Date of Report: December 29, 1995

**LCMR Research Work Program 1994-95**
**Final Status Report**



I. **Project Title:** Biological Control of Plant and Animal Pests

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A. Legal Citation: M.L. 93 Chpt. 172, Sect. 14, Subd. 3(a)

Total Biennial LCMR Budget: $880,000

Balance: $ -0-

This appropriation is from the oil overcharge money to the commissioner of administration for transfer to the commissioner of agriculture to develop, test and implement biological control agents to reduce the use of petroleum-based chemicals. 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. **LMIC Compatible Data Language:** Not applicable.

C. **Status of Match Requirement:** Not applicable.

II. **Project Summary:**

The overall goal of biological control of plant and animal pests is to identify, develop, test and implement biological control agents in Minnesota. This program focuses on effective integrated pest control with a reduction in chemical use and energy costs. Minnesota's cropland alone exceeds 36 million acres of which more than half is treated for pests. Biological control when implemented as part of an integrated pest management program would save an estimated $145 million in chemical costs and an additional $300 million in energy costs. Our research, experimental design, methodology, validation criteria and application costs developed in the past 4 to 6 years provide a solid base to refine most of our projects for implementation.

**Importation and establishment of natural enemies** has accomplished classical biological control of leafy spurge and will be extended to musk thistle, cereal leaf beetle, gypsy moth, filth flies and cabbage and broccoli pests.

**Environmental manipulation** using a broad range of techniques will be used for *Brassica* smother plants, cocklebur, scab and verticillium wilt of potato, sugarbeet root rot, alfalfa pests, corn rootworm, and arthropods in commercial greenhouse production.

**Periodic releases of natural enemies** will be used against the European corn borer and insect pests of small grains and forage crops.

**Preservation of existing natural enemy fauna** will encompass all of our projects.

III. **Statement of Objectives:**

1. Implementation of the newly discovered Canada thistle biocontrol agent, *Pseudomonas syringae* pv. *tagetis* for use in cropland and noncropland. Page 4.
2. Continuation of the development of dwarf *Brassica* smother plants for weed control in corn and soybeans. Page 8.
3. Biological control of cocklebur in Minnesota. Page 11.
4. Biological control of Musk thistle in Minnesota. Page 13.
5. Evaluate microbial entomopathogens for control of armyworm and grasshoppers in Minnesota. Page 16.
6. Release and evaluation of a microsporidian pathogen for biological control of the European corn borer. Page 18.
7. Biological control of scab and verticillium wilt of potato. Page 22.
8. Enhanced suppression of sugarbeet root rot pathogen. Page 24.
9. Enhancing biological and cultural control of alfalfa insect pests. Page 25.
10. Biological control of the western corn rootworm using an entomoparasitic nematode. Page 28.
11. Determine how production process influences the quality of *Trichogramma* used in biological control. Page 30.
12. Implement biological control of European corn borer by mass rearing and release of egg and larval parasitoids. Page 35.
13. Continuation of biological control for the cereal leaf beetle. Page 38.
14. Biological control of the insect pest complex in cabbage and broccoli: Evaluation and Implementation. Page 40.
15. Anticipatory Biological Control of Gypsy Moth: Evaluation and Implementation. Page 42.
16. Enhanced natural control of filth flies through winter augmentation and selective importation. Page 46.
17. Integrating biological control of arthropods into commercial greenhouse production. Page 48.

This program, begun in F.Y. 1988, is designed to investigate biological control in four broad areas.

**In the area of weed control** (Objectives 1-4), importation and establishment of two flea beetles, *Aphthona nigriscutis* and *A. cyparissae* to control leafy spurge, *Euphorbia esula* has been successful. *A. nigriscutis* is being redistributed to new farm sites in 1992 which will conclude this project.

A promi new bacterial biological control agent, *Pseudomonas syrin* *. tagetis*, for control of Canada thistle (*Cirsium arvense*) has been discovered and is being tested. Development of the technology to introduce this bio-herbicide and factors affecting its performance will be studied.

A smother plant system using *Brassica campestris* is being developed to control weeds in soybeans and corn. Promising smother plant lines will be evaluated against annual weeds and the development and yield of soybeans and corn. Biological control of cocklebur (*Xanthium strumarium*) is a new project that focuses on isolation, identification and characterization of microorganisms deleterious to the roots and seed pods and screen for anti microbial activities of the plant, plant residue and microorganisms. Biological control of musk thistle, *Carduus nutans*, using musk thistle weevils, *Rhinocyllus conicus* and *Coleophora klimeschiella* was begun in 1976 and was discontinued in 1980. This project will reintroduce and expand on site establishment of the weevils which have successfully controlled musk thistle in Missouri and Kansas.

**In the area of micro-biological control** (Objectives 5-8), virulence and persistence under laboratory and field conditions will be evaluated for *Nosema acridophagus* and *N. locustae* against grasshoppers (*Melanoplus sanguinipes, M. differentialis, M. bivittatus,* and *M. femurrubrum*) and *Vairimorpha necatrix* and *Nosema furnacalis* against armyworm, *Pseudaletia unipuncta. Nosema furnacalis*, a pathogen of Asian corn borer, *Ostrinia furnacalis* will be evaluated against the European corn borer, *Ostrinia nubilalis*. Current levels of *N. pyrausta*, a pathogen of Minnesota field populations of ECB, and mortality induced by *N. furnacalis* and *N. pyrausta* in the field will be assessed.

Microbial isolates of Streptomyces are being developed to suppress the causal soil microorganisms of scab and verticillium wilt of potato, *Streptomyces scabies* and *Verticillium albo-atrum*, and *V. dahliae*. This project is unique in its investigation of the possibility of controlling multiple pathogens simultaneously using a single biocontrol organism.

Finally a study is directed toward enhanced suppression of sugarbeet root rot pathogen, *Aphanomyces cochlioides* using green oat crop residue. This project will investigate how green crops can be managed to shift the biological balance in soil to preserve and enhance beneficial microorganisms and suppress plant pathogens.

**In the area of field and vegetable crop pest control** (Objectives 9-14), alfalfa pests, especially alfalfa weevil, *Hypera postica*, now have a complex of five parasitoid species, *Microctonus aethiopoides, M. colesi, Tetrastichus incertus, Bathyplectes curculionis, B. anurus* and a fungal disease, *Zoophthora phytonomi*. This project will integrate and manipulate cultural strategies with detailed knowledge of parasitoid phenology and ecological dynamics in on-farm sites.

Corn rootworm control using an entomoparasitic nematode is continuing. In 1992, field farm trials explored individual and interactive effects of nematode application rate and relative timing with significant effects. This study will be repeated in 1993 to determine optimum application timing and rate. In addition, virulence, size and host finding ability of the nematode will be studied. Parasitoids of the European corn borer are being investigated continuously. The egg parasitoid, *Trichogramma* has proven promising but varies considerably in its performance due to factors not entirely attributable to environmental variations. One part of the project will focus on production process influences on the . .ity of the parasitoid. The second part of this project is to mas. .oduce the parasitoids and test them in large scale field releases. By using parasitoids that attack different stages of the European corn borer, it will be possible to determine integrated impact of combinations of parasitoids.

The larval parasitoid, *Tetrastichus julis*, has been detected in trace numbers in Minnesota in the cereal leaf beetle. Development of field insectaries for release of this parasitoid is proposed with the assistance of USDA biological control lab in Niles, Michigan. Laboratory rearing of the egg parasitoid, *Anaphes flavipes* will continue along with investigation of two other larval parasitoids, *Lemophagus curtus* and *Diaparsis temporalis*.

Insect pest complex of cabbage and broccoli include the imported cabbageworm, the cabbage looper and the diamondback moth. The primary focus of this project is to assess the performance of new formulations of *Bacillus thuringiensis* var. *kurstaki* (Bt) and the impact of the parasitoids *Cotesia rubecula* and *C. glomeratus*. Impact of integration of Bt and the parasitoids will be studied.

**In the area of urban, livestock and commercial greenhouse pest control** (Objectives 15-17), Gypsy moth has been trapped in Minnesota for the past several years but is not known to be established. Anticipatory biological control is a viable strategy. Minnesota Department of Agriculture has been successful in the rearing and field release of an ichneumon wasp, *Coccygomimus disparis*, which has been established in native pest populations of tent caterpillars, *Malacosoma* spp., whitemarked tussock moth, *Orgyia leucostigma*, and the fall webworm, *Hyphantria cunea*. This project will expand and evaluate field release sites and in addition work with the existing tachinid parasite, *Compsilura concinnata*.

Enhanced natural control of filth flies has been an ongoing project. The house fly, *Musca domestica* and the stable fly, *Stomoxys calcitrans* are targeted for control with the wasp *Muscidifurax zaraptor*. This study will evaluate on farm effects of pre-release conditioning on survival and timing of spring emergence, which if proven efficient can lead to commercial applications.

Biological control of arthropods in commercial greenhouse production needs integration. A predator, *Delphastus pusillus*, of greenhouse whitefly, *Trialeurodes vaporariorum* on fuchsia and tomato, a parasitoid *Encarsia formosa* on alstroemeria will be evaluated in a commercial greenhouse. Newly constructed commercial greenhouses will be used to release and establish 2 predacious mites, *Neoseiulus cucumeris* and *N. barkeri* to control thrips combined with ladybeetles and a parasitoid, *Aphidius matricariae*, to control aphids on roses.

**IV. Research Objectives:** (Please refer to individual projects.)

**V. Evaluation:** For the F.Y. 94-95 biennium the program can be evaluated by its ability to: (1) reach implementation of about 40 percent of the proposed projects; (2) adequately assess the effectiveness and practicality of the biological control agents; (3) identify the areas where further research and field experimentation is needed to complement the ongoing studies; (4) transfer technology and educate the growers and measure success based on the number of crop producers that adopt the new technology.

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

**VI. Context within field:** (For more detail see individual projects.)

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

Each objective focuses on an important need in pest management that will result in reduced pesticide use through the incorporation of biological control alternatives. These objectives are unified by a common set of ecological theories that underlie biological control implementation. Results from project objectives are shared during meetings of the Enhanced Natural Control Working Group and is a result of our symposium, Ecological Interactions and Biological Control. Over 140 scientists from the United States, Canada, Israel, Korea, Mexico, and Europe attended the symposium. A book arising from this symposium dealing with the unifying ecological theories that relate to biological control will provide a further basis for interaction of our biological control scientists.

A direct result of these projects will be reduction in the amount of chemical pesticides used. This will have the impact of preserving whatever natural enemies already exist in the habitat.

Introduction of agents is an important emphasis and is mentioned in many objectives (eg. 4, 6, 9, 10, 11, 13, 14, 15, 16, 17).

Strategies for large scale releases and implementation need to be developed. For example, parasites of cereal leaf beetle need to be spread over large areas and in large numbers at a time when pest (CLB) numbers are lower and more easily controlled. Development and establishment of field insectaries is a logical next step.

Biological control approaches have a high probability of being implemented as part of an integrated pest management strategy where other techniques of control are used (eg. objectives 2, 3, 9, 10, 14, 17).

This program is organized to allow research, development and implementation of biological control in four broad areas because of separate disciplines which integrate into one interactive and inter-dependent team of scientists who regularly interact. A relationship has been established its with participants in this project. Continuation will result in useful biological control technology for Minnesota.

A significant amount of work will be done on working farms or the actual habitat of application (eg. objectives 1, 4, 5, 9, 12, 13, 14, 15, 16, 17). Wording changes have been made to better reflect this fact. In addition, a direct link exists between objectives 5 and 6, and 11 and 12. Implementation strategy via demonstration and extension education will produce a common link. Furthermore, coordinated linkages will occur for specific end user groups (eg. organic growers, livestock producers, greenhouse operators).

Our projects are diverse because of the diversity of pest management needs of Minnesota. Each project addresses an important area of need that will result in reduced pesticide use for the growers, producers and the public. All objectives are potentially successful although the length of time varies by objective.

**VII. Benefits:** Pest management has focused heavily on chemical control since the 40's. The public is now calling for alternative approaches that maintain the richness of animal and plant diversity including preservation of existing natural enemies while reducing chemical use and accommodating sustained biological control options. The potential benefits to U.S. agriculture alone could be in the billions of dollars.

Biological control will have significant beneficial impact on human health, surface and ground water, and food. Urban environments will have less toxic chemicals and pollution. Biological control will be an integral part of crop, pasture and non-cropland systems and will reduce the emergence of pesticide resistant strains.

Successful demonstration of biological control technology will serve to increase public awareness of biological control alternatives and help promote acceptance. Production and marketing of beneficial organisms by the private sector will be promoted.

The role of the program manager is to screen potential objectives for their applicability to the goals of this project in doing work that will lead to practical biological methods of pest control that can be integrated into the diverse "real world" pest management needs of Minnesota. The program manager is also responsible for articulating the purpose and value of this project and its objectives to decision-making groups and the public. The program manager works as a liaison between the agencies involved in this endeavor. The program manager also coordinates and reviews six month status reports for submission to LCMR.

**VIII. Dissemination:** Our research findings are regularly presented at national, regional and state scientific professional meetings. The Symposium, "Ecological Interactions and Biological Control" held in October 25-27, 1992 is a good example of sharing Minnesota's accomplishments in biological control.

Our research in the past four years 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.

**IX. Time:** . gical control research and implementation has been and co. s to be a long-term program primarily because of using living organisms and their complex ecological interactions. Some of the projects may take 2 years and others another two years. Projected completion by 1995: Projects 6, 7, 8, 9, 10, 12, 14 and 16; Introductions of biological control agents completed by 1995: Projects 4 and 13; Development, implementation and monitoring beyond 1995: Projects 1, 2, 3, 5, 11 and 15. The research and monitoring part of the program will continue for a minimum of 10 to 12 years.

**X. Cooperation:** This is a cooperative, multiagency and multidisciplinary program. Six years ago it started with 5 scientists; today this program has 26 scientists, 4 from MDA, 21 from U of M and 1 from Mankato State University. There will be at least another 40 people including research associates, lab technicians and students.

**XI. Reporting Requirements:** Semiannual status reports will be submitted not later than January 1, 1994, July 1, 1994, January 1, 1995 and a final status report by June 30, 1995.

**XII. Literature Cited:** (See individual projects.)

**IV. Research Objectives**



**OBJECTIVE 1.**

**A. Title of Objective:** Implementation of the newly discovered Canada thistle biocontrol agent, *Pseudomonas syringae* pv. *tagetis* for use in cropland and non-cropland.

**A.1. Activity:** Assess the impact of bioherbicide formulations of *Pseudomonas syringae* pv. *tagetis* on Canada thistle infestations in cropland and non-crop land areas. Data on bioherbicide efficacy will be collected at University of Minnesota Experiment Station, agricultural production and roadside sites where *Pseudomonas syringae* pv. *tagetis* bioherbicide has been applied. Evaluation of the bioherbicide in soybean was begun in FY91, roadside studies were started in FY92.

**A.1.a. Context within the project:** A promising new bacterial biological control agent for control of Canada thistle has been discovered and is being tested (Johnson and Wyse, 1991). Development of the technology necessary to use the organism as a bioherbicide is the next logical step. Preliminary data show that applications of the bioherbicide can control Canada thistle in corn and soybean. Further study is required to identify factors affecting bioherbicide performance.

**A.1.b. Methods.** *Pseudomonas syringae* pv. *tagetis* bioherbicide will be applied to crop and non-crop areas where Canada thistle has been intentionally introduced for experimental purposes or has become established naturally, as in roadsides or on farm production areas.

**Sites: Artificially infested soybean field**. A soybean field in Rosemount, MN will be artificially infested with Canada thistle. Thistles that are greenhouse-grown to flowering in 30 x 30 x 30 cm pots, will be cut back and transplanted into the field during May.

**Naturally infested sites.** Roadside sites infested with Canada thistle will be provided by the Minnesota Department of Transportation. Corn, soybean, small grain, and/or other agricultural production fields infested with Canada thistle will be provided by grower-cooperators in Minnesota.

**Bioherbicide.** The bioherbicide will be prepared using an isolate of *P. syringae* pv. *tagetis* cultured from naturally infected Canada thistle collected in Waseca, MN. The standard formulation will consist of a suspension of $10^9$ colony forming units (cfu)/ml in 10% v/v 0.01M phosphate buffer (pH 7) and 0.05% v/v organosilicone surfactant (Stevens and Zabkiewicz, 1988).

**A.1.c. Materials:** Materials necessary to accomplish this objective are an environmental gyratory shaker, sterile culture facilities and supplies, portable spray equipment, vehicles and agricultural machinery needed for soybean production. With the exception of the shaker and some supplies, the equipment is on hand at the University of Minnesota.

**A.1.d. Budget:** $18,667 **Balance:** $-0-

**A.1.e. Timeline:**

| | 7/93 | 1/94 | 6/94 | 1/95 | 6/95 |
|---|---|---|---|---|---|
| Artificially-infested soybean experiment: | | | | | |
| Thistles transplanted | ------- | | ------- | | ---------- |
| Thistles inoculated | ------ | | ------- | | ------- |
| Data analysis | | | | | ---------- |
| Naturally infested sites: | | | | | |
| Field inoculations | ---------- | | --------- | | ------ |
| Data analysis | ---------- | | | | |

**A.1.f. Status:** Final Status Report, September 1, 1995. Two replicated experiments in soybean are now complete. In both experiments, spring regrowth of field-transplanted Canada thistle was significantly reduced when single spray applications of the phytopathogenic bacterium *Pseudomonas syringae* pv. *tagetis* (Pst) were made anytime between late postemergence to early bud stage. The efficacy window for Pst application is now known to be relatively broad

In roadside studies, single and double treatments suppressed current season Canada thistle growth and significantly reduced following season regrowth. There was no detectable injury to grasses or woody ornamentals, even though these were contacted by spray. It is clear that *Pseudomonas syringae* pv. *tagetis* is most effective on Canada thistle where competition from other plants is high

**A.2. Activity:** Improve efficacy and shelf life of bioherbicide formulations. Test the effects of certain additives and production methods on efficacy and shelf life of the bioherbicide.

**A.2.a. Context within the project:** We would like to improve formulation and application technology to enhance herbicidal action, specificity, and shelf life, and thus create a useable product. Development of an effective, stabilized product formulation would facilitate use of this new biocontrol technology, resulting in decreased use of chemical herbicides. There is little risk of surface or ground water contamination with this bioherbicide, and the exposure of users to herbicides would be greatly reduced. Thus, the transition from chemical to biological weed control would significantly reduce human health risks as well as impacts on natural habitats of fish and wildlife.

Many properties of *Pseudomonas syringae* pv. *tagetis* have already been described (Mitchell and Durbin, 1981; Lukens and Durbin, 1985). It grows quickly in culture, causes injury to Canada thistle 4-7 days after application and has excellent potential as a bio-control agent (Johnson and Wyse, 1991). The addition of other components, such as plant growth regulators, uptake enhancing substances, or microbial nutrients may significantly increase activity of the bioherbicide. Storage, production, and stabilization methods will be developed to produce an effective product with extended shelf life.

**A.2.b. Methods:** Initial disease enhancement experiments will be conducted in the greenhouse. Potential formulation additives will be applied to seedlings with a standard inoculum formulation. Seedlings will be evaluated for disease incidence, severity and symptom duration. Promising additives will be field tested on a dense natural stand of Canada thistle.

Stabilization methods, such as low temperature liquid culture storage and freeze-drying will be evaluated. Shelf life will be measured by periodic serial dilution culturing to verify viability and plant inoculation to verify pathogenicity.

**A.2.c. Materials:** Materials necessary to accomplish this objective are greenhouse and growth chamber space, sterile culture facilities and supplies, a freeze dryer and basic laboratory equipment. All of these are available at the University of Minnesota.

**A.2.d. Budget:** $18,667 **Balance:** $-0-

**A.2.e. Timeline:**

| | 7/93 | 1/94 | 6/94 | 1/95 | 6/95 |
|---|---|---|---|---|---|
| Identify potential additives | ---------- | | | | |
| Greenhouse tests | ---------- | | | | |
| Field tests | | | ------ | | ------ |
| Stabilization tests | ---------- | | | | |

**A.2.f. Status:** Final Status Report, September 1, 1995. Freeze dried *P. syringae* pv. *tagetis* formulations are being developed in cooperation with Mycogen Corporation as a potential product formulation. Viability of freeze dried Pst is greater than 75%. Freeze dried material can be stored several months at 22-28 C and indefinitely at 2-3 C. In field trials, Pst survived more than 24 hours at 25 C in spray formulation, indicating the material remains active in the sprayer tank long enough for large-scale field application.

**A.3. Activity:** Determine host range of the *Pseudomonas syringae* pv. *tagetis* bioherbicide. Data on the host range of the *Pseudomonas syringae* pv. *tagetis* bioherbicide will be gathered in greenhouse and field studies.

**A.3.a. Context within the project:** The host range reported for *Pseudomonas syringae* pv. *tagetis* in scientific literature is incomplete and contradictory (Styer and Durbin, 1982; Shane and Baumer, 1984; Rhodehamel and Durbin, 1985). It is essential to document natural hosts of the bacterium. Preliminary data indicates host range of the bioherbicide formulation of *Pseudomonas syringae* pv. *tagetis* differs from the natural host range. New, useful applications of the bioherbicide technology might be discovered, and damage to non-target plants could be avoided by testing a wide selection of potential hosts.

**A.3.b. Methods:** Important weed species and a diverse collection of other potential hosts will be screened for reaction to the *Pseudomonas syringae* pv. *tagetis* bioherbicide. In greenhouse studies, seedlings will be quantitatively inoculated with the *Pseudomonas syringae* pv. *tagetis* bioherbicide and disease symptoms recorded. In field studies, weed

series and natural stands of weeds in crop and non-crop syst. will be treated and observed.

**A.3.c. Materials:** Materials necessary to accomplish this objective are greenhouse and growth chamber space, field sites, sterile culture facilities and supplies. All of these are available at the University of Minnesota. Existing and new weed nurseries at the Rosemount Experiment Station will be used to evaluate the *Pseudomonas syringae* pv. *tagetis* bioherbicide on weeds under field conditions. The bioherbicide will also be tested on naturally occurring weeds in crop and non-crop areas supplied by grower-cooperators, MN-DOT, or University of Minnesota Experiment Stations.

**A.3.d. Budget:** $18,666 **Balance:** $-0-

**A.3.e. Timeline:**

| | 7/93 | 1/94 | 6/94 | 1/95 | 6/95 |
|---|---|---|---|---|---|
| Greenhouse host range studies | ---------- | | | | |
| Weed Nursery host range studies | | | --------- | | ------ |
| Host range studies on naturally occurring weeds | | | --------- | | ------ |

**A.3.f. Status:** Final Status Report, September 1, 1995. In field studies, Corn and soybean were not injured by *Pseudomonas syringae* pv. *tagetis,* despite multiple applications. Cereal seedlings (oats, barley and wheat) were not injured by Pst in greenhouse studies, and older cereals (joint growth stage) were not injured by Pst in field trials. Weeds not injured by Pst include pigweed, leafy spurge, lambsquarters, bindweeds, nightshades, and most grasses. Some important weeds not naturally infected by the bacterium, such as cocklebur, horseweed, and musk thistle, are injured or killed when sprayed with inundative bacterial concentrations ($10^8$ cells/ml) in the spray formulation.

**V. Evaluation**: The program should be evaluated by advancement of the bacterium *Pseudomonas syringae* pv. *tagetis* towards a useful biological control agent. Criteria for evaluation are: 1) a detailed description of the host range for the bio-herbicide, especially regarding economically important plants; 2) discovery of formulation and/or production technologies that increase efficacy of shelf life; and 3) demonstration and documentation of herbicidal activity of the formulated bacterium.

**VI. Context within field:** The usefulness of introducing 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 certain weeds with *P. syringae* pv. *tagetis* resulted in typical apical chlorosis symptoms, but spray applications with aqueous bacterial suspensions failed to produce infection. A new inoculation method under development dramatically improved infection of weed by *P. syringae* pv. *tagetis*. Low pressure spray application of bacterial suspension in phosphate buffer with an organosilicone surfactant has reliably produced apical chlorosis symptoms of greater severity than those produced by needle inoculation (Johnson and Wyse, 1991; Stevens and Zabkiewicz, 1988). Unlike inoculation techniques requiring physical injury to the target plant, this spray application method could eas.. be adapted for field application. This technique needs to be ev..ated further to determine if problem weeds, such as Canada thistle, can be selectively controlled in crop and non-crop systems with spray applications.

Phytopathogenic fungi have been examined by many researchers as potential biological control agents, but these organisms are limited by their environmental requirements and characteristics in culture (Ormeno-nunez, et al., 1988; Sands et al., 1990; Smith R.J. Jr., 1986). Bacteria are ideally suited to large scale fermentation for industrial use, but little bio-control work has been done with phytopathogenic bacteria because they normally require wounds for successful host infection. Our current application technology precludes the requirement for wounding, thus enabling us to use the bacterium for biological control.

