current field experiments will determine optimal spray timing and surfactant rate for Canada thistle control, and provide additional host range information.

B.6. Benefits: The reliance on herbicides for control of Canada thistle along roadsides, on recreational land and farmland would be greatly reduced. This would reduce the impact of herbicides on surface and ground water.

C. Biological control of weeds in corn

Dr. Donald Wyse

C.1. Narrative: Widespread use of herbicides has often led to unwanted results such as increased production costs, polluted water resources, and herbicide-resistant weed populations (3). A cover crop or smother plant that provided acceptable weed control in a corn production system could potentially alleviate the above concerns, and provide the added benefit of soil erosion control. A number of researchers have attempted to use cover crops as a method of weed control in corn production systems. The majority of these efforts have focused on fall seeded winter rye or perennial legumes, such as the clovers and vetches. A few have dealt with cover crops planted simultaneously with the primary crop.

Fall planted winter rye killed with a herbicide in the spring has been reported to suppress annual weeds (8). However, a herbicide is required to terminate the growth of the winter rye, and its growth in the spring can substantially deplete soil moisture levels (7). Echert (2) has reported that the presence of a rye cover corp, killed by a herbicide at the time of corn planting, resulted in a reduced corn stand under some conditions.

Species such as hairy vetch, crownvetch, winter rape, and alfalfa have also been investigated for use as cover crops. Hartwig (4) reported that a perennial crownvetch (*Coronilla varia* L. 'Penngift') cover crop chemically suppressed early in the growing season, provided 60 to 100 percent control of yellow nutsedge (*Cyperus esculentus* L.). Mayer and Hartwig (5) observed that corn yields from plots containing a suppressed but living crownvetch mulch generally outyielded those plots on which a cover crop had never been used. However, the successful implementation of legume cover crops has generally occurred in the Southeast. In the North Central Region, winderhardiness of the potential legume cover crops has been a serious constraint.

A spring seeded smother plant in corn is essentially an intercropping system, and although the idea of controlling weeds using a smother plant seems rather novel, the use of intercrops for weed control has been practiced for many years in some parts of the world. In West Africa the eugsi melon (*Citrullus vulgaris* Schard.) is commonly intercropped with corn and other crops and functions as a smother plant.

Unamma et al. (6) compared the use of intersown cowpea and egusi melon against the use of two hand weedings or herbicides for a corn and cassava intercrop. Even though the smother crops failed to control weeds as effectively as hoeing or herbicides late in the growing season, crop yields were not depressed by weed competition since there was adequate weed suppression in the critical period four to eight weeds after planting. In each of two years, the unweeded cowpea/corn/cassava or melon/corn/cassava crop systems produced as much cassava and corn grain as weed-free monocultures of each crop. In contrast, across the two years in the corn/cassava system, uncontrolled weeds reduced cassava yield by 49 percent and corn grain yield by 53 percent. Economic returns for the different weed control systems were in the order: smother crops > herbicides > hand weeding. Akobundu (1) reported similar results using egusi melon and sweet potatoes as smother crops in an intercropping system.

The widespread use of companion crops and smother plants indicates that interspecific interference and competition can be managed, with benefical weed control and conservation results. However, the smother plant and the primary crop must have complementary growth and development characteristics. The focus of this study will be the determination of the particular traits essential to a successful smother plant for weed control in corn, and the development of a class of plants with those general traits. Based on the results of competition and cover crop studies found in the literature, we are proposing a smother plant with the following characteristics: rapid germination and development under cool conditions, broad leaves, short stature, and a lifecycle of four to five weeks. Non-adapated *Brassica* spp. possess these general characteristics and so will be the focus of the study.

C.1.a. Objectives

1. Develop additional short-term smother plants (dwarf-*Brassica* spp.) that have the potential to replace herbicides in corn and soybean production systems.
2. Determine the essential characteristics of a model smother plant system in corn.
3. Transfer dwarf-*Brassica* smother plant technology to the farming community.

C.2. Procedures

Procedures Objective 1

The goal of the plant breeding program is the development of a rapid cycling, short statured plant capable of covering the ground rapidly in early spring. A number of rapid cycling dwarf-*Brassica* spp. have been obtained from Paul Williams at the University of Wisconsin. A gibberellic acid mutant and a dominant dwarf phenotype will be the primary dwarf species used in the program. The rapid cycling dwarf populations will be crossed with locally adapted *Brassica* species in an attempt to obtain a rapid growing and leafy plant yet small statured and rapid cycling. The locally adapted species will be obtained from local seed houses and possibly from

other research institutions. The two rapid cycling species are *Brassica campestris*, and locally adapted species are *Brassica campestris*, *Brassica juncea*, and *Brassica napus*. Common names of the locally adapted *Brassica campestris* species are Chinese cabbage, mustard spinach, and pak choy. *Brassica juncea* species are broadleaf mustard, bok choy, southern giant curled mustard, red giant mustard, and green wave mustard. The two dwarf species and the locally adapted varieties will be grown in the greenhouse and reciprocally crossed in bee cages. The resulting seed will be planted in the greenhouse. Time of emergence, percent germination, height 28 days post planting, time of flowering, and harvest date will be recorded. Promising crosses will be identified, and the seed multiplied for evaluation under field conditions.

Procedures Objective 2

Two field experiments (A and B) designed to improve the selection criteria for the breeding program will be conducted at St. Paul, Jackson and Lamberton, Minnesota, during the 1991 and 1992 growing seasons. The following methods apply to the following two experiments. Hybrid field corn will be planted in rows spaced 76 cm apart, during the first week of May, with a four row planter in a conventional tillage system. Yellow mustard (*Brassica hirta*) will be seeded over the corn row immediately after the corn is planted. All plots will be a single row, with a 50 cm area between plots maintained weed-free. A randomized complete block split-split plot design with four replications will be used. Whole plot treatments will be weedy or weed-free. Propachlor at 3.0 kg/ha will be applied to the weed-free plots. Mustard height, corn height, and percent ground cover will be recorded weekly for the first 12 weeks postemergence. The number of corn collars will be recorded for each plot seven and ten weeks postemergence. Days to 50 percent silk emergence will be recorded. At harvest, grain yield and moisture from each plot will be determined. The stover from a 90 cm portion of each plot will be harvested, and stover per plant at harvest will be calculated. The data will be statistically analyzed to determine LSD values, and correlation between variables will be tested.

Experiment A is designed to determine the influence of yellow mustard height and length of lifecycle on corn and weed populations. Plots will be 4.5 m long. Mustard seed will be applied in a 25 cm band over the corn row at a density of 2150 seeds/m^2. A push-type seed bander will be used to seed *B. hirta*. Split plots will be composed of 2, 4, 6, and 8 week periods of mustard interference. The periods of interference, or simulated lifecycle lengths, will be regulated with an application of 2,4-D. Split-split plots will consist of a control with no mustard, 10 cm, and 20 cm mustard heights. Dry weights of corn, mustard, broadleaf weeds, and grasses from a .09 m^2 area will be determined at the time of herbicide application, five weeks after application, and at 11 weeks after mustard emergence.

Experiment B is designed to evaluate the effects of mustard planting pattern and seeding density on weed populations and on corn development. Plots will be 6 m long. Split plots will be mustard planting pattern. The effects of mustard planted in a 25 cm band over the corn row, in a single row directly over the corn row, and in three rows with one row directly over the corn row and a row 10 cm to either side of the corn row will be investigated. A Plantet Jr. will be used to seed the single rows of mustard. Split-split plot treatments will consist of eight mustard planting densities. The densities will be 0, 135, 270, 540, 1080, 2150, 4310, and 8610 seeds/m^2. An application of 2,4-D will be used to kill the mustard four weeks after emergence. Dry weights of corn, mustard, broadleaf weeds, and grasses from a .09 m^2 area will be determined five and eleven weeks postemergence.

Procedures Objective 3

Our research group has been successful in developing one new smother plant by crossing a dwarf *Brassica campestris* with chinese cabbage (*Brassica campestris* ssp. *pekinensis*). It was evaluated under field conditions during the 1990 growing season. Results from this research indicate that the dwarf-*Brassica* controls weeds in corn with only limited influence on corn development. We are now in the process of a limited seed increase of the dwarf-*Brassica*. This seed will be sent to Texas for a large seed increase for use in the 1991 adaptation and technology transfer project.

The technology transfer project will be conducted in Southwestern Minnesota with the cooperation of Sister Esther Nickel, Religious Sisters of Mercy, Jackson, Minnesota, and at the Koch Sustainable Agriculture Farm, Lamberton, Minnesota. These two locations are ideal because they will test the smother plant system under stressful environmental conditions (i.e. dry and windy) and both cooperators have a background in sustainable agriculture research and demonstration. We believe that interaction with sustainable agriculture farmers is important because this group of producers will be the first to adapt the new technology. Experiments will be designed to evaluate the dwarf-*Brassica* smother plant system under field conditions. Corn and soybean will be planted in 75 cm rows in early May. Research results from objective 2 will be used to determine the smother plant planting pattern and densities included in the field scale trials. If a large seed increase of the newly developed dwarf-*Brassica* line is obtained (it appears that we will), 0.5 to 1.0 acre fields will be used as test plots. In each case the appropriate chemical weed control treatments will be included for comparison. Commerical on-farm equipment will be used to seed the dwarf-*Brassica* smother plant. Data will be collected to describe the development of the dwarf-*Brassica*, weed control and corn yield. Data collected will be subjected to an analysis of variance.

C.3. Budget

	LCMR Funds
a. Amount Budgeted:	$30,000
b. Balance:	$-0-

C.4. Timeline for Products/Tasks

	July 91	Jan 92	June 92	Jan 93	June 93
Detail design	—				
Fieldwork (breeding)	———	———	———		
Laboratory & greenhouse (breeding)	———	———	———		
Data analysis & evaluation		———	———	———	
Reports	—	—	—	—	—

C.5. Status: Final status report June 30, 1993. A *Brassica spp.* smother plant was developed, and its effect on corn growth and yield was evaluated. F_1 progeny of crosses between rapid cycling, short statured *Brassica campestris* species and locally adapted *Brassica spp.* were evaluated in the greenhouse. One population, derived from a dwarf *Brassica* by Chinese cabbage cross,

was chosen for field evaluation as a spring seeded smother plant in corn. When grown in the field, the dwarf *Brassica* smother plant flowered 3 weeks after emergence, had a maximum height of 33 cm, and began to senesce 5 weeks after emergence. Field studies were conducted to determine the effect of dwarf *Brassica* smother plant seeding rates of 0, 530, 1060, 2120, and 4240 seeds m^{-2} on corn developement and yield. Corn silk emergence at St. Paul was not affected by smother plant seeding rates of 530 to 4240 seeds m^{-2}, but at Waseca the same range of seeding rates delayed silk emergence by 1.5 to 4.9 days. At St. Paul, corn grain yield was not affected by smother plant presence, but at Waseca smother plant seeding rates of 530 to 4240 seeds m^{-2} reduced grain yield by 11 to 24 percent. Environmental factors appeared to be responsible for the differential response of corn to smother plant presence across locations and years. These results indicate that it may be possible to develop spring seeded *Brassica spp.* smother plants that do not have a negative effect on corn development and yield.

C.6. Benefits: Reduced herbicide use will lower the potential for groundwater contamination from atrazine and alachlor; smother plants will provide rapid soil cover reducing soil erosion.

IV. Evaluation

Criteria for evaluation include the following: Document percent of control of leafy spurge at release sites. Assess increases in biomass of native forage. The impact of the disease on the long-term survival of Canada thistle will be evaluated and documented. Document impact of smother plants in the control of weeds in corn.

V. Context

LCMR funding for this project on weed control began in the 1989-90 biennium, $30,000. Beetles were obtained from Canada and USDA/APHIS gratis. Salaries, facilities and equipment and some research funds are provided by the University of Minnesota Agricultural Experiment Station and USDA-APHIS.

Importation of the new agents will involve the development of ties with International Institute for Biological Control in Europe. In kind contribution: Project Scientists 20 percent or $20,000 per biennium.

VI. Qualifications

Major Cooperators

1. Dr. Donald L. Wyse
   Professor, Department of Agronomy and Plant Genetics
   University of Minnesota
   Ph.D., Weed Science, Michigan State University, 1974
   M.S., Weed Science, Michigan State University, 1972

   Dr. Wyse is the project leader for research related to perennial weed control in agronomic crops and lead scientist on the impact of herbicides on water quality. Areas of research include: (a) influence of tillage on perennial weed development and control; (b) perennial weed biology; (c) mechanism of bud dormancy in perennial weeds; (d) use of biotechnology to develop herbicide tolerant crops; and (e) impact of herbicides on surface and ground water quality.

2. Dr. David W. Ragsdale
   Associate Professor, Department of Entomology
   University of Minnesota

   Ph.D., Entomology and Microbiology, Louisiana State University, 1980
   M.S., Entomology and Microbiology, Louisiana State University, 1977

   Dr. Ragsdale has been involved with integrated pest management and biological control of insect pests of agricultural crops for 15 years. His expertise is in pest management of insect vectors of plant disease agents and serological analysis. Current research projects include developing treatment decision aids for insect vectors of plant viruses on potato, incorporation of wild germplasm into cultivated potato to impart insect and virus resistance, and developing a diagnostic tool and pest management tactics for tracheal mites of honey bees. These projects are funded by the North Central Integrated Pest Management - Competitive Grants Program, the National Potato Council, and the Greater Minnesota Corporation. Dr. Ragsdale's primary role will be to assess the impact of arthropods on control of leafy spurge.

Project 2 Title: **Evaluate and Produce Microbial Pathogens for European Corn Borer, Grasshopper, Potato Scab Disease Control and Develop Monitoring Tools for European Corn Borer in Minnesota**

III. Objectives

A. **Isolate and improve European corn borer and grasshopper microsporidia, develop markers for detection and identification of strains, and, determine pathogen persistence in pest insects in the field.**
Dr. Timothy Kurtti, Dr. Ulrike Munderloh

A.1. Narrative: The goal of this research is to develop effective microbial pathogens for corn borer and grasshopper control. The microsporidia, a primitive phylum of spore forming protozoa, are natural suppressors of corn borer and grasshopper populations (Brooks, 1988). Previous studies have demonstrated the potential of these microorganisms to control insects. Although current strategies for the use of microsporidia are based on three decades of research, success in the field has been limited because available strains have low virulence or persistence. Techniques to isolate or identify strains that possess features important to the control of these major pest insects are restricted to bioassays using insects. More efficacious strains are needed not only for Minnesota but elsewhere e.g., areas of the world that are not accessible for

geographical or political reasons. Insect cell biotechnology (Kurtti and Munderloh, 1987) using cell culture provides us with powerful new tools for strain improvement and selection of microsporidia. We need strains that not only establish themselves permanently, but cause long term population suppression while doing so. To combat unexpected outbreaks, we need virulent strains that can be applied as "microbial insecticides." We are working with three microsporidia that are pathogenic for corn borers (*Nosema pyrausta, Nosema furnacalis,* and *Vairimorpha necatrix*), and one grasshopper pathogen (*Nosema locustae*). These will be characterized biologically and biochemically so that they can be quickly and reliably identified in the field. This will be essential to monitor specificity and persistence.

A.2. Procedures: Lepidopteran and grasshopper cell cultures will be used to isolate and select new strains of pathogens. We will isolate from heterogeneous populations of microsporidia natural variants having characteristics important for insect control, such as increased virulence and persistence. To achieve this goal, spores will be produced in insects or cell cultures, purified, activated (Kurtti et al., 1990) and cloned *in vitro* by limiting dilution or colony (plaque) isolation from infected cell cultures overlaid with solidified media (Ross, Munderloh and Kurtti, in preparation). Clones will be expanded *in vivo* and *in vitro*, and evaluated with respect to their pathogenicity for insects. If possible, we will establish a link between behavior *in vitro* (e.g., growth rate) or colony morphotype (e.g., invasive versus non-invasive) and virulence and persistence. We expect that strains and clones which multiply rapidly and which display an invasive colony morphotype will be more virulent. We will use biochemical techniques to characterize the DNA (agarose electrophoresis; pulsed field or horizontal; restriction endonuclease profiles) (Munderloh et al., 1990) and proteins (polyacrylamide gel electrophoresis) (Streett and Henry, 1985) of the clonal isolates. This information will enable us to identify molecular markers that distinguish clones from one another and allow us to monitor them in future field trials.

To permit year round studies, laboratory reared corn borers and grasshoppers will be used to evaluate potential pathogen strains. Spores mass-produced in insects or tissue cultures will be purified and used to infect colonized or field-collected insects. Standard bioassay techniques (Henry et al., 1979) will be used to determine and compare the virulence (as measured by the dose or time needed to kill or infect 50 percent of the insects, using Probit analysis) of the strains we isolate.

In collaboration with researchers at the Minnesota Department of Agriculture, promising microsporidian pathogens (*V. necatrix* and a *Nosema* species) identified by the procedures outlined above will be evaluated in field trials. Spores produced in corn borers will be purified, quantified and formulated with the addition of ultraviolet light protectants and stickers. Plots of both naturally and artificially infested corn will be treated with dilute spore suspensions using hand held sprayers (Laing and Jaques, 1984). The efficacy of this treatment in protecting plants against first and second generation corn borer infestation will be evaluated as follows: ears and stalks will be dissected and the number of holes and tunnels determined. Dead and live borers will be counted and microscopy used to detect insects infected with microsporidia. These data will also be used to calculate the prevalence of microsporidia in the population. The significance of differences between treatments and between treated and untreated plots will be determined by analysis of variance. The DNA and protein profiles of the microsporidia will be compared with those of the strains applied in the field to assess their identity.

A.3. Budget

a. Amount Budgeted:	$50,000
b. Balance:	$-0-

A.4. Timeline for Products/Tasks

	July 91	Jan 92	June 92	Jan 93	June 93
Design	Accomplished during previous funding period				
Lab Research					
Field Trials	By collective agreement				
Data Analyses	—	—	—	—	
Reports		—	—	—	—

A.5. Status: Final status report June 30, 1993. We improved in vitro cultivation of microsporidian pathogens of corn borers and grasshoppers, prerequisite for controlled production and commercialization of microsporidia as biological control agents.

Three grasshopper cells lines, MSE3, -4, and -8, were evaluated for their ability to support replication of *Nosema acridophagus.* Of the three *Melanoplus sanguinipes* cell lines, *N. acridophagus* spore yields were highest in MSE4. One 10 ml culture yielded an average of 550 million spores, compared to 15 million per caterpillar. In MSE4, *N. acridophagus* numbers doubled every 50 h, and spores developed normally as shown by electron microscopy. Methods were developed to harvest, purify and store spores produced in cell culture. Bioassays evaluating the infectivity and virulence of cultured *N. acridophagus* for grasshoppers and caterpillars will be done this summer. The lines MSE4, -7, and -8, were characterized using karyology and isozyme analysis. Comparisons with tissues taken from nymphal *M. sanguinipes* confirmed the identity of the MSE cell lines.

We continued our work with the microsporidia of corn borers. In vitro development of *Nosema furnacalis* spores was analysed and compared with spores produced in caterpillars. *Nosema furnacalis* formed mainly two types of spores in culture. One spore type had thick spore walls and longer polar filaments; these resembled spores formed in corn borers during the latter stages of pathogenesis. A second spore type, with thinner walls and shorter polar filaments, was similar to spores formed early after per os infection of the insect. Results with other nosemal species indicate that late spores are responsible for horizontal infections and germinate in the midgut of the insect. In contrast, the spores formed during early stages of the disease germinate intracellularly and are involved in tissue cross infection. *Nosema furnacalis* maintained in continuous culture for 70 transfers formed spores infective for *Ostrinia nubilalis.* However, after 40 transfers infectivity and virulence for borers declined due to the predominant formation of early spores. Identification of spores having different morphology, virulence and infectivity is important to the use of microsporidia as biological control agents as these features influence their efficacy and persistence in the field. Several new *O. nubilalis* cell lines were tested for their ability to support replication of *N. furnacalis* in line ONP22 was 31°C. None of four new lines supported continuous growth of *Nosema pyrausta.* An in vitro production system for *N. pyrausta* is still needed. The projects with the grasshopper microsporidian *N. acridophagus* were done in collaboration with Bozeman BioTech and supported in part by a

small business grant from the USDA. Our work on the microsporidia of corn borers was partially supported by a grant from the USDA-CSRS.

A.6. Benefits: Reduced use of chemical pesticides; production and marketing of beneficial and natural insect pathogens by the private sector; and reduced production costs for farmers using pathogens that recycle and sustain themselves in the field.

IV. Evaluation

The persistence of microsporidia in treated populations will be monitored by sampling the populations in subsequent months or years (1-3) and the prevalence of infection determined.

V. Context

LCMR funded this project $43,000 in 1987-88 and $34,000 in 1989-90. Additional support: Grasshopper IPM Project (USDA, APHIS, and PPQ), $40,000, 06/90-09/91; USDA, CSRS Competitive Grants Program, $150,000, 09/90-08/31/92. In kind contribution: Project Scientists 20 percent or $20,000 per biennium.

VI. Qualifications

Major Cooperators

1. Dr. Timothy Kurtti
Associate Professor, Department of Entomology
University of Minnesota

Ph.D., Entomology, University of Minnesota, 1974

Dr. Kurtti is an insect pathologist interested in microbial control of pest insects. He has experience with insect pathogenic microsporidia, viruses and bacteria, and has served on the editorial board of the Journal of Invertebrate Pathology. He advises graduate students whose thesis research involve microsporidia, specifically *V. nacatrix and N. pyrausta.* Dr. Kurtti's primary role will be to isolate and identify strains of microsporidia having potential to control corn borers and grasshoppers and to conduct the bioassays evaluating their efficacy.

2. Dr. Ulrike G. Munderloh
Research Associate, Department of Entomology
University of Minnesota

Ph.D., University of Munich, 1975
D.V.M., University of Munich, 1977

Dr. Munderloh has extensive research experience with microsporidia and other protozoa. In addition to invertebrate pathology she has worked with animal pathogens transmitted by

arthropods (mosquitoes and ticks). Her experience in arthropod tissue culture and molecular characterization of microorganisms associated with arthropods spans more than ten years. Dr. Munderloh's primary role will be the characterization and cultivation of the microsporidian pathogens.

B. Development of packaged microorganisms for biological control of potato scab disease
Dr. Neil Anderson, Dr. Janet Schottel

B.1. Narrative: Potato scab is an important disease in most of the potato production areas in Minnesota. The disease, caused by *Streptomyces scabies*, declined after 30 years of continuous potato cultivation in a Minnesota research plot. From tubers produced in this plot we have obtained non-pathogenic isolates of *Streptomyces* that produce antibiotics that are lethal to disease producing strains. A two year field study was made using three gallon pots to which the suppressive strains grown in a sterilized mixture of sand and peat was added at 1, 5, and 10 percent V/V to disease producing soil. Statistically significant disease reduction occurred at the 1 and 5 percent levels and the disease was eliminated at 10 percent. In the research proposed here we will develop the technology to package the suppressive strains or their anti-pathogen antibiotics for use in controlling the scab disease as an alternative to chemical control.

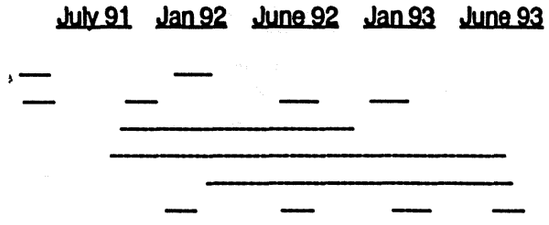
B.2. Procedures: In this research we will develop the technology to deliver suppressive isolates of *Streptomyces* and their anti-pathogen antibiotics to Minnesota potato fields and will assess the various methods for their effectiveness in controlling the disease. Spore suspensions and inexpensive nutrient substrates will be tried as the delivery system for applying the suppressive isolates to potato soils. The survival rate and spread of the suppressive isolates in the soil will also be noted using strains that have specific nutritional requirements. Several inoculations of the same field will be tried to enhance the development of the suppressive strains and increase disease control. Disease control using this procedure may be "permanent" as no scab was noted on tubers grown in the abandoned research plot after 18 years.

The technology for maximizing growth and antibiotic production by the suppressive isolates will be developed. The antibiotic/s will be tested against known pathogenic *Streptomyces* isolates and in disease control tests involving potato tubers. Procedures for isolating and purifying the antibiotic will be developed.

B.3. Budget

	LCMR Funds
a. Amount Budgeted:	$40,000
b. Balance:	$-0-

B.4. Timeline for Products/Tasks



B.5. Status: Final status report June 30, 1993. Three hundred strains of *Streptomyces* have been isolated from a potato scab plot that became suppressive after 30 years of potato monoculture. All of these strains were tested for antibiotic production against pathogenic strains of *Streptomyces scabies* and 64 were found to produce zones of growth inhibition greater than that produced by strain Pon SSII. Five strains were tested in the field. Strain Pon SSII was grown on vermiculite plus on oatmeal broth and added to scab conducive soil at a ratio of 1 percent V:V and placed in 16 L pots set into the soil. Scab was reduced 39, 61, 73, and 80 percent over a four year field test. Strain 93 is the most promising strain tested to date. In field trials approximating growers conditions scab was reduced 47 percent by strain Pon SSII, 29 percent by strain Pon R, 34 percent by strain 32, 43 percent by strain 15, and 57 percent by strain 93. Strains 15, 32, and 93 also produced antibiotics against a number of soil-borne fungal pathogens of potato and in 1993 biocontrol tests are in progress to evaluate their effect on the fungi that cause *Verticillium* wilt and silver scurf of potato. A second inoculum formulation using suppressive mycelium grown in fermentation tanks, freeze dried, and granulated is being evaluated in field tests for biocontrol of scab in 1993. Additional tests on different procedures to granulate the suppressive inoculum are in progress.

Work on purifying the antibiotic produced by strain Pon SSII is in progress. The strain is grown on NMM liquid medium and antibiotic production was monitored throughout the growth curve. Maximum antibiotic activity occurred as strain Pon SSII entered the stationary growth phase and the activity was rapidly degraded after 10 hours into the stationary phase. In purifying the antibiotic, nearly all the activity was recovered after the XAD-2 column chromatography step. After the next two steps, the reverse-phase TLC and cellulose TLC, about 8 percent of the starting activity was recovered. Nearly all of the material was recovered through the final C18 HPLC. The major peak that eluted at 7.84 min contains the antibiotic activity. Further purification will allow for eventual characterization of this compound.

B.6. Benefits: Potatoes are the most important vegetable crop produced in Minnesota. Potato tubers with scab have to be discarded. The disease is important in irrigated production areas and where potatoes have been grown continuously or in short rotations. Scab resistant cultivars are available but are not always the growers choice. Scab resistance in a potato breeding program imposes a genetic bottle-neck and limits the germplasm that could be utilized. At present, control of the scab disease is by fumigation using Vapam, or in furrow treatment with PCNB (pentachloronitrobenzene) or occasionally sulfur. The above research is designed to reduce the use of chemicals and will give growers another alternative in controlling this disease.

IV. Evaluation

Criteria for evaluation include whether we are able to: (1) provide the proper package (substrate) for the suppressive isolate to be delivered to field soils and control or significantly reduce the scab disease, (2) have developed the technology to maximize antibiotic production and have obtained a highly purified form of the antibiotic.

V. Context

The two laboratories involved in this proposal have been involved in cooperative work on *Streptomyces scabies* with emphasis on pathogenicity and biological control. We have isolated and tested suppressive isolates in laboratory, greenhouse and in two-year field studies (using three gallon pots) and have evidence that it was these isolates that were part of the natural phenomenon of disease decline that occurred in the Minnesota research plot. Our research objective is to develop the technology by which we can introduce these organisms into Minnesota soils and determine their effectiveness in controlling the disease. The chemical nature of the antibiotic is necessary before this method can be used as a biocontrol agent.

VI. Qualifications

Major Cooperators:

1. Dr. Neil A. Anderson
   Professor, Department of Plant Pathology, University of Minnesota
   Ph.D. Plant Pathology, University of Minnesota, 1960

   Dr. Anderson has taught Mycology and Genetics of Plant Pathogens and does research on potato pathogens. His pathology input have contributed to five improved potato cultivars released by the Minnesota Potato Breeding Program. His interests are in genetics and biological control, and research is going on in these areas. Dr. Anderson will direct the research on packaging the suppressive isolates and their application and effectiveness in potato soils to control the disease.

2. Dr. Janet L. Schottel
   Associate Professor, Department of Biochemistry, University of Minnesota

   Ph.D. Biology, Washington University, 1977
   Dr. Schottel does research in the area of molecular genetics and is interested in an extracellular esterase from pathogenic Streptomyces scabies that is inducible by zinc. Dr. Schottel will direct the research at purifying and eventually characterizing the antibiotic. Graduate students will do the research as part of their thesis experience.

C. **Develop serological assays to detect pathogens of European corn borer selected for field efficacy trials**
Dr. David Ragsdale

C.1. Narrative: Microbial pathogens are promising biorational agents (bacteria, fungi, protozoa, and viruses). Before any biological organism can be used as a bioinsecticide the environmental fate, efficacy, and effects on non-target species must be determined. It is particularly difficult to identify microsporidians based on gross morphology. Often host range, virulence on alternate hosts, and spore ultrastructure are needed to correctly identify a species. Serological analyses are commonly used to identify strains of pathogenic bacteria and plant and insect viruses. It is the goal of this research program to develop serological tools that can be used to identify *Nosema furnacalis, Nosema pyrausta* and *Vairimorpha necatrix*. Once the serological tests have been developed and characterized, they will be used to evaluate potential impact of microsporidians selected as promising bioinsecticides on non-target organisms, evaluate field efficacy trials, and determine the proportion of the European corn borer population that is currently infected with microsporidians.

C.2. Procedures:
Previous work: With funds provided in 1989-1990 by LCMR, we have grown all three microsporidian species mentioned above in insects and developed methods to insure successful inoculation of spore-free insects. We have purified spores of all three species and have on hand a sufficient quantity to complete the next phase of the study. We have: refined methods to germinate spores, harvested and characterized the sporoplasm, injected both mice and rabbits with sporoplasm for antibody production, and isolated immunoglobulin G (IgG) from these antisera. IgG was used to develop the enzyme linked immunosorbent assays (ELISA). One ELISA we developed and fully characterized reacts only with *Nosema furnacalis*, i.e., it is species specific and can detect infection in first instar larvae of European corn borers 48 hours after infection. Until now any serological based assay for microsporidian identification has always extensively cross reacted with other microsporidian species (Knell 1975, Greenstone 1986, Kawarabata and Hayasaka 1987, Ke et al. 1990).

Proposed work: A major effort will be to complete development of serological assays for *N. pyrausta* and *Vairimorpha necatrix*. A goal of this proposal is to develop the tools that will allow us to determine the environmental fate of microsporidians identified as candidate bioinsecticides. Although we did not need to produce monoclonal antibodies to achieve species specificity with the *N. furnacalis* assay, we have planned for monoclonal antibody development by immunizing mice as well as rabbits. We routinely produce monoclonal antibodies in the laboratory for other serological tests and are technically capable of producing monoclonal antibodies if needed (Ragsdale and Furgala 1987, Ragsdale and Kjer 1989).

Characterization of ELISAs: European corn borers can be infected with each microsporidian species. To determine the sensitivity of the ELISA we will simultaneously infect 400 larvae with each microsporidian species. Ten larvae will be confined per 50 ml diet cup (modified Van der Zant-Adkisson diet) with ten cups per replication. We will inoculate each cup with 1×10^5 spores, and harvest one cup per replication at random every 48 hours. Larvae within each cup will be pooled and spore counts and ELISA will be conducted. This will determine how soon a microsporidian infection can be detected by our ELISA and compare spore concentration to the ELISA absorption values.

Non-Target Organisms: There have been reports of apparent infection of corn borer parasite tissue with microsporidians (Andreadis 1982, Sajap and Lewis 1988). Some have suggested that microsporidian infection may be a contributing factor in the disappearance of *M. grandii* from an area where they once were established (Andreadis 1982). The ELISA assays we are developing react only to germinated spores, thus only active infection is detected and not mere spore presence. We will parasitize microsporidian (*N. pyrausta*) infected European corn borers with *Macrocentrus grandii* and test parasite larvae, pupae and adults for active microsporidian infections. *M. grandii* is currently being cultured in an adjoining lab (R. L. Jones).

We also wish to determine the host range of the exotic microsporidian *N. furnacalis*. Characterization of the host range of this microsporidian must be determined before this species can be approved for field release. We will attempt to infect a wide variety of caterpillars with this microsporidian and use the ELISA to confirm infection by dissecting silk glands and preparing smears from these organs for microscopic examination. The lepidoptera species we plan to test are *Manduca sexta*, *Heliocoverpa zea*, and *Danaus plexippus* (others will be tested as opportunity arises). We will also feed various predators infected corn borer larvae and determine if these non-target species develop an active infection. We will use *Hippodamia convergens, Coleomegilla maculata, Chrysopa carnea, Orius insidiosus* and several *Nabis* spp. for this phase of the study. All of the above predators are common in corn fields and readily consume corn borer larvae.

Screen Minnesota European corn borer populations for microsporidians: We will cooperate with the Minnesota Department of Agriculture when they conduct their annual survey for corn borer parasites. They collect thousands of larvae from throughout the state to determine the parasitization level. Many of these insects die during the process and these cadavers plus all surviving corn borers will be tested for microsporidians using the ELISAs developed above. Cadavers can be kept frozen until the assays are fully developed and tested. This information will be useful in determining what proportion of the natural field population is currently infected with microsporidians. If microsporidians can cause local extinction of exotic parasites, then areas where there are only low levels of microsporidian infection will be targeted for future parasite releases.

C.3. Budget:

a. Amount budgeted:	$40,000
b. Balance:	$-0-

C.4. Timeline for Product/Tasks:

	July 91	Jan 92	June 92	Jan 93	June 93
Purify pathogens	———				
ELISA development & characterization	———	———	———		
Non-target effects				———	———
Screen native corn borer population				———	———
Reports		———	———	———	———

C.5. Status: Final status report June 30, 1993. *Nosema furnacalis* is a microsporidian pathogen originally described from the Asian corn borer, *Ostrinia furnacalis*. It has also been found to infect the European corn borer, *O. nubilalis*, a major pest of corn in the United States. Before a pahtogen such as *N. furnacalis* can be released in a classical biological program for the European corn borer, we must have a method to monitor this microsporidian in the field, and be able to distinguish it from the native microsporidian, *N. pyrausta*. We have developed a species-specific serological assay for *N. furnacalis* that detects only the vegetative stage of this pathogen (Olen and Ragsdale, 1992). We can document the presence of infection in European corn borer within 48 hours of inoculation. Until now, any serological assay for microsporidian identification has always extensively cross reacted with other microsporidian species. In addition, we have tested nine generalist predator species and one parasitoid species for susceptibility to *N. furnacalis* (Olen and Ragsdale, 1993). We found that none of the predators fed *N. furnacalis* spores or *N. furnacalis* infected European corn borers developed an infection, demonstrating that these predators are not potential hosts for this microsporidian.

Serogical assays for the other two species of microsporidian have been discontinued. Monoclonal antibodies raised against *N. pyrausta* have proven unable to distinguish between *N. pyrausta* and *N. furnacalis*. Since *Vairimorpha necatrix* is seldom found in field populations and is morphologically distinct from *N. pyrausta* and *N. furnacalis*, a serological assay for this species was abandoned.

In cooperation with the Minnesota Department of Agriculture, we screened European corn borers collected from throughout Minnesota for microsporidia. Of the 53 counties tested in Minnesota, 37 had *N. pyrausta* spores present. Even with the small number of individuals tested from the various sites, initial data shows that this species is found throughout Minnesota.

Studies are presently being conducted to ascertain if the parasitoid *M. grandii* can become infected with *N. furnacalis*, and the effects of infection on fertility, fecundity, and longevity. In addition, we plan to study at least three other parasitoids of European corn borer for susceptibility to *N. furnacalis*. Also, we are studying the ability of these parasitoids to transmit *N. furnacalis* to uninfected European corn borers, and the degree of any resultant infection. If this occurs, these parasitoids could play an important role in the horizontal transmission of *N. furnacalis* within a European corn borer population. We will again cooperate with the Minnesota Department of Agriculture in their annual survey of Minnesota populations of European corn borer to determine the current level of infection by *N. pyrausta*. We will again look for areas of Minnesota that are free from infection by *N. furnacalis* in a field population of European corn borer. Most of the previous work already cited has been a prelude to release of *N. furnacalis* in the field. This research will be small in scale, in accordance with Environmental Protection Agency requirements, but will provide some much needed information on the viability of *N. furnacalis* in a Minnesota field environment. It will also give us a better understanding of the suitability of *N. furnacalis* as a potential biological control agent of the European corn borer.