**VII. Benefits:** Development of the *Pseudomonas syringae* pv. *tagetis* bioherbicide would have significant beneficial impact on human health, environmental pollution, and agricultural competitiveness. This bioherbicide would be much less toxic than chemical alternatives, and thus greatly reduce impacts on surface and ground water and protect the health and safety of the users and consumers. This product would be especially useful in areas where conventional herbicide application is impractical because of water contamination of spray drift hazards. Successful demonstration of this technology will serve to increase public awareness of biological control alternatives and help to promote acceptance of future biocontrol alternatives.

**VIII. Dissemination:** Results from this project will be presented at national, regional, and state scientific professional meetings. The same results will later be published in major peer-reviewed plant science journals. Results of this project will also be shared with potential future users of the technology and to the general public. Presentations have already been made at Minnesota Department of Transportation Herbicide Applicator Training sessions, at the Minnesota Science Museum, and in course lectures in the Department of Plant Pathology, University of Minnesota. New information about biological control generated by the project will be incorporated in weed science courses taught in the Department of Agronomy and Plant Genetics and in guest lectures given in other departments at the University of Minnesota.

**IX. Time:** Development of useful biological control technology requires a long term investment of time and effort. With the advent of low-input sustainable agriculture, we anticipate a need for continued research to further develop this and other bio-control technologies. Funding beyond the FY94-95 will be requested from LCMR.

**X. Cooperation:**

| Name | Description | Role |
|---|---|---|
| Dr. David R. Johnson<br>Agronomy/Plant Genetics | Plant Pathologist | Design/conduct experiments, analyze data, write reports |
| Jim Byron<br>RR 1 Box 61 | Soybean, corn<br>small grain farmer | Provide test sites |

| | | |
|---|---|---|
| Leo Holm<br>Minnesota Department<br>of Transportation | Agricultural Engineer | Provide test sites |

**XI. Reporting Requirements:** Semiannual status reports will be submitted not later than January 1, 1994, January 1, 1995, and a final status report by June 30, 1995.

**XII. References (See Detailed Work Program)**

**Qualifications:**

Dr. Donald L. Wyse, **Principal Investigator**

**Professional Position:** Professor - University of Minnesota
Department of Agronomy and Plant Genetics
411 Borlaug Hall, 1991 Buford Circle
St. Paul, MN 55108
612-625-1232

**Education:** Ohio State University; B.S., Agronomy, 1970
Michigan State University; M.S., Crop Science (Weed Science), 1972
Michigan State University; Ph.D., Crop Science (Weed Science), 1974

**Research Experience:** Dr. Wyse is the project leader for research related to perennial weed control in agronomic crops and lead scientists 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) mechanisms of bud dormancy in perennial weeds; d) impact of herbicides on surface and ground water quality; e) development of smother plants for weed control.

**Selected Publications: (See Detailed Work Program)**

**Name:** Dr. David R. Johnson

**Professional Position:** Research Associate-University of Minnesota
Department of Agronomy and Plant Genetics
411 Borlaug Hall, 1991 Buford Circle
St. Paul, MN 55108
612-625-1232

**Education:** University of Minnesota; B.S., Plant Health Techology, 1980
University of Minnesota; M.S., Plant Pathology, 1986
University of Minnesota; Ph.D., Plant Pathology, 1991

**Professional Organizations:** American Phytopathological Society
Gamma Sigma Delta
Sigma XI

**Research Interests:** Dr. Johnson is currently employed as a post-doctoral Research Associate working on biological control of Canada thistle. Previous research dealt with plant tissue culture, *in vitro* selection of wild rice with fungal pathotoxins, and genetic disease resistance in *Avena* spp. of diverse origins.

**Selected Publications: (See Detailed Work Program)**

**OBJECTIVE 2:**

**A. Title of Objective:** Continuation of the development of dwarf Brassica smother plants for weed control in corn and soybeans.

**A.1. Activity:** Develop additional short term smother plants (dwarf-*Brassica sp.*) that have the potential to replace herbicides in corn, soybean and other crops as part of an integrated pest management system.

**A.1.a. Context within the project:** The goal of the project is to develop a smother plant system that will control weeds in soybean and corn. Since the smother plants are not currently available for evaluation, they must be developed through plant breeding.

**A.1.b. Methods:** Two rapid cycling, short statured *Brassica campestris* lines from the Crucifer Genetics Cooperative will be used as parental lines for the breeding program.

**A.1.c. Materials:** All greenhouse, laboratory and field materials necessary to accomplish this objective are available at the University of Minnesota.

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

**A.1.e. Timeline:**

| | 7/93 | 1/94 | 6/94 | 1/95 | 6/95 |
|---|---|---|---|---|---|
| Design Experiments | -------- | | | | |
| Breeding additional smother plants | ---------- | ---------- | ---------- | | |
| Produce bulk seed | | | ---------- | ---------- | |

**A.1.f. Status:** Final Status Report, September 1, 1995. The *Brassica* breeding program was continued in the greenhouse during the 1994-95 winter. The focus was on the development of one or more dwarf *Brassica* winter annuals suitable for use as a smother plant by crossing commercially available winter annual lines with compatible dwarf, rapid-cycling lines. After seed from each of the crosses had been harvested, the populations were evaluated; at this time the breeding work has progressed through the F1 generation of plants. The breeding program will continue during the winter of 1995-96 with other funding.

**A.2. Activity:** Field evaluation of dwarf *Brassica* smother plants.

**A.2.a. Context within the project:** In our program we are concentrating on several *Brassica sp.* that exhibit the general characteristics necessary for short-term smother plants. As promising smother plant lines are developed in the breeding program, field studies are conducted to determine their effect on the development of annual weeds, and in the development and yield of corn and soybean.

**A.2.b. Methods:** The dwarf *Brassica* smother plants developed in the breeding program will be evaluated in the field at the University of Minnesota St. Paul and Waseca Experiment Stations.

The experimental design will be randomized complete block with a split plot restriction on randomization with four replications. Main plot treatments are weedy or weed-free. Propachlor (2-chloro-*N*-(1-Methylethyl)-*N*-phenylacetamide) will be applied preemergence at 3.4 kg ai $ha^{-1}$ to control annual grass and broadleaf weeds in the weed-free plots. Weed-free plots will be hand weeded throughout the growing season, as needed. Subplot treatments will be dwarf-*Brassica* seeding rates of 0, 530, 1060, 2102, or 4240 seeds $m^{-2}$.

Crop height, smother plant height, and an estimation of smother plant ground cover will be recorded weekly for each subplot during the first 11 weeks of the growing season. Eleven weeks after smother plant emergence a .01 $m^{-2}$ area will be harvested from each subplot, and dry weights of grass and broadleaf weeds, smother plant, and crop will be recorded. At maturity, the crop in the center 5.5 m of each subplot will be harvested and grain yield recorded.

**A.2.c. Materials:** All material required to conduct the field trials are available at the University of Minnesota.

**A.2.d. Budget:** $20,000 **Balance:** $-0-

**A.2.e. Timeline:**

| | 7/93 | 1/94 | 6/94 | 1/95 | 6/95 |
|---|---|---|---|---|---|
| Select field sites | --------- | | | | |
| Conduct field experiments | ---------- | ---------- | | | |
| Prepare summary of results | -- | -- | -- | -- | -- |

**A.2.f. Status:** Final Status Report, September 1, 1995. Two different *Brassica* spp. were evaluated under field conditions for use as a spring-seeded smother plant; a dwarf, rapid-cycling mustard and commercially available winter annuals. The dwarf *Brassica* controlled weeds yearly in the growing season, but did not reduce late season weed biomass. The dwarf *Brassica* did not reduce soybean yields. There was a consistent trend towards reduced corn yields with increasing dwarf *Brassica* populations, but most of these yield reductions were not significant. Winter annual *Brassica* provided good weed control through the entire season in both corn and soybeans. Both *Brassica* spp. need further evaluation under a wider range of environmental conditions. Development of multiple smother crop lines for field situations with different critical periods is a promising area for further research.

**A.3. Activity:** Transfer dwarf-*Brassica* smother plant technology to the farming community.

**A.3.a. Context within the Project:** The successful development of the smother plant technology to replace current weed control practices will take an equally successful education program. The field-study sites will be focal points for demonstrating to farmers the objectives and agronomic management technique necessary for the successful use of short term smother plants.

**A.3.b. Methods:** The technology transfer project will be conducted in southwestern Minnesota with the cooperation of Sister Esther Nickel, Religious Sisters of Mercy, Jackson, MN 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). 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 A 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* lines can be obtained 0.25 acre areas will be used as test plots. The treatments will be replicated. In each case the appropriate chemical weed control treatments will be included for comparison. Commercial 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 crop yield. Data collected will be subjected to an analysis of variance.

**A.3.c. Materials:** The materials required to conduct the on-farm trials are available at the University of Minnesota or can be provided by the cooperating farmers.

**A.3.d. Budget:** $16,000 **Balance:** $12,220

**A.3.e. Timeline:**

| | 7/93 | 1/94 | 6/94 | 1/95 | 6/95 |
|---|---|---|---|---|---|
| Conduct on-farm trials | ---------- | | | | |
| Prepare reports | -- | -- | -- | -- | -- |

**A.3.f. Status:** Third Status Report, September 1, 1995. All of the field research was conducted in cooperation with farmers and the University of Minnesota Branch Experiment Stations. Four farmers were involved in the design and facilitation of the on-farm research and were involved in the summary of the research data. The farmer cooperators concluded that although they had a very strong interest in the cover-crop technology they were convinced that more effective cover crops needed to be developed before this technology could be used in their cropping systems.

V. **Evaluation:** The early success of the project can be evaluated by the number of dwarf-*Brassica* lines we develop that meet the criteria of the ideal smother plant. In the long run the best practical measure of the program's success will be based on the number of crop producers that adopt the new technology. The magnitude of the economic benefit could be obtained by analyzing and comparing the enterprise budgets of farmers who adopted the new technology with budgets of those who retained the herbicide-based weed control strategies.

VI. **Context Within Field:** Widespread use of herbicides has often led to unwanted results such as increased production costs, polluted water resources, and herbicide-resistant weed populations (Enache and Ilnicke, 1986). A cover crop or smother plant that provided acceptable weed control in a crop 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 (Williams and Wicks, 1978). 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 (Warnes, 1988). Echert (1988) has reported that the presence of a rye cover crop, 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 (1977) 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 (1986) 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, winterhardiness 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 egusi melon (*Citrullus vulgaris* Schard.) is commonly intercropped with corn and other crops and functions as a smother plant.

Unamma et al. (1986) 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 weeks 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 (1980) reported similar results using egusi melon and sweet potatoes as smother crops in an intercropping system.

The wi[illegible]ad use of companion crops and smother plants indicated th[illegible]erspecific interference and competition can be managed, with beneficial 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 soybean, 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-adapted *Brassica sp.* possess these general characteristics and so will be the focus of our research.

**VII. Benefits:** The widespread use of dwarf-*Brassica* smother plants could have numerous environmental benefits. First, herbicide use would be tempered, reducing the potential for herbicide contamination of ground water and surface water. Second, the smother plants would help protect soil from wind and water erosion. A reduction in water runoff would also reduce the movement of pesticides and fertilizers into streams and lakes.

**VII. Dissemination:** Results from this project will be presented at national, regional and state scientific professional meetings. The same results will be published in major peer reviewed plant science journals. Results of this project will also be shared with potential future users of the technology and to the general public.

**IX. Time:** The development of breeding material with the desired characteristics for smother plant development is a long term project. The intent of the project is to develop the background information that will allow us to determine the feasibility of developing the smother plant technology. Funding beyond the FY94-95 biennium will be requested from LCMR and other sources.

**X. Cooperation:**

1. Dr. Nancy Jo Ehlke
   Plant Breeder
   Assistant Professor
   Department of Agronomy and Plant Genetics
   University of Minnesota

2. Dr. William Lueschen
   Agronomist
   University of Minnesota
   Southern Experiment Station
   Waseca, MN

3. Sister Esthe[illegible]kel
   Agronomist
   Religious Sisters of Mercy
   Jackson, MN

**XI. Reporting Requirements:** Semiannual status reports will be submitted not later than January 1, 1994, July 1, 1994, January 1, 1995, and a final report by June 30, 1995.

**XII. Literature Cited. (See Detailed Work Program)**

**OBJECTIVE 3:**

**A. Title of Objective:** Biological Control of Cocklebur in Minnesota

**A.1. Activity:** To identify, isolate and characterize potential biocontrol mechanisms by screening the roots of cocklebur seedlings for microorganisms deleterious to the weed but not to corn or soybeans, and examining seed pods for microorganisms capable of penetrating the pod and infecting the seeds, thus diminishing the cocklebur seed bank in the soil.

**A.1.a. Context within the Project:** The cocklebur is a noxious weed common to at least 25% of corn and soybean fields. The weed is highly competitive and its seed can remain dormant in the soil for years. A recent literature search suggests that very little is being done to identify biological control mechanisms for cocklebur infestations in corn and soybean fields. The cocklebur plant would be most susceptible to biological control measures during germination and the seedling stage. The moist environment needed for germination and the fleshy tissue of early growth are desirable conditions for microbial invasion and infection.

Current control relies upon pre-emergent herbicides, which are costly (approximately $22 million annually based upon 2.3 milllion acres at $8 per acre) and environmentally damaging. Current costs specifically for controlling cocklebur are estimated at $2.5-3 million, exclusive of any environmental damage. It is possible that some microorganisms will enhance germination and cocklebur seedling growth because of their ability to produce plant hormones (IAA and others) involved in stimulating seed germination and/or the initiation of the disease process.

**A.1.b. Methods:** Seedlings: Cocklebur plants and some related species such as sunflower will be collected repeatedly from corn and soybean fields throughout Southern Minnesota to ensure the greatest biodiversity of plants, microflora and soil types.

Evaluation of the inoculated seedlings in the Falcon plates will include root length measurements(cm), and a visual evaluation of disease characteristics based on a scale of 1 (most deleterious) to 3 (normal). Deleterious isolates will be saved in the refrigerator and in greenhouse cocklebur pot cultures (to ensure retention of pathogenicity) for additional tests with cocklebur as well as tests on corn and soybeans. Plant growth-promoting organisms will also be saved because of their importance as potential plant hormone producers for further studies of disease enhancement and antimicrobial activity.

Seed Pods: Cocklebur seed pods with ungerminated seeds will be collected from field soils. Seeds will be removed from pods, surface sterilized for 8 minutes and plated. Evaluation will be done by surface sterilizing the seeds and pods, opening them, and enumerating the organisms capable of seed penetration.

**A.1.c. Materials:** All equipment to complete this activity is available within the Department. The requested budget includes mileage to collect samples and the purchase of expendables, including agars, chemicals, and some glassware.

**A.1.d. Budget:** $31,000 **Balance:** $-0-

**A.1.e. Timeline:**

| | 7/93 | 1/94 | 6/94 | 1/95 | 6/95 |
|---|---|---|---|---|---|
| Screen for potential biocontrol agents | ----- | ----- | ----- | ----- | ----- |
| Test for biological activity to cocklebur | ----- | ----- | ----- | ----- | ----- |
| Test for biological activity to crop plants | | ----- | ----- | ----- | ----- |
| Greenhouse pot testing | | | ----- | ----- | ----- |
| Data analysis and reports | ----- | ----- | ----- | ----- | ----- |

**A.1.f. Status:** Final Status Report, September 1, 1995. Approximately 33 DRB and 12 fungal isolates were selected from the second testing phase of approximately 200 isolates. The selected isolates were tested in greenhouse conditions for the final selection phase. During the three-week treatments with each isolate grown independently in full soil pot cultures of either cocklebur and soybeans, none of the isolates demonstrated the biological activity necessary to control the cocklebur. Greenhouse results showed that DRB and fungal isolates did affect primary root development in the cocklebur. The primary roots were frequently stubby with abnormal growth, and secondary root development was reduced in some areas of the primary root . Total cocklebur growth was retarded during week one and somewhat during week two compared to the control plants. However during the second and third week, secondary roots were able to overcome the potential biocontrol agent and normal growth of the cocklebur resumed. The DRB isolates did not appear to affect the soybean plants. The fungal isolates (*Fusarium* spp., *Cephlosporium* spp., and *Alternaria* spp.) generally showed increased lesions to cocklebur roots when compared to the bacteria, but they also showed greater biological activity to soybean plants as well. Additional screening continued for fungal isolated obtained from Illinois.

**A.2. Activity:** Screen for antimicrobial activities of the plant, plant residue and microorganisms and specific interactions that could enhance biocontrol mechanisms.

**A.2.a. Context within the Project:** In order for the biological control process to function successfully, the allelopathic metabolites of the cocklebur seedling, seed pod and seed, as well as microbial metabolites (antibiosis) must be fully understood so that they can be optimally applied to enhance the growth and virulence of biocontrol organisms.

**A.2.b. Methods:** Fresh cocklebur roots will be collected, washed, chopped into 1 cm pieces, and weighed into six 100g (wet wt) samples. Each root sample will then be placed in either 100mL of water or 100mL of ethanol for 24 hours to extract the water soluble and alcohol-soluble leachates. The water and alcohol extracts will be evaporated dry and reconstituted to a volume of 10 mL for the purpose of saturating 5 mm absorbent pads. The

s will then be dried and placed on pre-inoculated (12-hours est organism) nutrient agar plates to determine the antimicrobial activity of the plant extracts on common root microfloral organisms. Antimicrobial evaluation will be done by measuring clear zones of inhibition (mm) around the pads. Colorimetric (Folin-Denis method) and chromatographic analysis of the leachates will determine total phenolic content and other compounds (flavonoid and alkaloid) present (Kremer et al. 1984 Agron J 76:745-9).

The water and alcohol-soluble extracts will also be tested for inhibition to corn, soybeans and alfalfa. Evaluation will be done by measuring root growth. The same procedure will be used to determine the impact of seed pod allelopathic compounds on common soil microbes and crop seeds.

The antibiosis bioassay will determine the influence plant growth-promoting and deleterious rhizosplane bacteria and fungi have on each other in competing for the plant nutrients of the rhizosphere. The bioassay will be accomplished by preparing pour-plates of bacterial-minimal medium containing known levels of bacterial or fungal cultures. The pour-plates will be grown for 12 hours at 28°C and then 0.1 microliter drops of the test organism will be strategically added to the plates. The plates will be re-incubated for 24 hours and the antibiosis or antagonism between organisms will be evaluated by measuring the clear zones of inhibition around the points where the second organism has been added.

**A.2.c. Materials:** All equipment to complete this activity is available within the Department. The requested budget includes mileage to collect samples and the purchase of expendables, including agars, chemicals, and some glassware.

**A.2.d. Budget:** $8,000 **Balance:** $-0-

**A.2.e. Timeline:**

| | 7/93 | 1/94 | 6/94 | 1/95 | 6/95 |
|---|---|---|---|---|---|
| Screen allelopathic effects | | ----- | ----- | ----- | ----- |
| Screen antibiosis activity | | ----- | ----- | ----- | ----- |
| Test bioactivity to crop plants | | | ----- | ----- | ----- |
| Data analysis and reports | | ----- | ----- | ----- | ----- |

**A.2.f. Status:** Final Status Report, September 1, 1995. This objective was completed and reported on in the Third Status Report.

**V. Evaluation:** Ideally, 0.5-1.0% of the isolates tested will have favorable characteristics with potential for some role in biological control mechanisms. A successful project conclusion will be the identification of one or more organisms that will have potential as a biological control agent for cocklebur seedlings in greenhouse tests as well as one or more biocontrol organisms that can act as a pathogen on cocklebur seeds. In addition to identifying these agents, the analysis of antimicrobial interactions in the total environment will be extremely important to maximizing the effectiveness of individual biological control organisms that may be identified by this research.

**VI. Context within** : This project addresses biological control in a comprehensive approach that couples the natural effect of organisms on the weed seed bank, seed germination, seedling growth and any allelopathic affect from crop residue.

**VII. Benefits:** Biocontrol agents capable of degrading cocklebur seed will allow fields with high seedbank counts to be treated with biocontrol organisms to reduce the weed pressures to manageable levels. The moist environment of the germinating seed favors microbial activity, coinciding with the cocklebur seed's optimal developmental stage for biocontrol (i.e., its fleshy tissues are vunerable to pathogens). Identifying a biological control mechanism to control this noxious weed will replace the need for pre-emergent herbicides, thereby reducing costs and improving environmental quality.

**VIII. Dissemination:** All aspects of the project will be fully documented in report form to LCMR, shared freely among colleagues, and submitted for publication in farm and agricultural trade journals and in professional journals as information becomes available.

**IX. Time:** This is a new project in an area that has not previously received funding. This project does not involve field testing and implementation.

**X. Cooperation:**

Dr. Donald Wyse
Department of Agronomy and Plant Genetics
University of Minnesota
St. Paul, MN

**XI. Reporting:** Semiannual status reports will be submitted not later than January 1, 1994, July 1, 1994, January 1, 1995 and a final status report by June 30, 1995.

**XII. Literature Cited: (See Detailed Work Plan)**

**Qualifications:**

Dr. John E. Frey, **Principal Investigator**
Professor
Department of Biological Sciences
Mankato State University

D.A. Botany, University of Northern Colorado, 1972
M.S. Biology, Emporia State University, 1967

Research Interest

My research interest is in the area of plant root-microbial interactions as it relates to root diseases and vesicular-arbuscular mycorrhyizae (VAMF). During the 1990-91 academic year I worked on the relationship of soil properties to the response of VAMF species and soybeans.

During the past two years, my research focus has shifted to the rhizosphere of weeds; and in the past year, it has become further refined to focus on the deleterious microorganisms that have potential as biological control organisms for cockleburs.

Recent Publications and Papers. (See Detailed Work Plan)



**OBJECTIVE 4:**

**A. Title of Objective:** Biological Control of Musk Thistle (*Carduus nutans*) in Minnesota.

**A.1. Activity:** Surveys will be conducted in southeast and southwest Minnesota to determine the density of musk thistle and the establishment of musk thistle biological control agents from previous releases in Minnesota. Musk thistle bio-control agents will be released and redistributed as populations increase.

**A.1.a. Context within the Project:** The successful implementation of biological control of musk thistle in Minnesota will decrease the management costs of pasture and other low-input lands, both public and private, by reducing herbicide or mechanical control means; aid in the control of a noxious weed; and demonstrate new weed management techniques using biological control agents.

**A.1.b. Methods:** Four counties in southwest Minnesota and four counties in southeast Minnesota will be surveyed for musk and plumeless thistles and musk thistle weevil in the first year. Four more counties will be surveyed in the second year and another four in the third year.

Plant densities and percent infestation of seed heads by musk thistle weevil will be determined progressively in the 4, 8, and 12 counties in the next 3 years.

Rosette and musk thistle weevils will be introduced in Jackson county in southwest Minnesota and Olmsted county in southeast Minnesota the first year. Introductions will occur in 4 counties the second year and 6 counties the third year. Lab rearing possibilities as well as field insectaries for the weevils will be explored.

Surveys will be conducted from June through September (4 months) each year.

**A.1.c. Materials:** One seasonal employee will be needed for survey, collections, and releases.

MDA will provide a systematist year round. Dr. John Luhman is a taxonomist-entomologist as well as a biological control scientist.

**A.1.d. Budget:** $40,000 **Balance:** $-0-

**A.1.e. Timeline:**

| | 7/93 | 1/94 | 6/94 | 1/95 | 6/95 |
|---|---|---|---|---|---|
| Survey for musk thistle | ----- | | --------- | | ------ |
| Collect seedheads | ----- | ----- | | | |
| Identify musk thistle weevils & other biological control agents | | | -------- | ---------- | |
| Release weevils | ----- | | ----- | | ------ |

**Status:** Final Status Report, September 1, 1995. From to 1980 nearly 10,000 musk thistle weevils (*Rhinocyllus conicus*) were released in Minnesota in an effort to establish biological control of musk thistle. An estimate of the extent of musk and plumeless thistle populations was first made in Minnesota in 1976 as reported by O.E. Strand, Extension Agronomist, University of Minnesota (An update of Minnesota's musk thistle weevil release project). Initial release of musk thistle weevil occurred in Minnesota in 1975 with subsequent releases in '77, '78, '79, and 1980. Weevils have been released in Fillmore, Houston, Olmsted and Winona counties in southeast and Jackson, Pipestone and Rock counties in southwest Minnesota. These releases were a joint effort by APHIS, MDA, and the University of Minnesota. Monitoring of the spread and establishment of the weevil and moths was last done in 1983.

During 1993 surveys detected musk thistle head weevil, *Rhinocyllus conicus*, at three sites in Olmsted county in which releases had been made periodically from 1975 to 1980. Musk thistle head weevils were also found at a prior release site in Blue Mound State Park near Luverne in Rock county on plumeless thistle.

Musk thistle head weevils were released at two sites in Fillmore county (~ 500/site) and one site in Olmsted county (~ 500) in southeast Minnesota, one site in Washington county (~ 1000) in east central Minnesota and at one site in Rock county (~500) in southwest Minnesota in 1993. Musk thistle head weevils were released at the Fillmore county sites on May 28 & June 4, at the Washington county site on June 18 and at the Rock county site on June 1, 1993.