C.6. Benefits: The serological assays are powerful tools because they can be made species specific and are relatively easy to run since they do not require any specialized equipment. Serological detection systems will allow us to determine the environmental fate of these entomopathogens once they are released in the field. Without these types of probes persistence of released pathogens is difficult to document. Characterizing infection levels in different European corn borer populations will allow exotic parasites to become established more readily.

V. Context

Funds for this project from LCMR began in 1989, $43,000 for the 1990-1991 biennium. The work is supported by the University of Minnesota Agriculture Experiment Station with salaries, equipment, space, and research funds. In kind contribution: Project scientists: 20 percent or $20,000 per biennium.

VI. Qualifications

Major Cooperators

1. Dr. David W. Ragsdale
   Associate Professor, Department of Entomology
   University of Minnesota

   Ph.D., Entomology and Microbiology, Louisiana State University, 1980
   M.S., Entomology and Microbiology, Louisiana State University, 1977

   Dr. Ragsdale has been involved with integrated pest management and biological control of insect pests of agricultural crops for 15 years. His expertise is in pest management of insect vectors of plant disease agents and serological analysis. Current research projects include developing treatment decision aids for insect vectors of plant viruses on potato, incorporation of wild germplasm into cultivated potato to impart insect and virus resistance, and developing a diagnostic tool and pest management tactics for tracheal mites of honey bees. These projects are funded by the North Central Integrated Pest Management - Competitive Grants Program, the National Potato Council and the Greater Minnesota Corporation. Dr. Ragsdale's primary role will be to develop and field test the immunological assays for detection of microsporidians of the European corn borer (See objective A).

D. **Develop monitoring tools for parasites of European corn borer**
Dr. Richard Jones

D.1. Narrative: Assessment of biological control programs requires effective monitoring techniques. We are identifying, synthesizing and field testing aggregating pheromones and sex pheromones of parasites of the European corn borer. Sex pheromones are ideal monitoring

tools because of their specificity and biological activity. We have identified one compound of an apparent multicomponent sex pheromone of Macrocentrus grandii and have spectral analysis of another parasite Eriborus terebrans completed.

D.2. Procedures: Identification of the active stereoisomer out of a possible eight of an aggregation pheromone produced by both male and female M. grandii will be completed. We will use open column, liquid and gas chromatography, and NMR spectroscopy to complete this characterization.

D.3. Budget

a. Amount Budgeted:	$5,000
b. Balance:	$ -0-

D.4. Timeline for Products/Tasks

	July 91	Jan 92	June 92	Jan 93	June 93
Chemical and spectral analysis	———				
Laboratory bioassays	———		Project ended		
Status Report - Final		—	December 31, 1991		

D.5. Status: **Final status report January 1, 1992.** Understanding the chemical mechanisms that govern insect behavior provides a means for controlling pest species and enhancing beneficial species in a biorational and environmentally safe way. The objectives of this study were to isolate, identify and bioassay the sex pheromone components for *Macrocentrus grandii* and *Eriborus terebrans*, larval parasitoids of the European corn borer. Knowledge of their sex pheromones could lead to enhanced efficacy of these beneficial parasitoids, biological control of the European corn borer and therefore reduce pesticide usage.

For the parasitoid *Macrocentrus grandii* our objectives have been completed. We have [illegible]ted that female *Macrocentrus grandii* possess a sex pheromone with three active [illegible]ents. One component consists of a series of (*Z,Z*)-9,13 hydrocarbon dienes of 27-41 carbon atoms that are attractive to males in wind tunnel and field bioassays. We have synthesized two of these dienes and found the synthetics attractive. A second component, (*Z*)-4-tridecenal, an air oxidation product of each diene, is active alone and it is probably responsible for the pheromonal activity of the (*Z,Z*)-9,13 dienes. We have synthesized this aldehyde component and found the synthetic attractive in wind tunnel and field bioassays. In addition, a third component elicits upwind flight by males in a wind tunnel as well as synergizes the (*Z,Z*)-9,13 dienes and (*Z*)-4 tridecenal in bioassays. This component has been identified as "(3R*,5S*,6R*)-3,5-dimethyl-6-(methylethyl)-3,4,5,6-tetrahydropyran-2-one. We are currently in a cooperative project with the Department of Chemistry proceeding with the synthesis of the active isomer. Due to the complexity of the molecule the synthesis has proceeded slower than initially expected. However, we expect completion by summer of 1992. A combination of (*Z*)-4-tridecenal and the synergist should prove useful in monitoring *M. grandii* populations and establishment and assessment of parasite release programs.

The following manuscripts are completed and have been submitted to the *Journal of Chemical Ecology*. "A multicomponent sex pheromone in *Macrocentrus grandii* (Goidanich) (Hymenoptera: Braconidae)" and "(*Z*)-4 Tridecenal, a pheromonally active air oxidation product from a series of (*Z,Z*)-9,13 dienes in *Macrocentrus grandii* (Goidanich) (Hymenoptera: Braconidae)." A third manuscript "(3R*,5S*,6R*)-3,5-dimethyl-6-(methylethyl)-3,4,5,6-tetrahydropyran-2-one, a third sex pheromone component for *Macrocentrus grandii* (Goidanich) (Hymenoptera: Braconidae)" will be submitted early in 1992. If the synthesis is completed as expected, a manuscript will be delivered describing the absolute configuration and synthesis of the synergistic pheromone component of *M. grandii* and a final paper field bioassaying all the components.

For the parasitoid *Eriborus terebrans* our objectives have been partially completed. This project is part of a student's thesis and will continue. Our study of the sex pheromone components for *Eriborus terebrans* has demonstrated two active chemical fractions. A nonpolar fraction and a very polar fraction that are attractive to male wasps in laboratory bioassays. The nonpolar component is comprised of at least two hydrocarbons. One component has been identified as (*Z*)-9-pentacosene. This component has been synthesized and found attractive to male wasps in a wind tunnel. A second unrelated hydrocarbon has been characterized. Its synthesis is currently under investigation. Both of the hydrocarbon components apparently synergize the polar component. This project will continue through March 1992 at which time results will be described.

D.6. Benefits: Monitoring tools based on sex and aggregation pheromones will increase efficacy of biological control implementation by determining if a previous release in a specific geographical area was successful. Assessment of parasite release without such monitoring tools is extraordinarily difficult and small populations of parasites are easily overlooked. Focusing additional parasite releases on areas where no parasites exist will maximize inoculative release efforts. Establishing European corn borer parasites over a wider geographical area will reduce pesticide use.

IV. Evaluation:

The program can be evaluated by: (1) development of immunological assays that can unequivocally identify a particular microsporidian species, (2) having two components of the aggregating and sex pheromones of two parasites of European corn borer identified and synthesized.

Major Cooperators

Dr. Richard L. Jones
Professor and Head, Department of Entomology
University of Minnesota

Ph.D., Entomology, Insect Toxicology, University of California, Riverside, 1968
M.S., Entomology, Mississippi State University, 1965

Dr. Jones, in addition to his administrative duties, conducts research with semiochemicals and parasitoid behavior and has taught courses in insect behavior and physiology.

Project 3 Title: **Biological Control of the Alfalfa Weevil: Assessing the Effectiveness of the Parasitoid Complex.**
Dr. Edward Radcliffe, Dr. Kathy Flanders

III. Objectives

A. **Assess relative importance of alfalfa weevil parasitoids and parasitoid phenology in relation to alfalfa management strategies**

A.1. Narrative: LCMR funded research is in progress to quantify the effect of natural enemies on alfalfa insect pests, to refine economic thresholds for alfalfa weevil and to assess the impact of insecticides on the alfalfa ecosystem. The project proposed here is intended to provide the additional ecological information necessary for implementation of alfalfa pest management strategies that effectively exploit the benefits of biological control.

A.2. Procedures:

A.2.a. Background: The alfalfa weevil and its natural enemies in the United States. The alfalfa weevil, *Hypera postica* (Gyllenhal), was introduced from Eurasia into Utah in 1904, Arizona in 1939, and Maryland in 1953. Efforts were made to introduce natural enemies from Europe, especially after the introduction of the eastern strain. Among the parasitoids introduced were the larval parasitoids *Bathyplectes curculionis* (Thomson), *Bathyplectes anurus* (Thomson), and *Tetrastichus insertus* (Ratzeburg); the adult parasitoids, *Microctonus aethiopoides* Loan; and *Microctonus colesi* Drea, parasitize late instar larvae and mature in the adult weevil (Day 1981). There is also an insect pathogen, *Zoophthora phytonomi*, which attacks alfalfa weevil larvae (Harcourt et al. 1974).

Alfalfa weevils overwinter as adults and, in some areas, eggs. *Bathyplectes* spp. and *T. insertus* overwinter as pupae in the alfalfa field. *Microctonus* spp. overwinter as larvae within the overwintering weevil (Dysart and Day 1976).

The alfalfa weevil parasitoid complex has been responsible for reducing alfalfa weevil populations in Northeast by 70 percent. Among the parasitoids, *M. aethiopoides* and *B. anurus* are the most effective parasitoids. In areas where it has been introduced, *B. anurus* tends to displace *B. curculionis* (Harcourt 1990).

A.2.b. Background: Alfalfa weevil and its natural enemies in Minnesota. The Maryland, or Eastern strain of alfalfa weevil reached Minnesota in 1970 (Radcliffe and Chiang 1972). *B. curculionis* arrived in Minnesota with its host. *M. aethiopoides* was introduced into Minnesota in 1978-80 by our laboratory (Radcliffe et al. 1983). Subsequent releases in Minnesota were made by USDA, APHIS in cooperation with the Minnesota Department

of Agriculture. In 1985, *M. aethiopoides* was recovered from 45 Minnesota counties (Krueger and Radcliffe, 1986). *M. colesi*, and *T. insertus* were recently established by APHIS, and occurred in one and three counties respectively in 1989 (USDA APHIS did not sample for alfalfa weevil parasitoids in Minnesota in 1990. Apparently, APHIS was unsuccessful in establishing *Bathyplectes anurus* (Niles Biological Control Laboratory 1991). *Z. phytonomi* has never been officially reported in Minnesota, but we observed it in Rosemount in 1984 following a rainy spring.

A.2.c. Context of proposed research. The past 20 years, our laboratory has studied pea aphid and spotted alfalfa aphid natural enemies (Amaya 1980), the alfalfa ecosystem in general (Radcliffe et al. 1976), and has developed economic thresholds for potato leafhopper and pea aphid on alfalfa (Cuperus et al. 1982, Cuperus et al. 1983). These studies have increased our knowledge of the major alfalfa pests in Minnesota, but local information on the alfalfa weevil and its parasitoids is still lacking. No information on phenology of the host and its parasitoids in Minnesota is available. Recent introductions of parasitoids make a statewide survey necessary to determine their distribution and relative abundance. We do not know the extent of the distribution of *Z. phytonomi*. This information is needed in order to make environmentally and economically acceptable management decisions. Research emphasis at the USDA, APHIS Niles Biological Control Laboratory has been shifted away from alfalfa weevil, therefore, further work must be done locally. Work funded by LCMR in 1989-1990 examined alfalfa weevil parasitism in Rosemount. Thus far, only *M. aethiopoides* and *B. curculionis* have been recovered. Parasitism rates were over 50 percent for *M. aethiopoides*, and ranged from 35 to 50 percent for *B. curculionis*.

A.2.d. Research Objectives. Research is planned for the 1991 and 1992 field seasons with the following objectives:

1) Determine distribution of alfalfa weevil parasitoids and *Z. phytonomi* in Minnesota,
2) Determine relative abundance of the parasitoids,
3) Determine percent larval and adult parasitism rates,
4) Determine phenology of parasitoids relative to heat unit accumulations and alfalfa and weevil phenology, and
5) Release *Bathyplectes anurus* into nursery sites in Minnesota, with goal of statewide establishment of this parasitoid.

A.2.e. General methods. Population densities of alfalfa weevil and associated parasitoids will be monitored at 26 locations in the state. Six sites will be monitored intensively on a degree-day schedule to determine host and parasitoid phenology. Twenty sites will be monitored extensively to determine relative importance and distribution of the alfalfa weevil parasitoids. Alfalfa weevil larvae and adults will be field collected, brought to the laboratory, and reared for parasitoid emergence and identification, examined for fungal infection, or dissected to determine parasitism rate. Fields will be located near weather stations for monitoring heat unit accumulations and rainfall. Detailed field histories will be maintained. Nursery sites for release of *B. anurus* will be established at two locations.

A.2.f. Location of sampling sites. Sampling sites (fields) will be located along a 300 mile transect running from Houston County in southwestern Minnesota northwest to Wadena County. This transect will pass through the major alfalfa growing regions within the state. Brief fact sheets explaining the alfalfa weevil, its natural enemies, and the purpose of the project will be mailed in late January 1991 to cooperative extension agents in each county along the transect. Agents will be asked to cooperate by contacting farmers within their county and identifying 2-3 alfalfa fields that could be sampled. Fields will need to be second crop year or older. Fields to be monitored will be chosen from this list of fields. To determine distributions of alfalfa weevil natural enemies, host populations must be high enough in sampled fields to allow for collection of adequate sample sizes, however, detailed histories will be collected for all fields identified for use in this study that cannot be used because of low densities. Results of the surveys will be sent to both county agents and participating farmers.

A.2.g. Extensive monitoring for parasitoid distribution and relative abundance. Twenty fields will be chosen along the transect for this study. Each field will be visited three times: when most larvae are in early instars, and 10 and 20 days later. These intervals should allow us to detect presence of the major parasitoids and *E. phytonomi*. Populations permitting, 1000 alfalfa weevil larvae and 100 adults will be collected from each field each date by sweep-net sampling. These will be held in the laboratory until parasite emergence. Fifty adults and 50 larvae will be collected and frozen, pending dissection to determine parasitism rates. Fifty overwintering adults will be collected in September to determine parasitism of that generation.

A.2.h. Intensive sampling to determine phenology. Six locations along the transect will be monitored every 70 Fahrenheit degree-days, base 48°F from early May through mid-June (approximately 12 dates). Sites will be located near National Oceanic and Atmospheric Administration weather stations or University of Minnesota weather stations to allow access to accurate meteorological data. Fifty adults and 50 larvae will be collected and frozen, pending dissection to determine parasitism rates. Another 50 larvae will be collected and examined for fungal infection. Density and age structure of alfalfa weevil larval populations will be determined each date. One thousand weevil larvae and 100 adults will be collected on three dates and held until parasite emergence.

A.2.i. Insect rearing methods. Standard operating procedures developed by the Niles, Michigan USDA, APHIS alfalfa weevil project will be used. Adults will be placed in rearing cages and held for emergence of *M. aethiopoides* pupae. Alfalfa weevil larvae will be placed in paper bags and fed until pupation. At this time, samples will be allowed to dry down, then bags will be torn apart and examined for parasitoid pupae and fungal infection. 100 healthy alfalfa weevil pupae from the last collection from each field will be reared to adults, and then dissected in order to detect *Microctonus colesi* larvae. Each year, insect rearing will be completed by mid-July.

A.2.j. Dissection methods. Standard operating procedures developed by the Niles, Michigan USDA APHIS alfalfa weevil project will be used. Larvae and adults will be frozen until they can be dissected. Percent of individuals parasitized, and growth stages of the parasitoids will be recorded. Each year, dissection of samples from the intensive and extensive monitoring study will take one person approximately three months.

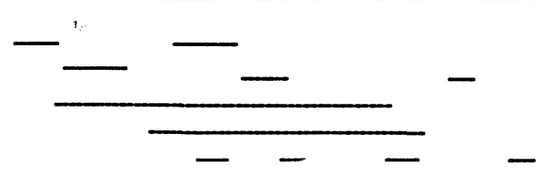
A.2.k. Data analysis. Principal parasitoids in each field will be determined by abundance. Percent parasitized larvae or adults will be used to assess effectiveness of the parasite. Field histories will be examined to see if there is a pattern to alfalfa weevil abundance or parasitism rates. The data collected will not permit detailed association with field history, but will be useful in suggesting future experiments. Likewise, the data may suggest interspecies interference within the parasitoid complex, which could be examined in future experiments. Published base temperatures for development will be tested to see if heat unit accumulations are useful in detecting first and peak occurrence of alfalfa weevil larvae, first and peak emergence of *M. aethiopoides* from weevil adults, and first and peak parasitization of alfalfa weevil larvae. Amount of fungal infection will be examined in conjunction with rainfall amounts and heat unit accumulations. These data can be combined to make specific management recommendations for each alfalfa growing region along the transect.

A.2.l. Introduction of Bathyplectes anurus. Permission to import alfalfa weevil larvae from areas where *B. anurus* occurs into Minnesota will be obtained from the Minnesota Department of Agriculture. Alfalfa weevil larvae, a proportion of which will be parasitized by *B. anurus*, will be obtained from cooperators in the NC-193 alfalfa project. For maximum chance of recovery, approximately 2000 *B. anurus* should be released into each site (M. D. Bryan, USDA APHIS, personal communication). Nursery sites will be chosen in 1991 that provide wooded refuge for alfalfa weevil adults on at least two sides. First harvest will be delayed until after *B. anurus* has pupated. No insecticides will be used. First crop alfalfa will be chosen, in hope that the alfalfa field can be maintained for at least three years. One such site has already been located at Rosemount, Minnesota. The second will presumably be located at another state experiment station. Releases into nursery sites will be made each year, with first recovery sampling in spring 1993.

A.3. Budget

a. Amount Budgeted:	$66,000
b. Balance:	$-0-

A.4 Timeline for Products/Tasks



[a]Field work will actually begin in May 1991. Recovery sampling for *B. anurus* will be only field sampling in spring 1993.

A.5. Status: Final status report June 30, 1993. Alfalfa fields sampled are located along a 300 mile transect from Houston Co. in southeast Minnesota to Wadena Co. in Northwest Minnesota. Six (1991) or seven (1992) fields were sampled intensively to determine parasitoid phenology. Each year, 20 fields were extensively sampled to determine parasitoid distribution.

Host density: Alfalfa weevil occurred at moderate (southeast) to very low (northwest) densities in 1992. Cool, dry conditions permitted many alfalfa weevil eggs and larvae to survive first alfalfa harvest. Atypically, alfalfa weevil larvae were common during the fourth harvest in September 1992.

Parasitoid distribution: The adult parasitoid *Microctonus aethiopoides* (Braconidae); the larval parasitoids *Bathyplectes curculionis* (Ichneumonidae), and *Tetrastichus incertus* (Eulophidae); and the fungal pathogen *Zoophthora phytonomi* (Entomophthoraceae) were common throughout the survey area. *Bathyplectes anurus* is now established in southeastern Minnesota, but present only in low densities. *Microctonus colesii* was not recovered.

Parasitoid phenology and importance: *Microctonus aethiopoides* parasitism rates were lower in 1992 than in 1991. *M. aethiopoides* apparently overwinters as a late instar larva in Minnesota, and completes at least two generations per year. When first alfalfa harvest occurs on a normal schedule (as in 1992), most *M. aethiopoides* are still larvae within their hosts. Harvest causes the weevils to leave the alfalfa - usually moving to nearby woodlots where *Microctonus* larvae emerge, pupate, then parasitize new hosts. Delayed harvest (as in 1991) results in *Microctonus* completing development and pupating within the alfalfa field. When the weevils subsequently depart, the parasitoid and its host are spatially separated. Leaving strips or borders might retain alfalfa weevil adults and their parasitoids, thus enhancing the level of biological control. *Tetrastichus incertus* was most common during second and third harvests, with almost 100% of alfalfa weevil larvae parasitized. *Bathyplectes curculionis* has two generations per year, with greatest impact during the second cutting. *Zoophthora phytonomi* has its greatest impact early in second harvest. Epizootics caused collapse of larval populations in 1991, but did not occur in 1992.

Our 1991-92 survey for distribution of alfalfa weevil parasitoids resulted in a new state record for occurrence of the larval parasitoid, *Bathyplectes anurus*, and numerous county records for other parasitoids. Three parasitoids of alfalfa weevil, as well as one pathogen, are widely distributed and collectively reduce alfalfa weevil populations by 90% or more. Dissections of alfalfa weevil show that the timing of occurrence of the parasitoid species is predictable when plotted on a degree-day scale. More work is needed to confirm parasitoid phenology, and to determine effect of management practices on alfalfa weevil natural enemies.

A.6. Benefits: Alfalfa is grown on over 2 million acres of Minnesota cropland. Since the primary use of alfalfa is as feed for dairy animals it is important to minimize use of insecticides. In Minnesota, biological control usually provides adequate levels of pest control. Nevertheless, annual losses to alfalfa insects still run in the millions of dollars and in some years tens of millions. Research will suggest alfalfa management strategies, e.g., cutting and spray schedules, most complementary to biological control of alfalfa weevil.

Our research product is intended to be the establishment of an additional alfalfa weevil parasitoid and the formulation of alfalfa pest management strategies that enhance effectiveness of natural enemies.

IV. Evaluation

Criteria for evaluation include whether we are able to: 1) document the distribution and relative importance of alfalfa weevil parasitoids in different regions of the state, 2) determine parasitoid and pathogen phenology, and 3) provide farmers with recommendations regarding alfalfa management strategies that enhance or maintain effectiveness of alfalfa weevil natural enemies.

V. Context

A. Our laboratory was responsible for the establishment of *Microctonus aethiopoides* in Minnesota. This parasitoid now occurs throughout the state and is a major cause of mortality in alfalfa weevil adults. *Bathyplectes curculionis* arrived in Minnesota with the alfalfa weevil and is a major cause of larval mortality. Two other parasitoids, *Microctonus colesi* and *Tetrastichus insertus* were recently established by APHIS, but still occur only locally. Apparently, APHIS was unsuccessful in establishing a sixth parasitoid, *Bathyplectes anurus*. We intend to release *B. anurus*, and believe establishment should be successful if initial releases are made in favorable sites specifically managed to favor the parasitoid. The research objectives of this project are to assess the relative contribution of each parasitoid species to regulation of alfalfa weevil numbers and determine best alfalfa management practices based on natural enemy distribution and phenology.

LCMR provided $36,000 funding for the first phase (1989-1991) of this project. An additional $30,000 was provided by the Minnesota AES. The proposed second phase of this research (1991-1993) will need to be initiated prior to the LCMR fiscal year. During this time, costs of approximately $20,000 will be borne by our MAES alfalfa project. An additional in kind contribution of $10,000 per biennium (Project Scientist salary 10 percent) will be provided.

VI. Qualifications

Major Cooperators:

1. Dr. Edward B. Radcliffe
   Professor, Department of Entomology, University of Minnesota

   Ph.D. Entomology, University of Wisconsin, 1963
   M.S. Entomology, University of Wisconsin, 1961
   B.S.A. Entomology, University of Manitoba, Winnipeg, Canada, 1959

Assistant Professor, University of Minnesota, 1965-70
Associate Professor, University of Minnesota, 1970-76
Professor, University of Minnesota, 1976-present

Radcliffe has 30 years of experience in integrated pest management research. His interests and expertise include studies of biological control agents on alfalfa, development of action thresholds for alfalfa and potato pests, studies on the spread of aphid-borne viruses in potato, selection and breeding for host plant resistance to insects, and research on natural product insecticides. Currently, research is underway in each of these areas. Dr. Radcliffe's primary role will be in experimental design and project oversight.

2. Dr. Kathy L Flanders
Research Associate, Department of Entomology, University of Minnesota

Ph.D. Entomology, University of Minnesota, 1989
M.S. Entomology, University of Minnesota, 1986
B.S. Plant Protection, Cornell University, 1980

Research Assistant, University of Maine, 1981-82.
Research Associate, University of Maine, 1983.
Graduate Research Assistant, University of Minnesota, 1984-89.
Graduate Teaching Assistant, University of Minnesota, 1984-85.
Research Associate, University of Minnesota, August1989-present.

Dr. Flanders is currently studying the impact of insecticides on the alfalfa ecosystem, an LCMR funded project. Past research, in cooperation with Dr. Radcliffe, has included developing action thresholds for green peach aphids in seed potato production, determining temporal and spatial dynamics of potato leafhoppers, and evaluating wild potato accessions for resistance to potato leafhopper and potato flea beetle. Dr. Flanders attended the workshop on alfalfa weevil biological control techniques at USDA APHIS in Niles, MI in 1990. Dr. Flanders' primary role will be to conduct field research involved in this project and to analyze the results.

Project 4 Title: **Biological Control of Corn Rootworms in Minnesota**
Dr. Kenneth Ostlie, Ms. Ann Journey

III. Objectives

A. **Selection and field evaluation of nematode strains and natural plant product baits.**

A.1. Narrative: While integrated pest management (IPM) tactics have reduced insecticide use by 50 percent over the last ten years, more insecticide is used annually on corn rootworm than any other insect pest in Minnesota. Applied to the soil, these insecticides pose a potential threat to water quality, nontarget organisms, and applicator health. Previous work has evaluated effectiveness of nematodes as a biological control option. Strains with greater virulence and better host location abilities are currently under selection. Research has also shown that certain natural plant products, such as cucurbitacins, selectively attract corn rootworm adults and stimulate feeding. When combined with an insecticide or pathogens, these lethal baits could control adults and eliminate the need for soil insecticides. This project will focus on completion of selection and field evaluation of nematode strains, resolve problems in the nematode rearing process, and explore use of baits to effectively control adult corn rootworms.

A.2. Procedures: Nematode strains will be bioassayed for their virulence and stability through rearing procedures. Strains will be improved through recurrent selection against corn rootworm larvae and through host location tests in soil arenas. These efforts are aimed at producing more virulent nematodes with improved host locating behavior. Field experiments will evaluate strain effectiveness and explore use criteria (e.g., application rate). Cucurbitacin baits containing trace amounts of insecticide will be compared in field trials to two current tactics for corn rootworm management: broadcast insecticide application for adult control, and soil insecticide application for larval control.

Note: Considering the implementation hurdles already identified, I am reluctant to devote resources at a molecular level for two reasons. First, reliable performance of any strain under field conditions is lacking. Second, the nematode *Steinernema feltiae* is exempt currently from environmental risk assessments required by EPA under FIFRA. As proposed, the artificial selection component of this project will not change that exempt status but release of a genetically engineered strain of the nematode/bacterial system would trigger an environmental risk assessment. A number of research articles on *Steinernema feltiae* and its associated bacterium *Xenorhabdus nematophilous* have addressed the feasibility of genetically improving the nematode or bacterial components. Artificial selection or hybridization is more appropriate for the nematode. The bacterium *X. nematophilus* could be genetically engineered but currently its extreme virulence and broad host range suggest that the bacterium hardly needs modification. Thus, the weak link at this point is nematode's delivery of the bacterium to the corn rootworm. Improving the nematode's performance does not require work at the molecular genetics level.

A.3. Budget

	LCMR Funds
a. Amount Budgeted:	$40,000
b. Balance:	*$ -0-

* The project will continue uninterrupted by moving funds from other projects. This is a common bookkeeping situation.

A.4. Timeline for Products/Tasks

	July 91	Jan 92	June 92	Jan 93	June 93
Expt. design	—				
Strain selection & lab bioassays	——	——	——	——	——
Field studies with nematode	—		——		——
Field studies with bait	—		——		——
Data analysis & evaluation	—		—	—	——
Reports		—	—	—	—

A.5. Status: Final status report June 30, 1993. During the summer of 1992, a field trial at the Rosemount Agricultural Experiment Station evaluated the efficacy of *Steinernema carpocapsae,* All strain, as a biological control agent for the western corn rootworm (WCR), *Diabrotica virgifera virgifera.* This experiment investigated the effects of rate and timing of nematode application relative to rootworm development. Nematodes significantly reduced root injury in 4 treatments: 10^6 and 10^7 nematodes/row-ft applied to early third instar WCR larvae, and 10^6 and 10^7 nematodes/row-ft applied to early second instar larvae. Root injury remained below the economic threshold (root rating of 3.0) in 3 treatments: 10^7 nematodes/row-ft applied to early second or third instar larvae, and 10^6 nematodes/row-ft applied to early thirds. Although nematodes provide adequate root protection, they did not prevent WCR larval feeding altogether in 1992. Root ratings were slightly above those observed with effective soil insecticides.

- Adult WCR emergence declined significantly from the agar controls only when 10^7 nematodes/row-ft were applied to early third instar WCR. Within this rate treatments, all nematode applications made after "egg stage" significantly reduced adult emergence relative to that early application. In general, later applications were more effective at reducing adult emergence. However, emergence was complicated by competition for food among rootworm larvae; beetles collected last summer are being sexed and weighed to provide some quantitative means by which to separate the effects of nematode mortality and competition. Root and yield samples are also being processed. Extensive biomass measurements are being made on the roots in order to develop a more descriptive root injury rating system than the Iowa 1-6 scale. Yield samples are being counted and weighed; unfortunately, it may be impossible to distinguish between the effects of rootworm feeding and those of last summer's cool, wet weather.

The 1993 nematode field trial is underway, but there are no data to report. This trial explores the effects of nematode species and application rate on WCR control. *Steinernema riobravis* became available for field work this summer; its performance will be compared to our benchmark *Steinemema carpocapsae,* All strain. Although *S. riobravis* is considerably more virulent than All strain against corn rootworm larvae in laboratory bioassays, its field performance has not been evaluated. Corn was planted in a timely manner (May 14), but unfavorable weather has delayed its development. The plants reached a stage suitable for rootworm egg infestation (2-leaf) on the 11th of June. Nematodes will be applied when the rootworm population reaches its most-vulnerable early third instar. Five replicates of 4 nematode rates (10^4, 10^5, 10^6, and 10^7 nematodes/row-ft), an agar control, and an insecticide control will be used. WCR emergence cages will be placed in the plots after nematode application, and will be monitored weekly until the end of adult emergence. Roots will be washed and rated using the Iowa 1-6 root injury scale in late August. Half of each 60-plant plot is reserved for harvest.

A.6. Benefits: Developing viable adult and/or larval control alternatives would reduce soil insecticide use and associated environmental and health risks. Additionally, this research would diversify our control options.

IV. Evaluation

For the FY 92-93 biennium, this research program can be judged by its accomplishment of the following objectives:

1. Establish baseline virulence information on nematode strains.
2. Evaluate effects of recurrent selection through corn rootworm larvae on virulence.
3. Quantify host location
4. Demonstrate nematode performance against corn rootworms under field conditions.
5. Explore potential of cucurbitacin baits as an adult management tool.

V. Context

Developing viable management options for corn rootworms is an integral part of research efforts that have already reduced soil insecticide use by 50 percent since 1978. Adult scouting coupled with reduced rates could further lower use of soil insecticides on 20 percent of Minnesota's corn acreage. Growing concerns about soil insecticide use (wildlife, water quality, health) and evidence that crop rotation may not be effective against northern corn rootworms necessitate the development of new management options.

Funding by LCMR for this project began in 1987: $51,000 for 88-89 and $40,000 for 90-91. The research is cooperative between University of Minnesota, USDA-ARS at Brookings, South Dakota and the Minnesota Department of Agriculture. All three agencies support salaries, space, and some research costs. In kind contribution: Project scientist 10 percent or $10,000 per biennium.

VI. Qualifications

Major Cooperators

1. Dr. Kenneth Ostlie
   Associate Professor, Department of Entomology
   University of Minnesota

   Ph.D., Entomology, Iowa State University, 1984
   M.S., Ecology, Utah State University, 1980

Dr. Ostlie's primary expertise is the integrated pest management of corn insect pests, including corn rootworms. His work with corn rootworms encompasses basic biology, the influence of cultural practices (e.g., tillage), economic thresholds, yield losses and insecticide performance. Recently, his work documented a new wrinkle in corn rootworm management, the ability of northern corn rootworms to circumvent crop rotation. Besides research, he has more than six years of experience with Minnesota Extension Service in delivering this research information and helping farmers improve their management of corn rootworms.

Project 5 Title: **Biological Control of European Corn Borer In Sweet Corn**
Dr. David Andow, Dr. D. M. Olson

III. Objectives

A. **Develop improved strains of *Trichogramma* for control of European corn borer in sweet corn.**

A.1. Narrative: Researchers in Europe, China, and the Soviet Union have claimed that *Trichogramma* wasps can be used effectively to control European corn borer in field and seed corn, however, there has been relatively little work with these parasitoids in sweet corn. Indeed, our evaluation of the scientific literature published on the efficacy of *Trichogramma* in these countries is that it is not completely conclusive. This literature suggests that *Trichogramma* is likely to be an effective biological control agent in sweet corn. Our approach is to identify the major factors that limit the effectivess of *Trichogramma* in sweet corn and then look for species or select for strains that overcome these limitations.

Sweet corn receives the second highest amount of insecticide of all crops in Minnesota. Previous work has demonstrated that *Trichogramma* markedly reduced ECB populations, but high numbers were required (400,000/acre). Control is nearly adequate for processed corn, but not for fresh market corn, because a high proportion of ear tips were damaged by a few larvae. Appropriate *Trichogramma* release densities and the major practical limitations of one species have been determined. These limitations are that host finding is inefficient and survival in the field is poor. The focus of this objective is to import, develop and evaluate more efficient strains and species of *Trichogramma*.

A.2. Procedures: We will establish laboratory colonies of native *Trichogramma* species, select strains of our laboratory colonies, and possibly import new species of *Trichogramma* from abroad. We will evaluate host finding ability of several strains or species of *Trichogramma* on a variety of simulated and real plant surfaces in the laboratory and the field to find strains or species that have better searching efficiency than the one we have been evaluating. We will begin investigation into the factors that affect adult mortality in the laboratory and the field.

A. 3. Budget

	LCMR Funds
a. Amount Budgeted:	$60,000
b. Balance:	$ -0-

A.4. Timeline for Products/Tasks

	July 91	Jan 92	June 92	Jan 93	June 93
Field experiments	—		——		——
Lab experiments		——		——	
Data analyses		——		——	
Reports		—	—	—	—

A.5. Status: Final status report June 30, 1993. Continuing research on host handling behavior of *Trichogramma* and preliminary results from sibling matings and the assays of the progeny of *T. nubilale* suggest that there is little genetic influence in percent parasitism and number of eggs parasitized although there appears to be a significant maternal effect on these parameters. Further investigation will hopefully indicate the source of this enviromental influence on egg handling behavior. Also, egg handling behavior on irradiated versus non-irradiated ECB eggs are being investigated to determine the effect of the embryo on the oviposition behavior and the possible mechanism of host size assessment for *T. nubilale* on ECB egg masses.

In addition, final results of 3 *Trichogramma nubilale* releases against European corn borer (ECB) from our cooperative work with the Ag Research Department of Pillsbury/Green Giant during the summer of 1992 are: (1) Corn plant variety had no effect on parasitism rates of ECB. (2) Parasitsm rates were low in the snap bean releases of *T. nubilale* most likely because of cold weather and resulting delayed emergence of the wasps. (3) Shorter corn plants had higher parasitism in both the first and second generation of ECB infestations even when releases of wasps occurred at the same time on corn planted at different times. This suggests that our previous observations of higher parasitsm on corn at anthesis than in the whorl stage was not due to the differences in corn stage or other environmental factors in the field such as the wind, but to the quality differences between the wasps that were released in the first generation and the second generation of ECB flight. Future emphasis will be placed on the quality of the released parasitoids and factors influenceing the persistance of this quality.

A.6. Benefits: Reduction in insecticide use and creation of demand for cottage industry in beneficial insect production.

IV. Evaluation

Does our protocol provide a solid basis for evaluating *Trichogramma* where other experimental methods do not?

V. Context

LCMR funding for this project began in 1987; $118,000 for 87-88 and $45,000 for 89-90. Salaries, equipment, and facilities are contributed by Minnesota Department of Agriculture and

the University of Minnesota MAES. In kind contributions: Sweet corn industry in the form of producer's fields, Project scientist 10 percent or $10,000 per biennium.

VI. Qualifications

Major Cooperators

1. Dr. David A. Andow
Associate Professor, Department of Entomology
University of Minnesota

Ph.D., Ecology, Cornell University, 1982
Dr. Andow's interests and expertise range over several areas of sustainable agriculture, and he has a particular interest in biological control. He has published extensively on the ecology of *Trichogramma*, has over six years experience working with these organisms, and has supervised a post-doctoral associate, who conducted several experiments on the feasibility of *Trichogramma*.