The two sites in Fillmore county (ChatÞeld and Fountain) were surveyed on August 4 and musk thistle seed-head samples taken. Twenty-five seed heads from ChatÞeld and 112 seed heads from Fountain were collected. Twenty percent of the seed heads from ChatÞeld and 9% of those from Fountain were infested. Six beetles were found at ChatÞeld and 15 larvae at Fountain. Approximately 30% of plumeless thistle seed heads at the Rock county site were infested with an average of 3 weevil larvae per infested seedhead.

Musk thistle head weevil release sites in ChatÞeld and Fountain were visited on May 2, 1994. Musk thistle plants were in rosette growth; no weevils were recovered. The Blue Mound State Park site in southwest Minnesota was visited on May 4, 1994; no weevils were recovered. On May 9, 1994 plots were established at ChatÞeld and Fountain and sprayed with *Pseudomonas syringae* pv. *tagetis*.

Approximately 250 weevils from Kansas were released at the Fountain site on June 10, 1994.

A new release of musk thistle head weevil was conducted at Courtland, MN in Nicollet county on June 24, 1994. Approximately 300 weevils were released.

Musk thistle weevils were collected in Plumeless thistle seedheads at Blue Mounds State Park, Luverne, MN on June 29, 1994. There was an average of 2 weevils per infested seed head. Forty seven musk thistle weevils recovered from the Luverne site were released at a site in Hennepin county on August 12, 1994. Agricultural Inspectors were contacted in Southwest and Southeast Districts to determine reported musk thistle infestations. Musk thistle infestations were mapped in Rock, Jackson, Pipestone and Murray counties in the southwest Minnesota and Winona county in southeast Minnesota. Musk thistle weevils were recovered from musk thistle at a site near Cazenovia, in Pipestone county. This site will be monitored next spring as a possible recovery site for re-release at other musk thistle sites.

A site west of St. Charles in Winona county was surveyed for musk thistle weevils and will be a release site in 1995. This site has a moderate musk thistle infestation on 20 acres at one location and scattered infestations on adjacent sites. No musk thistle weevils were recovered at this location. Surveys at Fountain and ChatÞeld in Fillmore county did not recover any musk thistle head weevils in 1994.

The 1993 release site in Blue Mounds State Park was located along a hiking trail and was very visible to the public. Park administration determined that plumeless thistle on the site should be controlled with herbicides. On July 11, 1995 plumeless thistle seedheads were collected from the site. The head weevils were in the pupal stage at this time and would develop into adults. These seedheads were brought to the MDA Biocontrol Laboratory in St. Paul to allow the adult weevils to emerge. From the 1545 seedheads collected approximately 2200 weevils emerged. Four hundred weevils were released at a new location at Blue Mounds State Park on August 8 and 200 weevils were released at a site in Murray county. The remaining weevils will be released during September '95.

Surveys for musk thistle infestations and presence of musk thistle head weevils were conducted in the southwestern counties of Pipestone, Nobles, Cottonwood, Jackson and Murray and the southeastern counties of Winona, Houston and Fillmore in 1995. Weevils from releases prior to MDA involvement were found in all southwestern counties but only Winona county in the southeast had weevils from prior releases. The percent of seedheads infested with musk thistle weevils was 19% in Cottonwood, 12% in Jackson, 11% in Murray and 4% in Nobles counties in the southwest. In the southeast, Winona county musk thistle seedhead samples had 5% infestation but no weevils were found in samples taken from Houston and Fillmore counties.

Releases of rosette weevils collected in Kansas were also conducted in Winona and Jackson counties. Approximately 500 rosette weevils were released in Winona county on June 8, 1995 and about 470 rosette weevils were released in Jackson county on June 27, 1995.

Musk thistle weevil parasites were recovered from the plumeless thistle seedheads that were collected from Blue Mounds State Park. Two species of Braconidae and 2 species

of Pteromalidae were identified. About 7% of the weevils collected from the Blue Mounds site were parasitized. Parasites were not found in any other samples.

V. **Evaluation:** Results will provide detailed information on musk thistle density in southeast and southwest Minnesota and the impact of biological control through the release of the thistle weevils. Musk thistle and plumeless thistle control will be increased through the use of these biological control agents.

VI. **Context within field:** From 1975 to 1980 nearly 10,000 musk thistle weevils (*Rhinocyllus conicus*) and about 100 coleophorid casebearer moths (*Coleophora klimeschiella* and *parthenica*) were introduced in an effort to establish biological control of musk thistle. An estimate of the extent of musk and plumeless thistle populations was first made in Minnesota in 1976 as reported by O.E. Strand, Extension Agronomist, University of Minnesota (An update of Minnesota's musk thistle weevil release project). Initial introduction of musk thistle weevil occurred in Minnesota in 1975 with subsequent releases in '77, '78, '79, and 1980. Weevils have been introduced in Fillmore, Houston, Olmsted and Winona counties in southeast and Jackson, Pipestone and Rock counties in southwest Minnesota. These releases were a joint effort by APHIS, MDA, and the University of Minnesota. Monitoring of the spread and establishment of the weevil and moths was last done in 1983.

In the 1960's, Europeans explored the possibility of using the musk thistle weevil to control musk and plumeless thistles in Europe. In the 1970's, musk weevil populations were established in Missouri, Montana, Nebraska, and Virginia that significantly reduced musk thistle seed production. Musk thistle in Minnesota renders infested pastures unproductive for livestock. Control measures, such as cutting and repeated herbicide applications, are difficult and time consuming in steep and rough areas of southeastern and southwestern Minnesota where musk thistle is a problem weed. Thus, the musk thistle weevil will be a valuable biological control agent in these and other infested areas.

VII. **Benefits:** Musk thistle weevil (*Rhinocyllus conicus*) has been used successfully in Europe and in several states to control musk thistle (*Carduus nutans*) in pasture and non-croplands. In the 1970-80's the weevil was released and established in areas of southern Minnesota. We want to expand the distribution of the weevil, especially into areas that are difficult to manage because of the terrain. As a declared noxious weed, land owners must control musk thistle even if control measures are time consuming, difficult, or costly. The herbicide 2,4-D is widely used for musk thistle control. Since 2,4-D is injurious to other broadleaf plants its reduced use for musk thistle control would decrease the opportunities for damage to non-target broadleaf plants such as desirable native species and crops.

Biological control of musk thistle will result in reduced use of herbicides and reduced labor cost for pasture and non-cropland thistle management and increased use and production on low-input pasture and parkland infested with thistle.

VIII. **Dissemination:** Project results will be summarized in the annual Plant Protection Division report. Poster and/or oral presentation of results will be made at North Central Weed Science Society meetings and other appropriate professional society meetings.

IX. **Time:** Two years, plus the need for continued monitoring and augmentative releases for another two. Redistribution of weevils will be on going.

X. **Cooperation:** We will cooperate with APHIS and other sources of musk thistle weevils. The Department of Agronomy and Plant Genetics, University of Minnesota will be a resource.

XI. **Reporting requirements:** Semiannual status reports will be submitted not later than Jan. 1, 1994, July 1, 1994, Jan. 1, 1995 and a final status report by June 30, 1995.

XII. **Selected References: (See Detailed Work Program)**

**Qualifications:**

Dr. L. Neville Wilson, **Principal Investigator**
Agronomist
Plant Pest Survey, Detection and Biological Control Program
Plant Protection Division
Minnesota Department of Agriculture

Oklahoma State University; M.S., Agronomy (Crop Physiology), 1975
University of Minnesota, Ph.D., 1993.

Dr. Wilson has been coordinating statewide survey and detection of economic plant pests, primarily plant diseases, weeds and insects for the past 4 years. The Plant Pest Survey, Detection and Biological Control Program also surveys statewide for beneficial organisms and assists in the recovery and establishment of biological control agents. He will manage lab and field operations necessary for parasite release, data collection, evaluation and recovery.

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

University of California - Riverside; Ph.D., Entomology, 1986
University of Minnesota; M.S., Entomology, 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.

**OBJECTIVE 5:**

**A. Title of Objective:** Evaluate microbial entomopathogens for control of armyworm and grasshoppers in Minnesota.

**A.1. Activity:** Microorganisms appropriate for microbiological control of armyworms and grasshoppers in Minnesota will be identified. Data on the virulence and persistence of these microbes under laboratory and field conditions will be collected. *Nosema acridophagus* and *Nosema locustae* will be evaluated as microbiological control agent of grasshoppers. *Vairimorpha necatrix* and *Nosema furnacalis* will be evaluated as microbial insecticides of armyworms, *Pseudaletia unipuncta.*

**A.1.a. Context within the Project:** Natural control of outbreaks of armyworms and grasshoppers requires the use of microbes with insecticidal and biological control characteristics. Data on the use of disease causing microbes of insects to control armyworms and grasshoppers in Minnesota are lacking. Armyworms, which migrate from Texas and Oklahoma each spring, are a periodic problem in Minnesota. Outbreaks in small grains occur approximately once every 4 years and typically encompass 0.5 million acres which are treated with chemical insecticides (parathion). Natural epizootics of *V. necatrix* have been observed in armyworms in the field (Tanada and Chang, 1962) but its efficacy in control programs for armyworms has not been tested.

*Nosema locustae* is currently marketed as a microbial insecticide against grasshoppers, but is lacking the traits that would qualify it for being labelled as such (high virulence and fast killing action). It is now being proposed as a biological control agent of grasshoppers, but its potential for that role has not been documented. *Nosema acridophagus* is a newly identified grasshopper pathogen which promises to be a good candidate for use as either a microbial insecticide or a biological control agent of grasshoppers. Whether it will be useful under conditions prevalent in Minnesota needs to be explored.

**A.1.b. Methods:** Laboratory trials: Laboratory colonies of armyworms (*P. unipuncta*) and grasshoppers (*Melanoplus sanguinipes*) will be reared on artificial diet to permit year round trials. Spores of *N. acridophagus, N. locustae, V. necatrix,* and *N. furnacalis* will be mass produced in insects. Infected insects will be homogenized and the spores concentrated and washed by differential centrifugation. Partially purified spores will be centrifuged through Percoll gradients to separate them from contaminating insect material and microbes.

Virulence and infectivity of spores will be quantified using standard bioassay techniques. We will measure time and dose needed to kill various proportions of the target insect. Probit analysis will be used to calculate LT50 and LT90 or LD50 and LD90.
Field Trials: Field trials will be conducted to answer the questions: Does the pathogen cause epizootics in the target pest insect population after inoculative or innundative release? Does the pathogen spread effectively if it is introduced in limited quantities to form epicenters? Does the pathogen persist in the population under conditions found in Minnesota? These projects will be done in collaboration with researchers at the Minnesota Department of Agriculture. Pathogen species and strains showing promise in the laboratory will be field evaluated. For inoculation or innundation of field plots, spores produced in insects will be purified, quantified and formulated with the addition of adhesives and UV protectants. Naturally infested plots will be treated with known doses of spores. Pre- and post-treatment sampling of plants will be done to quantify insect numbers and determine their stage. Representative numbers of insects will be dissected and examined microscopically to determine the prevalence and peristence of pathogens in the population. Significance of differences between treatments and between treated and untreated plots will be determined by analysis of variance.

The ability of *N. furnacalis* and *V. necatrix* to control armyworms in naturally infested plots (peas or barley) will be evaluated. Treatment plots will be arranged in randomized block design with 3 or 4 replicates and an untreated check. Pre and post treatment counts and larval stage determinations of insects will be made. Spores will be applied with a "back pack" or hand held sprayer at concentrations determined to be effective by laboratory trials. The number of larvae per $ft^2$, and disease prevalence in 3-5 separate sampling areas per replicate will be determined. Sampling may be done by sweeping or shaking plants over trays to capture insects. To analyse for secondary cycling, treated sites will be revisited the second year, armyworms and/or other caterpillars present will be collected and the prevalence of *V. necatrix* determined (*V. necatrix* has a wide host range and may persist in other Lepdioptera even if armyworms are not present). ANOVA will be done to determine if significant differences exist among treatments, and to determine how effective treatments were in reducing armyworm numbers.

*Nosema locustae* and *N. acridophagus* will be evaluated for potential as biological control agents of grasshoppers. With the help of the Minnesota Department of Agriculture heavily infested areas will be identified. Spores will be mixed with bran flakes and applied at rates exceeding $5X10^9$ spores/ha so as to create epicenters of disease. The population density of grasshoppers will be determined 2-3 days before inoculation. Samples will be collected at selected time post treatment to determine species composition of the grasshoppers, their age structure and prevalence of microsporidia. Treated plots will be sampled one year later and grasshoppers evaluated for *N. locustae* infection.

The dynamics of microsporidian growth and dispersal in populations of grasshoppers or armyworms will be analyzed following experimental strategies similar to those used by Evans and Allaway (1983). Briefly, insects will be infected in the laboratory with lethal doses of spores and then introduced into plots naturally infested with either armyworms or grasshoppers. Known numbers of infected insects will be released at selected foci. Spore dispersal and growth within the surrounding pest population will be quantified. Insect numbers will be determined pre- and post introduction of infected insects along with the prevalence of insects infected with microsporidia.

**A.1.c. Materials:** Laboratory materials include insect rearing containers and diet, biochemical supplies for purifying and characterizing pathogens. Cell culture glassware

and media are needed for growing cells and pathogens in culture. Field materials include rental and maintenace fees for experimental plots, insects needed for infesting field crops.

**A.1.d. Budget:** $54,000 **Balance:** $-0-

**A.1.e. Timeline:**

| | 7/93 | 1/94 | 6/94 | 1/95 | 6/95 |
|---|---|---|---|---|---|
| Lab. Analyses | ---------- | | | | |
| Field Trials | -------- | | | ------ | |
| Evaluate Data | | ---------- | | ---------- | |
| Reports | | ---- | ---- | ---- | ---- |

**A.1.f. Status:** Final Status Report, September 1, 1995. A laboratory colony of 3 day-old armyworms, *Pseudaletia unipuncta,* was challenged with *Nosema furnacalis* spores ($10^5$ to $10^7$ spores). *N. furnacalis* did not infect the armyworms thus field tests with this organism against armyworms were not conducted. Laboratory evaluations with *Vairimorpha necatrix* are under way. To detect field released microsporidia that infect caterpillars and grasshoppers we used a method, developed by scientists at the CDC in Atlanta, Georgia, to distinguish their DNA. Species specific PCR primers, based on DNA sequences found in the small subunit ribosomal RNA gene, delineated between *N. furnacalis, N. pyrausta, and N. acridophagus*. Results revealed that sequences found in the insect pathogenic microsporidia were quite different from microsporidia infecting humans and domestic animals.

**V. Evaluation:** Commercialization of any of the strains we identify as efficacious and their utilization by growers or government agencies will be the best way to evaluate the outcome of this project. However, to improve the performance of these biological control agents more basic information is needed. Precocious implementation is likely to lead to negative results which will undermine clientele confidence in use of biological control agents to control pest insects. The results of our trials, whether positive or negative, will provide an important knowledge base for future trials and the eventual implementation of these agents under less controlled conditions. Practical implementation protcocols would be another outgrowth of these studies. These studies should enable us to make a recommendation regarding the use of these microorganisms as microbial insecticides or microbiological control agents within the environment prevalent in Minnesota.

**VI. Context within field:** Microbial entomopathogens are important natural control agents of insects. Two basic approaches are used in their application: 1) inundative release of microbes as insecticides as part of an IPM program, or 2) introduction of native (indigenous) and/or foreign (exotic) pathogens into native pest insect populations as biological control agents. Most emphasis has been placed on pathogen use as biological insecticides, but more research is needed on their application as biological control agents. Our project considers both strategies in the control of armyworms and grasshoppers.

Biological control agents that can act fast when used in the inundative approach are badly needed to protect high-value crops from pest insects. *Nosema acridophagus* and *Vairimorpha necatrix* may have the necessary virulence and speed. Grasshopper problems are exacerbated by chem-fallow/no till methods and Conservation Reserve Programs which increase the breeding areas for grasshoppers. *Nosema locustae,* the only commercially available agent at this time, is largely ineffective when used as an insecticide; it acts too slowly and its biocontrol potential has not been adequately documented. Microbiological control agents have the potential to persist and spread beyond the pests originally treated. This potential for *V. necatrix* and *N. acridophagus*, both which have wide pest insect host range, needs further examination. Grasshopper pathogens, such as *N. locustae* and *N. acridophagus* have a worldwide distribution and new pathogens species and strains are currently being discovered. *Nosema acridophagus* (Henry et al. 1985) is a new isolate that is more virulent than *N. locustae. Nosema acridophagus* infects the 4 most economically important grasshoppers in the U.S.: *Melanoplus sanguinipes, M. differentialis, M. bivittatus,* and *M. femur-rubrum*. Its host range against pest lepidoptera needs further evaluation. *Vairimorpha necatrix* infects more than 40 different species in 9 families of Lepidoptera. Methods to mass produce these agents for field test applications, and commercialization require more research.

This research objective complements projects of Drs. Kurtti and Munderloh have been directing for several years. These include grants funded by the USDA-APHIS Grasshopper IPM Program (Project Title: Cell culture systems for production of host cell dependent grasshopper pathogens), USDA-CSRS Competitive Research Grants Program (Project Title: Strain improvement of microsporidia for use in corn pest management systems), and the USDA-CSRS Small Business Innovation Research Program (Project Title: A fast acting microsporidia for grasshopper control, with Bozeman BioTech, Bozeman, MT). Funds for these projects have totaled more than $200,000.

This research objective will also complement part of the national research biological control initiative. The investigators contribute to a regional project (S240: Development of entomopathogens as control agents for insect pests) which includes a national membership with members from industry, USDA and universities. The goals of this group are to characterize entomopathogens for use in regional pest management systems, monitor environmental fate of naturally occurring and introduced pathogens, and to evaluate efficacy and establish criteria for use of entomopathogens in regional pest management systems.

**VII. Benefits:** Identification of appropriate agents for the biological control of armyworm and grasshoppers. Establishing these agents in natural populations of these pests could reduce pesticide dependency and eliminate dollars spent on chemical treatment. The use of natural pathogens such as these will reduce the emergence of pesticide resistant strains of grasshoppers and armyworms. Government benefit from this project would be reduced cost of grasshopper suppression on public lands and enhanced natural habitats. Another potential benefit of this project would be the production and marketing of beneficial microorganisms by the private sector.

**VIII. Dissemination:** Our findings will be published in peer reviewed scientific journals. We will also present our results at international, national or local scientific meetings or workshops. Scientists at the Minnesota Department of Agriculture and the Minnesota Extension Service will also be made

aware of ings that will enable them to make better informed recommendations to farmers and growers needing to control grasshopper or armyworm infestations using natural methods of control.

IX. **Time:** The use of these microbials as biological control agents will require us to monitor the environment in which these organisms have been introduced. It is anticipated that the laboratory and field trials will require 4 years during which additional federal and LCMR funding will be sought.

X. **Cooperation:** Dr. Dharma Sreenivasam, Minnesota Department of Agriculture.

XI. **Reporting Requirements:** Semiannual status reports will be submitted not later than Jan. 1, 1994, July 1, 1994, Jan. 1, 1995 and a final status report by June 30, 1995.

XII. **Literature Cited: (See Detailed Work Program)**

**Qualifications:**

Dr. Timothy J. Kurtti, **Principal Investigator**
Associate Professor of Entomology
University of Minnesota

PhD: Entomology, University of Minnesota, 1974

Dr. Kurtti is an insect microbiologist with experience in microbial control of pest insects and the transmission of human and animal pathogens by vector arthropods. His current research centers around the development of microsporidia for pest insect control and the transmission of the Lyme disease spirochete by the deer tick. His research support over the past 10 years has been derived from the NIH, USDA, LCMR, WHO, AID, and private industry. Dr. Kurtti is the author or coauthor on more than 70 publications in insect pathology, medical entomology, and cell biology. He has broad experience with the microsporidia and related protozoa.

Dr. Ulrike G. Munderloh

Research associate, Department of Entomology
College of Agriculture, University of Minnesota

Ph.D., Veterinary Medicine, University of Munich, 1977

Dr. Munderloh is an arthropod microbiologists with experience in tropical veterinary medicine and invertebrate pathology and medical/veterinary entomology. She has worked with the cultivation of entomopathogens in vitro. Her current research centers around the development of microsporidia for pest insect control and the transmission of the Lyme disease spirochete by the deer tick. Her research support over the past 10 years has been derived from the NIH, USDA, LCMR, WHO, AID, and private industry. Dr. Munderloh is the author or coauthor on more than 35 publications in parasitology, insect pathology, medical entomology, and cell biology.



**OBJECTIVE 6:**

**A. Title of Objective:** Release and evaluation of a microsporidian pathogen for biological control of the European corn borer.

**A.1. Activity:** Laboratory studies with *Nosema furnacalis*.

**A.1.a. Context within the Project:** The European corn borer, *Ostrinia nubilalis*, was first introduced into the United States in 1917 and became established in Minnesota in 1943. Twenty-four exotic insect parasitoids have been released in the United States as biological control agents for the European corn borer. Only three species (*Macrocentrus grandii*, *Lydella thompsoni*, and *Eriborus terebrans*) became widely distributed in the North Central United States. Today, none of these species are a major mortality factor of European corn borers in Minnesota. In a recent study of European corn borer parasitism, *E. terebrans* and *M. grandii* parasitism rates in Minnesota were 1.1% and 1.4%, respectively. *L. thompsoni* has all but disappeared from the Upper Midwest. One of the most important European corn borer mortality factors is the microsporidian, *N. pyrausta*. Infection levels as high as 100% have been reported. *N. furnacalis* is an exotic microsporidian closely related to *N. pyrausta* that was originally described from the Asian corn borer, *O. furnacalis*. *N. furnacalis* is also pathogenic to the *O. nubilalis*. Our goal is to determine the host range of *N. furnacalis*, to determine if it can become established in Minnesota, and its effect on *O. nubilalis* populations in Minnesota. Our hypothesis is that supplementing the natural enemy complex with *N. furnacalis* will reduce the overall abundance of the European corn borer and thereby limit European corn borer damage potential in field and sweet corn. However, before we can release *N. furnacalis* in the field, we must obtain approval of the Environment Protection Agency (EPA). Before EPA approval is granted to release an exotic insect pathogen, we must assess its impact on nontarget species. The most important nontarget species are known predators and parasitoids of the European corn borer. Impacts on nine predator species have been completed and no predator became infected with *N. furnacalis*. We propose to evaluate effects of *N. furnacalis* infection on three introduced larval parasitoids of the European corn borer, *M. grandii*, *E. terebrans*, and *L. thompsoni* and one introduced egg parasitoid, *Trichogramma nubilale*.

**A.1.b. Methods:** European corn borer larvae will be reared on a modified Van der Zant-Adkisson diet. To inoculate European corn borer larvae with microsporidians, a suspension of *N. furnacalis* spores is spread onto the diet surface. Ten 1st instar larvae will be confined in a 30 ml diet cup. Infection levels can be varied by using different spore dosages (e.g., $10^2$, $10^3$, and $10^4$ spores/50 ml) applied to the diet surface. *M. grandii* and *E. terebrans* adults will be given access to 2nd or 3rd instar larvae. *L. thompsoni* adults will be allowed to larviposit on moist filter paper and a 1st instar maggot will be placed on the dorsum of a 4th instar European corn borer larva. We will compare fertility, fecundity, longevity, and developmental rates of *M. grandii*, *E. terebrans* and *L. thompsoni* reared on *N. furnacalis* infected and uninfected European corn borers. *T. nubilale* will be given access to infected and uninfected European corn borer egg masses. Percent emergence, adult longevity and

spore density in adults will be determined. Results will be analyzed using analysis of variance (ANOVA).

**A.1.c. Materials:** All equipment necessary to conduct this research is available. Expendable laboratory supplies for insect diet, serological analysis, and insect rearing are needed.

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

**A.1.e. Timeline:**

| | 7/93 | 1/94 | 6/94 | 1/95 | 6/95 |
|---|---|---|---|---|---|
| *M. grandii* assays | ------- | ----- | ----- | ------ | ------ |
| *L. thompsoni* assays | | ------ discontinued | | | |
| *T. nubilale* assays | | | ------- | | |
| *E. terebrans* assays | | | | ------ | ------ |

**A.1.f. Status:** Final Status Report, September 1, 1995. Laboratory colonies of microsporidian-free and infected *Macrocentrus grandii* were reestablished. A chronically infected *M. grandii* colony was maintained for a total of seven generations. Performance data suggest that a chronic infection has a small but significant negative effect on fecundity, longevity and general fitness of *M. grandii*. The propensity of *N. furnacalis* to chronically infect *M. grandii* suggest that chronically infected parasites might be useful in establishing *N. furnacalis*. Such a strategy would enable a more careful introduction of this pathogen. In particular, using a specific parasite would reduce the risk associated with using a spray solution of suspended spores that might drift off the intended target site (corn plants infested with European corn borer larvae).

**A.2. Activity:** Field Studies: Assessment of current levels of *N. pyrausta* in field populations in Minnesota.

**A.2.a. Context within the project:** There are no survey data available for Minnesota to document the distribution of the native microsporidian, *N. pyrausta*. Each fall, Minnesota Department of Agriculture (MDA) personnel collect European corn borers throughout Minnesota. The purpose of this survey is to determine population densities of both corn borers and parasitoids. It is known that *N. pyrausta* does infect *M. grandii* and *T. nubilale*; yet, no data are available to document the incidence of *N. pyrausta* in the parasitoid populations. We will assist MDA personnel in determining the levels of *N. pyrausta* infection in the corn borer and parasitoid populations. These data will enable us to assess the risk of releasing an exotic microsporidian for European corn borer control.

**A.2.b. Methods:** Diapausing European corn borer larvae are collected each fall, held at 4° C until diapause is broken, placed at 27° C for 3 to 4 weeks to allow parasitoids and European corn borers to emerge. Once an adult European corn borers or parasitoids have emerged, these insects will be frozen for subsequent microscopic examination for presence of microsporidian infection.

**A.2.c. Materials:** All the necessary equipment to conduct this research is available.

**A.2.d. Budget:** $7,500 **Balance:** $-0-

**A.2.e. Timeline:**

| | 7/93 | 1/94 | 6/94 | 1/95 | 6/95 |
|---|---|---|---|---|---|
| Fall ECB collection | | ----- | ------ | | |
| Microscopic examinations | | ------ | | ------ | |

**A.2.f. Status:** Final Status Report, September 1, 1995. Samples of European corn borers collected in the Fall of 1994 from 93 counties throughout Minnesota by personnel with the Minnesota Department of Agriculture, were evaluated for presence of microsporidia. A total of 1,722 larvae, pupae or adults were examined for microsporidia. A statewide average incidence was 27.1%. Data from individual counties showed that infection ranged from 0 to 90.2%. One trend that appears in these data is the relatively high incidence of microsporidia in corn borers collected in the Red River Valley. In the 15 counties located in the Valley where European corn borers have a single generation per year, average incidence of *N. pyrausta* was nearly double the statewide average (50.5%, range 10.5-90.2%). Moreover, counties where corn production is the most intense (southern 1/3 of the state, 50 counties) average infection was only 13.5% (range 0-61.2%). This low level of *N. pyrausta* may have contributed to the damaging populations found in southern Minnesota in the 1995 field season.

**A.3. Activity:** Field studies: European corn borer mortality induced by *N. furnacalis* and *N. pyrausta*.

**A.3.a. Context within the Project:** To assess the efficacy of *N. furnacalis* in the field, we will determine survival of European corn borers inoculated with *N. furnacalis*, *N. pyrausta*, and uninoculated controls.

**A.3.b. Methods:** Plots will be planted using a sweet corn hybrid Jubilee. This hybrid is widely planted and is not resistant to European corn borers. Plots will consist of a single row with ten plants spaced at 30 cm. Infected first instar larvae will be obtained by confining them on inoculated diet for 48 h. Fifteen to 20 infected second instar larvae will be placed in the whorl of each plant at the 5-6 leaf stage. The experiment will be a 3 x 2 x 2 factorial arrangement of treatments with four replications. Treatments are the two microsporidians *N. pyrausta* and *N. furnacalis* and an uninoculated control, a high ($10^5$) and a low ($10^3$) dose of microsporidians, and two evaluation dates corresponding to late 3rd instar and late 5th instar larvae. Response variables include number of larvae per stalk and cavity length. Data will be analyzed using ANOVA.

**A.3.c. Materials:** All equipment necessary to conduct this research is available. Expendable field supplies include land rental, plot maintenance, transportation to research sites, and purchase of European corn borer egg masses.

..d. **Budget:** $13,750 **Balance:** $-0-

**A.3.e. Timeline:**

| | 7/93 | 1/94 | 6/94 | 1/95 | 6/95 |
|---|---|---|---|---|---|
| Field studies | -------- | | --------- | | |
| Lab studies | | -------- | | | ------ |

**A.3.f. Status:** Final Status Report, September 1, 1995. The second year of field study was completed to assess the level of overwintering mortality induced by varying inoculation levels of *N. furnacalis* in European corn borer. There was no significant difference between controls and inoculated larvae. Uninoculated controls had an average survival rate of 94.8% while low and high levels of inoculum had average survival rate of 91.2 and 93.0%, respectively. These data suggest that as with *N. pyrausta,* effects of infection are primarily reduced fecundity and adult longevity.

**A.4. Activity:** Field studies: Horizontal transmission of *N. furnacalis* and *N. pyrausta.*

**A.4.a. Context:** Experiments will be designed to determine the extent of horizontal transmission (larva to larva). New infections can be caused by ingestion of spore contaiminated plant material. Horizontal transmission is one of the ways microsporidians are maintained in the European corn borer population. The importance of larval movement from plant to plant in horizontal transmission under field conditions is currently unknown. Vertical transmission (adult to offspring) is currently being studied in the laboratory.

**A.4.b. Methods:** Plots will be planted using a sweet corn hybrid Jubilee. Plots will consist of a single row with nine plants spaced at 30 cm. Two uninfected egg masses in the blackhead stage will be pinned to the underside of the youngest fully expanded leaf at the 5-6 leaf stage. Ten *N. furnacalis* infected 3nd instar larvae will be placed in the whorl of the center plant. The experiment will be a 3 x 2 factorial arrangement of treatments with 5 replications. Treatments are 3 levels of *N. furnacalis* infection and two dissection dates corresponding to when larvae from egg masses should be in the 3rd instar and 5th instar larvae (approximately day 17 and 30). Cumulative day-degrees will be used to determine dissection dates. All larvae collected will be assayed for *N. furnacalis* with an enzyme-linked immunosorbent assay (ELISA) developed in this laboratory. Number of *N. furnacalis* infected larvae per plant and the distance *N. furnacalis* spread from the center plant will be measured. By using 3rd instar larvae as the infected source and egg masses as the susceptible larvae, larval size can be used to distinguish inoculated larvae from those infected by horizontal transmission.

**A.4.c. Materials:** All equipment necessary to conduct this research is available. Expendable supplies include land rental, plot maintenance, transportation to research sites, purchase of European corn borer egg masses, and disposable supplies for the ELISA.

**A.4.d. Budget:** $13,750 **Balance:** $-0-

**A.4.e. ..eline:**

| | 7/93 | 1/94 | 6/.. | 1/95 | 6/95 |
|---|---|---|---|---|---|
| Field studies | -------- | | ------- | | |
| Lab studies | | -------- | | | ------ |

**A.4.f. Status:** Final Status Report, September 1, 1995. Table 1. represents data from a second year of field study to assess the degree of horizontal transmission of *N. furnacalis* in the European corn borer. Infected ECB larvae were placed on a single plant in mid-whorl stage. The remaining plants were inoculated with uninfected corn borers. Ten plants on either side of the inoculated center (within the same row) were harvested and larvae removed. On average, 17.8% of the larvae became infected with microsporidia (193/1089). These data demonstrate that horizontal transmission does occur. However, there is no apparent pattern in the spread. Plants as far as 10 stalks away from the inoculated center were as likely to contain an infected larva as those stalks adjacent to the stalk where infected larvae were placed. From these data it is apparent that ECB larvae will either readily move from plant to plant or that some other method is moving spores from one stalk to another.

Table 1. Horizontal spread of *N. furnacalis* from a central inoculated plant (0) in replicated field plots.

| ***Plant Number (Absolute Value)*** | ***Percent Infected (Low)*** | ***Percent Infected (High)*** |
|---|---|---|
| 0 | 12.0 | 35.3 |
| 1 | 10.0 | 22.5 |
| 2 | 13.1 | 17.4 |
| 3 | 30.9 | 19.7 |
| 4 | 16.5 | 10.6 |
| 5 | 25.8 | 11.0 |
| 6 | 17.8 | 17.1 |
| 7 | 7.9 | 19.7 |
| 8 | 9.5 | 12.2 |
| 9 | 13.6 | 6.2 |
| 10 | 7.6 | 18.1 |
| **Average** | 16.5 | 19.0 |

**V. Evaluation:** Criteria for evaluation will include a laboratory assessment of the effect *N. furnacalis* has on four European corn borer parasitoid species. Specifically, we will evaluate effects of *N. furnacalis* on parasitoid longevity, fecundity, fertility and developmental rates. We will document the level of *N. pyrausta* infection in field collected European corn borers and parasitoids in Minnesota. If *N. furnacalis* can be demonstrated to reduce European corn borer survival, to be transmitted horizontally, and have a limited effect on established biological control agents, *N. furnacalis* should be considered for widespread release.

**VI. Context within field:** Of the 24 exotic parasitoids released for biological control of European corn borer, only a few have become established. More importantly, none of the established parasitoids have become significant mortality factors of the European corn borer population. Indeed, one parasitoid, *L. thompsoni* after becoming widely distributed, has all but disappeared from the Upper Midwest. Many attribute its decline to *N. pyrausta.* However, this is unlikely as recent work has shown this parasitoid is unaffected by *N. pyrausta.* In long term studies, the most consistent biological mortality factor of the European corn borer has been *N. pyrausta.* If *N. furnacalis* is released, this will be only the second time an exotic microsporidian has been intentionally released as a classical biological control agent. More importantly, work previously done in this laboratory has developed a powerful serological diagnostic tool that will enable us to closely monitor movement of this exotic microsporidian in the field. Thus, we can readily document mortality due to the exotic microsporidian in both target and nontarget species.

**VII. Benefits:** Information from this study will: 1) determine the effects on four parasitoid species, 2) how this microsporidian will cycle in the European corn borer popoulation, and 3) develop strategies to effciently distribute this pathogen. The European corn borer is a major pest of both sweet and fied corn in Minnesota. In outbreak years losses due to reduce yield and insecticide costs are enormous and can reach $200 million. Because this insect in not native to North America, the natural enemy complex was left behind in continental Europe when the corn borer was accidentally introduced into the U.S. in the early 1900's. Re-establishing biological control has proven difficult for this species and full economic control has not yet been realized. By introducing an exotic pathogen we expect this pathogen to reduce winter survival of the European corn borer and limit the number of eggs an infected female can produce, thus diminishing the damage potential of this pest.

**VIII. Dissemination:** Information generated from these studies will be published in peer review scientific journals and presented orally at scientific meetings, seminars and workshops.

**IX. Time:** All objectives can be completed within the two year time frame. If EPA approval to field release *N. furnacalis* is not approved, simulated field trials can be conducted in the greenhouse.

**X. Cooperation:** Dr. Dharma Sreenivasam, Minnesota Department of Agriculture: The annual survey MDA conducts for determining population density of European corn borers and parasitism levels will be the source of the insects we will examine for microsporidians.

Dr. David Andow: Dr. Andow's laboratory has agreed to provided us with corn borer egg masses.

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

**Qualifications:**

Dr. David W. Ragsdale, **Principal Investigator**
Associate Professor, University of Minnesota
Department of Entomology

EDUCATION

| | | | |
|---|---|---|---|
| Ph.D. | Louisiana State University | Entomology, Microbiology | 1980 |
| M.S. | Louisiana State University | Entomology, Microbiology | 1977 |
| B.S. | Point Loma College | Biology, Chemistry | 1974 |

PROFESSIONAL EXPERIENCE

| | |
|---|---|
| 1994-present | Professor, University of Minnesota |
| 1987-1994 | Associate Professor, University of Minnesota |
| 1981-1987 | Assistant Professor, University of Minnesota |
| 1979-1981 | Research Associate, Louisiana State University |
| 1975-1979 | Graduate Research Assistant, Louisiana State University |

SELECTED PUBLICATIONS (See Detailed Work Program)

**OBJECT 7:**

**A. Title of Objective:** Biological Control of Scab and Verticillium Wilt of Potato

**A.1. Activity:** This project will develop procedures to effectively use isolates of *Streptomyces* to suppress the organisms that cause scab and verticillium wilt of potato.

**A.1.a. Context within the Project:** Potatoes are the most important vegetable crop produced in Minnesota. Scab and verticillium wilt, caused by the soil microorganisms *Streptomyces scabies* and two *Verticillium* species, respectively, are two of the four most important diseases of potato in Minnesota and the U.S. Verticillium wilt is important in all potato growing areas of the state, while scab is a problem primarily in the sandy irrigated production areas. Some scab resistant potato cultivars are available, though not always preferred by growers. Verticillium wilt resistant cultivars are very few. Soil fumigation with Vapam (sodium MDCB) is used to control both wilt and scab. Scab is sometimes controlled with PCNB (a chlorinated hydrocarbon and a potential carcinogen). Biological control of these diseases would avoid the risks of soil and water contamination posed by toxic chemical applications and give the grower another alternative for controlling these diseases.

**A.1.b. Methods:** Current control strategies for scab and verticillium wilt on potatoes are inadequate and present a potential threat to the environment. Twenty-six strains of *Streptomyces* spp., in addition to our original PonSSII suppressive isolate (Lorang, J.M., M.S. thesis, Univ. of MN, 1988), have been identified that produce antibiotics. Of these 26 suppressive strains, six produce antibiotics active against the scab-causing organism *Streptomyces scabies* and two other species of gram positive bacteria. These strains also produce antibiotics against the wilt organisms *Verticillium albo-atrum* and *V. dahliae*. Knowledge of the chemical structure and activity of the antibiotics produced by these strains will allow an assessment of their effects on other pathogens and on beneficial microbes present on potato roots as well as their use as biological control agents for scab and verticillium wilt diseases.

**A.1.c. Materials:** For the field and greenhouse tests, the materials required include bacterial growth medium, controlled environmental shakers and incubators, a gas chromatography system, fermentors, lyophilizers, growth chambers, greenhouse facilities, and field plots. For characterization of the antibiotics, additional materials utilized are a spectrophotometer, TLC, HPLC, gas chromatography/mass spectroscopy, and assays to monitor antibiotic activity.

**A.1.d. Budget:** $67,000 **Balance:** $-0-

**A.1.e. Timeline:**

| | 7/93 | 1/94 | 6/94 | 1/95 | 6/95 |
|---|---|---|---|---|---|
| Characterize antibiotics | ---------- | | | | |
| Field and greenhouse trials | ---------- | ---------- | | | |
| Package microorganisms | | | ---------- | ---------- | |
| Data analysis | | | | ---------- | ---------- |
| Reports | ---- | ---- | ---- | ---- | ---- |

**A.1.f. Status:** Final Status Report, September 1, 1995. 1. Characterization of the antibiotic: Further progress has not been made.

2. Field and greenhouse trials: In the field, biocontrol efficacy, and disease, are aggregated in space. Biocontrol becomes more uniform in space over time. Levels of biocontrol in adjacent microplots in the field are positively correlated, but not correlated at larger scales. This suggests that factors associated with soil movement, for example movement of the pathogen population, may be less important to influencing biocontrol than the physical environment (especially moisture levels) at different locations in the field. Further investigation of the spatial patterns of disease and biocontrol will provide important insight into factors that may be critical to biocontrol success.

**V. Evaluation:** Criteria for evaluation include: 1) purification and structural identification of the antibiotics, 2) efficacy of disease control (scab, *Verticillium*, *Fusarium* and *Rhizoctonia*) using different suppressive *Streptomyces* strains, and 3) commercial prospects of delivery system.

**VI. Context within field:** Biological control of soil-borne plant diseases is the objective of many research groups world-wide. This project is exciting and unique because it investigates the possibility of controlling multiple pathogens simultaneously using a single biocontrol organism. In addition, this project is one of only a few in which the chemical nature of substances involved in biocontrol is being investigated. The laboratories involved in this proposal have been involved in cooperative work on *Streptomyces scabies* with emphasis on pathogenicity, biocontrol, biochemistry, and ecology for a number of years. Our research objective is to develop the technology by which we can introduce these organisms into Minnesota soils for controlling plant diseases.

**VII. Benefits:** Biological control of these diseases would avoid the risks of soil and water contamination and the threat to human health posed by toxic chemical applications.

**VIII. Dissemination:** Research results will be published in scientific and industry journals. Dr. Roger Jones (Univ. of MN Extension Plant Pathologist) has cooperated with us in establishing plots in growers' fields and will play an important role in communicating our results to the growers.

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

**X. Cooperation:** Dr. Janet Schottel, Department of Biochemistry, University of Minnesota; Dr. Linda Kinkel, Department of Plant Pathology, University of Minnesota.

Dr. Schottel will work on the purification and characterization of the antibiotics produced by the biocontrol organisms as well as physiology of these organisms.

Dr. Kinkel will focus on tracking introduced biocontrol agents in the field and on investigation of the influences of colonization ability on biocontrol effectiveness.

XI. **Reporting Requirements:** Semiannual status reports will be submitted not later than Jan. 1, 1994, July 1, 1994, Jan. 1, 1995, and a final status report by June 30, 1995.

**Qualifications:**

Dr. Neil A. Anderson, **Principal Investigator**
Professor
Department of Plant Pathology
University of Minnesota

**Education**

| | | | |
|---|---|---|---|
| B.S. | Forestry | University of Minnesota | 1951 |
| M.S. | Plant Pathology and Plant Genetics | University of Minnesota | 1957 |
| Ph.D. | Plant Pathology and Plant Genetics | University of Minnesota | 1960 |

**Research Interests**

Dr. Anderson's research interests are in the genetics of plant pathogens and in biological control.

**Relevant Publications (See Detailed Work Program)**

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

**Education**

| | | | |
|---|---|---|---|
| B.A. | Microbiology | University of Missouri<br>Columbia, MO | 1972 |
| Ph.D. | Biology | Washington University<br>St. Louis, MO | 1977 |
| Postdoctoral | Molecular Biology | Stanford University<br>Palo Alto, CA | 1981 |

**Research Interests**

Dr. Schottel is a molecular geneticist and biochemist interested in the mechanism of *S. scabies* pathogenicity on potato.

**Relevant Publications (See Detailed Work Program)**

**Dr. Linda L. Kinkel**
Assistant Professor
Department of Plant Pathology
University of Minnesota

**Education**

| | | | |
|---|---|---|---|
| B.A. (honors) | Biology | St. Olaf College<br>Northfield, MN | 1981 |
| M.S. | Plant Pathology | University of Wisconsin<br>Madison, WI | 1985 |
| M.S. | Biometry | University of Wisconsin<br>Madison, WI | 1987 |
| Ph.D. | Plant Pathology | University of Wisconsin<br>Madison, WI | 1988 |

**Research Interests**

Dr. Kinkel's research program focuses on the ecology of microorganisms on plant surfaces, including both pathogens and non-pathogens.

**Relevant Publications See (Detailed Work Program)**

**OBJE\ £ 8:**

**A.1.d. . get:** $77,000 **Balance:** $-0-

**A. Title of Objective:** Enhanced suppression of sugarbeet root rot pathogen.

**A.1. Activity**: Field soils collected in sugarbeet-growing regions of Minnesota will be tested for effectiveness of oats in reducing Aphanomyces root rot of sugarbeet. Disease reduction will be correlated with populations of soil microorganisms that are antagonistic to *Aphanomyces cochlioides* and to detrimental effects of oat residue decomposition on *A. cochlioides*.

**A.1.a. Context within the Project:** In greenhouse studies by the P.I., soil-incorporation of green oats reduces Aphanomyces root rot of sugarbeet. It is unknown how oats affects *A. cochlioides*, so it is uncertain how to best manage oats for maximum reduction of Aphanomyces root rot in the field. Additionally, soil type, populations and species of soil microorganisms, and local environmental conditions differ among fields and regions.

**A.1.b. Methods:** Farm fields that are naturally infested with *A. cochlioides*, and that represent a range of soil types, will be sampled in the sugarbeet-growing areas of Minnesota. The number of fields sampled will be determined by the work load, since the proposed research is labor-intensive. Soil from each field will be fertilized for optimal sugarbeet production and evaluated in the greenhouse under identical conditions, as described in Steps I-III.

In Step I, soil will be prepared, dispensed into pots, and the following treatments applied: one-third planted to 'Starter' oats, one-third planted to 'Maribo Ultramono' sugarbeets (= a control) and one-third not planted (= a "fallow" control). Treatments will be arranged in a randomized block design and replicated at least 16 times.

In Step II, after 4 wk of growth, oat and sugarbeet plants will be severed at the soil line, weighed, cut into pieces, and reincorporated into soil in which each crop was grown. These amended soils and fallow soils will be moistened and incubated at 25 C for 3 wk to allow crop residue to partially decompose.

In Step III, the oat- and sugarbeet-amended soils and fallow soil will be planted to sugarbeet. After emergence, temperatures will be increased to 28±2 C to favor Aphanomyces root rot. Dying seedlings will be assayed for infection by several potential pathogens. There are no culture media for quantification of *A. cochlioides* populations in soil, so relative population levels are based on number of dying sugarbeet seedlings infected by the fungus. Four weeks after planting, surviving plants will be rated for root rot severity.

**A.1.c. Materials:** The P.I. has a greenhouse with temperature, light, and photoperiod controls; and laboratory equipment: rotary shaker, colony counter, centrifuge, incubators, Millipore filters, ultraviolet lamp, compound microscope with epifluorescence, and an ultra low freezer. Materials needed include expendable laboratory and greenhouse supplies and desiccators.

**A.1.e. Timeline:**

| | 7/93 | 1/94 | 6/94 | 1/95 | 6/95 |
|---|---|---|---|---|---|
| Sample fields | ----- | | ------ | | |
| Greenhouse trials | ---------- | | ---------- | | |
| Microbe isolations | ---------- | | ---------- | | |
| Test microbes against *Aphanomyces* | | | ----- | ------ | |
| Test oat decomposition on *Aphanomyces* | ---------- | | ---------- | | |
| Data analysis | ---------- | | ---------- | | |

**A.1.f. Status:** Final status report, September 1, 1995. When an experiment was inadvertently fertilized with Peters solution (N:P:K+micronutrients), Aphanomyces root rot (RR=0-100 scale) was reduced in soils precropped with oat (RR=15) or fallowed (RR=15) compared to nonfertilized fallow controls (RR=93). In subsequent trials (averaged for three soil sources), root rot was reduced in soils precropped to oat and fertilized (RR=26), precropped to oat alone (RR=26), or fallowed and fertilized (RR=41) compared to fallow alone (RR=94). These results suggest that an oat precrop, as well as Peters solution, contribute nutrient(s) (likely micronutrient[s]) that reduce disease. *Trichoderma* and fluorescent pseudomonads increased in some soils precropped to oat. Research on mechanisms of disease suppression continue (see attachment).

**V. Evaluation:** Success of this project will be determined by the ability of a green oat crop to 1) reduce Aphanomyces root rot in soil from fields differing in characteristics and 2) correlate the level of disease reduction to populations of specific groups or species of microorganisms that are antagonistic to *A. cochlioides* or with oat decomposition products that are inhibitory to *A. cochlioides*. In the long-term, success will be realized when a green oat crop can be managed in the field to optimize the microbes or oat decomposition products that inhibit disease.

**VI. Context within field:** Previous research has shown that green crop residues can reduce, or even increase, disease caused by several soilborne plant pathogens (Cook and Baker, 1983; Lumsden et al., 1983). Whether a disease is enhanced or suppressed by a green crop depends upon many factors. Populations of soil microorganisms that are antagonistic to a pathogen are affected by the type and maturity of the crop, kinds and amounts of decomposition products, proportion of available nutrients in soil in relationship to resistant components in plant residue, and carbon:nitrogen ratio of soil. Moreover, plant pathogenic fungi produce infective and survival spores that may differ in sensitivity to other microorganisms, or to plant decomposition compounds. Understanding how green crops can be managed to shift the biological balance in soil to enhance beneficial microorganisms and to suppress plant pathogens will benefit crop production, while promoting ecologically sound preservation of the environment.

**VII. Benefits:** The incorporation of green oats into soil enhances and exploits naturally occurring biological control, is environmentally safe, and is compatible with other sugarbeet production practices. It will add a much needed component to a limited arsenal of available control practices.

VIII. **Dissemination:** Results will be presented to peers at national and regional meetings and to sugarbeet producers and industry personnel at local meetings. Written results will be published in peer-review journals and in semi-technical annual reports that are distributed to 3,000 sugarbeet producers and industry personnel.

IX. **Time:** My intent is to understand how oats reduces Aphanomyces root rot of sugarbeet. Subsequent studies will concentrate on management of oat production in the field to optimize the mechanisms that result in reduction of disease (funding for this phase will be through commodity dollars).

X. **Cooperators:** Dr. R. Watkins, American Crystal Sugar Company, Moorhead, MN; Mr. T. Knudsen, Minn-Dak Farmers Cooperative, Southern Red River Valley; and Dr. J. Widner, Southern Minnesota Beet Sugar Cooperative, Renville, MN will advise in the selection of appropriate fields for sampling.

XI. **Reporting Requirements:** Semiannual status reports will be submitted not later than January 1, 1994; July 1, 1994; January 1, 1995; and a final status report by June 30, 1995.