Project 6 Title: **Biological Control of the Cereal Leaf Beetle, the European Corn Borer and Selected Species of Grasshoppers**
Dr. Dharma Sreenivasam, Dr. John Luhman

III. Objectives

A. **Assess and enhance biological control agents for the cereal leaf beetle *Oulema melanopus* (L.) in Minnesota.**

A.1. Narrative: Minnesota Department of Agriculture detected the cereal leaf beetle in 1986 for the first time in Fillmore County, southeast Minnesota. By 1989 cereal leaf beetle has spread westward into 20 counties with populations increasing at a slow rate. Approximately 30 percent of the state's small grain crops valued at $40 million are harvested annually from this 20 county region. Burger and Holmes in 1971 stated that this region offers the greatest potential for cereal leaf beetle to reach pest status in Minnesota.

A.2. Procedures: Two species of parasitoids have proven effective as biological control agents in Michigan and Indiana where chemical control is not used anymore. *Anaphes flavipes* (Foerster) attacks eggs and *Tetrastichus julis* (Walker) attacks larvae. The egg parasitoids can be mass reared in the laboratory while the larval parasitoid can only be reared in field insectaries. USDA Niles, Michigan Biological Control Laboratory discontinued rearing cereal leaf beetle parasites in 1982.

Field collected parasitized eggs will be obtained from Indiana and reared in the lab in year 1. Rearing procedures developed by Niles Laboratory will be used to mass produce *A. flavipes* for release in year 2. Five Minnesota counties have traces of the larval parasitoid *T. julis*. Extensive cereal leaf beetle larval collections will be taken, reared in the laboratory and released in two fields selected for insectaries.

A.3. Experimental design: Ten small grain fields will be selected, located in five counties. Site selection will be based on topography, cropping history, soil type, farming practices, precipitation, degree day accumulations and no insecticide use. Cereal leaf beetle eggs, larvae and adults will be collected from five fields in the first year and five fields in the second year. Parasitoid releases will be conducted in the first five fields at the end of second year, and the second five fields at the end of third year. Intensive monitoring and sampling will continue for four years.

Budget

	LCMR Funds
a. Amount Budgeted	$20,000
b. Balance	$5,007

A.4. Analysis: Parasitoid abundance, percent parasitism, and rates of parasitization and spread over time will be determined. Relationships between degree day accumulations, occurrence of cereal leaf beetle life stages and parasitoids will be examined to determine parasitoid and beetle phenology.

Timeline for Products/Tasks	July 91	Jan 92	June 92	Jan 93	June 93
Lab rearing	———	———	———	———	———
Field work	——		——		——
Data entry & analysis		——		——	——
Reports		—	—	—	—

A.5. Status: Final status report June 30, 1993. Cereal Leaf Beetle. No parasites, but several adults were reared from 20 eggs received from Indiana in May. Populations were very low in 1992. Our surveys found one small CLB larva and traces of adult and larval feeding in Wabasha Co. The Niles, MI, laboratory is reentering the CLB project to rear CLB parasites. We are listed as cooperators for 1994 season.

B. **Biological Control of the European corn borer and evaluate promising microsporidian pathogens identified in Project 2A for the European corn borer and grasshoppers.**

B.1. Narrative: Previous studies have demonstrated the potential of microsporidia to suppress corn borer and grasshopper populations. Success has been .limited because of low virulence and persistence of the microsporidia. Kurtti and Munderloh, 1987, have developed a technique for strain improvement and selection of microsporidia. They will be working with three microsporidia *Nosema pyrausta*, *N. furnacalis* and *Vairimorpha necatrix* that are pathogenic to corn borers, and *Nosema locustae*, pathogenic to grasshoppers.

B.2. Procedures: Three species of grasshoppers, the twostriped (*Melanoplus bivittatus*), the migratory (*M. sanguinipes*) and the redlegged, *M. femurrubrum* will be reared in the laboratory. European corn borer colony is already being maintained in the lab. Several life stages of these colonies will be used to evaluate potential pathogen strains, using standard bioassay techniques. Promising microsporidian pathogens will be evaluated in field trials. Plots will be treated with dilute spore suspensions using hand held sprayers.

B.3. Experimental design: Four one-acre plots: Two sweet corn and two field corn selected without insecticide to test effect on both generations of naturally occurring European corn borer; Two one-acre plots each of small grains, soybeans and alfalfa without insecticide to test effect on the grasshoppers. All plots will have one control and one treated field.

Budget

	LCMR Funds
a. Amount Budgeted	$20,000
b. Balance	$-0-

B.4. Analysis: For the corn borer study, ears and stalks will be dissected and the number of holes and tunnels counted. Dead and live borers will be subjected to microscopy to detect insects infected with microsporidia. This data will also help to calculate the prevalence of microsporidia in the European corn borer population. For grasshopper study, feeding damage will be quantified, densities will be determined and the percent microsporidial infection. Analysis of variance will be calculated to determine the significance of differences between treatments and between treated and untreated plots.

Timeline for Products/Tasks	July 91	Jan 92	June 92	Jan 93	June 93
Lab rearing	———	———	———	———	———
Field work	——		——		——
Data entry & analysis		——		——	——
Reports		—	—	—	—

B.5. Status: Final status report June 30, 1993. European Corn Borer. Large-scale releases of the ECB egg parasitoid *Trichogramma nubilale* were made in sweet corn fields of six organic growers in the summer of 1992. Releases were made in June-July for control of first generation ECB and in August-September for second ECB generation control. The release rate ranged from 125,000 to 168,000 females per acre. First generation parasitism levels of egg baits ranged from 65 to 89% in the release areas. There was evidence that the wasps had dispersed considerably from the original release points. No ECB were observed in corn stalks collected during the first generation from either control or release plots. Second generation parasitism levels of egg baits ranged from 35 to 88% with a 40 to 66% reduction in infestation levels in release plots compared to control plots.

Laboratory Production

Rearing of European Corn Borer (ECB) egg parasitoid, *Trichogramma nubilale* continued at the MDA laboratory, at the Dakota Co. Technical College. In the past two years, the rearing process has been streamlined so 14 million ECB eggs can be produced in one field season. Part of this has been accomplished by improving on procedure such as the synchronization of the parasitoid exposure days, refrigeration of the parasitized eggs, and by doubling the cage life through improved feeding methods of the ECB adults.

The number of ECB larvae per diet dish was maximized which in turn maximizes the number of moths per cage, therefore increasing ECB egg production. Other observations have included: tolerance of *T. nubilale* in late larval and pupal stages; refrigeration of ECB eggs for both ECB and parasitoid production; and effect on longevity and fecundity by feeding moths sucrose.

Overall in 1991 and 1992 *T. nubilale* production was estimated through the number of ECB egg sheets used and reached over eight million parasitoids. Potential parasitoid production, based on unused egg mass weight, was about an additional six million. This production was from over 30 cages with an average of six cage set ups per week; more in the summer and fewer in winter. Each cage containing three ECB pupal rings, each of these rings releasing approximately 400 moths, half of which are females, totaling 600 females per cage. Each female is able to produce approximately 250 eggs, therefore permitting a potential average of 125,000 eggs per cage per day.

Field Releases

1991 releases of the egg parasitoid, *T. nubilale,* were made in June. These resulted in considerable reduction of ECB damage to corn plants and ears. The number of tunnels per plant in release plots averaged 0.66; that of the control plots, 2.26. The quality of the corn reflected these lower rates of infestation. Overall undamaged length of ears averaged 6.06 inches in the release plot; that of the control, 5.35 inches. Baits of ECB egg masses were placed in the plots in an attempt to recover progeny from earlier releases. Percentages of egg parasitism in the release plots was typically 10-50%; that of the control 0-2%.

1992 mass releases of the egg parasitoid, *T. nubilale,* were made late June to early July for 1st generation ECB, and the last 3 weeks of August for 2nd generation ECB. These releases were made in sweet corn fields of six organic growers from five counties: Wabasha, Goodhue, Dakota, Scott and Morrison. The release rate ranged from 125,000 to 168,000 females per acre. ECB infestation was reduced 40-60% in release plots compared to that of the control. Egg parasitism was 65-89% during the 1st generation, and 35-89% during the 2nd. There was no parasitism of ECB egg masses in control plots. There was evidence that the wasps had dispersed considerably from the original release points.

1993 releases of *T. nubilale,* started on Wednesday, June 23 in Farmington, Dakota County. Between 100,000 and 150,000 T. nubilale will be released per acre during a three week period. Thursday, parasitoids will be released in Goodhue and Wabasha counties.

European Corn Borer parasite surveys from fall 1990 to 1992.

In the fall of 1990, 1991 and 1992, 2485, 2114 and 1028 European Corn Borer (ECB) larvae were collected, respectively, from 66 to 69 counties as part of the continuing ECB survey in Minnesota. *Beauvaria bassiana* is the most common disease in larvae and four species of parasitoids are recovered from year to year: ichneumonid *Eriborus terebrans* (Gravenhorst); braconid *Macrocentrus grandii* (Goidanich); tachinid *Eumea caesar* (Aldrich) and the eulophid *Symplesis viridula* (Thomson).

Parasitism killed an average 10 % to 20 % in 1990 and 1991, adding a few counties to the list. *E. terebrans* was recorded for the first time in McLeod and Rice counties in 1990, and in Pennington and Pipestone counties in 1991. *E. caesar* was recorded in 1990 from Mashall and Wadena counties. In 1992, six new records of recovery were established for *E. terebrans*: the counties of Kittson, Clearwater, Mahnomen, Clay, Swift, Lincoln, and Wabasha. *M. grandii* was recorded for the first time in Pennington county which is quite remarkable because of the distance from its normal distribution in Minnesota. *E. terebrans* is often recovered from the south and west, while *M. grandii* is found mainly in the south east. *E. caesar* and *S. viridula* were recorded for the first time in Norman and Nobles counties, respectively. A first time record was *Agrypon prismaticum* Norton, an ichneumonid, in Isanti county. As a general pattern, the range of distribution of the four main parasitoids has been increasing since the recoveries first began in 1977.

In 1992, approximately 20% of the larvae were killed by disease or parasitism, the remaining emerged as healthy ECB adults. Overall, 40% of the mortality in the collected ECB larvae was from *Beauveria bassiana*. *E. terebrans* and *M. grandii* account for 25% and 20% of the ECB mortality, respectively. Unknown causes of death reached 10 %. All *E. terebrans* and *M. grandii* reared from the ECB larvae were added to their respective colonies for production of more parasitoids that will be used during the 1993 summer for field releases.

Grasshopper Rearing. Our lab at Dakota Co. Technical College continued rearing redlegged, migratory, differential, and twostriped grasshoppers in conjunction with experiments at the University of Minnesota on microbial pathogens. Next season we will continue surveys for tachinid fly parasites of grasshopper nymphs and adults from live collections. We will also survey for egg parasites and predators by setting out grasshopper egg baits as sentinels.

We have completed a study to determine the effect *Nosema furnacalis* has on the predator populations of European corn borer. Using microscopic examination and a species-specific ELISA we found 17 of 554 predators tested weakly positive with ELISA, and few spores were found. These data suggest that *N. furnacalis* cannot establish an active infection in any of these predators. Initial data indicates that at least one parasitoid of European corn borer may be susceptible to *N. furnacalis*.

Data from the cooperative study with the University of Minnesota Department show that *N. pyrausta* was present in 37 of 53 counties surveyed in Minnesota, with spores being distributed throughout the entire sampling area.

B.6. Benefits: Development and implementation of biological control agents as a major control strategy; transfer rearing technology to private industry; and promote reduced use of chemical controls.

IV. Evaluation

Experimental release fields will be monitored for two or three growing seasons to determine establishment of biological control agents and their effectiveness for a wider application. Cost of biological control will be compared with other alternative controls on a long term basis.

V. Context

Partial funding from LCMR in 1987-88 $49,000 and 1989-90 $49,000; Minnesota Department of Agriculture supported 15 percent or $5,000 and 50 percent or $25,000 staff time for the two bienniums respectively. Change level requests by Minnesota Department of Agriculture have been turned down. Dakota County Technical College at Rosemount provides greenhouse and lab facilities. Student Ag. Technicians and student interns from College Internship Program are hired seasonally.

VI. Qualifications:

LCMR Program Manager

1. Dr. Dharma D. Sreenivasam
   Entomologist and Supervisor, Minnesota Department of Agriculture
   Associate Professor, Adjunct, Department of Entomology, University of Minnesota

   Ph.D., Entomology, University of Wisconsin, 1970
   M.S., Entomology, Purdue University, 1966
   M.S., Zoology, University of Calcutta, 1957

   Dr. Sreenivasam has worked with pests of agricultural crops and urban forests for the past 16 years. He supervises statewide pest surveys, coordinates data collection, interpretation and dissemination. As program manager for LCMR in 1987 he initiated the Minnesota Department of Agriculture-University of Minnesota comprehensive biological pest control research program working toward implementation. He has been involved in cooperative programs with USDA-APHIS in the release of natural enemies of alfalfa weevil, European corn borer and the gypsy moth, and in the development of state and national pest survey data and distribution network. Dr. Sreenivasam's primary role will be to integrate research findings on biological control funded by LCMR and develop implementation strategies for a wider scale application in Minnesota.

2. Dr. John C. Luhman
   Biological Control Scientist, Plant Industry Division
   Minnesota Department of Agriculture

Ph.D., Entomology University of California, Riverside, 1986
M.S., Entomology, University of Minnesota, St. Paul, 1980

Dr. Luhman has been involved with parasitic Hymenoptera systematics and biological control for 12 years. His background includes four years of experience in extension entomology, and over 20 of general insect systematics. He now oversees the daily operations of the MDA biological control program. He will manage lab and field operations necessary for parasite release, data collection, evaluation and recovery.

Project 7 Title: **Biological Control of the Gypsy Moth in Minnesota**
Dr. Herbert Kulman, Dr. Willis Schaupp, Jr.

III. Objectives

A. **Anticipatory biological control of the gypsy moth with native and introduced multi-host parasites**

A.1. Narrative: The gypsy moth is the most destructive hardwood defoliator in North America. Turmoil accompanies this exotic pest, as citizens objecting to defoliation conflict with citizens objecting to chemical control. As the gypsy moth migrates westward, newly infested areas characteristically incur the highest levels of tree mortality and the lowest levels of parasitism.

Our objectives are: (1) to establish gypsy moth parasites in Minnesota prior to general infestation by the gypsy moth and (2) to survey native defoliator pests for their parasites and determine which might attack the gypsy moth. Both objectives are currently being pursued, funded primarily by LCMR.

Candidate parasites necessarily are multi-host (polyphagous) in order to establish in the absence of gypsy moth. Two such exotic multi-host parasites are currently available, *Coccygomimus disparis* and *Compsilura concinnata*. *C. disparis*, established recently in New England, has been obtained from Korea and China. Released in Minnesota in 1989 and 1990, it has been recovered. *C. concinnata* was established in New England in 1909 from European stock. A few specimens were recovered in 1989 during our survey.

The release and recovery attempts with *C. disparis* will continue. Additional parasite species will be released if available.

A.2. Procedures: *C. disparis* will be raised in the laboratory and released into populations of susceptible native defoliators in areas likely to first become infested with gypsy moth, such as the Twin Cities. Attempts to recover *C. disparis* from previous release points will continue. Predators attacking defoliators in the field will be preserved and identified. A list of the predator and parasite species recovered will be compared with information on native parasites known to attack the gypsy moth elsewhere in North America. Particular attention will be paid to recoveries of *C. concinnata*.

A.3. Budget

	LCMR Funds
a. Amount Budgeted:	$15,000
b. Balance:	$ -0-

A.4. Timeline for Product/Tasks

	July 91	Jan 92	June 92	Jan 93	June 93
Detail design	—				
Field work	———*				
Lab & library work	————		Project ended		
Analysis	————		December 1991.		
Status Report		—			

A.5. Status: **Final status report January 1, 1992.** Field releases of the stingless ichneumon wasp *Coccygomimus disparis* continued. Three release sites were used in 1991. One new site was located in each of the Twin Cities. The third site had been used for releases in 1989. Because no recovery was made there the following two years, a different strain was released this fall. This is the only instance of more than one strain being released at one site. Detailed documentation of all releases has been provided to appropriate Federal and State agencies. The colony of *C. disparis* at the University of Minnesota was turned over to the Minnesota Department of Agriculture, along with technical guidance for its maintenance and possible future use. The 1991 *C. disparis* releases increased to over 4,000 the total number of females of this multi-host gypsy moth parasite that we raised and liberated in Minnesota from 1989-1991.

Recovery attempts continued at all 1989 and 1990 *C. disparis* release sites. Positive recovery was made at two sites. Our identification of the recovered insects as *C. disparis* was confirmed by specialists at the Systematic Entomology Laboratory-Taxanomic Services Unit, USDA-Agricultural Research Service. The recovery at the Fort Snelling State Park site occurred in July. *C. disparis* was recovered at the site near Hope (Steele County) from June through September. This is strong evidence that permanent establishment may have occurred. In both cases, the strain from the Peoples' Republic of China was recovered. It may be better adapted to Minnesota and/or have been released at more suitable sites than the other strain of *C. disparis* that we released, which originated in Korea. While no Korean wasps were recovered, some may have survived. We expect that *C. disparis* will persist and spread across the state without further introductions, though additional releases would further insure and hasten establishment and spread. We have met one of our objectives, to establish gypsy moth parasites in Minnesota on native forest pests prior to general infestation by the gypsy moth.

Field work toward our second objective, a survey of three hardwood defoliator species for their natural enemies, has been completed. Approximately 3,000 parasitic wasps and flies have been tentatively identified. The process of submitting representative specimens to taxanomic specialists for verification and improvement of our identifications is well underway, although final results may not be available until summer 1992. Dr. John Luhman (currently with MDA-Plant Industry Division) provided many of the parasite identifications. Preliminary results indicate that several insect parasite species recovered in Minnesota can be expected to attack the gypsy

moth. These include the ichneumon wasps *Coccygomimus pedalis* and *Itoplectis conquisitor* and the tachinid fly *Compsilura concinnata*. Only 6 percent of the parasitic flies we reared were *C. concinnata*, despite focused attempts to recover this species. The rarity of *C. concinnata* is troublesome, because it is a very important gypsy moth enemy elsewhere and the only other exotic multi-host gypsy moth enemy currently available. Future efforts should seek to ensure that adequate, climate-adapted strains of *C. concinnata* are present in Minnesota.