XII. **Literature Cited: (See Detailed Work Program)**

**Qualifications:**

Dr. Carol E. Windels, **Principal Investigator**
Associate Professor of Plant Pathology
Northwest Experiment Station
University of Minnesota
Crookston, MN 56716 (218) 281-6510, Ext. 468



**OBJECTIVE 9:**

**A. Title of Objective:** Enhancing biological and cultural control of alfalfa insect pests.

**A.1. Activity:** This project will develop the knowledge base needed to improve cultural strategies for alfalfa management to more effectively exploit the benefits of biological control of alfalfa weevil, *Hypera postica* (Gyllenhal) as part of an IPM approach. Detailed knowledge of parasitoid phenology and the ecological dynamics of parasitoid association with its host are essential to achievement of this objective.

**A.1.a. Context within the Project:** Minnesota farmers grow almost 2 million acres of alfalfa and an additional 0.5 million acres of alfalfa-grass mixtures. Since the primary use of alfalfa is as feed for dairy animals, it is important to minimize use of insecticides. Suppression of alfalfa weevil is one of the outstanding success stories in the annals of biological control in the United States. Despite the great progress that has resulted from biological control efforts, alfalfa weevil still ranks as one of the most damaging insect pests of alfalfa. Local failure of biological control or delay of harvest occasionally result in serious outbreaks that must be controlled by the application of insecticidal sprays. A complex of five parasitoid species and a fungal disease are now established in Minnesota and becoming increasingly widely distributed (Flanders and Radcliffe, results from LCMR 1991-1993 project). But, maximization of the benefits of biological control agents may require modifications of cultural management practices for the crop, especially as relating to cutting.

Agronomic considerations dictate that the optimal timing of alfalfa harvest is in the bud to early bloom stage. Fortunately, this also appears to be optimal in terms of management of the parasitoids we have studied (Flanders and Radcliffe, data from LCMR Project, 1991-1993), especially for *Microctonus aethiopoides* Loan, *Tetrastichus incertus* (Ratzeburg), and *Bathyplectes curculionis* (Thompson). When alfalfa is cut on this schedule, most *M. aethiopoides* are in the larval stage within adult alfalfa weevils. Clean harvest of alfalfa leaves a stubble that is an inhospitable environment for alfalfa weevil, most larvae die and adults leave the field. In the case of those adult weevils that are parasitized with *M. aethiopoides* this removes the parasitoids from the alfalfa field. The consequences of this behavior on the overall benefits of *M. aethiopoides* are unknown. Clearly, it contributes to the rapid spread of the parasite to new environments, but what is the effect in the field where the parasitoid originated? Do parasitized adult weevils live to return to the regrowth or are the parasitoids effectively lost? In the case of the larval parasitoids *B. curculionis* and *T. incertus*, harvesting would clearly be detrimental if the parasitoids are still present within the alfalfa weevil larvae. When alfalfa is harvested on schedule, most *B. curculionis* and *T. incertus* parasitoids are in the pupal stage within the soil and litter in the alfalfa field. Even so, upon emergence, they may have difficulty finding a host. This interference with the spatial dynamics of the host-parasitoid relationship might be alleviated by leaving unharvested strips of alfalfa as refugia for the parasitoids. Strip-cropping has often been proposed as a means of enhancing biological control (Stern 1969), but finds little grower acceptance because it is inconvenient, sometimes more costly, may reduce overall yield,

may not appeal to the growers sense of aesthetics. We pro[illegible]o test a variation on this strategy, leaving unharvested a border strip only on field margins. This is considerably simpler than usual strip crop practices and may prove much more acceptable to growers.

Alfalfa weevil adults utilize nearby woodland during summer diapause, and as shelter during the winter. The parasite *M. aethiopoides* is carried back and forth with the weevils. Seasonal utilization of woodland by alfalfa weevils has not been studied in Minnesota, and indeed, contradictory information exists from studies in other states.

**A.1.b. Methods:** We will continue to assess the relative importance of alfalfa weevil parasitoids in Minnesota, and determine the incidence of the pathogen, *Zoophthora phytonomi* (Arthur). We will determine the phenology of the natural enemies in relation to host phenology, heat unit accumulations, and rainfall. We will determine the effect of leaving unharvested alfalfa strips as refugia on biological control of the alfalfa weevil. We will also assess seasonal utilization of wood lots by alfalfa weevil adults.

**A.1.c. Materials:** A vehicle is required for travelling to the farm sampling sites as approximately 12,000 miles will be driven during the 1993 survey. Field sampling equipment includes weather data loggers ($1500 plus cost of software), sweep-nets, replacement sweep-net bags, sieves, stubble sampler, forceps, aspirators, Falcon seal tight dishes, and plastic bags. Laboratory equipment includes three dissecting microscopes, two with transmitted light stands, one equipped with a camera for documenting results; jewelers forceps; Boerner slides; magnifying glass; computer equipped with modem; database management software; desktop publishing software; 35 mm camera equipped with bellows and ring flash; photographic film; and computer diskettes. Labor during the field season (May through August) will average 160 h per week.

**A.1.d. Budget:** $57,000 **Balance:** $-0-

**A.1.e. Timeline:**

| | 7/93 | 1/94 | 7/94 | 1/95 | 7/95 |
|---|---|---|---|---|---|
| Detail experimental design | | ------ | | | |
| Collect samples | ------- | | ------ | ------ | |
| Process samples | ------- | | ------ | ---- | |
| Prepare manuscripts for publication | | ------ | | ------ | |
| Reports | | ---- | ---- | ---- | ------ |

**A.1.f. Status:** Final Status Report, September 1, 1995. Field resesearch on this project was completed in1993. Data entry and statistical analysis were completed in 1994. Research seminars were presented by Radcliffe in the departments of Horticultural Science and Entomology in 1995. An MAES fact sheet on alfalfa weevil is in preparation to include information on biological control (Hutchison,. Flanders and Radcliff, *Univ. Minn., Agric. Ext. Serv. Agric. Fact Sheet AG-FS-1026*). A second manuscript intended for submission to a peer-reviewed journal is in preparation. Completion of both been delayed by the recent move of the senior PI (Flanders) to a new position. [illegible]he first manuscript from this research is now published: F[illegible]ers, K. L., E. B. Radcliffe, and C. A. Krueger. 1994. Natural enemies of alfalfa weevil, *Hypera postica* (Coleoptera: Curculionidae), in Minnesota. *Great Lakes Entomologist* 27 (1): 7-18.

**V. Evaluation:** Criteria for evaluation include whether we are able to: 1) document the relative importance of alfalfa weevil parasitoids in different regions of the state, 2) determine parasitoid and pathogen phenology, 3) determine the effectiveness of unharvested alfalfa borders as refugia for alfalfa weevil parasitoids, and 4) provide producers with recommendations regarding alfalfa management strategies that enhance or maintain effectiveness of alfalfa weevil natural enemies.

**VI. Context within field:** Our laboratory has researched species interactions in the alfalfa ecosystem (Radcliffe *et al.* 1976), parasitoids of pea aphid and spotted alfalfa aphid (Amaya 1980), economic thresholds for potato leafhopper and pea aphid on alfalfa (Cuperus *et al.* 1982, 1983), cutting management for potato leafhopper control (Cuperus *et al.* 1986), effects of insecticidal sprays on pea aphid parasitoids (Cuperus and Radcliffe 1984), dispersal behavior of the potato leafhopper (Flanders *et al.* 1989), and alfalfa stress interactions (Chen *et al.* 1990). Previous LCMR funding (1989-91) was used to study the interacting effects of cultivar and insecticides on pea aphid and alfalfa weevil natural enemies. Current LCMR funding (1991-93) is being used to determine alfalfa weevil natural enemy distribution and phenology.

The alfalfa weevil 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 *B. curculionis*, *B. anurus* (Thompson), and *T. incertus*; the adult parasitoid, *M. aethiopoides*; and *M. colesi* Drea, which parasitizes late instar larvae matures in the adult weevil (Day 1981). Alfalfa weevil overwinters as adults and, in some areas, eggs. *Bathyplectes* spp. and *T. incertus* overwinter as pupae in the alfalfa field. *Microctonus* spp. overwinter as larvae within the overwintering weevil. In areas where it has been introduced, *B. anurus* is a more effective parasitoid than *B. curculionis* (Harcourt 1990). There is also an insect pathogen, *Z. phytonomi*, which attacks alfalfa weevil larvae (Harcourt et al. 1974). The Maryland, or Eastern strain, reached Minnesota in 1970 (Radcliffe and Chiang 1972). With it arrived *B. curculionis*.

Our laboratory was responsible for the introduction of *M. aethiopoides* in Minnesota (Radcliffe et al. 1983). Subsequent releases in Minnesota were made by USDA APHIS in cooperation with the Minnesota Department of Agriculture. By 1985, *M. aethiopoides* was recovered from 46 Minnesota counties (Krueger and Radcliffe, unpublished data). *M. aethiopoides* is a major cause of mortality in alfalfa weevil adults. *M. colesi* and *T. incertus* were recently established by APHIS, but their known distribution prior to 1991 was limited. Apparently, APHIS was unsuccessful in establishing *B. anurus* (Niles Biological Control Laboratory 1991). *Z. phytonomi* had never been officially reported in Minnesota, but was observed in Rosemount in 1984 following a rainy spring (Flanders, unpublished). Our research in 1991-1992 found *Z. phytonomi* and *T. incertus* to be widely distributed. *B. anurus* has been recovered from four counties in southeastern Minnesota. *M. colesi* was not recovered.

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 pest management practices based on natural enemy distribution and phenology. Cutting at, or just prior to, 1/10th bloom is recommended for best forage quality. Alfalfa harvest interferes with biological control in that it removes most of the host insects, via mortality to the weevil larvae, and by causing adult weevils to disperse. Biological control might be enhanced if small numbers of weevil adults and larvae were conserved at the time of harvest. An example might be to leave uncut borders around the alfalfa field. This would retain alfalfa weevil adults that contain *M. aethiopoides* larvae.

**VII. Benefits:**

**A.** Increase understanding of role of natural enemies in regulating populations of insect pests of alfalfa.

**B.** Reduce insecticide use on alfalfa by developing management strategies that incorporate contribution of biological control agents to suppression of insect pest numbers. Alfalfa is grown on over 2 million acres of Minnesota cropland. Research will suggest alfalfa management practices most complementary to biological control of alfalfa weevil.

**VIII. Dissemination:** As in the past, survey results will be made available to county extension agents and cooperating producers in the winter following the survey. Significant results will be presented in real time to county agents and producers via The Plant Pest Newsletter. Results will be presented to peers at branch and national meetings of the Entomological Society of America, and published in appropriate journals. Data will be presented to the public when opportunities arise. In the past year, presentations have been made to the Weed Science Society of America, to Minnesota's outstanding farm families at the farm family banquet, the North Central Branch of the Entomological Society of America, the Minnesota Science Museum, the Staples Irrigation Center field day, and the University of Minnesota Agri-Growth Tour. Data from the survey will be incorporated into the next revision of the Minnesota Cooperative Extension service fact sheet on alfalfa weevil.

**IX. Time:** The research proposed here will be completed within the biennium. Further requests to LCMR may be made to test other management strategies.

**X. Cooperation:** Dr. Kathy L. Flanders, Department of Entomology, University of Minnesota. Producers in Wadena, Morrison, Sherburne, Scott, Dakota, Olmsted, and Houston Counties.

**XI. Reporting Requirements:** Semiannual status reports will be submitted not later than Jan. 1, 1992, July 1, 1994, Jan. 1, 1995, and a final status report by June 30, 1995.

**XII. Literature Cited (See Detailed Work Program)**

**Qualifications:**

Dr. Edward B. Radcliffe, **Principal Investigator**
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

Dr. 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 under way in each of these areas. Dr. Radcliffe's primary role will be in experimental design and project oversight.

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

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 assessing impact of cultivar and insecticides on alfalfa weevil and pea aphid natural enemies, 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 conduct field research involved in this project and to analyze the results.

**OBJECTIVE 10:**

**A. Title of Objective:** Biological control of the western corn rootworm using an entomoparasitic nematode.

**A.1. Activity:** To determine optimum application timing and introduction rate of the nematode *Steinernema carpocapsae* for corn rootworm control, to determine whether *S. carpocapsae* exhibits directional preference when locating a host; and to modify virulence, size, and host-finding ability of *S. carpocapsae* via serial passage through the intended host.

**A.1.a. Context within the Project:** Field trials testing the efficacy of the entomoparasitic nematode, *S. carpocapsae,* as a biological control agent for western corn rootworm (WCR), *Diabrotica virgifera virgifera,* have yielded inconsistent results (Munson and Helms, 1970; Poinar, et al, 1983; Rohrbach, 1969). This inconsistency may reflect overly low application rates, or variation in virulence among nematode strains and insect ages (Jackson, 1985). Understanding the behavior of this nematode in the soil is critical to proper soil placement. Previous studies indicate that *S. carpocapsae* tends to move upwards when inoculated below the surface of a soil or sand column (Georgis and Poinar, 1983; Kondo and Ishibashi, 1986a; Kondo and Ishibashi, 1986b; Moyle and Kaya, 1981; Schroeder and Beavers, 1987).

Certain characteristics of *S. carpocapsae* which are important to its performance as a biological control agent can be changed by rearing the nematode in the desired host. The host-finding ability and virulence of *S. carpocapsae* has been significantly improved by rearing nematodes in scarab beetle and gypsy moth larvae for several generations (Gaugler and Campbell, 1991; Shapiro, et al., 1985). Nematode size decreased when granary weevil and WCR larvae were substituted for the usual rearing host, wax moth larvae (Alikhan, et al., 1985; Jackson, 1985). First and early second instar WCR larvae, which cause far less damage to corn than late seconds and thirds, are less susceptible to *S. carpocapsae* attack than mature larvae, in large part due to the small size of the insect relative to that of the nematode.

**A.1.b. Methods:** The field trial will take place on the Rosemount Experiment Station. A factorial design consisting of 4 replicates of 4 nematode application timings (egg, first instar, second instar, and early third instar) and 5 rates (0, $10^4$, $10^5$, $10^6$, and $10^7$ nematodes/row-ft) will be used, with a total of 80 plots.

Nematode strain "improvement" will be attempted using All strain *S. carpocapsae.* The nematodes will be raised in wax moth larvae (*Galleria mellonella*) and third instar WCR larvae. Virulence to third instar WCR larvae will be assessed with the bioassay developed by Jackson (1985) after each passage through the hosts. Host-finding ability is indirectly tested by this assay, as well. Trends in this data will be analyzed by regression analysis; ANOVA will be used to address bulk differences. $LD_{50}$'s for third instar WCR will be computed using Probit analysis for each generation/host. The POLO software package will be used for this analysis.

**A.1.c. Materials:** Nematodes will be obtained from BIOSYS; western corn rootworm eggs will be purchased from French Agricultural Research Service. Wax moth larvae will be purchased from Sunfish Bait. A short-season corn hybrid (Northrup-King 3624, relative maturity 95 days) has been used in 1991-2 with good results, and will again be used in 1993-4.

**A.1.d. Budget:** $36,000 **Balance:** $-0-

**A.1.e. Timeline:**

| | 7/93 | 1/94 | 6/94 | 1/95 | 6/95 |
|---|---|---|---|---|---|
| Field test nematodes | ---------- | ---------- | | | |
| Laboratory bioassays | | ---------- | ---------- | ---------- | |
| Data analysis | ---------- | ---------- | ---------- | ---------- | ---------- |

**A.1.f. Status:** Final Status Report, September 1, 1995. The principal objective of our research over the past four years has been to determine optimum application timing and introduction rate of entomoparasitic nematodes for larval western corn rootworm (*Diabrotica virgifera virgifera* LeConte) control. Initial studies used *Steinernema carpocapsae* (Weiser), All strain, the most commercially reliable nematode available. These experiments have been highly successful. Reduction in western corn rootworm adult emergence has been striking (up to 85%) and consistent. Corn root injury protection has been somewhat variable, but high rates of nematodes have routinely provided protection equal to that afforded by the best-performing chemical insecticides. Effective nematode introductions occur when corn rootworm larvae are in their late second to mid-third instar.

The optimal application rate for *S. carpocapsae* (approximately 500,000 nematodes/row-ft) is cost-prohibitive, but can be reduced by selection of more appropriate nematodes. Two highly virulent species were compared to *S. carpocapsae* in field trials. *Steinernema riobravis* (Poinar) and *Heterorhabditis bacteriophora* (Poinar), New Jersey strain, outperformed *S. carpocapsae* in corn rootworm adult emergence reduction, and provided equivalent root injury protection. Both species are undergoing further testing in 1995 against a naturally-occurring, mixed population of western and northern corn rootworms.

Secondary objectives have been to determine whether *S. carpocapsae*, All strain, exhibits directional preference in host location, and to modify virulence, size, and host-finding ability of this nematode via serial passage through an intended host. Both objectives were abandoned, based on results of field trials and developments in commercial nematode formulations. All strain *S. carpocapsae* is the least virulent nematode assayed against corn rootworm larvae; furthermore, an effective immune response can be mounted by corn rootworms against this strain. In addition, the behavior of this species in the soil may make it less likely to contact corn rootworm larvae than *S. riobravis* and *H. bacteriophora.* Recently, these species became available in water-dissolvable granules, alleviating handling and shelf-life concerns, and enhancing the possibility that economically feasible control of larval corn rootworms with nematodes can be achieved.

**Effects of Nematode Application Rate, Relative Application Timing, and Species on Western Corn Rootworm Control**

| Year | Treatment/ Species | Rate (#/row-ft) | WCR Instar Targeted | Avg. Root Rating | Emergence (WCR/plant) | %Reduction Emergence |
|---|---|---|---|---|---|---|
| 1991 | Agar Control | | | 2.95 | 29 | |
| | *S. carpocapsae* | $10^4$ | 1.5 | 2.95 | 21 | 26 |
| | | $10^5$ | | 2.83 | 18 | 39 |
| | | $10^6$ | | 2.60 | 14 | 54 |
| | | $10^7$ | | 2.04 | 5 | 85 |
| 1992 | Agar Control | | | 4.11 | 30 | |
| | *S. carpocapsae* | $10^4$ | Egg | 3.89 | 37 | 25 |
| | | $10^5$ | | 4.20 | 22 | 25 |
| | | $10^6$ | | 3.60 | 26 | 11 |
| | | $10^7$ | | 3.99 | 47 | -61 |
| | Agar Control | | | 4.11 | 27 | |
| | | $10^4$ | 1 | 3.91 | 31 | -15 |
| | | $10^5$ | | 4.04 | 42 | -57 |
| | | $10^6$ | | 3.89 | 41 | -53 |
| | | $10^7$ | | 4.09 | 32 | -18 |
| | Agar Control | | | 4.08 | 40 | |
| | *S. carpocapsae* | $10^4$ | 2 | 4.08 | 45 | -14 |
| | | $10^5$ | | 4.13 | 29 | 26 |
| | | $10^6$ | | 3.16 | 23 | 42 |
| | | $10^7$ | | 2.76 | 17 | 56 |
| | Agar Control | | | 4.11 | 38 | |
| | *S. carpocapsae* | $10^4$ | 3 | 3.99 | 29 | 25 |
| | | $10^5$ | | 3.59 | 29 | 25 |
| | | $10^6$ | | 2.86 | 17 | 56 |
| | | $10^7$ | | 2.58 | 6 | 84 |
| 1993 | Agar Control | | | 3.29 | 26 | |
| | *S. carpocapsae* | $10^4$ | 3 | 3.09 | 23 | 10 |
| | | $10^5$ | | 3.03 | 14 | 45 |
| | | $10^6$ | | 2.94 | 7 | 74 |
| | | $10^7$ | | 2.70 | 3 | 87 |
| | *S. riobravis* | $10^3$ | | 3.11 | 24 | 8 |
| | | $10^4$ | | 3.10 | 17 | 35 |
| | | $10^5$ | | 3.07 | 16 | 38 |
| | | $10^6$ | | 2.81 | 5 | 81 |
| 1994 | Untreated | | | 2.80 | 23 | |
| | Furadan 4F | | | 2.10 | 3 | 85 |
| | *S. carpocapsae* | $10^3$ | 3 | 2.85 | 20 | 10 |
| | | $10^4$ | | 2.78 | 19 | 14 |
| | | $10^5$ | | 2.65 | 13 | 42 |
| | | $10^5$, spray | | 2.70 | 9 | 58 |
| | | $10^6$ | | 2.36 | 6 | 73 |
| | *S. riobravis* | $10^3$ | 3 | 2.60 | 13 | 40 |
| | | $10^4$ | | 2.89 | 15 | 33 |
| | | $10^5$ | | 2.69 | 5 | 78 |
| | | $10^5$, spray | | 2.53 | 7 | 70 |
| | | $10^6$ | | 2.44 | 4 | 83 |
| | *H. bacteriophora* | $10^3$ | 3 | 2.66 | 18 | 18 |
| | | $10^4$ | | 2.63 | 10 | 55 |
| | | $10^5$ | | 2.50 | 3 | 89 |
| | | $10^5$, spray | | 2.58 | 3 | 87 |
| | | $10^6$ | | 2.40 | 2 | 89 |

**V. Evaluation:** In the field trials, a clear demonstration of rate effect, timing effect, and/or interaction will be considered a success, particularly if the data generally agree with 1991-2 results. To date, most field data concerning rootworm control by *Steinernema carpocapsae* have been inconclusive; many of the studies conducted have been "screens," as opposed to the "field bioassay" approach taken here. The laboratory studies should be evaluated on the basis of their ability to improve virulence and host-finding ability in nematodes and to provide meaningful information on nematode movement, all of which could be used toward improving the performance of *S. carpocapsae* under field conditions.

**VI. Context:** The corn rootworm is the number one target of insecticide use in Minnesota, with over 1.2 million acres treated annually at a cost of $14 million. Control options are currently limited to soil insecticides (e.g., Counter, Lorsban, Thimet, Force, and Dyfonate) and crop rotation. Control limitations are increasing, as not all presently available chemicals (e.g. Furadan) are being re-registered with the EPA.

**VII. Benefits:** Developing viable IPM options for corn rootworm is an integral part of research that has already reduced soil insecticide use by 50%. The use of nematodes as a biological control agent would diversify our control options, and further reduce the need for soil insecticides at a time when the impacts of these chemicals on public health, water quality, and avian toxicity are being questioned.

**VIII. Dissemination:** Farmers and agricultural professionals who advise them will be informed through the farm media and presentations at the Crop Pest Management Shortcourse, Ag Professional Updates and county extension meetings. Results from this study will be presented to fellow entomologists at North Central Branch and national meetings of the Entomological Society of America, and will be published as they become available in Insecticide and Acaricide Tests. Publication in refereed journals will occur after study completion.

**IX. Time:** Two years.

**X. Cooperation:** Dr. Jan Jackson, USDA-ARS, Northern Grain Insects Research Laboratory, will assist in experimental design.

**XI. Reporti. equirements:** Semiannual status reports will be submitted . er than Jan. 1, 1994, July 1, 1994, Jan. 1, 1995 and a final status report by June 30, 1995.

**XII. Literature Cited: (See Detailed Work Program)**

**Qualifications:**

Dr. Kenneth Ostlie, **Principal Investigator**
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 in the integrated pest management of corn insect pests, including corn rootworms, European corn borer, armyworms and black cutworm. His work with corn rootworm encompasses basic biology, the influence of cultural practices (tillage, crop rotation, crop resistance), scouting and economic thresholds, yield losses and insecticide performance. His research expertise is complemented by more than 8 years experience with the Minnesota Extension Service in delivering research information to farmers and helping them improve their corn insect management.

**Cooperating Scientist:**

Jan Jackson
Research Entomologist
Northern Grains Insect Research Laboratory, USDA-ARS
Brookings, SD

Ph.D. Entomology, 1985, University of Minnesota
M.S. Biology, 1973, South Dakota State University

Dr. Jacksons's research had investigated corn rootworm biology and management for the past 16 years. Currently, his work focuses on discovery, development, and evaluation of biological control technologies, including insect parasitic nematodes.

**OBJECTIVE 1i.**

**A. Title of Objective:** Determine how production process influences the quality of *Trichogramma* used in biological control.

**A.1. Activity:** The influence of several factors, including adult parasitoid feeding history, rearing host species and other factors, on parasitoid host handling and parasitoid host finding will be determined.

**A.1.a. Context within the Project:** During our research on the efficacy of *Trichogramma nubilale* as a biological control agent for control of European corn borer on sweet corn, we observed considerable variation in the performance of the parasitoid that could not be entirely attributable to variation in the environmental conditions at the time of release. In addition, informal observations on the behavior and longevity of the parasitoids in our production colonies has indicated considerable variation that appears to depend on the rearing conditions, i.e., that the production process may influence parasitoid efficacy. These observations imply that further research on the production of *Trichogramma* is necessary to develop rearing processes and quality control evaluations that will guarantee uniform, high quality production of parasitoids.