We continued to take every opportunity to inform the scientific community and the public of our effort and how it was supported. A feature story aired on Minnesota Public Radio in late July. The USDA journal Agricultural Research had several pages describing our project (vol. 39, no. 10, pgs. 7-8, October 1991). This resulted from our use of HMC as a release site and an interview we gave to their Public Relations Department. Interpretive materials were provided to the naturalist at Fort Snelling State Park, one of our release sites. Correspondence, public contacts and attendance at meetings further spread awareness of our work.

Our article entitled "Attack behavior and host utilization by Coccygomimus diaparis (Hymenoptera:Ichneumonidae) in the laboratory," is in press and will appear in Environmental Entomology. When all identification work is completed, a list of parasite species recovered and an assessment of their impact upon gypsy moth will be provided to state personnel. Other manuscripts will result from this data, with provisional titles listed in the LCMR Final Status Report of July 30, 1991. Deposition of voucher specimens will be made in the University of Minnesota insect museum, providing a permanent record of these beneficial insects.

Final status report June 30, 1993. In the Fall of 1991, the MDA-PPD took over the colony of *Coccygomimus disparis* from Dr. Willis Schaupp, Department of Entomology, University of Minnesota. Production of *disparis* continued in our lab at the Dakota County Technical College, Rosemount in cocoons of European corn borer and in wax moth. In 1992 we continued releases of the wasp, adding 5 new sites. We released over 500 female *C. disparis* in 7 sites in 6 counties: Chisago, Goodhue, Hennepin, Ramsey, Steele, and Winona. Four of these were the state parks Ft. Snelling (Hennepin Co.), Frontenac (Goodhue Co.), Interstate (Taylor's Falls, Chisago Co.), and Kipp (Winona Co.). The target host in these parks was fall webworm. There were 2 new release sites in the TC Metro area. These included 2 boulevard groves, all with linden trees infested with whitemarked tussock moth larvae. One site was in downtown Minneapolis on South 2d St. The other was at Rosedale in Roseville. In addition to these releases, we supplied wasps to the USDA-ARS in Delaware to start up their *disparis* colony, and to a researcher developing biological insecticides from parasitic wasp venoms. In June, 1993, we released 100 female *disparis* each in Frontenac St. Pk., the St. Croix Infm. Center on West I-94, and 2 new sites—Afton State Park (Washington Co.) and a residential area in northwestern Minnetonka where gypsy moth adults and eggs were found. Throughout the summer releases will continue at all 1992 sites plus 2 new ones. To date, over 5000 C. disparis females have been released in Minnesota since 1989.

We recovered *Coccygomimus disparis* and the tachinid fly *Compsilura concinnata* from whitemarked tussock moth July and August, 1992, at the Roseville (Rosedale) site. We will continue to monitor all 1989-1992 release sites for establishment of*disparis* and the spread of *concinnata*.

Several strategies were used in determining release sites of gypsy moth parasites. State Parks along Minnesota's border with Wisconsin are important as potential infestation sites of gypsy moth by tourists. The forests in these parks are mixed hardwoods with oaks—a preferred tree of gypsy moth. These sites were also generally unsprayed and provide a quality environment for released wasps. A second priority are sites in the Greater TC area where gypsy moths have been trapped or egg masses found. As great a priority are sites with heavy infestations of alternate hosts of *C. disparis* (such as whitemarked tussock moth larvae) in public areas. These are important as educational, demonstration sites as to the feasibility of biological control to show to the public. Augmentative releases are done at previous release sites of special importance. Other sites include rest stops or groves along interstate highways. In early summer we look for sites with tent caterpillars; in mid summer, sites with whitemarked tussock moth; in late summer and Fall,sites with fall webworm, an overwintering host.

In the lab*C. disparis* was reared in the cocoons of European corn borer. These are already being reared in our facility in the Dakota County Technical College, Rosemount, to supply host stages for our colonies of egg and larval parasites of corn borer. By refrigerating parasitized corn borer cocoons, we can produce disparis all Fall and winter long for mass releases in spring and summer. In the summer months, we produce up to 500 females per week.

Publicity of our biological efforts against gypsy moth continue at every oppurtunity. Our rearing of *disparis* was part of poster displays of biological control at the LCMR sponsored symposium on biological control in October, 1992, on the Minneapolis Campus of the University of Minnesota. It was also part of our poster display at the national Entomological Society of America Meetings in December, 1992, at Baltimore, MD. Last March, it was part of the Plant Protection Division's display for the MDA open house. Several articles have appeared in the Minnesota Pest Report telling about our activities (May 8, 28, July 17, 1992; May 28, 1993). There were also articles in the MDA publication Overstorey (May-June, 1992, Vol. 7[1]) and in the DNR Newsletter, July, 1992. We have distributed information and reports on our rearing and release activities to personnel at state parks, the City of Minnetonka, Agricutlure Canada, and to the Gypsy Moth Ad Hoc Meeting in January, 1993. We have also presented several displays of biological control agents, including *disparis*, at the St. Paul Arts and Sciences Museum. Voucher specimens of recovered and lab reared *disparis* and *Compsilura concinnata* have been accessioned into the University of Minnesota Insect Collection.

A.6. Benefits: When the gypsy moth establishes here, we will have parasites in place on a reservoir of native pests that will also attack the gypsy moth, slowing its expansion and damage. Increased levels of biological control will reduce the need to use chemical sprays in urban areas. The new survey information on the parasites of native pests of hardwoods will facilitate management of those pests and identify windows of opportunity for parasite introductions against them.

This project performs the dual functions of determining which beneficial insect species are already present and increasing their number and effectiveness. This can serve as the basis for an ecologically sound, economically beneficial and environmentally safe pest management tactic. Fostering public awareness of and confidence in natural and biological control may lessen the turmoil typical in urban areas under pressure from dense gypsy moth populations.

Dr. Schaupp served as a mentor to a Minnesota high school student through the Howard Hughes Medical Institute Young Scholars Summer Research Program, administered through the College of Biological Sciences. He also sponsored a student through the Undergraduate Research Opportunity Program. Both students worked on the gypsy moth project, thus extending the benefits of LCMR funding to include the development of human resources. He and Dr. Kulman intend to continue to seek such benefits and to promote public awareness of the project whenever possible.

IV. Evaluation

The program can be evaluated by its ability to: (1) provide release information on one exotic species of gypsy moth parasite; (2) provide inventory information on the native natural enemies of certain native hardwood defoliating insect species; (3) provide pest management personnel with a list of these enemies which can attack the gypsy moth.

V. Context

A. Since most imported enemies of the gypsy moth are specific, they arrive last to newly infested areas. *C. disparis* is not yet established in Minnesota. The resistance of Minnesota's forested environments to the gypsy moth can be increased prior to infestation.

B. This project was funded in 1989 by LCMR at $50,000 for the 89-90 biennium. As such, continuation of the project will be with the benefit of experience gained. Salaries, space and some expenses are provided by the University of Minnesota Agricultural Experiment Station. The in kind contribution amounts to at least 10 percent. This will enable three full summers of field research to be derived from each biennial funding instead of one, as a more timely schedule can be maintained for the salaried Research Associate. The program manager, by participating in the unfunded NE-143 program, is able to draw upon the experience of those in the northeastern US working with gypsy moth natural enemies and to coordinate efforts with members from the upper Midwest facing similar infestation.

VI. Qualifications

Major Cooperators:

1. Herbert M. Kulman
   Professor, Department of Entomology
   University of Minnesota, St. Paul, MN 55108

   Ph.D. Entomology, University of Minnesota, 1960
   M.F. Forestry, Duke University, 1955

   Dr. Kulman has held a tenured faculty position at the University of Minnesota since 1966. He is author or co-author of over 70 scientific publications in refereed journals; distinguished forest entomologists are among the many graduate students he has trained. His extensive research has focused upon the biological control and natural enemies of many of Minnesota's forest pests. He is a participant in the Cooperative States Research Service project NE-143 entitled,"The Gypsy Moth and its Natural Enemies: Behavioral and Population Determinants" and has recently supervised a study on the development of a gypsy moth risk-rating system for Minnesota's forests.

2. Willis C. Schaupp, Jr.
   Research Associate, Department of Entomology
   University of Minnesota, St. Paul, MN 55108

   Ph.D. Entomology, University of California-Berkeley, 1988.

   Dr. Schaupp has been involved in research upon insect parasites for over ten years. He is an expert on the biology and identification of natural enemies of defoliating insects and has been responsible for the Minnesota survey and release activities undertaken in anticipation of the gypsy moth since the inception of the project in early 1989. In addition to research commitments, he has sought to inform the public regarding the LCMR funded effort against the gypsy moth, including a presentation to the 1990 convention of the Minnesota Nursery and Landscape Association. He is preparing journal articles based upon his research in Minnesota and presented a poster on this work at the annual meeting of the Entomological Society of America in 1989.

Project 8 Title: New Strategies for Field Implementation of Biological Control
Dr. Roger Moon, Ms. Valerie Cervenka

III. Objectives

A. Winter augmentation for biological control of summer filth flies

A.1. Narrative: A new augmentative release strategy holds promise for biological control of stable fly, *Stomoxys calcitrans* [L.] and house fly *Musca domestica* L. These flies are a perennial source of summer discomfort to livestock, humans and pets in rural and urban environments. Native parasitic wasps alone are insufficient because populations die back each winter, leaving too few in late spring to override the reproductive capacity of the flies. Summer augmentation outdoors with *Spalangia endius* Walker and *Muscidifurax raptor* Girault & Sanders has failed in other states (CA, NE) apparently because numbers required once summer populations were large greatly exceeded capacity to produce and release wasps. An alternative approach would be to augment the overwintering reservoir during winter. Research in winter 1989-90 showed that released *M. zaraptor* Kogan & Legner overwintered exclusively indoors at ground level where the cool, non-freezing microclimate permitted survival but prevented midwinter emergence. These findings indicate winter livestock housing might be viewed as a refrigerator—but not freezer—in which wasps could be released, accumulating through May to emerge en masse as fly reproduction begins. The work proposed here will evaluate this new approach.

A.2. Design, Methods & Procedures:

Goal 1. Compare the impacts on summer fly populations of winter and conventional augmentation strategies at semi-heated livestock facilities. Nine dairies or stables (hereafter, sites) south and west of the Twin Cities will be blocked in sets of three by location, size and fly densities judged from summer 1991 censuses. Cooperators will be coached on preventive sanitation, and will commit to avoid fly sprays during the 2-year study. One site in each block will receive no parasites (untreated controls), the second will receive weekly releases indoors from 9/1/91-5/30/92 and outdoors from 6/1/91-9/15/92, and the third will receive releases just during summer, from 6/1-9/1/92. Treatments will be assigned at random. We will use a Minnesota strain of *M. zaraptor* produced at the University of Minnesota. This strain has a superior reproductive capacity. Releases will be in the form of parasitized house fly puparia packed in mesh bags for field placement inside wooden release stations that exclude mice and other animals. Releases will be weekly, at a rate of one parasite per ten target fly pupae, based on pre-release censuses of pupal fly densities at each site during summer '91. The pre-release censuses of wild fly pupae will use area-stratified core sampling and flotation-extraction.

Response variables will be weekly measures of adult house fly abundance, of adult stable fly abundance, and of wasp attack rates at each of the nine sites from 5/15-9/15/92. Census methods used in this study are the same as those used by colleagues in California, Nebraska, Kansas and New York. For house flies, we will use ten fixed station sticky traps indoors and ten more outdoors on each date. For stable flies, we will record instantaneous counts of flies taking blood meals at hosts' legs (n=30 or more) during permissive weather, and we will also use daily catch rates from five fixed-station "Williams" traps around each site. We will index ovipositional activity by free living wasps on each premise by exposing ten replicated bags of one-day old house fly pupae placed in breeding media indoors and out. After exposure for one week, the bags will be returned to the lab to rear out and count offspring from parasites that attacked the pupae while in the field. For each of the response variables (as monthly and season-long means), treatment effects will be analyzed by ANOVA in a randomized block design (three treatments in each of three sets of sites). Planned contrasts are for all possible pairwise combinations of treatments.

Goal 2. Evaluate overwintering survival and phenology of *M. zaraptor* released at the three winter release sites, and develop mathematical models to predict survival, development and spring emergence of the wasps.

Response variables will be estimated survival rates and developmental progress, inferred by dissection of aliquots (n=100) of parasitized puparia retrieved at weekly intervals from the release stations. From week to week after release, changes in frequencies of living and dead parasite larvae, pupae, adults and exited adults reflect survival and development in each batch of wasps. Corresponding temperatures will be monitored continuously with thermistors placed in the release stations. These records will be used to infer through nonlinear regression the effect of temperature on stage-specific weekly rate of survival, and to validate temperature-dependent phenology models for termination of diapause in the survivors and subsequent emergence by overwintered wasps.

A.3 Budget:

a. Amount budgeted: $70,000
b. Balance: $-0-

A.4. Timeline for Project Tasks:

	July 91	Jan 92	Jun 92	Jan 93	Jun 93
Pre-release censuses	———				
Parasite rearing & release	———	———	———		
Field evaluations	———	———	———		
Sample processing	———	———	———	———	
Data entry & analysis	———	———	———	———	———
Reports		—	—	—	—

A.5. Status: Final status report June 30, 1992. Pre-release sampling: In May, 1991, we located 12 horse stables in Scott and Dakota Counties with owners willing to participate and facilities otherwise suitable for the study. At each stable, we measured fly abundance and age structure on a weekly basis from June through September, and evaluated extent of natural parasitism prior to release of beneficial fly parasites (*Muscidifurax zaraptor*). Resulting data were analyzed to judge degree of geographic isolation among populations at adjacent and distant farms. This information was needed to design the mass release experiment which began in November 1991. Abundance was measured with 3,300 sticky traps, fly age structure was measured through age-grading of 4,500 flies, and parasitism was rated by exposing a total of 360 sentinel pupal bags to natural parasites.

Results and final experimental design: These pre-release measurements indicated fly populations at the 12 stables likely originated from breeding sites on or near each premise. Abundance at a given farm was independent of abundance at other farms. Attack rates by natural parasites were virtually zero. Flies at two of the stables were too scarce to evaluate effects of parasite releases, so they were dropped from further consideration. Of the remaining ten stables, three were designated to receive indoor releases that began in November 1991, three will receive summer releases starting June 1, 1992, and the remaining four will serve as untreated controls, receiving no parasites. No blocking by geographic location was employed in the final experimental design.

Mass culturing methods: Insect colonies were extended to produce parasites for field release. Fly and parasite rearing materials and space were procured, and colonies were expanded to production scale to yield 32,000 parasites for release each week at the three winter-release stables, beginning November 11. Parasites (in fly puparia) are being released into screen enclosures in protected parts of the stables. Thermometers nearby are being checked daily by cooperators. Subsamples from each week's release batch are being retrieved at monthly intervals from each stable to assess the initial quality and overwintering fate of the released parasites.

Nine private horse stables in Scott and Dakota Counties were studied in 1991 and 1992 to test two parasite release schedules for augmentative biological control of noxious house flies and

stable flies. Three of the stables received parasites on a Winter+Summer (W+S) schedule. Releases were of *Muscidifurax zaraptor* at a rate of 300 parasites per horse per week, indoors from November, 1991 through May, 1992, and then outdoors at the same rate from June through September, 1992. A second set of 3 stables received parasites at the same rate during summer only (—S), from June onward in 1992. A final set of 3 stables were left untreated. A grand total of 3.2 million parasites were produced from our UM insectary. Before and during the releases in both years, we measured summer fly abundance by trapping on a weekly basis, and measured rates of natural parasitism with sentinel house fly pupae placed in the field.

The pre-release measurements in 1991 indicated fly populations at the 9 stables ranged from 2 to 5 flies per trap day, and attack rates by natural parasites were virtually nil (0.4%). During the release year (1992), catch rates at the W+S and —S sites were reduced by 45 and 55%, respectively, compared to rates in 1991, but remained the same at the untreated sites. The odds that a fly would be parasitized at the W+S and —S sites were the same, being 36-fold greater than at the average untreated site. These results indicated augmentation during summer reduced the level of fly nuisance by increasing levels of biological control, but that the two release schedules were equivalent.

Aliquot samples from each weeks releases were retrieved at monthly intervals to see how many of the wasps survived to emerge after release. Dissections of 28,000 fly pupae indicated that parasites released before April, 1992 died before emerging, and extent of late winter survival was related to barn temperature. Surviving parasites emerged in late May. Survival of parasites released from June onward exceeded 80%. These results indicated that the W+S and —S schedules produced equivalent effects on fly abundance because wasp mortality during winter killed the parasites that had been released during winter.

A.6. Benefits: Annual losses attributable to filth flies in Minnesota were $38M in 1983, third behind European corn borer and *Aedes vexans* Meigen. Approximately half of Minnesota's 98,000 farms have one form or another of animal confinement facility that produces filth flies. Urban encroachment into rural areas, and the state's new solid waste management law that bans lawn clippings from land fills in the metropolitan area are bringing the public into increasing contact with filth flies. At the same time, the public is becoming averse to insecticide use, and flies are becoming resistant to the few insecticides on the market for fly control. Commercial interest in biological control of flies is evident, both among livestock producers and vendors, but efficacy through summer augmentation has not been successful in the Midwest. If our attempt with the new winter release approach will work, then livestock producers and yard waste operations would have a viable alternative to insecticidal fly control.

IV. Evaluation

As described in section A.2 above, efficacy of winter *M. zaraptor* releases inside confinement facilities will be evaluated by comparing subsequent fly densities at winter release sites with those at sites where none have been released, and where parasites were released in summer alone. Earlier work in our program indicated a Minnesota strain of *M. zaraptor* has a superior ovipositional rate, and that overwintering by this species occurred exclusively as diapausing larvae inside (but not outside) one confinement facility. The present study would extend these findings to test the idea that augmentation of the overwintering reservoir can enhance biological control in a subsequent fly reproduction season.

Detailed analysis of survival and development of the winter inoculum in the proposed study will document the fate of the winter released wasps in a greater variety of facilities. Mathematical models of survival and development that arise from the analysis of microclimate and parasite demography will provide insight into reasons underlying the release experiment's success or failure.