**A.1.b. Methods:** *Trichogramma* will be reared on either European corn borers or tobacco hornworm eggs under controlled environments. The ratio of parasitoids to unparasitized hosts will be modified so that parasitoids will be reared under either crowded or uncrowded conditions. Prior to testing, parasitoids will be provided with an adult food (dilute honey, or some other sugar source) or not supplied with an adult food. Parasitoids will be tested individually in laboratory environments on eggs of tobacco hornworm or egg masses of European corn borer.

The ability to parasitize multiple hosts, such as in egg masses of European corn borer, the duration of the oviposition events, the number of eggs oviposited (if possible), and the sex of the offspring (if possible) will be measured to determine how production conditions may influence the host handling abilities of the parasitoids. These affects are particularly important because European corn borer lays eggs in egg masses, and it will be important to ensure that released parasitoids can parasitize all or nearly all of the eggs in an egg mass.

**A.1.c. Materials:** Production of *Trichogramma* is essential, and will be accomplished similar to the previous 8 years. Microscopes, video tapes, experimental test containers, computers, and other miscellaneous laboratory supplies will be used.

**A.1.d. Budget:** Please see section A.2.d.

**A.1.e. Timeline:**

| | 7/93 | 1/94 | 6/94 | 1/95 | 6/95 |
|---|---|---|---|---|---|
| Conduct host handling expt | ---------- | | | | |
| Conduct host finding expts | | | ---------- | | |

**A.1.f. Status:** First status report, December 28, 1993. A half-sib mating design was used to determine the relative importance of genetic and environmental factors on foraging behavior of *Trichogramma nubilale*. There was no additive genetic variance for any of the foraging behaviors measured, but about 14% of the variation could be attributed to maternal effects. Experiments on the details of oviposition behavior showed that females drill near the embryos of developing host eggs, they prefer the eggs on the perimeter of egg masses to those in the center. Females are unable to identify if an egg has an embryo or not, and cannot distinguish between young eggs without embryos and older eggs with embryos. Offspring survived very poorly when oviposited in an egg without an embryo or far from the embryo.

Second status report, June 30, 1994. Preliminary results had indicated that females avoid superparasitism, perhaps by marking parasitized eggs on the surface. This surface mark (if it exists) is not completely effective because many females redrill eggs that they themselves have parasitized. Most females will reject eggs that have been parasitized, suggesting that there is an internal mark that allows females to discriminate between parasitized and unparasitized eggs. Although there are differences in the exposed surface area of eggs within an egg mass, females do not appear to use this information in their oviposition decisions.

Third status report, December 27, 1994. Statistical analysis has been completed for all of the results mentioned in the previous reports. Investigations on the factors that cause a female to leave an egg mass have not identified any particular cause. Specifically, theoretical predictions have not been sustained, and an alternative explanation needs to be found.

Final status report, September 1, 1995. I have been researching various aspects of host handling of female *Trichogramma nubilale* to determine to what extent predictions could be made about the rate of parasitism, and the amount of time spent and eggs oviposited while exploiting a single European corn borer (ECB) egg mass. I have also observed the oviposition behavior of females on an ECB egg mass for patterns that might predict how females perceive and handle this host species. We have found that (1) females prefer the eggs on the periphery of the egg mass where progeny survival is significantly higher than the internal eggs, (2) host eggs that have not as yet reached the first cleavage stage are killed by oviposition but cannot support parasitoid progeny although they are parasitized significantly more than more developed eggs, (3) females prefer drilling in the depressions of the egg mass caused by the overlap of 2-3 eggs, (4) females receive important information after drilling because they reject eggs significantly more often when they have drilled > 1 mm from the embryo and when the egg is already parasitized (5) when eggs are oviposited > 1 mm from the embryo, progeny survival is significantly reduced and the host develops successfully (6) females do not use exposed surface areas of the eggs in a mass to measure egg size, and they probably do not use egg curvature because the eggs are not spherical, (7) there are significant maternal effects associated with the number of eggs parasitized in an egg mass (8) some females will oviposit 2 female eggs per host egg with varying frequencies (from 1-60% of their ovipositions on the first egg mss encountered), even when many nonparasitized eggs are available within the egg mass. There is no influence from the exposed surface area of the eggs or the location of the egg in the mass (peripheral or internal) in a female's decision to oviposit 2 eggs in a single egg.

Although the information on host usage is useful in determining some of the influences on oviposition behavior, there remains a great deal of variation even within families in the number of eggs parasitized, the time spent in egg mass exploitation, the pattern of movement on the egg mass, and the number of eggs a female will place in a single ECB egg. For the latter, it appears that there are 2 distinct oviposition behaviors in the colony, and I have been focusing on the source of this variation. I have incorporated dynamic optimality models (Iwasa et al. 1984) and some principles of game theory (Smith 1985 & Sigmund 1988) to determine the potential evolution and stability of 2 oviposition behaviors of the laboratory colony of female *T. nubilale*, and potential affects on their fitness and efficacy of control of ECB in the field.

Recent results of my research of ***Trichogramma nubilale***, an egg parasitoid of European corn borer, *Ostrinia nubilalis* (Hubner) indicate that 2 ovipositional strategies exist within my colony. Most females oviposit strictly 1 egg per host egg, but approximately 37% of the females oviposit 2 eggs per host egg some of the time and a single egg the remainder of the time with the frequency of expression of double egg-laying possibly varying continuously. Other researchers (e.g. Bigler, 1994, personal communication) report that their colonies of *Trichogramma* spp. are comprised of mostly females that oviposit 2 eggs per oviposition. When females lay three eggs per host egg, per capita survival is reduced substantially and when they lay two eggs per host egg, progeny size is reduced. The size of the ovipositing female significantly affects total fecundity and longevity (Table 1-4). However, females depositing 2 eggs per host egg can produce almost twice the amount of progeny than females depositing a single egg for significanltly less amount of time spent exploiting an egg mass (Kolmogorov-Smirnov Test for differences in distributions of 2 samples of continuous observations, P=0.000) and they have a significantly higher progeny survival rate (Figure 1, Table 5). There is no difference in the primary sex ratio between these two types of females (86% females), so manipulation of the sex ratio is not the cause for these behaviors. With higher rates of survival and parasitism for those females depositing 2 eggs per oviposition, it is surprising that this type of behavior has not increased in the population. Females within the laboratory colony are subject to a variable number of hosts and conspecifics over time and without a carbohydrate source they live 2-3 days. It is possible that these factors influence the relative fitness of the two behavioral types and their resulting frequencies in the population.

## ...ie Theory

In a situation where what a female does is dependent on what others around her are doing, the optimal solution to oviposition might not be obvious. A sequence of decisions or strategies at the individual level will have some payoff associated with it. It is possible that game theory , the mathematics of conflicting interests (Sigmund, 1993), might be useful in predicting the frequency and stability of the behavioral types within the population. The two main concepts of game theory are payoff and strategy. The two oviposition behaviors or "strategies" of female *T. nubilale* are the mixed strategy of ovipositing 2 eggs per oviposition with varying frequencies, and the pure strategy of ovipositing a single egg per oviposition..

When two alternative strategies exist, there are only 3 possible dynamics for the competing strategies (Sigmund, 1993). The first alternative is for one strategy to dominate the other, where one strategy always does better no matter if it encounters a copy of itself or the other strategy. This suggests that the less dominant or maladaptive strategy will eventually go extinct, and the population will become constrained to a single behavioral strategy.

The second alternative considers a bi-stable game where each strategy is the best reply against itself. There are 3 equilibria for the population, where either strategy goes to fixation, and where their frequencies are approximately equal. The actual equilibrium attained is dependent on initial frequencies so only at fixation is the equilibrium stable.

The last alternative is coexistence of strategies where each is the best reply against the other but not against itself, therefore it cannot take over the population. The population would consist of a mixed polymorphism maintained by frequency dependent selection. In this case, the lower the frequency of behavioral strategies like myself, the better I fare in that population. When the frequency of my more rare type begins to increase, selection against me will cause my frequency to decline.

Other possible sources of behavioral variation are conditional strategies where expression of a behavior will depend on some factor(s) affecting the individual female, for example, where a female changes her behavior with temporal variations in host availability or when she is time limited. The resulting strategy is not necessarily a random strategy.

## Characterization of Behavioral Types

To distinguish between the possible alternatives and prior to all possible pairwise interactions between the various behavioral strategies, I have begun selection experiments to characterize the behaviors. The behaviors must be stable enough to accurately determine pay-offs under the various pairwise interaction regimes. After 5 generations I have increased the frequency of females that express the mixed behavior in a single line from 25% to 42% of females suggesting that the trait is genetically determined. Because the mixed strategy appears to vary continuously, a stabilizing selection ...periment has been started to isolate females that express the ...avior for 50% of their ovipositions, and directional selection experiments have been started for females that express the behavior 100% of the time. Because a mixed strategy may be expressed either randomly or conditionally, the selection experiments will help to distinguish between the two possible modes of expression, and the limit to the frequency of expression. How the trait is expressed will determine the fitness values to assign in a pay-off matrix of pairwise interactions.

Females that are stable for 50% expression under a specific set of conditions will be assigned fitness values based on progeny produced in all pairwise interactions with other females expressing similarly, and females that lay single eggs. The conditions will then be changed so females are either host or time limited or both (ie. laboratory colony conditions) and their fitness determined. Predictions about the evolution and the stability of the behaviors can then be made. If the lines do not stabilize to a particular frequency of expression over time, the expression of the mixed strategy could be random. Random expression of a behavior is the best a female can do when interactions between females are repeated many times and she has incomplete information. Genotypically, this means that females have a gene(s) for random expression of a mixed strategy and I cannot select for 50% expression. Assigning fitness values to these females will be more difficult but a computer software program on game theory currently on campus may be useful in determining the outcome of pairwise interactions in this case, and I am currently investigating this possibility. In all situations the fitness values will be frequency and density dependent, and this is where the models of the software program could be most useful.

## Adaptive Value of Behaviors

The adaptive value of each behavior is dependent on the total reproduction of small and large females. I have determined that under conditions of no hosts or food, hosts and no food, food only and hosts and food, that the longevity and fecundity of large females (females not sharing an egg) is significantly higher than small females (Tables 1- 4). Hind tibia lengths are proportional to the number of eggs laid, and females that do not share an egg, females that share an egg with a male and females that share an egg with another female are .18 mm, .16 mm and .15 mm, respectively. The size of females will be considered when determining a female's pay-off when interacting with other females. I am currenly investigating possible adaptive values of the 2 behaviors under various scenarios such as time and host limitations. A consequence of laying double eggs when females cannot count the number of eggs already within an egg is that females that lay single eggs will superparasitize eggs with frequencies dependent on the hosts available. Females that exhibit double egg-laying behavior do not superparasitize eggs. When a female superparasitizes an egg already parasitized by a female that has double egg laid, she loses a single progeny to the latter female's 2 progeny. Interaction experiments with various frequecies of each behavior and density of hosts will determine the outcome to using each behavior when other females are present.

Genetic influences

I have established 100 lines of females from which to select the behavior of double egg-laying; I want at least 7 lines to begin selecting behaviors and currently 7% of the population expresses this behavior at a frequency of 50% of their ovipositions. I am currently selecting 5 females from each of the resulting 7 lines to reproduce for the next generation. I have determined the expected frequency of expression of the behavior given that there is a single or 2 loci gene involved with either dominant or recessive expression. I will choose 5 progeny from the F2 generation to reproduce from all lines because if thare is a 2 loci gene involved that is recessive, I would not see the behavior in the F1 but selection in the F2 will fix the trait by the F3 generation. I will select only females expressing the trait at 50% frequency after the 1st generation to attempt to obtain the desired 50% and 100% expression. If, and when the traits are fixed, I can begin backcrosses to verify the line has fixed for the trait.

Implications for biological control

Females that have mixed behaviors leave the egg mass sooner than fermales that lay single eggs only. The adaptive value to the mixed behavior might not be high in the field where eggs are difficult to find. Females used as biological control agents in inundative releases would be most useful when they maximally exploit a single egg mass discovered by remaining on the egg mass for greater amounts of time. An increase in the frequency of the mixed behavior, especially at higher frequencies of expression within a single female, would probabaly not be desired in the laboratory colony. I am investigating the possibility of evolution of the mixed behavior for future studies of the potential impact on the efficacy of control in the field.

**A.2. Activity:** Determine how some environmental conditions influence the performance of *Trichogramma.*

**A.2.a. Context within the Project:** During our research on the efficacy of *Trichogramma nubilale* as a biological control agent for control of European corn borer on sweet corn, we observed considerable variation in the performance of the parasitoid that may have been related to environmental factors. To the extent that these environmental factors mitigate the efficacy of *Trichogramma*, they will limit the potential applications for inundative release against European corn borer in sweet corn. In particular, we have observed relatively high levels of control of European corn borer by released *Trichogramma* during the second flight of the corn borer, but we have observed relatively poor control during the first flight. Among the more obvious differences during these two flight periods are weather patterns (cooler during the first flight), corn plant size (shorter and smaller plants during the first flight), and variation in potential food resources (no pollen and fewer sugar resources during the first flight). We propose to conduct studies to determine if there are any environmental factors limiting the efficacy of *Trichogramma* during the first flight of European corn borer. In addition, there are a large number of corn varieties used in sweet corn production, and we will also investigate whether or not corn variety can mitigate efficacy of *Trichogramma.*

**A.2.b. Methods:** Two field experiments will be conducted. The first will determine if the development stage of corn can mitigate the efficacy of *Trichogramma.* Sweet corn will be planted at two times during the growing season so that during the normal second flight period, the corn will be at mid-whorl (the late planting) or just post-anthesis (the early planting). Plots will be aproximately 10 x 10 m and replicated several times. *Trihcogramma* will be released and efficacy will be monitored using sentinal egg masses of European corn borer from our laboratory colony. The second experiment will evaluate the influence of corn variety. Between 3 and 5 varieties, representing several agronomic types will be planted in small relicated field plots. *Trichogramma* will be released and efficacy will be monitored using sentinal egg masses of European corn borer from our laboratory colony. Depending on the results, the behavioral basis of the influence of corn development stage or corn variety may be examined.

**A.2.c. Materials:** Production of *Trichogramma* is essential, and will be accomplished similar to the previous 8 years. Egg masses of European conr borer will be produced in our laboratory. Rental of field plots and equipment for corn production will be necessary. Seed, fertilizer, plot markers, and miscellaneous field supplies will be purchased.

**A.2.d. Budget:** $40,000 **Balance:** $-0-

**A.2.e. Timeline:**

| | 7/93 | 1/94 | 6/94 | 1/95 | 6/95 |
|---|---|---|---|---|---|
| Conduct corn development stage expts | ----- | | ----- | | |
| Conduct corn variety expts | ----- | | ----- | | |
| Analyze data | | ---------- | | ---------- | |
| Plan future work | | -------- | | ---------- | |

**A.2.f. Status:** Final Status Report, September 1, 1995. See A.1.f.

**A.3. Activity:** Continue construction of controlled environment rooms for production of insects.

**A.3.a. Context within the Project:** Construction of four controlled environment chambers was initiated under previous LCMR awards. Because of cutbacks in the size of the awards and the increased cost of construction with inflation and piecing the work together, we have not yet been able to complete the job. Two chambers are fully operational, and the other two are partially completed. They require installation of humidity equipment, lighting, water supply and controls.

**A.3.b and c. Methods and Materials:** Using previous blueprints and plans, we will complete as much of the remaing project as is possible, given current levels of funding.

**A.3.d. Budget:** $12,000 **Balance:** $0

**e. Timeline:**

| | 7/93 | 6/94 | 1/95 | 6/95 |
|---|---|---|---|---|
| Request & receive cost ests. | ------ | | | |
| Construct chambers | | ------- | | |
| Test controls | | | ------- | |

**A.3.f. Status:** Final Status Report, September 1, 1995. See A.1.f.

V. **Evaluation:** This project can be evaluated first on our ability to produce uniformly effective parasitic wasps for use in field trials. In addition, we should understand some of the major factors that influence the efficacy of the parasitoids in the field, so the project might also be evaluated on our ability to improve the performance of parasitic wasps. Finally, we may be able to partially specify the scope of environmental conditions under which the parasitic wasps might be effective.

VI. **Context within field:** Research on *Trichogramma* as a biological control agent has extended back about 90 years and has been conducted in nearly every major agricultural locality. During the latter half of this period, researchers have focused increasingly on the use of *Trichogramma* as an inundative biological control tactic. Although *Trichogramma* has been used widely on corn in China and the old Soviet Union, and in scattered localities in Europe, it has not been used very much in the United States. Several factors contribute to this, including the availability of relatively inexpensive, registered insecticides, lower tolerance for arthropod pest injury, and variable performance of *Trichogramma* in field trials (Andow and Olson 1992). In our past four years of work we have demonstrated that there are no naturally occurring species of *Trichogramma* that parasitize significant proportions of European corn borer egg masses (Andow 1992, unpublished data), that *Trichogramma nubilale* might be a suitable candidate as a biological control agent (Hintz and Andow 1990), and that under some circumstances, *Trichogramma nubilale* exhibited good control of European corn borer in sweet corn, but in other cases control was poor (Prokrym and Andow 1992a, 1992b, Andow and Olson 1992). We have shown that two factors that limit parasitism are high mortality/dispersal rates and relatively poor searching ability (Andow and Prokrym 1991) that may be related to the physical structure of the corn plant (Andow and Prokrym 1990). Recently we have observed considerable variation among individual wasps in their oviposition behavior, but we do not yet know the source of this variation.

For *Trichogramma* to be able to be used as a control agent, it is essential to be able to produce uniform quality wasps. Although a variety of rearing methods have been investigated and established to this end, relatively few studies have searched for behavioral variation (most look at gross reproductive potential or gross morphology of adults). Yet this variation is the variation that is most likely to cause variation in performance in the field. It is possible that behavioral variation could account partially for the relative lack of success of *Trichogramma* in the field.

Moreover, it will be essential to determine how to improve *Trichogramma* in the laboratory to be able to develop even more efficacious parasitoids. Most of the previous work has focused on selection among existing strains of *Trichogramma*, and relatively little work has been conducted on improvement. We have shown that significant genetic variation for searching behavior on complex surface structures exists in our laboratory populations (unpublished data), so it should be possible to improve strains. [illegible], this seems to be a potentially productive line of research to develop better biological control agents.

VII. **Benefits:** In Minnesota, sweet corn receives over $3 million worth of insecticides/ year, primarily for the control of European corn borer. Sweet corn is one of the largest consumers of insecticides in the state. If biological controls can be made competitive, they could be substituted for insecticides, thereby reducing dependence on insecticides. This could result in greater worker health safety, lower contamination of foods with insecticide residues, and less disruption of the environment. Current insecticide controls cost about $25-40/ acre/ year. Our work with *Trichogramma* suggests that comparable control by *Trichogramma* might cost as little as $50/ acre/ year, when the parasitoids prove efficacious. If we can stabilize the variability in performance, and bring down the cost of production slightly, it may be possible to eliminate considerable pesticide use. If this occurs, an additional benefit would be an increased demand for insect natural enemies, which should spawn a cottage industry to supply this demand.

VIII. **Dissemination:** Results from this project will be presented at national and regional scientific meetings to peers in the field, and at relevant state meetings to local scientists, extension agents and producers. In addition, a poster and live demonstrations will be prepared for presentation in cooperation with the Minnesota Science Museum so that school children will become aware of work in Minnesota on biological control. Following these presentations, the results will be published as peer-reviewed articles in major national and international journals.

Data will be publicly available upon request and hard copies will be archived after publication at the University of Minnesota. Insect specimens will be prepared as vouchers and deposited in the entomological collection at the Department of Entomology at the University of Minnesota.

IX. **Cooperators:** There are no cooperators on this project.

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

**Qualifications:**

Dr. David A. Andow, **Principal Investigator**
Associate Professor, Department of Entomology
University of Minnesota
St. Paul, MN 55108

Education:

Syracuse University, NSF High School Program, 1971
Brown University, Providence, Rhode Island, B.S., 1977, Biology magna cum laude
Cornell University, Ithaca, New York, Ph.D. 1982. Ecology

**OBJECTIVE 12:**

**A. Title of Objective:** Implement biological control of European corn borer by mass rearing and release of egg and larval parasitoids.

**A.1. Activity:** Implementation strategies will be developed for biological control of European corn borer using the egg parasitoid *Trichogramma nubilale* and the larval parasitoid *Macrocentrus grandii.* Mass introductions of the parasitoids will be made in sweet corn fields of organic growers in different counties in Minnesota. By using parasitoids that attack different stages of European corn borer development, it will be possible to determine whether combinations of parasitoids are better at controlling the pest than a single parasitoid species.

**A.1.a. Context within the Project:** European corn borer is a major pest in sweet corn which receives the second highest amount of insecticides of all crops in Minnesota. While available literature indicates that parasitoids have potential for control of European corn borer, strategies to be adopted for large scale releases are not known. If implementation strategies are available for mass release of insect parasitoids, growers can protect their sweet corn from insect damage by using parasitoids instead of expensive insecticides which are harmful to the environment.

**A.1.b. Methods:** In 1988-1989 field releases of *T. nubilale* were made to determine their efficacy in controlling European corn borer (Prokrym et al. 1992). In 1991, releases were made in an organic grower's field in Dakota County, Minesota. The results indicated that the parasitoid was effective in reducing European corn borer larvae by 90 percent. With our facilities we have the capacity to increase the operation several fold. In 1993-1995 at least ten organic growers will be included in the project.

Organic growers will be contacted and their sweet corn fields will be surveyed soon after planting to select release and control areas. Selected areas will consist of one-acre plots where parasitoids will be released and an additional control plot located at least 600 ft away. Searching area available to *T. nubilale* appears to be a major factor that needs to be considered while calculating release rates (Kanour and Burbutis 1984) hence information on plant density will be recorded and leaf surface area of corn plants in the plots will be determined before each release to calculate release densities in terms of a surface area index (Andow and Prokrym 1991) . Pre-counts of European corn borer egg masses will be recorded by randomly sampling sweet corn plants in the fields before releasing *T. nubilale* to estimate levels of European corn borer infestation.

*T. nubilale* will be reared in large numbers in the laboratory using eggs of the European corn borer using techniques described by Burbutis and Goldstein (1983). After exposure to *T. nubilale*, egg masses will be randomly sampled to estimate % parasitism and sex ratio of the laboratory colony. Releases of the parasitoid will be made by placing parasitized egg masses randomly in the field for 3-4 consecutive weeks starting from the second week of June for control of first generation European corn borer and in late July-early August for control of the second generation. Parasitoids will be released in the pupal stage at a release rate that will yield 150,000 females per acre. Parasitized egg masses will be placed in paper cartons and released at appropriate locations on the plant since *T. nubilale* searches in a distinctive manner on corn (Burbutis et al. 1977). To monitor the parasitoid in the field, sentinel egg masses will be placed in the field. These will be recovered in three days and examined under the microscope for evidence of parasitism by *T. nubilale*. Prior to harvest, 50 corn plants per acre will be sampled for stalk and ear damage.

It is not known whether *T. nubilale* can overwinter in Minnesota. In Delaware it overwinters by hibernating in the pupal stage in eggs of European corn borer (Burbutis et al. 1976; Curl and Burbutis 1977). Since large-scale releases are planned in diverse locations in the state, it will be possible to determine whether specific habitats favor its survival through the winter in Minnesota. To determine whether *T. nubilale* can overwinter in Minnesota, sentinel eggs will be placed in the same field in the following year at the start of the season. These will be recovered in three days and examined microscopically for presence of *T. nubilale* or any other egg parasitoid.

The larval parasitoid *Macrocentrus grandii* has several characteristics that indicate its potential as a good biological control agent (Udayagiri and Jones, 1992). However, it has not been evaluated for its efficiency in controlling European corn borer in the field. At present it is being reared in the laboratory using procedures described by Ding et al. (1989). Attempts will be made to simplify rearing procedures to develop economic techniques for rearing it in large numbers. In 1993-95 releases will be made in three of the sweet corn fields selected for release of *T. nubilale. M. grandii* adults will be released from paper cartons which will be placed randomly in the field. Release schedules will be developed to synchronize release of *M. grandii* with the presence of European corn borer larvae in the field. Prior to harvest, corn plants will be sampled in control and release areas and the number of European corn borer larvae will be determined to estimate parasitism by *M. grandii.*

Data will be analyzed statistically to determine whether there is any difference in egg parasitism and numbers of European corn borer larvae between release and control areas. Comparisons will be made on control of European corn borer by *T. nubilale* alone and by a combination of *T. nubilale* and *M. grandii.*

**A.1.c. Materials:** Materials required include vehicles, rearing cages, growth chambers, materials for rearing European corn borer, *T. nubilale* and *M. grandii.*

**A.1.d. Budget:** $60,000 **Budget:** $-0-

**A.1.e. Timeline:**

| | 7/93 | 1/94 | 6/94 | 1/95 | 6/95 |
|---|---|---|---|---|---|
| Insect rearing | ---------- | | | | |
| Field work | -------- | | --------- | | |
| Data analyses | ----- | | ----- | | |
| Reports | ---- | ---- | ---- | ---- | |

**.f. Status:** Final Status Report, September 1, 1995.

Laboratory Production

During 1993 the number of European corn borer (ECB) egg parasitoids was tripled, *Trichogramma ostriniae* and *T. minutum* joined *T. nubilale* at the MDA laboratory. The rearing process has been streamlined so more ECB eggs can be produced in one field season. Part of this has been accomplished by improving on procedure such as the preparation of the diet for ECB, amount of diet per dish, and by improving the feeding methods of the ECB adults. The number of ECB pupal rings placed in each cage has been increased from three to eight rings— increasing ECB egg production per cage. Eggs are now harvested daily and the rearing capacity of the ECB Vollrath has been tripled.

Total 1993 *T. nubilale* production was estimated through the number of ECB egg sheets used. Over nine million parasitoids were produced. This production was from an average of eight cage set ups per week during the summer production period. Production and release of the parasitoids were much lower due to cooling system failure in both the Percival and Vollrath. The production of *Macrocentrus grandii* was slowed down drastically while the production of *Eriborus terebrans* was stopped completely by the excessive heat in their respective rearing areas.

Rearing of European corn borer (ECB) egg parasitoids, *Trichogramma nubilale, T. ostriniae* and *T. minutum* started early in 1994 to release *Trichogramma* wasps at fifteen different locations covering approximately 25 acres. Taking into account the changes made in the rearing process for ECB and the wasps, and the increase participation of the growers in the release and monitoring aspects of the program last year, this goal was reached. Rearing of ECB and the wasps and educational aspects of the program was maintained by the staff working at the laboratory. The release and monitoring part of the program were maintained by the growers which benefited from the program. Together, we increased the number of releases and the total acreage of sweet corn managed by this program, which definitively increased our knowledge of the factors affecting the releases by causing variation in the percentage of control of ECB. This permitted us to improve on procedure such as the synchronization of the presence of ECB eggs in the field and parasitoid release.

Another part of my time was delegated to set up a slide set which was used at a later date as a teaching tool for the growers involved in the *Trichogramma* wasps release program. The slide set consisted of photographs of insect life stages, rearing processes and equipment necessary for the entire process and was accompanied by word slides explaining each of these steps. A tool like this one was definitively needed to increase the ease of transfer of information and it was also set up very inexpensively. In addition to the slide show, a hard copy information package was prepared and distributed to each grower participating in the program. These growers had to attend a one day workshop at no monetary cost and was very beneficial to gain a good knowledge of this system. The packag[illegible]plained degree-days, monitoring tools for the pest and the w[illegible]s, rearing and releasing the wasps and some samples to recognize the pest and the wasp.

Field Releases

In 1993, releases of *T. nubilale*, started on Wednesday, June 23 in Farmington, Dakota County. Seven organic growers of sweet corn participated in the program which consisted of 17 research plots at nine locations distributed from south of Red Wing to west of Alexandria. A few of our research plots were flooded either totally or in parts. Release density varied from 40,000 to 150,000 *T. nubilale* per acre during a three to four week period according to research plot experimental objective and ECB generation. The low release density objective was to determine if *T. nubilale* could establish itself at least for one season with one or two small releases.

In research plots where density of ECB were moderately high *T. nubilale* parasitized between 30 and 80 percent of the eggs from the egg baits placed in the release areas. It also reduced the number of ECB larvae found in the corn plants by 79 to 85 percent producing an average gain of 15 percent in the weight of the husked corn ears. Releases of *T. nubilale* and *T. ostriniae* together and *T. ostriniae* alone showed results comparable to the high range of the former results. Small acreage releases of *T. minutum*, a native of Minnesota, on corn earworm eggs in sweet corn fields showed slight reduction in corn earworm in the release area although their density was very low that season.

In the 1994 season, the biological control program for the reduction of European corn borer (ECB) in organically grown sweet corn covered over 25 acres in 11 counties. Approximately 15 growers were involved with the release of parasitoids and the collection of egg baits. The egg parasitoids, *Trichogramma nubilale*, *T. ostriniae* and *T. minutum*, and the larval parasitoids, *Macrocentrus grandii* and *Eriborus terebrans* were reared in ECB eggs and larvae. The egg parasitoids were released weekly while the larval parasitoids were released during the presence of ECB larval stages in the field.

ECB numbers were much lower in 1993 than 1994 based on light trap catches and infestation levels in the field. Parasitization by *T. nubilale* and *T. ostriniae* was significantly different from the control. Pooled data showed an average of 2.5 larvae per plant, and 70 percent of the plants infested in 1994. The impact of both *T. nubilale* and *T. ostriniae* resulted in significantly fewer ECB larvae. Only *T. ostriniae* caused significantly lower ECB infestations. *T. ostriniae* caused significantly higher larval mortality than *T. nubilale*.

Total parasitism of the egg baits did not show any significant differences between the two species. There was however a significant difference in the parasitism in the control plots. This difference was lessened due to some wasps dispersing into the control plot from release sites. The percent of egg masses having at least one egg parasitized was approximately the same for the two species and showed the same trend for total parasitism.

The variation in naturally occurring infestation levels of *O. nubilalis* in sweet corn is derived from the normal shift in population size throughout its life cycle. Although the naturally occurring disease, predation and parasitism keep ECB population somewhat in check, augmentative release of *Trichogramma* can help in keeping the population close to or below the economic threshold. The reduction in the number of ECB larvae from the control to the release plot with *T. nubilale* as observed had also been observed by Prokrym et al. (1992). *T. ostriniae* was added to our field study to determine any differences in efficacy. Patterns of efficiency according to release rate which were used in our experiment had been proposed by Andow and Prokrym (1991).

Parasitism does not show any significant trends for either of the species. In the field, wild *O. nubilalis* egg masses tend to show a greater percentage of parasitism than the egg baits from the laboratory. This might be explained by the host selection process by *T. nubilale* (Hintz & Andow 1990) and/or by the patterns of host exploitation by *T. minutum* (Bai & Smith 1994).

At lower ECB density (1993) the efficacy of *T. nubilale* and *T. ostriniae* does not vary significantly. At higher ECB density, *T. ostriniae* significantly reduces the number of emerging larvae and plant infestations, compared to *T. nubilale.* In both cases, the movement of the parasitoids from the release plots to the control plots statistically reduces the efficacy of the *Trichogramma* wasps. If the control plots are located further away from the release plots it would definitely decrease the percentage of infestation.

PRODUCTION COST ESTIMATES

Estimates based on *Trichogramma* spp. reared on *O. nubilalis* eggs. Costs are estimated for a release rate of 150,000 females per acre at a 3:1, female:male ratio.

| | |
|---|---|
| Diet costs: | |
| Ingredients | $13.00 |
| Materials | $ 4.00 |
| Per batch cost | $17.00 |
| | |
| Females / batch | 300,000+ |
| Total *Trichogramma* cost | $8.50 / acre |

Other costs not included:
Labor (approx. 2 hours/acre), equipment, *Trichogramma* needed for producing the next generation, shipping and release time.

**V. Evaluation:** The program can be evaluated by determining the reduction in European corn borer populations in participating growers fields located in diverse regions in Minnesota. The success of the project can also be assessed from the number of growers that indicate an interest in being included in the project and by the reduction in pesticide use for European corn borer control.

**VI. Context within the field:** Biological control in agricultural crops is receiving greater attention with increasing public awareness of the negative aspects of pesticide use. Several parasitoids of European corn borer have been studied in the laboratory. In this project we attempt to transfer the technology from the laboratory to the field and demonstrate to growers how parasitoids can be released efficiently for controlling European corn borer in their fields.

**VII. Benefits:** This project aims at controlling European corn borers using an approach that is environmentally safe and one that reduces the use of chemical pesticides. It is expected to provide growers with implementation strategies for release of natural enemies in their fields. If economical rearing procedures are developed, the technology can be transferred to private industry.

**VIII. Dissemination:** Results of this project will be summarized in an annual report that will be circulated to various organizations in the state. Results will be presented at the branch and national meetings of the Entomological Society of America and at Biological Control symposia. Subsequently these will be published in peer-reviewed journals to disseminate the information to entomologists in the U. S. and other countries. Results will also be communicated to participating growers and other sweet corn growers in Minnesota.

**IX. Time:** The objectives are likely to be achieved in the two years of the project.

**X. Cooperation:** Organic growers in Minnesota will be the primary cooperators. They will provide appropriate release and control plots of sweet corn for our study on biological control of European corn borer.

**XI. Reporting Requirements:** Semiannual status reports will be submitted not later than January 1, 1994, July 1, 1994, January 1, 1995, and a final status report by June 30, 1995.

**XII. Literature cited: (See Detailed Work Program)**

**Qualifications:**

Richard Gagné, **Principal Investigator**
Biological Control Scientist
Plant Protection Division, Minnesota Department of Agriculture

M.S. Entomology, University of Missouri, 1993
B.Sc. Agriculture (Major Entomology), University of Guelph, 1989

Richard Gagné worked on the biological control of apple leafminers with native parasitoids in Missouri orchards for his Master's thesis. He has also studied the biodiversity of big-headed flies (parasitoids of leafhoppers and closely related insects) in Missouri natural areas to determine their use as a bio-indicator. While an undergraduate, he studied the effects of bacteria and various organic materials on corn rootworms. His primary responsibilities are the supervision of the Rosemount biological control laboratory, parasitoid releases, and data collection and evaluation.

Relevant publications authored by P.I. and cooperators.

Gagné, . and B. A. Barrett. 1993. Seasonal occurrence of *Phyllono*. spp. (Lepidoptera: Gracillariidae) and major parasitoids in Missouri apple orchards. Environ. Entomol. (In press).



**OBJECTIVE 1.**

**A. Title of Objective:** Continuation of biological control for the cereal leaf beetle.

**A.1. Activity**: Five Minnesota counties have traces of the parasite *Tetrastichus julis* which attacks larvae of the cereal leaf beetle (CLB). Development of field insectaries for this parasite will be continued along with the laboratory rearing and release of the egg parasite *Anaphes flavipes*. Investigations of two other larval parasites, *Lemophagus curtus* and *Diaparsis temporalis*, will be continued.

**A.1.a. Context within the Project:** Cereal leaf beetle occurs in 20 counties of Minnesota. About 30 percent of the state's small grain crops valued at $40 million are harvested annually from this 20 county region. Populations have been monitored since 1986. Four effective parasites have been established to control CLB in states east and south of Minnesota, and rearing of them continues at a USDA rearing facility in Indiana. Studies (Anderson & Paschke, 1968) have shown that egg parasite *Anaphes flavipes* will attack related leaf beetles, such as the spiderwort beetle, spotted asparagus beetle, and species of *Lema*. Field insectaries with populations of host and parasites are the most efficient way of propagating CLB parasites. In these sites parasites can be established and then recovered by rearing field collected CLB eggs and larvae in the lab. Parasites from these rearings can be introduced at additional sites. Mass rearing and release of the egg parasite and development of field insectaries for one to three parasites are essential for establishing biological control of the cereal leaf beetle.

**A.1.b. Methods**: Ten small grain fields in five counties selected in 1991-92 will be used in this project. Baseline data is available and will include cropping history, cultivars, stand density and previous use of pesticides. Intensive sampling and monitoring will continue through the 1995 season. Collections of CLB larvae will be dissected following the procedures developed by USDA , APHIS at Niles, Michigan. Also, rearing methods for CLB as well as *Anaphes* are being followed (personal communication from Niles, MI).

Analysis. Parasite abundance, percent parasitism, rates of parasitization and distribution over time will be determined. Relationships between degree day accumulations, occurrence of CLB life stages and parasites will be analyzed to establish parasite and beetle phenology. Any incidental disease-causing agents will be further investigated.

**A.1.c. Materials**: One full-time seasonal person and a vehicle are required. Laboratory equipment includes growth chambers, microscopes, camera, computer including software.

**A.1.d. Budget**: $30,000 **Balance:** $-0-

**A.1.e. Timeline:**

| | 7/93 | 1/94 | 6/94 | 1/95 | 6/95 |
|---|---|---|---|---|---|
| Field work | ------ | | ------ | | ------ |
| Lab rearing | ---------- | | | | |
| Data entry & analysis | | -------- | | --------- | |
| Reports | | --- | --- | --- | ---- |

**A.1.f. Status:** Final Status Report, September 1, 1995. Eight counties in the southeast crop district and eight counties in south central district have been surveyed twice during the '94 and '95 crop season. Ten experimental plots have been set up in Houston, Winona, and Olmsted counties. CLB population has been sparse during 94-95 seasons. A total of 75 cereal leaf beetle (CLB) larvae have been collected and reared in the lab for parasite emergence. Approximately 10% of the CLB larvae were parasitized with the wasp, larval parasite, *Tetrastichus julis*. *T. Julis* was then released in cages containing imported willow leaf beetles and asparagus beetles. None of these beetles were parasitized which proved that they are not alternate hosts even when they are coincidental with CLB. Degree day accumulations at base 44°F, averaged for 1994 and 1995 for larval development ranged from 650-1200 in Houston and Olmsted County plots, and from 750-1350 in Winona County plots. The larval stages occurred from June 5 to June 26 in the two-year study. The best release times for larval parasites should occur between June 5 to June 26. The egg parasite, *Anaphes flavipes*, has yet to be confirmed in Minnesota CLB populations.

**V. Evaluation:** Experimental release fields will be monitored to determine establishment and effectiveness of the parasites; farmer-producers will be provided with integrated management recommendations that will allow enhancement and maintenance of biological control agents.

**VI. Context within field**: The natural spread of CLB larval parasite *T. julis* has been slow and rates of parasitism remained well below that needed to maintain CLB populations at subeconomic levels (Harcourt *et. al.*, 1977). Considering the fact that CLB reached epidemic levels in Michigan in about a decade (Haynes and Gage, 1981) the first discovery in 1986 in Minnesota points to the need for enhancing biological control. *T. julis* has been confirmed in trace levels in 5 Minnesota counties (Minnesota Pest Report, 1989). This provides an excellent opportunity to augment the parasite population and develop field insectaries.

**VII. Benefits**: Biological control of CLB has been successful in Michigan and Indiana because of the concerted effort in releasing and establishing parasites. This is a sound approach to control the CLB in Minnesota without having to use chemical pesticides and contaminating the environment needlessly. Establishment of CLB egg and larval parasites would preclude additional use of Furadan (carbofuran), malathion, Penncap-M (methyl parathion), parathion, and Dylox (trichlorfon). This will also allow the end user, the farmer, to subscribe to biological control that works.

**VIII. Dissemination**: Minnesota Department of Agriculture cooperates with the federal network computers for information distribution. The Minnesota Pest Report produced by the MDA reaches all county agriculture clientele. Membership and participation in professional organizations provides opportunity for publication.

**IX. Time**: Two years and continued monitoring for another two years.

**X. Cooperation**: USDA, APHIS Biological Control Laboratory, Niles, Michigan.

**XI. Reporting Requirements:** Semiannual reports will be submitted not later than January 1, 1994, July 1, 1994, January 1, 1995, and a final status report by June 30, 1995.

**XII. Literature Cited: (See Detailed Work Program)**

**Qualifications:**

Dr. Dharma Sreenivasam, **Principal Investigator**
Entomologist and Supervisor
Plant Protection Division, Minnesota Department of Agriculture

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

Dr. Sreenivasam initiated the comprehensive biological pest control research program in 1987 with LCMR funding. This program has evolved into a viable network of cooperation between MDA and several University of Minnesota disciplines and scientists. Some projects have yielded results for implementation of biological controls.

Dr. Sreenivasam has worked with pests of agricultural crops and urban forests for the past 17 years. He supervises statewide pest surveys, coordinates data collection, interpretation and dissemination. He has been involved in cooperative programs with USDA-APHIS in the release of natural enemies for alfalfa weevil, European corn borer and the gypsy moth, and in the development of state and national pest survey data and distribution network. He is an Adjunct Associate Professor in the Department of Entomology, University of Minnesota. Dr. Sreenivasam's primary role has been to integrate research findings on biological control funded by LCMR and develop implementation strategies for a wider scale application in Minnesota.

**OBJEC  E 14:**

**A. Title of Objective:** Biological Control of the Insect Pest Complex in Cabbage & Broccoli: Evaluation & Implementation

**A.1. Activity:** The primary focus of this project is to document the performance of new formulations of *Bacillus thuringiensis* var. *kurstaki* (*Bt*), a bacterium specific to moth and butterfly larvae, such as the imported cabbageworm (ICW), and how microbial control may be used to compliment the impact of native or introduced parasitoid fauna. In addition to ICW, the cabbage looper (CL) and diamondback moth (DBM) are also considered key pests of cabbage and broccoli in Minnesota.

**A.1.a. Context within the Project:** Our preliminary data indicate a diverse array of native parasitoids attacking ICW, CL, and DBM, as well as acceptable performance by several *Bt* products. We also propose to continue to introduce a new beneficial parasitoid, *Cotesia rubecula* (Braconidae), that has been successfully established in Massachusetts and Michigan. This parasite has a primary advantage over the native ICW parasite, *C. glomeratus*, in that it parasitizes early-instar larvae and kills the ICW before it reaches the 5th-instar (> 1" in length), where most of the feeding damage occurs.

In contrast to the broad-spectrum conventional insecticides which also kill non-target beneficial insects, *Bt* is unique in that it is specific only to lepidopteran larvae and poses no safety risk to people, wildlife or the environment (e.g, Kovach et al. 1992). One new *Bt* product of interest, MVP (Mycogen Corp.), has recently been developed that uses another bacterium to naturally encapsulate the *Bt* toxin. This approach is less susceptible to breakdown by UV radiation, and should thereby provide longer residual control in the field. Specific goals of these studies are to determine when ICW, CL and DBM infestations develop, optimum timing of *Bt* applications, persistence of *Bt* applications in the field and the impact of *C. glomeratus* and *C. rubecula* on ICW, as well as other native parasitoids on CL and DBM. This information will be evaluated to determine how *Bt* and the biological control fauna can be integrated most efficiently to manage the pest complex for both processing and fresh-market industries.

Looking at the non-target impacts of Bt is beyond the scope of this objective. Only past lepidopteran species are associated with cabbage/broccoli production.

**A.1.b. Methods:** Field plots will be established at the University of Minnesota Experiment Station at Rosemount and in at least one organic-certified field under commercial production practices. The Rosemount location will be used to evaluate new *Bt* treatments and to release the new ICW parasitoid in collards (leafy, fresh-market cole crop) that are not treated with insecticides. This crop provides a suitable host that is attractive to ICW throughout the season, and thereby a constant supply of ICW larvae for the parasites to colonize and increase their probability of establishment. The grower field will also be used to demonstrate the complimentary, integrated use of *Bt* and release of new ICW parasitoids in a commercial production setting.

All *Bt* e.. ..cy and timing treatments will be replicated four times. Two l... ..s of *Bt* evaluations will be conducted; (a) detailed pest survivorship analysis (sampling twice/week) of three selected *Bt* formulations, and less intensive bi-weekly sampling for a variety of experimental *Bt* formulations. Survivorship analysis of pest populations exposed to *Bt*, conventional insecticide and no treatment will be compared using the method of Hogg & Nordheim (1983). A minimum of 40 plants/treatment will be sampled on each sampling date for the detailed survivorship study; a minimum of 20 plants per treatment will be sampled for the less intensive experimental *Bt* products. The insect pest complex, and all beneficial arthropods (insects and spiders) will be monitored twice per week, with one at-harvest destructive sample taken to evaluate final insect damage and marketability of the final product (i.e., damage to heads and wrapper leaves). Damage analysis will include marketability for fresh market and processing (primarily cole-slaw in Minnesota). Parasitism rates will be determined approximately every two weeks throughout the growing season by collecting mid- to late-instar larvae of each pest, and returning the specimens to the laboratory for emergence.

**A.1.c. Materials:** Primary materials needed for the project include a vehicle for the summer months; cabbage, broccoli and collard transplants (locally grown), transplanter and all necessary production inputs; diet cups, and artificial diet for collection and rearing of CL larvae for parasitism. Cabbage leaves will be used to rear DBM and ICW larvae for parasitism and or disease incidence. Transplanter and portable scale have been purchased for the project with matching funds. *C. rubecula* parasites will be obtained from cooperators in Massachusetts and Michigan.

**A.1.d. Budget:** $54,000 **Balance:** $-0-

**A.1.e. Timeline:**

| | 4/93 | 7/93 | 1/94 | 6/94 | 1/95 | 6/95 |
|---|---|---|---|---|---|---|
| Develop field plans, experimental design | ------ | | ------ | | ------ | |
| Develop collard, nurse crops for parasites | ---------- | | | ------ | | ---------- |
| Conduct field trials in cabbage | ---------- | | ---------- | | | ---------- |
| Evaluate results | ---------- | | | | | |
| Reports | | | ---- | ---- | ---- | ---- |

**A.1.f. Status:** Final Status Report, September 1, 1995. Considerable progress was made during this two year study in achieving the following objectives: 1) development of new action thresholds (based on presence/absence of cabbage looper eggs and/or larvae/plant), and 2) potential of reduced rates of the insect-specific bacterium, *Bacillus thuringiensis* (BT), to enhance parasitism levels of naturally occurring parasitic wasps and thereby reduce BT use and BT costs. In contrast to 1993, cabbage looper (CL) populations in 1994 developed much later in the season, with imported cabbageworm (ICW) becoming the predominant pest species in both studies. For Obj. 1, we found that a variety of BT action threshold resulted in 50% reductions in BT use (e.g., 4 vs. 8 sprays in 1993; and 3 vs. 6 sprays in 1994), with no significant difference in marketability ratings (i.e., both equally and highly marketable for fresh-market or processing). Because CL infestations were lower in

1994, this study will need to be repeated in 1995 (3rd year of data). Thus final recommendations to growers will not be published until the third year of data are analyzed. For Obj. 2, we observed much higher levels of parasitism (ca. 80%) for all pest species, compared with previous years. For both BT formulations (Dipel and Javelin), reduced rates (down to ½ the labelled rate) + naturally occurring parasitism, resulted in acceptable marketability non-significant differences from the full-rate BT treatments or permethrin.

The results of this study were summarized by Ms. Patricia Bolin (Ph.D. student) at the Annual Minn. Fruit & Vegetable Growers Assoc. meeting in February, 1995. Despite not having final recommendations in place, we are aware of at least two commercial "conventional" growers who are now using BT, for some applications, for cabbage insect pest management. One of these growers is permitting us to demonstrate the use of the new action thresholds on his farm this summer in a controlled experiment; we will be responsible for all insect treatment decisions on 4 1-acre blocks and compare our results with 4 1-acre traditionally managed blocks (usual grower decision-making). Both refereed journal articles and extension publications are in preparation, with anticipated completion during fall/winter, 1995.

V. **Evaluation:** Detailed sampling protocols for the *Bt* timing and efficacy studies will demonstrate whether or not these treatments provide statistically significant differences among *Bt* products and traditional insecticides for control of the lepidopteran pest complex. Survivorship studies outlined for CL and ICW will also be useful for quantitative statistical evaluation of how and why *Bt* and other mortality factors are effective, or how we can better incorporate new biological control agents to provide additional mortality. During the 1993 field season we will be able to evaluate whether *C. rubecula* was able to successfully overwinter and establish itself on ICW. Additional releases are planned for both 1993-1995. As indicated in the methods section, all treatments will be evaluated using standard criteria for marketable fresh-market or processing product.

VI. **Context within field:** Cabbage, broccoli and cauliflower are presently grown on about 2,500 acres, with an annual Twin Cities fresh market value of $2.5 million. Several conventional, petroleum-based insecticides are applied each year to protect these vegetables from an array of insect pests. During the growing season, nerve-toxin insecticides are applied to these crops on a 7-10 day schedule. Of the leaf-feeding insects, the imported cabbageworm (ICW) and the cabbage looper (CL) are the most serious pests of cabbage in Minnesota. However, other pests such as the diamondback moth (DBM) are also present and do significant damage to untreated fields. 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 alternative *Bt* products are available commercially, very little quantitative research has been done to demonstrate which products provide consistent control, and what combination of biological control tactics will be economically feasible over a broad range of pest infestation levels, likely to be encountered by commercial growers.

Because of the relative effectiveness of several new *Bt* formulations on the pest complex in Minnesota cabbage (Bartels et al. 1992), and the presence of a diverse fauna of parasitoids and predaceous arthropods (Weires & Chaing 1973), the cabbage agroecosystem provides a model system for implementing a biological control program. However, since the late 1960's (Weires & Chaing 1973), an in-depth study of the population dynamics of the cabbage pest/natural enemy complex has not been undertaken in Minnesota. Preliminary results from intensive sampling at Rosemount, Minnesota during 1991-1992 indicates an interesting difference in the pest species complex attacking cabbage and broccoli. For example, Weires and Chaing (1973) found that ICW was often the most dominant species in Minnesota, whereas we have found that CL has been the most abundant pest during the 1991 and 1992 seasons (also at Rosemount; WDH, unpublished data). If this trend continues, it will be significant because, of the three key pests, CL is usually the most difficult to control with biological alternatives such as *Bt* (Bartels et al. 1992) or parasitoids (e.g., Lingren and Green 1984).