Future research and development: If winter augmentation succeeds in creating a "wave" of spring parasite activity, and it causes a measurable reduction in summer fly densities, then the next step in research is to adjust the schedule and quantity of winter-spring releases, both indoors and out, with the criteria that one wants to get the most "fly control" for the lowest cost. Depending on the extent of success, the concept may be sufficiently established for commercialization. Further, success with winter augmentation may stimulate use of the approach in other host-parasite systems, where applicable. On the other hand, a next step if winter augmentation fails would be to explore possible ways of protecting winter releases to enhance survival into the next summer. The models generated in the proposed study will permit development through simulation of variations on the winter release approach in a wide variety of habitats and locations.

V. Context

Funding by LCMR for biological control of filth flies began with $51,000 in 1987-89 and $40,000 in 1989-91. Work to date has documented presence of seven filth fly parasite species in Minnesota, surveyed their natural field prevalence (or lack thereof), compared the oviposition rates of ten different species and strains of parasites, and examined influence of field release location and date on overwintering success of released specimens. The present proposal will extend past work to test an emerging winter release strategy, one which might capitalize on the opportunity created by the ubiquity of winter livestock housing in Minnesota. Other University support for this project includes 1/2 FTE technician ($15,000) from AES and other sources, in kind contributions for the project scientist (30 percent FTE = $30,000), and laboratory facilities for parasite production and sample evaluations. Private cooperators will allow access to field facilities, avoid use of insecticides, and accommodate experimental activities on their premises.

VI. Qualifications

Major Cooperators:

1. Dr. Roger D. Moon
   Associate Professor, Department of Entomology
   University of Minnesota

   B.S. Entomology, University of California, Davis, 1975
   Ph.D. Entomology, University of California, Davis, 1979

Dr. Moon has 15 years research and teaching experience in population dynamics, pest management of veterinary and medically important arthropods, and the biology and biological control of filth flies in Minnesota, Nebraska, and California. He currently leads research projects in filth fly biological control, beef cattle IPM, and mosquito ecology. His long term interests and expertise are in biological control; ecology, management and computer modeling of insect populations; and interactions between arthropod parasites and their mammalian hosts. His role in the present project will be to design and supervise the study, and to analyze the resulting data.

2. Ms. Valerie J. Cervenka
Junior Scientist, Department of Entomology
University of Minnesota

B.S. Horticulture, University of Minnesota, 1982
M.S. Entomology, University of Minnesota, 1987

Ms. Cervenka has seven years experience in rearing and identification of parasitic Hymenoptera and muscoid flies. She is in charge of daily operations of the livestock insects laboratory and associated research. Her long term interests and expertise are in insect identification, rearing of beneficial insects, and forensic applications of insect biology. Her role in the present project will be to manage the laboratory and field operations necessary for mass release of parasites and evaluation of parasite efficacy and fate.

B. Integrating biological control of arthropods into commercial greenhouse production in Minnesota
Dr. Mark Ascerno

B.1. Narrative: Resistance of arthropod pests to pesticides coupled with the loss of registered products is creating severe problems for greenhouse production of quality floriculture and bedding plant crops. Although there is a great deal of interest in using biological control, there are currently no functioning biological control programs related to greenhouse production in Minnesota. Part of the reason related to the reluctance of commercial operators to rely on unproven programs that are perceived to carry a high risk of failure. In addition, implementation research, even on a small scale, is lacking.

B.2. Design, Methods & Procedures: The objective of the proposed work is to successfully integrate a biological control program for selected pests into a commercial greenhouse. To this end a commercial greenhouse operator growing Alstomeria will be enlisted as a cooperator. Alstomeria will be used because only a part of the plant (cut flower) is marketed. Encarsia formosa, a parasitoid of the whitefly, Trialeurodes vaporariorum and Phytoseilus persimilis, a predator of the mite, Tetranychus urticae will be released. Release rates, time and frequency of introductions, humidity, temperature, lighting, and crop stage will be monitored to find the combinations that produce acceptable pest control while necessary, active ingredients, rate, and application procedure will be evaluated for impact on biological control agents. Results will be summarized and presented at commercial flower growers conferences.

B.3. Budget:

	LCMR Funds
a. Amount Budgeted:	$40,000
b. Balance:	$-0-

B.4. Timeline for Products/Tasks

	July 91	Jan 92	June 92	Jan 93	June 93
Design	———				
Greenhouse research		———	———	———	
Data analysis and evaluation			———	———	
Reports		—	—	—	—

B.5. Status: Final status report June 30, 1993. Newly constructed houses for commercial rose production were used to evaluate the efficacy of the thrips predators *Neoseiulus cucumeris* and *N. barker*, aphid predator *Aphidoletes aphidimyza*, and aphid parasitoid *Aphidius matricariae*. Results indicated that *N. cucumeris* and *N. barkeri* may have been effective at controlling thrips, while aphid biological agents looked less promising. Future introductions of these agents will take place in spring of 1993.

An alternative study using *Delphastus pusillus*, a whitefly predator, will be conducted at St. Paul Campus, University greenhouses. Studies conducted in 1993 will focus on screening toxicity of various greenhouse pesticides to *D. pusillus*.

Integrating biological control into commercial greenhouses has proven more difficult than anticipated. Insecticide testing has documented long-term (2 months) mortality on *Delphastus pusillus* confirming recommendations that crops should be free of pesticide residues before releasing biological control organisms. Supplier-related problems have also hampered our efforts. Shipment schedules were erratic and often contained dead organisms upon arrival. Inability of suppliers to consistently deliver viable biological control agents makes precise timing and consistent releases of biocontrol agents impossible. We plan to continue monitoring the viability of commercially available biological control agents and report on our findings to growers and the scientific community.

The low tolerance of growers for pests often lead to decisions regarding pest controls that give little thought to their impact on biological control success. Education to increase grower tolerance is needed for success of these programs.

Presence of more than one pest species is a barrier to establishing biological control in rose. Providing simultaneous biological control of all pest species has proven to be difficult. If one pest species exceeds damage thresholds, the necessary chemical control measures to reduce that population disrupt the entire biological control agent complex. A parallel project is being developed in alstromeria. The different cropping system of alstromeria should enable us to determine if the problems we are experiencing are crop, biological control agent, and/or grower-related.

B.6. Benefits: Demonstrate the principles as well as the applicability and viability of integrating biological control into floriculture and bedding plant operations. Reduced reliance and use of pesticides for insect and mite management in greenhouses.

IV. Evaluation

Evaluation will be based on the degree to which biological control agents can be incorporated into a commercial greenhouse operation. Criteria will include: parasite/predator establishment, level of control, reduced pesticide use, costs, and the ability of the program to be carried out by the commercial operators.

V. Context

This project requests funding by reallocation from LCMR biological control program. No funds have been requested from LCMR in previous years. Additional support: commercial greenhouse facilities including crop, water, heat, lights, labor $249,600. In kind contribution: 10 percent of $14,000 per biennium.

VI. Qualifications

Major Cooperator

1. Dr. Mark E. Ascerno
Professor and Extension Entomologist
Department of Entomology
University of Minnesota

Ph.D., Entomology, Penn State University, 1976.
M.S., Entomology, Oregon State University, 1969.

Dr. Ascerno has been involved in entomological research related to ornamental plants for over 20 years. His research has included biological and integrated control of these pests. He is nationally known for his expertise in floricultural entomology contributing regularly to national symposiums and writing for a national floriculture trade publication. Dr. Ascerno has 15 years of experience with the Minnesota Extension Service developing and delivering research information for transfer to commercial operators. Dr. Ascerno is also Acting Head of the Department of Entomology and Director of the Dial U Insect and Plant Information Service at the University of Minnesota.

C. Biological control of the insect pest complex in commercial cabbage and broccoli production in Minnesota: evaluation & implementation

Dr. William Hutchison

C.1. Narrative: Cabbage and broccoli are presently grown on about 2,000 acres with an annual Twin Cities fresh market value of $2.5 million. Several conventional insecticides are applied each year to p[illegible] these vegetables from an array of insect pests. During the months of June through September, nerve-toxin insecticides are applied to these crops on a 7-10 day scedule. Of the leaf-feeding insects, the imported cabbageworm (ICW) is the most serious pest of cabbage and broccoli in Minnesota. As the public's demand for insecticide-free food has increased, there has also been an increasing number of vegetable producers who are interested in meeting this demand and reducing pesticide use on their farms. Although several new alternatives to conventional insecticides are available commercially, very little quantitative research has been done to indicate which products provide consistent control and what combination of biological control tactics will be economically feasible over a range of pest infestation levels.

Two promising options for a biorational approach to ICW management include: a new formulation of *Bacillus thuringiensis* var. *kurstaki (Btk)*, a bacterium specific to moth and butterfly larvae (worm stage) and a naturally occurring parasitic wasp, *Apanteles glomeratus*, that attacks ICW larvae. In contrast to the broad-spectrum conventional insecticides which also kill non-target beneficial insects, *Btk* is unique in that it is specific only to lepidopterous larvae and poses no safety risk to people, wildlife or the environment! A new *Btk* has recently been formulated that is micro-encapsulated. This new formulation provides longer insect control in the field because it is less susceptible to breakdown by UV-rays or extreme temperature fluctuations.

C.2. Design, Methods & Procedures: The primary objectives of this study is to determine when ICW infestations develop, optimum timing of *Btk* applications, persistance of *Btk* applications in the field and the impact of the *A. glomeratus*. Field plots will be established at the University of Minnesota Experiment Station at Rosemount and in at least one cooperating grower field. The Rosemount location will be used to evaluate new *Btk* treatments while the grower site will be used to demonstrate the use of known Bt timing scenarios for demonstration and educational purposes. One or more late-planted varieties will be selected and transplanted to two-row plots, about 40 feet long. All Bt and timing treatments will be replicated four times. ICW and the insect pest complex will be monitored twice per week, with one mid-season and one at-harvest destructive sample taken to evaluate final insect damage and marketability of the heads. Analysis will include marketability for fresh market and processing (primarily cole-slaw in Minnesota). Results will be summarized and presented at annual Vegetable Grower meetings, newsletter articles and in scientific journals.

C.3. Budget:

	LCMR Funds
a. Amount budgeted:	$19,000
b. Balance:	$-0-

C.4. Timeline for Products/Tasks

	July 91	Jan 92	June 92	Jan 93	June 93
Design		—			
Fieldwork		———		———	
Lab Research		———		———	
Data Analysis & Evaluation		———	———	———	———
Reports		—	—	—	—

C.5. Status: Final status report June 30, 1993. The purpose of this project is to document the performance of new formulations of *Bacillus thuringiensis*, and how microbial control may be complemented by native or introduced parasitoids to control the imported cabbageworm (ICW), *Pieris rapae*, the diamondback moth (DBM), *Plutella xylostella*, and the cabbage looper (CL), *Trichoplusia ni*. As in 1991, intensive sampling was done during 1992 to document the abundance and phenology of the pest complex and natural enemies in untreated cabbage (and collards), and in cabbage treated with *Bt*, and insecticides (permethrin). During both years of study, CL was the dominant pest species followed by ICW, and DBM as the next most abundant species in 1991, and 1992, respectively. Several *Bt* formulations, with or without low rates of permethrin, significantly reduced seasonal infestations of the pest complex. In 1991, larval/pupal parasitism averaged 29.4 and 28.6 percent for ICW and CL, respectively. Several new parasites of ICW and CL were recorded in 1992. During 1992, *Cotesia rubecula* (Marshall), a solitary larval parasitoid of ICW, was introduced to enhance the current level of ICW control.

Two years of field data have now been collected to document the impact of the bacterium, *Bacillus thuringiensis*, and naturally occurring parasitoids. Several treatments were found to be effective at significantly reducing larval infestations of the three major pests of cabbage in Minnesota: imported cabbageworm (ICW), diamondback moth (DBM) and cabbage looper (CL). One *B.t.* formulation, Javelin WG, was particularly effective; at-harvest larval infestations of all pests and product quality ratings were not significantly different from those of permethrin, a commonly used conventional insecticide. A total of 9 native parasitoid species were recovered from the pest complex during 1991-1992. Total parasitism for ICW was 21-32% and 21-28% for CL. However, parasitism for DBM averaged about 70%. A new parasitoid against the imported cabbageworm (ICW) was introduced in 1992. Surveys will be done in 1993 to document establishment; additional releases will be made in 1993.

Results of this work have been published in the Minnesota Fruit & Vegetable Grower Newsletter and are in preparation for scientific journals. To encourage more use of B.t. products by growers, more work is needed to demonstrate different timing scenarios and to develop action thresholds that are practical for *B.t.* use.

C.6. Benefits:

a) Reduced use of conventional broad-spectrum insecticides will result in less potential for insecticide residue on vegetable foliage, exposure to farm workers and non-target organisms such as wildlife and fish.
b) Reduced use of insecticides will allow natural ICW parasite populations to build-up during the course of the season to provide an additional level of biological control, which is currently impossible in present insecticide-based management programs.
c) Proper timing of B.t. applications, based on the biology of the ICW and ICW infestation-damage relationships, will help encourage minimum use of B.t. and thereby maximize net profits.
d) Laboratory studies will allow us to determine the relationship between ICW leaf consumption in relation to temperature and the relative susceptibility of each larval instar (stages between molts) to B.t. products, both of which will provide a basis for refinement of application timing strategies in the field.

IV. Evaluation:

Assessment of *Btk* control and parasitism rates will be determined throughout the growing season by sampling cabbage and/or broccoli plants 1-2 times/week. A variety of *Btk* treatments will be used to determine the minimum number of *Btk* treatments necessary to control Lepidopteran pest complex below economically damaging levels. Evaluations will be conducted for cabbage and broccoli grown for fresh market and cabbage grown for processing in Minnesota. Final results will form the basis of educational materials designed to assist producers and consultants in implementing biological control programs.

V. Context:

This is a new project requesting funding by reallocation from LCMR biological control projects. Although no funds have been requested from LCMR in previous years, a preliminary field study was initiated in 1991; the 1991 data will be used to refine techniques and guide priorities for future research support. Additional support: biological control (Btk)/agrichemical industry @ $10,000/year; field plot space, equipment, herbicide, fertilizer, labor @ $60,000. In kind contribution @ 10 percent or $14,000 per biennium.

VI. Qualifications:

Major Cooperator

1. Dr. William D. Hutchison
Assistant Professor & Extension Entomologist
Department of Entomology
University of Minnesota

Ph.D., Entomology, University of Wisconsin-Madison, 1984
M.S., Entomology, Mississippi State University, 1980
B.S., Agronomy, University of Arizona, 1977

Dr. Hutchison has been active in the areas of applied insect pest management and biological control research for the past 11 years. Prior to coming to the University of Minnesota in 1989, he had conducted research on alternatives to conventional insecticide use for key insect pests in cotton and alfalfa. Dr. Hutchison has authored or co-authored over 20 scientific articles and book chapters on various aspects of integrated pest management of arthropods. After accepting his current position in Extension, he has expanded his research focus to include applied aspects of insect pest management in vegetables, information that can readily be used by growers and processors of vegetable crops in Minnesota. To facilitate implementation of new information, Dr. Hutchison has become a regular speaker at the annual Minnesota Fruit & Vegetable Growers meeting, Midwest Food Processor meetings and regularly contributes to the University of Minnesota "Plant Pest Newsletter." In addition, during the summer of 1991, he has organized the first Vegetable Crop Field Day, highlighting *Btk* and other insecticide alternatives for insect pest management in cabbage, sweet corn and potatoes.

VII. Reporting Requirements

Semiannual status reports will be submitted not later than January 1, 1992; July 1, 1992; January 1, 1993; and final status reports by June 30, 1993.

Project 9 Title: **Biological Control Symposium**
Dr. Dharma Sreenivasam, Dr. Mark Ascerno

A.1. Narrative: Several agencies, state and federal, have been involved in biological control of pest organisms. Exchange of information between scientists and all others involved in pesticide alternatives is needed at this time. The proposed symposium will provide a forum for this exchange and a recognition that Minnesota is actively pursuing this goal to reduce use of chemical pesticides.

A.2. Procedure: A two-day symposium is proposed.

1.a. Successes in Biological Control and integration of Biological Control into pest management programs.
b. Biological Control and its relationship to sustainable agriculture and Integrated Pest Management (IPM) Program.
c. Business opportunities - mass rearing, greenhouse industry in California, Europe.
d. Future direction of Biological Control.

2.a. Genetic Engineering impacts on Biological Control, applications of plant pathogens for control, and modifications of insect pathogens (microsporidians, fungal and viral).
b. Fungal toxins for weed control.
c. Industrial viewpoint and future direction.

3.a. Limitations to Biological Control, pest density, agricultural practices.
b. Stability of biocontrol agents.
c. Where do we get biocontrol organisms - foreign exploration, Commonwealth Institute of Biological Control (CIBC) and other international and federal agencies.
d. Product formulation and application technology.

4.a. Invited experts from key disciplines:
Plant Pathology, Entomology and Weed Science.
One-half day will be presentations for the general public.

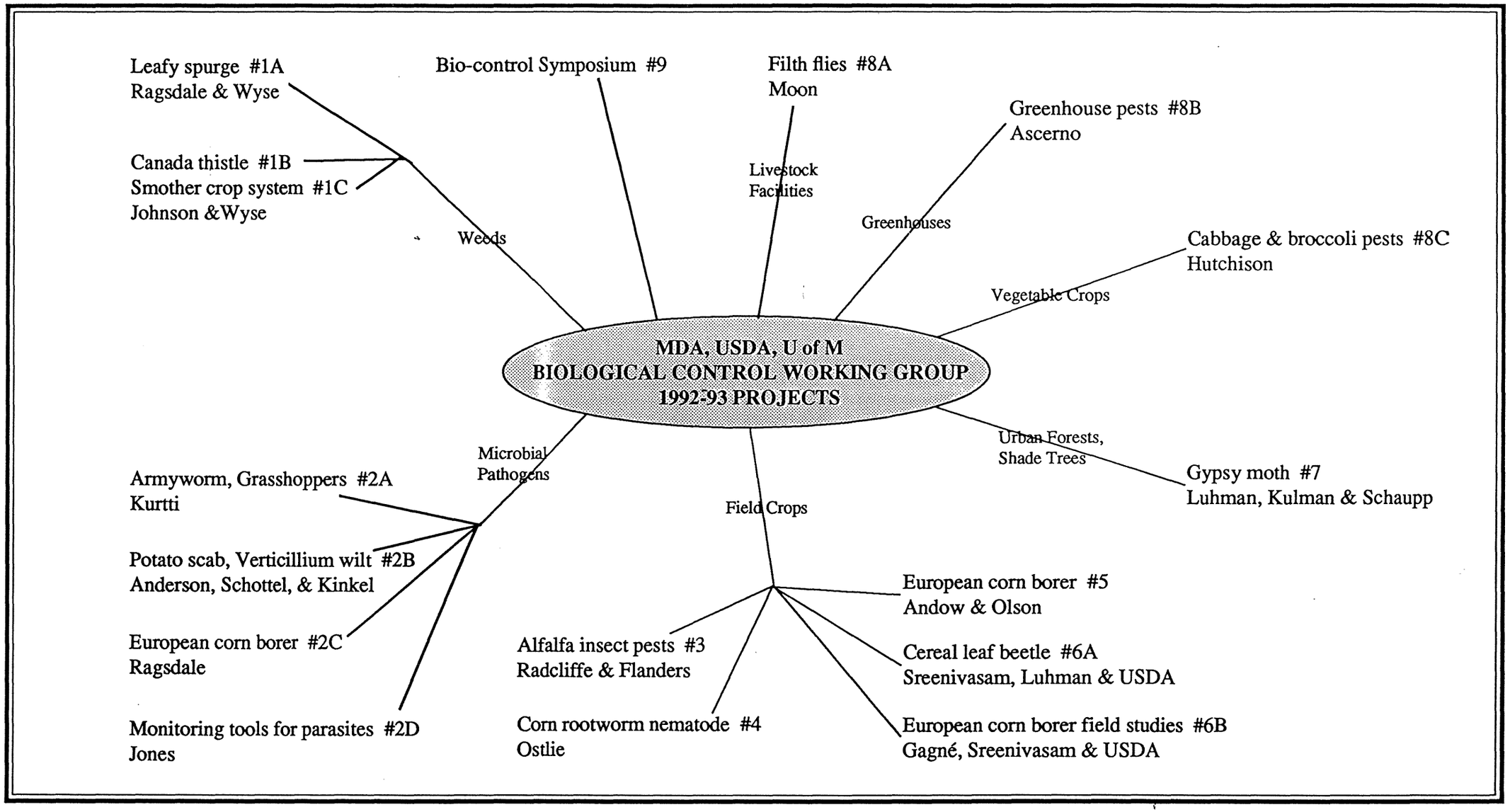
A.3. Budget

	LCMR Funds
a. Amount Requested:	$20,000
b. Balance:	$ -0-

A.4. Timeline: mposium proposed for October 25-27,1992.

A.5. Status: Final status report June 30, 1993. "*Ecological Interactions and Biological Control*", was held 25-27 October 1992. Over 140 scientists from the United States, Canada, Israel, Korea, Mexico, and Europe attended the symposium. The program began on Sunday 25 October with presentations for each project funded as part of "Biological Control of Pests" through the LCMR process . Monday and Tuesday consisted of presentations by an international array of biological control experts. In addition, 35 posters were displayed from Sunday afternoon to Tuesday noon. Round table discussions were held on Monday evening to provide an informal setting for information development and exchange. A book based on presented papers, round table discussions, and posters will be published by Westview Publishing in late 1993 or early 1994. Copies of this book will be given to the LCMR staff, participants in the conference, and sold to others. The symposium achieved its goals of making people aware of the significant biological control efforts in Minnesota, exchanging information among biological control practioners, providing a forum for discussion, and showcasing the outstanding interest and support of the State of Minnesota for biological control. Requests to hold a similar symposium in the future have been received and will be considered.

A.6. Benefits: Minnesotans will have scientific information relative to biological control and its application. There will be renewed interest in biological control for a wider use to protect the environment.


MDA, USDA, U of M
BIOLOGICAL CONTROL WORKING GROUP
1992-93 PROJECTS
Weeds
Leafy spurge #1A
Ragsdale & Wyse
Canada thistle #1B
Smother crop system #1C
Johnson &Wyse
Bio-control Symposium #9
Livestock Facilities
Filth flies #8A
Moon
Greenhouses
Greenhouse pests #8B
Ascerno
Vegetable Crops
Cabbage & broccoli pests #8C
Hutchison
Urban Forests, Shade Trees
Gypsy moth #7
Luhman, Kulman & Schaupp
Field Crops
European corn borer #5
Andow & Olson
Cereal leaf beetle #6A
Sreenivasam, Luhman & USDA
European corn borer field studies #6B
Gagné, Sreenivasam & USDA
Alfalfa insect pests #3
Radcliffe & Flanders
Corn rootworm nematode #4
Ostlie
Microbial Pathogens
Armyworm, Grasshoppers #2A
Kurtti
Potato scab, Verticillium wilt #2B
Anderson, Schottel, & Kinkel
European corn borer #2C
Ragsdale
Monitoring tools for parasites #2D
Jones

LCMR - Agriculture 2,
Program Title: Biological Control of Pests
Program Manager: Dharma D. Sreenivasam, MDA
July 1, 1993

STATEMENT OF OBJECTIVES

1. Development of Biological Control for Leafy Spurge, Canada Thistle and Smother Plant System for Control of Weeds in Corn - Donald L. Wyse (Agronomy) and David W. Ragsdale (Entomology).

- Three species of exotic flea beetles will be investigated for establishment and control of leafy spurge.
- Causal agent of "thistle apical chlorosis" disease isolated and identified as the bacterium *Pseudomonas syringae* pv. *tagetis*. Reduces winter survival and seed production. Develop an effective method of field-inoculation.
- Short-lived, dwarf *Brassica* spp. have been shown to be useful in establishing corn. Smother weeds and reduce wind erosion. A new Brassica hybrid is being developed and selection is proceeding to improve adaptation.

2. Evaluate and Produce Microbial Pathogens for European Corn Borer, Grasshopper and Potato Scab Disease Control - Timothy J. Kurtti and Ulrike G. Munderloh (Entomology), Neil A. Anderson (Plant Pathology), and Janet L. Schottel (Biochemistry).

- Three microbial pathogens (*Nosema pyrausta*, *N. furnacalis*, and *Vairimorpha necatrix*) are in laboratory culture and undergoing selection for virulence against corn borer and grasshopper. A method of infecting grasshopper cells with an entomopox virus is under development. Permits wil be requested for field testing.
- Non-pathogenic isolates of *Streptomyces* have been found that suppress potato scab and effect "permanent" control. Delivery technology is being developed.
- A serological assay specific for *Nosema furnacalis* has been developed. Next assays will be developed for *N. pyrausta* and *Vairimorpha necatrix*. These assays will be used to monitor infections of these pathogens in European corn borer and determine specificity to both pest and natural enemies.
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4. Biological Control of Corn Rootworms - Kenneth R. Ostlie (Entomology)

- The nematode *Steinernema feltiae* and its associated bacterium have been shown to be effective against larval northern corn rootworm. Mass rearing procedures will be developed and selection made for nematode strains with greater host-finding capability.

- Baits containing cucurbitacin (a natural plant product) and trace amounts of insecticide will be tested against adult corn rootworms.

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- Egg parasitism by *Trichogramma nubilale* was shown to be strongly influenced by habitat structure in controlled environments. Selection and release of *Trichogramma* strains/species will be tested and additional studies of biology will be conducted.

6. Biological Control of the Cereal Leaf Beetle, the European Corn Borer and Selected Species of Grasshoppers - Dharma D. Sreenivasam (MDA)

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- The multi-host parasitoid *Coccygomimus disparis* has been released and recovered. This gypsy moth parasitoid can attack larvae of tent caterpillar and other forest pests. Ecological needs for establishment will be studied.

- The tachinid *Compsilura* has been recovered from white-marked tussock moth. Better adapted strain will be imported if indicated. Prior establishment of these parasitoids should lessen gypsy moth impact if this species becomes established in Minnesota.

8. New Strategies for Biological Control of Filth Flies - Roger D. Moon and Valerie J. Cervenka (Entomology). Integrating Biological Control of Arthropods into Commercial Greenhouse Production in Minnesota - Mark Ascerno (Entomology). Biological Control of the Insect Pest Complex in Commercial Cabbage and Broccoli Production in Minnesota: Evaluation and implementation - William Hutchison (Entomology).

- Naturally occurring parasitoids do not become active until mid-summer, too late to adequately control stable flies and house flies. Flies breed so rapidly in summer that it is impractical in terms of costs and rearing capacity to produce enough parasitoids to effect control.

- A new strategy of releasing parasites is proposed. Parasitoids would be released inside dairies during fall and spring. The idea is that if control can be effected early in the fly-breeding season, summer populations may increase to overwhelming numbers. This will be tested in 9 dairies.

- Integrate a biological control program for selected pests into a commercial greenhouse production for quality floriculture and bedding plants.

- Two promising approaches for biological control of cabbage and broccoli pests will be field tested; use of a bacterium specific to moth and butterfly larvae and a naturally occurring parasitic wasp.

The LCMR-Biological Control Project research involves 15 scientists from 2 colleges and 5 departments of the University of Minnesota and 4 scientists from the Minnesota Department of Agriculture.

OVERALL PROJECT RESULTS

In the area of weed control:

Biological control of leafy spurge using three exotic flea beetles has been successful. Redistribution of the beetles is in progress and they will be available to resource managers in two years. The project will continue with funding from MDA and the U. of M. The bacterium isolated and being patented will be field tested against Canada thistle and its application methodology worked out. A smother crop system using dwarf mustard species needs more work.

In the area of microbiological control:

Cultivation of microsporidian pathogens in tissues (*in vitro*) has been improved for corn borers and grasshoppers. Spore production increased from 15 million to 550 million per caterpillar. Suppressive isolates tested against potato scab yielded up to 80% scab reduction over a four year field test. A species specific serological assay for *Nosema furnacalis*, a pathogen of the European corn borer, can document the infection in 48 hours.

In the area of field and vegetable crop control:

Three parasitoids of the alfalfa weevil, as well as one pathogen, are widely distributed and collectively reduce alfalfa weevil populations by 90% or more. The timing of occurrence of the parasitoids is predictable when plotted on a degree-day scale. Field trial experiments investigated the effects of rate and timing of nematode application relative to corn rootworm development and found that nematodes significantly reduced corn root injury. Other factors, such as competition for food among rootworm larvae and how to quantify effects of nematode mortality and competition need further study. The European corn borer biological control continues to present problems and promises. Production and field testing of egg parasite species (3 strains) have improved. We now know the optimum numbers needed for release, the amount of parasitism expected and the impact on corn yields by these parasites. The strain efficacies affecting quality of the parasite, and the combined impact of egg, larval and pupal parasites need further study before wider scale applications.

Two years of field data documented the impact of the bacterium, *Bacillus thuringiensis* and naturally occurring parasitoids. Several treatments were found to be effective at significantly reducing larval infestations by the imported cabbageworm, diamondback moth and cabbage looper.

In the area of urban, livestock and commercial greenhouse pest control:

A gypsy moth parasite being reared in the MDA lab has been released at 9 sites and has been recovered from the three native host species — whitemarked tussock moth, eastern and forest tent caterpillar and fall webworm. The European corn borer is used to rear this parasite as well as all other corn borer parasites. A grand total of 3.2 million parasites were produced for release against houseflies and stable flies. Measurement of summer fly abundance and natural parasitism showed a 36-fold increase than average untreated site. Parasite survival over winter needs further study.

Newly constructed greenhouses for commercial rose production were used to evaluate the efficacy of thrips predators, aphid predators and parasites. The predators look promising.

PROJECT RESULTS USE AND DISSEMINATION

A symposium, "Ecological Interactions and Biological Control" was held October 25-26, 1992. Over 140 scientists from the United States, Canada, Israel, Korea, Mexico and Europe attended the symposium. In addition, 35 posters were displayed.

Our research in the past four years (1990-93) has yielded 37 publications in peer reviewed scientific journals, 1 Ph.D. dissertation, 10 presentations at national and international conferences, and one commercial vegetable pest management guide.

LCMR - Agriculture 2,
Program Title: Biological Control of Pests
Program Manager: Dharma D. Sreenivasam, MDA
July 1, 1993

STATEMENT OF OBJECTIVES

1. Development of Biological Control for Leafy Spurge, Canada Thistle and Smother Plant System for Control of Weeds in Corn - Donald L. Wyse (Agronomy) and David W. Ragsdale (Entomology).

- Three species of exotic flea beetles will be investigated for establishment and control of leafy spurge.

- Causal agent of "thistle apical chlorosis" disease isolated and identified as the bacterium *Pseudomonas syringae* pv. *tagetis*. Reduces winter survival and seed production. Develop an effective method of field-inoculation.

- Short-lived, dwarf *Brassica* spp. have been shown to be useful in establishing corn. Smother weeds and reduce wind erosion. A new Brassica hybrid is being developed and selection is proceeding to improve adaptation.

2. Evaluate and Produce Microbial Pathogens for European Corn Borer, Grasshopper and Potato Scab Disease Control - Timothy J. Kurtti and Ulrike G. Munderloh (Entomology), Neil A. Anderson (Plant Pathology), and Janet L. Schottel (Biochemistry).

- Three microbial pathogens (*Nosema pyrausta*, *N. furnacalis*, and *Vairimorpha necatrix*) are in laboratory culture and undergoing selection for virulence against corn borer and grasshopper. A method of infecting grasshopper cells with an entomopox virus is under development. Permits will be requested for field testing.

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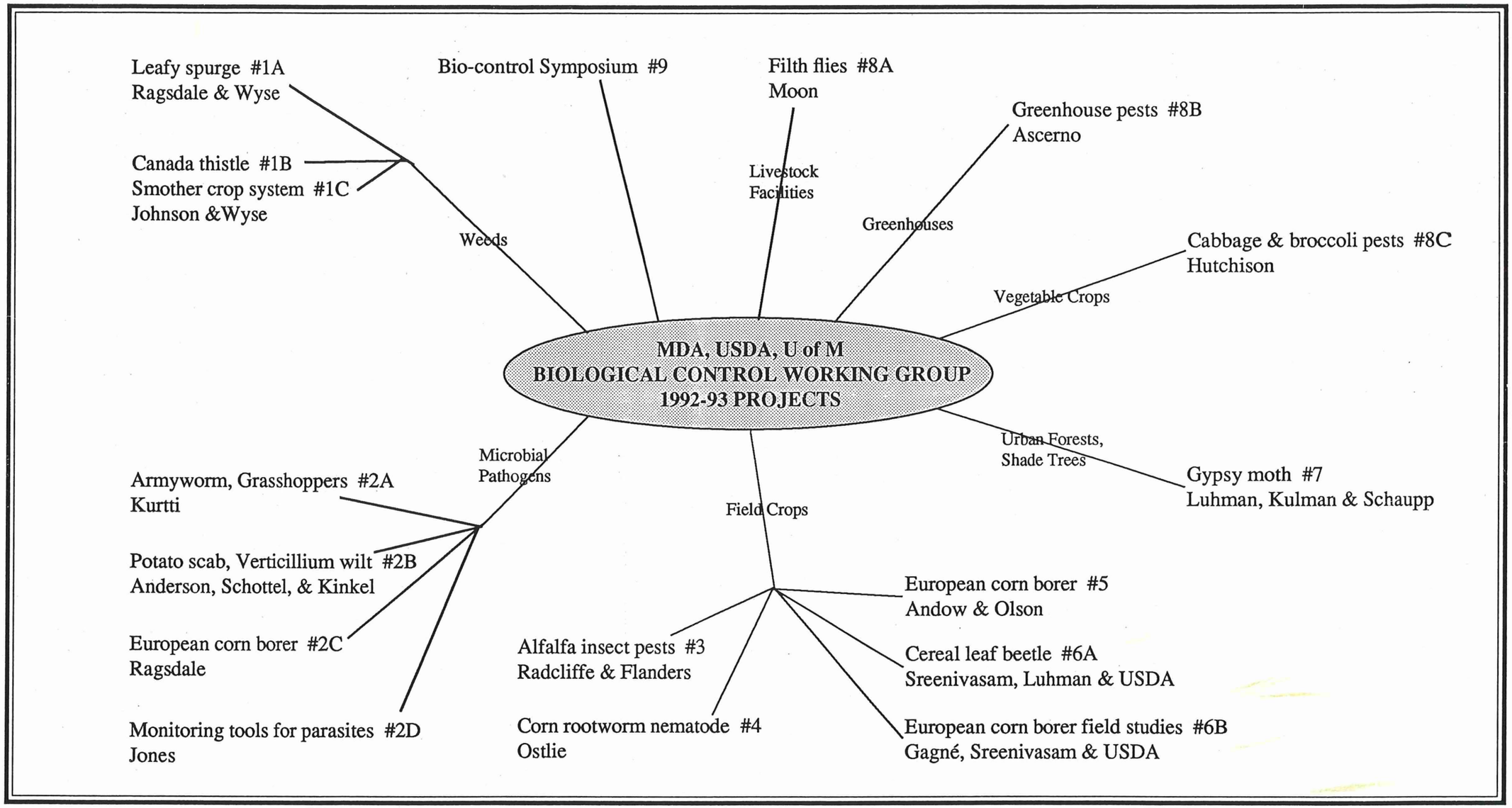
MDA, USDA, U of M
BIOLOGICAL CONTROL WORKING GROUP
1992-93 PROJECTS
Weeds
Leafy spurge #1A
Ragsdale & Wyse
Canada thistle #1B
Smother crop system #1C
Johnson &Wyse
Bio-control Symposium #9
Livestock Facilities
Filth flies #8A
Moon
Greenhouses
Greenhouse pests #8B
Ascerno
Vegetable Crops
Cabbage & broccoli pests #8C
Hutchison
Urban Forests, Shade Trees
Gypsy moth #7
Luhman, Kulman & Schaupp
Field Crops
European corn borer #5
Andow & Olson
Cereal leaf beetle #6A
Sreenivasam, Luhman & USDA
European corn borer field studies #6B
Gagné, Sreenivasam & USDA
Alfalfa insect pests #3
Radcliffe & Flanders
Corn rootworm nematode #4
Ostlie
Microbial Pathogens
Armyworm, Grasshoppers #2A
Kurtti
Potato scab, Verticillium wilt #2B
Anderson, Schottel, & Kinkel
European corn borer #2C
Ragsdale
Monitoring tools for parasites #2D
Jones