However, despite the high numbers of CL in our *Bt* experiments in Minnesota, we are finding little damage and/or larval contaminants in the final product (head or wrapper leaves) at harvest. It appears that some modification of CL larval behavior, due to microclimate and/or biological control agents, may be occurring that has not been accounted for in these types of studies (e.g., Hoy et al. 1989). Thus more detailed studies on CL need to be done to better understand why and how various biocontrol tactics are working. The detailed survivorship analysis proposed in this study (Hogg and Nordheim 1983), along with the observations of larval location on the plant, will provide the field data necessary for answering these questions. A long-term commitment to this project is necessary to verify the timing and magnitude of the dynamic pest complex of cabbage and broccoli in Minnesota, to allow enough time to evaluate colonization and establishment of the new ICW parasitoid or other biological control agents. The baseline data collected to date (1991-1992) has provided a good foundation to build on.

VII. **Benefits:** Integrating a biological control program of *Bacillus thuringiensis* var. *kurstaki* (*Btk*) and new parasitoids for cabbage and broccoli production will promote the following:

a) Reduced use of conventional broad-spectrum insecticides; less potential for exposure to farm workers and non-target organisms such as wildlife and fish. Of critical importance to many Minnesota producers, is the desire to minimize the potential for insecticide residue on final product.

b) Reduced use of insecticides will allow natural 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) Reduced use of insecticides will permit the build-up of a newly introduced parasitoid (*C. rubecula*), recently established in the eastern U.S.

d) Proper timing of *Bt* applications, based on the biology of the ICW and infestation-damage relationships, will help encourage minimum use of *Bt* and thereby maximize net profits.

VIII. **Dissemination:** The primary thrust of the outreach component of this project will be the timely presentation of the results of this research at vegetable grower meetings and on-farm demonstrations of biological control projects. Educational meetings will be sponsored by the Minnesota Fruit & Vegetable Growers Assoc. and the Minnesota Extension Service, University of Minnesota. To

compli .nis effort, articles will be written for the MFVGA newslett. .iling list over 6,000). We also anticipate that this research will contribute significantly to our knowledge of the complexity of the cabbage agroecosystem and be published in scientific journals.

IX. **Time:** Project will begin in late-April of 1993, continuing with current LCMR (1991-1993) funding, and continue from July 1993 to June 1995 with LCMR funding.

X. **Cooperation:** Contacts have been made with Dr. Roy Van Driesche, University of Massachusetts, and Dr. Ed Grafius, Michigan State University, to supply two different strains of *C. rubecula* for release against ICW larvae. In addition, personnel at the Rosemount Agric. Experiment Station, University of Minnesota, and participating fresh market and organic-certified vegetable growers will be involved.

XI. **Reporting Requirements:** Semiannual status reports will be submitted not later than January 1, 1994, July 1, 1994, January 1, 1995 and a final status report by June 30, 1995.

XII. **Literature Cited: (See Detailed Work Program)**

**Qualifications:**

Dr. William D. Hutchison, **Principal Investigator**
Assistant Professor and 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. Dr. Hutchison has authored or co-authored over 20 scientific articles and book chapters on various aspects of integrated pest management of insect pests of economic importance. 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 and Midwest Food Processor meetings. During the 1991 growing season, he organized the first Vegetable Crop IPM Field Day for central Minnesota, using demonstration plots to demonstrate how *Btk*, Biological Control and other alternatives to insecticides can be implemented for vegetable insect pest management.



**OBJECTIVE 1.**

**A. Title of Objective:** Biological control of Gypsy Moth: Evaluation and Implementation.

**A.1. Activity:** Evaluate and implement anticipatory biological control of gypsy moth through introduction and establishment of parasites *Coccygomimus disparis* and *Compsilura concinnata* on susceptible native defoliators, tussock moth, tent caterpillar, and fall webworm. Mass rearing will be done in the lab on European corn borer as host insect.

**A.1.a. Context within the Project:** This project will demonstrate the feasibility of anticipatory biological control of gypsy moth and will promote public awareness of the application and benefits of biological control against other native tree pests.

**A.1.b. Methods:** The procedures for introduction, recovery, and monitoring of field release sites during the fiscal years1990-1992 will continue in FY 93-95. Release sites are chosen that have infestations of native hosts, particularly tent caterpillars (*Malacosoma* spp.), whitemarked tussock moth (*Orgyia leucostigma*), and fall webworm (*Hyphantria cunea*). Preferred sites are those in state parks with oak stands on the eastern edge of Minnesota. Other sites of importance are areas near interstate highways, such as rest stops or the edges of groves or forests.

The tachinid fly *Compsilura concinnata* will be obtained for release from a USDA lab in the eastern US. We will attempt to rear it in the lab on whitemarked tussock moth caterpillars. These can be reared on a combination of the ECB diet, and on corn leaves. Procedures for rearing, release, and monitoring will be similar to those of *C. disparis*.

Introductions. *C. disparis* adults are conditioned to target host artifacts before release. These include silk, frass, chewed leaves, and moth remains. These host artifacts are obtained from field collected or lab reared caterpillars. Releases are made directly onto the trees with the target host.

**A.1.c. Materials:** Our laboratory rearing facility at Rosemount includes the following: 2 Vollrath walk-in growth chambers for rearing parasitized host material; a Percival growth chamber for rearing European corn borer larvae; 2 refrigerators for storing cocoons and parasites; several screen and glass emergence and exposure cages for parasites; screen cages for rearing ECB; plastic diet dishes for rearing ECB larvae parasitized ECB or other cocoons; supplies for making the artificial (meridic) diet, cardboard rings for ECB pupation, and other materials for watering and feeding adult ECB and parasites; and hardware cloth containers for setting out sentinels in the field.

**A.1.d. Budget:** $30,000 **Balance:** $-0-

**A.1.e. Timeline:**

| | 7/93 | 1/94 | 6/94 | 1/95 | 6/95 |
|---|---|---|---|---|---|
| Lab rearing | ---------- | | | | |
| Field releases | ----- | | ------ | | -------- |
| Evaluation | ------- | | -------- | | ------ |
| Reports | | --- | --- | --- | ---- |

**A.1.f. Status:** Final Status Report, September 1, 1995. Progress and Results: In the Fall of 1991, the MDA-PPD took over the gypsy moth parasite colony of *Coccygomimus disparis* from Dr. Willis Schaupp, Department of Entomology, University of Minnesota. From 1989 to 1991 he began the gypsy moth, anticipatory biological control project by studying native, multi-host parasites, and began rearing and release of the ichneumonid wasp C. *disparis*. Production of *disparis* continued in our lab at the Dakota County Technical College, Rosemount, and since June, 1994, at our new lab space near the MDA building in St. Paul. The number of release sites has been increased, and the use of *disparis* has been expanded for controlling local outbreaks of whitemarked tussock moth and tent caterpillars. We have confirmed the establishment of *C. disparis* and the tachinid fly *Compsilura concinnata* by rearing out host caterpillars and cocoons collected in the field. In 1994 we obtained permission to release *Ooencyrtus kuvanae*, an encyrtid wasp parasitic on gypsy moth eggs. *Ooencyrtus* was reared in the lab in whitemarked tussock moth eggs. Nearly 3,000 adults were released between June and the end of September in 11 sites in 5 counties. All but two were adjacent to areas where gypsy moth was trapped earlier in the season.

In all releases of *C. disparis*, the target host was whitemarked tussock moth, tent caterpillars, or fall webworm. In urban areas, tussock moth infested areas were targeted, to control tussock moth and to establish gypsy moth parasites. In rural areas and in state parks, tent caterpillars were targeted in early summer; fall webworm, late summer. In addition to these releases, we supplied wasps to the USDA-ARS in Delaware to start up their *disparis* colony, and to a researcher in Utah developing biological insecticides from parasitic wasp venoms. This past April, a forest pest researcher from Chile toured our lab to observe our rearing procedures for *disparis* to use in rearing a parasite of European pine shoot moth.

Since 1989 about 9000 *C. disparis* females have been released in Minnesota. From 1992 to August, 1995, nearly 4,000 *Coccygomimus disparis* females (and over 1,500 M) were released in 15 counties in over 30 sites:1992—500 females [F] in 7 sites , 6 counties; 1993—1,100 F (and about 800 males [M]) in 14 sites, 9 counties, including 5 state parks; 1994—1,300 F, 500 M in 18 sites, 7 counties, including 4 state parks; 1995 (to date)—700 F, 200 M in 12 sites, 10 counties. Details of these releases are listed in the table following the text.

We have recovered *Coccygomimus disparis* from field-collected whitemarked tussock moth cocoons and eastern tent caterpillar cocoons each of the past three years. It appears that *disparis* can overwinter and is now established in Minnesota. The overwintering host is probably fall webworm which is present August through September. The tachinid fly*Compsilura concinnata* , a gypsy moth caterpillar parasite, has also been recovered each of the past three seasons. This fly was released in Minnesota in 1937, 1971-1977, and 1983 released in Minnesota. The fly is commonly recovered from mid-summer tussock moth cocoons.

Publicity of our biological efforts against gypsy moth continue at every opportunity. 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 1992 at Baltimore, MD, and the ESA North Central Branch meetings March,1995, at Lexington, KY. In March, 1993, it was part of the Plant Protection Division's display for the MDA open house. Articles have periodically appeared in the Minnesota Pest Report telling reporting our release and recovery activities. There were also articles in the MDA publication Overstorey (May-June, 1992, Vol. 7[1]), a DNR Newsletter, July, 1992, and last month in the Chanhassan Villager. June, 1994, Minnesota Public Radio featured a 10 minute story on our releases of biological control agents to control tree pests. We have distributed information and reports on our rearing and release activities to DNR personnel at state parks, the city foresters of Apple Valley, Minnetonka, and Hutchinson, MN DOT, Agriculture Canada, and to the Gypsy Moth Ad Hoc Meetings. We have also presented several displays of biological control agents including *disparis* at the St. Paul Arts and Sciences Museum, the North Central Forestry Conference in Rochester, a meeting of the Minnesota Master Gardener's, the National Association of Science Teachers convention in Minneapolis, the Manitou Days Environmental Fair in White Bear Lake, an environmental fair at Champlin High School, and several grade school talks with displays in the TC Metropolitan Area. Voucher specimens of recovered and lab reared *disparis*, *Compsilura concinnata* , and *Ooencyrtus kuvanae* have been accessioned into the University of Minnesota Insect Collection in the Entomology Department.

Summary of releases by the MDA, 1992-1995. The following lists the number *Coccygomimus disparis* ( #F/#M= # female / estimated # male) and *Ooencyrtus kuvanae* (in parentheses) released in different sites by county. Counties in the greater Twin Cities Metropolitan Area are grouped together.

| **County**: Site | 1992 | 1993 | 1994 | (*Ooencyrtus*) | 1995 |
|---|---|---|---|---|---|
| (Greater Twin Cities area counties) | | | | | |
| **Carver:** | | | | | |
| Chanhassan-UM Arboretum | | | | | 100/5 |
| **Chisago:** | | | | | |
| Interstate St. Pk. | 70/25 | 100/100 | | | |
| **Dakota**: | | | | | |
| Apple Valley | | | 220/100 | (425) | 75/25 |

| | | | | | |
|---|---|---|---|---|---|
| ...isville | | | 125/50 | (125) | |
| Eagan | | | | | 75/25 |
| Lakeville | | | 50/10 | (275) | |
| Rosemount | | 10/5 | | | |
| | | | | | |
| **Hennepin:** | | | | | |
| Edina | | | 30/10 | (550) | |
| Ft. Snelling St. Pk. | 16/12 | | | | |
| Maple Grove | | | 12/5 | (25) | |
| Minneapolis | 80/20 | 130/60 | 185/85 | (655) | |
| Minnetonka | | 150/110 | 25/5 | (325) | |
| | | | | | |
| **Ramsey:** | | | | | |
| Maplewood | | 100/100 | 210/100 | (450) | |
| Roseville | 85/25 | 35/32 | 75/30 | | |
| St. Paul | | | 150/75 | | 75/40 |
| | | | | | |
| **Washington:** | | | | | |
| Afton St. Pk. | | 80/20 | 35/10 | | |
| I-94 Infm. Center | | 100/100 | | | 100/5 |
| Woodbury | | | 25/10 | (100) | |
| | | | | | |
| (Outstate counties) | | | | | |
| **Dodge:** W. Concord | | | | | 50/20 |
| **Douglas:** Alexandria | | | | | 50/10 |
| **Goodhue:** | | | | | |
| Frontenac St. Pk. | 90/10 | 100/100 | 120/50 | | |
| St. Hwy. #56 | | | | | 55/35 |
| **Istanti**: Melon Patch Herbs | | | | | 25/20 |
| **McCleod:** Hutchinson | | | 65/40 | (15) | |
| **Ottertail**: Richville | | | | | 50/10 |
| **Rice:** Little Chicago | | 0/20 | | | |
| **Steele**: Hope Exit I-35 | 15/5 | | | | |
| **Winona**: Kipp St. Pk. | | 160/80 | 75/25 | | |

V. **Evaluation:** Introductions are monitored for effectiveness and establishment by rearing cocoons either collected or exposed ("sentinels") at the site. To recover *C. concinnata*, a larval parasite, host larvae and cocoons will be collected from release sites and reared in the lab. Attempts at recovery are repeated at release sites for at least 3 years. Effectiveness, or percent parasitism, can be estimated by sampling and rearing host cocoons from release sites. A trained taxonomist can identify parasites that emerge, or remains of those that die in the host cocoon. Counts from recovery samples are made of the following: *C. disparis*, Tachinidae (Diptera), *Compsilura concinnata* (Tachinidae), Pteromalidae (Hymenoptera), other parasitic Hymenoptera, dead from unknown causes, and moths. Parasites reared are either used to enhance lab stock, are released, or retained as voucher specimens. Release and recovery data will be entered into an LCMR report file directly from data sheets. If a technician has made identifications, these will be confirmed by a qualified taxonomist.

For the FY 93-95 biennium the project will be successful if recoveries of *C. disparis* and *C. concinnata* are made from sentinels or collected host cocoons from release sites 2 seasons following a release. Host cocoons should include any of the following: fall webworm, tent caterpillars, or whitemarked tussock moth. Long term success will be measured by persistent recovery of the parasites from regions around release sites infested with any of the above defoliators. Additionally, the percent of parasitism should add substantially to that of the most efficient native parasites.

VI. **Context within field:** Gypsy moth is the most destructive hardwood defoliator in North America. As the gypsy moth migrates westward, newly infested areas characteristically incur the highest levels of tree mortality and the lowest levels of parasitism. Thus it is important to establish gypsy moth parasites prior to a general infestation (Schaupp, 1991). From 1905 to 1960 a USDA parasite introduction program resulted in the establishment of 10 parasites of gypsy moth eggs, larvae, and pupae, and one predaceous beetle, all from western Europe (USDA, 1981). Among the parasitic wasps and tachinid flies was *Compsilura concinnata*. From 1963-77 17 new gypsy moth parasites were imported and released by several state departments of agriculture and the USDA for establishment. Most of these species are found in Asia in areas native to gypsy moth and other *Lymantria* species. Among these species was *Coccygomimus disparis*.

*Compsilura concinnata* was released in Minnesota in the 1970's by the USDA APHIS, and in 1983 by the MDA. Specimens were recovered at 2 different sites by Schaupp in 1989. During 1989-1991 *Coccygomimus disparis* was released at 5 sites in Minnesota as part of an LCMR biological control program against gypsy moth. In 1990 a recovery was made at one of the sites. Over 5,000 *C. disparis* and several hundred *C. concinnata* have been released in Minnesota. The pupal parasite *C. disparis* has been recovered from tussock moth, tent caterpillar, and fall webworm cocoons. *C. concinnata* has been recovered from fall webworm cocoons.

Difficulties in establishing introduced parasites involve overwintering conditions and hosts, suitable alternate hosts before and after suitable gypsy moth stages are available, longevity, univoltinism, and host searching ability at low host density (Gupta, 1983; Sabrosky & Reardon, 1976; Fuester et al., 1983, 1988; Hoy, 1976). *Coccygomimus disparis* and *Compsilura concinnata* are promising parasites of gypsy moth because they are multi-host (polyphagous), multivoltine, long lived, they can survive Minnesota winters in alternate hosts, and they are available for rearing (Schaupp, 1991, 1992). We have taken over W. Schaupp's colony of *C. disparis* and are mass rearing it on pupae of both ECB and greater wax moth.

VII. **Benefits:** Continued releases and monitoring of these gypsy moth parasites are needed to complement ongoing gypsy moth detection and treatment programs. Enhancement of gypsy moth parasites on other native hosts will act as reservoirs should gypsy moth infest Minnesota. It will augment USDA-APHIS gypsy moth projects. This project will demonstrate the feasibility of anticipatory biological control by establishing biological control agents on alternate, susceptible pest species before infestation by the gypsy moth occurs.

Successful establishment of effective gypsy moth parasites would eliminate the use of chemical insecticides Dimilin (diflubenzuron), Sevin 4-oil (carbaryl), Orthene (acephate), and Dylox (trichlorfon). It would also reduce the use of bactericides Dipel, Foray, Thuricide, and Condor (*Bacillus thuringiensis* var. *kurstaki*) which affect other nontarget species.

VIII. **Dissemination:** News releases and radio programs have been generated locally about releases of *C. disparis* against infestations of whitemarked tussock moth in public areas. We have put on displays of this and other biological control projects at the St. Paul Arts and Science Museum. W. Schaupp presented a poster on his accomplishments at the national meetings of the Entomological Society of America. Several pages of his work have appeared in the federal publication *ARS Research.* Gypsy moth parasites can be used in the context of school talks on insects. Seminars at the Department of Entomology at the University of Minnesota, St. Paul, have and will continue to provide a forum of accomplishments and findings of gypsy moth biological control. Publications in professional journals have and will continue to summarize research and release findings. Parasites from rearings of gypsy moth parasites have and continue to make valuable contributions of voucher and collection material to the University of Minnesota Insect Collection in the Department of Entomology. Material in this collection is accessible to researchers throughout the world. We see that there are ample opportunities to present to the public and the scientific community what we have already accomplished, what we plan to do, and how we are supported: radio, newspapers, newsletters, public displays in schools and science centers, university seminars, publications in scientific journals, and additions of specimens to museum insect collections. In addition, we are able to share rearing information and exchange reared material with other state and federal agencies involved with gypsy moth parasites.

IX. **Time:** Two years, plus the need for continued monitoring for another two.

X. **Cooperation:** The Department of Natural Resources and Minnesota Department of Transportation have permitted release of the parasites in sites they oversee. We use library and some lab facilities of the UM Department of Entomology. USDA APHIS is an important source of new parasite material for our colonies as well as rearing and release information about *C. disparis* and *C. concinnata.*

XI. **Reporting Requirements:** Semiannual status reports will be submitted not later than January 1, 1994, July 1, 1994, January 1, 1995, and a final status report by June 30, 1995.

XII. **Literature Cited: (See Detailed Work Program)**

**Qualifications:**

Dr. John C. Luhman, **Principal Investigator**
Biological Control Scientist
Plant Protection Division, Minnesota Department of Agriculture

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

Dr. Luhman has been involved with parasitic Hymenoptera systematics and biological control for 12 years. His biological control education and experience were acquired at the Department of Entomology, University of California, Riverside. His background includes four years in extension entomology in the Department of Entomology, University of Minnesota, St. Paul, and over 20 of general insect systematics. He taught and managed the insect museum collection in the Department of Entomology, Pennsylvania State University. Dr. Luhman is a biological control scientist in the Plant Protection Division, Minnesota Department of Agriculture. He is also an Adjunct Assistant Professor in the Department of Entomology, University of Minnesota, St. Paul. He will manage lab and field operations necessary for parasite production, release and recovery, data collection, analyses and evaluation. He will be assisting with all biological control implementation programs.

**OBJEC  16:**

**A. Title of Objective:** Enhanced natural control of filth flies through winter augmentation and selective importation

**A.1. Activity:** Evaluate effects of pre-release conditioning on survival and timing of spring emergence by stingless wasps following winter introduction into unheated animal confinement facilities.

**A.1.a. Context within the Project:** House fly, *Musca domestica* (L.), and stable fly, *Stomoxys calcitrans* (L.) cause substantial annoyance around domestic animal housing and human refuse dumps. Current control methods rely on insecticides and sanitation, neither of which provide satisfactory control. Progress toward implementing biological control has been substantial. Field surveys at Rosemount and St. Paul revealed that seven species of native wasps are too scarce in spring to control spring-summer breeding flies (Cervenka and Moon, 1990). One solution is to introduce mass reared wasps as eggs or larvae during winter to augment spring populations. Field experiments demonstrated winter survival of one kind of wasp, *Muscidifurax zaraptor* (Kogan & Legner), was greater inside than outside animal confinement buildings (Moon, 1990). A field-scale project now in progress at nine commercial horse stables is evaluating the viability of winter augmentation. Results thus far indicate some wasps released weekly between November '91 and April '92 survived to emerge in spring '92. However, some mortality was also evident. The next question is whether winter survival can be increased by pre-release conditioning and placement during winter. If so, winter augmentation might be made efficient enough for commercial application.

**A.1.b. Methods:** Two experiments are proposed. The first one will measure developmental rates and survival of preadult stages of *M. zaraptor* over a range of rearing temperatures. Results will be used to plan an optimal winter release program, and to schedule activities in a second, main experiment. We will produce cohorts of wasp eggs, and then rear them at 7 different temperatures from 8 to 32mC. At 2-5 day intervals, depending on temperature, we will then retrieve sequentially subsamples of 200-400 puparia from each cohort and dissect them to assess the survival and developmental progress of the wasps. The entire design will be replicated twice in a randomized blocks design.

The second, main experiment will simultaneously test different methods for conditioning wasp larvae prior to winter release, and different methods for releasing the wasps into occupied, unheated horse stables. Our goals are to find rearing and release methods that will time field emergence after release to coincide with onset of fly reproduction, and that will keep wasp mortality to a minimum.

**A.1.c. Materials:** For both experiments, we will produce cohorts of wasp eggs by exposing 1-day old house fly pupae in a cage of ovipositing *M. zaraptor*. The wasps will be free of *Nosema sp.*, and will have been in mass culture for less than 3 years. For the first experim  we will divide resulting eggs (inside puparia) into lots of 500  ch (=experimental units) and house them in moist sand packed into ventilated containers. Retrieved puparia will be dissected with aid of Wilde stereoscopes with fiber optic illuminators. Logistic regressions will be done with GLIM on the University's VAX, using a logistic link function and binomial or poisson errors. Least-squares regression, non-linear estimations, and MANOVAs will be done using SYSTAT. Residuals will be checked by graphical inspection to check assumptions.

**A.1.d. Budget:** $58,000 **Balance:** $-0-

**A.1.e. Timeline:**

| | 7/93 | 1/94 | 6/94 | 1/95 | 7/95 |
|---|---|---|---|---|---|
| Insect rearing | ---------- | | | | |
| Dissections | ------- | --------- | | --------- | |
| Data entry and analysis | ---- | --------- | | --------- | |
| Progress reports | | ---- | ---- | ---- | ---- |

**A.1.f. Status:** Final Status Report, September 1, 1995. Field and lab work for this objective was completed in May, and data are being analyzed. Indications are that more preconditioned than non-preconditioned 4th instar larvae of *M. zaraptor*, released during winter in unheated barns, survived winter exposure and emerged in spring. Preconditioning was by storage at temperatures near freezing for 2 wks before release. Younger larvae failed to survive exposure, regardless of preconditioning. These results show that this beneficial insect, if preconditioned by storage at near freezing temperatures, can be released as late instar larvae in unheated barns during winter in an effort to increase natural biological control the following spring.

**A.2. Activity:** Identify sites in the Old World (Europe and Asia) for foreign exploration for natural enemies of introduced dung breeding flies.

**A.2.a. Context within the Project:** In Minnesota pastures, face flies and horn flies are major sources of irritation to dairy and beef cattle. The flies breed only in cattle dung pats. Field studies of insects in Minnesota dung pats (Cervenka and Moon, 1991; Light and Moon, 1991) indicate beneficial insects now present are able to find and kill native dung breeding flies, but not the two pest flies, both of which were accidentally introduced from Europe. Given their foreign origin, theory predicts that natural enemies in the pests' native range will be better than Minnesota species at finding and killing the introduced pests. The time is right to seek enemies in the pests' native range, and the proposed activity will produce a plan for subsequent foreign exploration. Candidates for selective introduction should occur exclusively in cattle dung pats, be known to attack face fly or horn fly, and occur in areas of the world that are climatically similar to Minnesota.

**A.2.b. Methods:** This activity will synthesize literature and museum records on parasitic natural enemies known to attack face fly and horn fly or close relatives, and yield a map indicating regions of the Old World that are similar climatically to Minnesota. These

products will serve to formulate a plan for foreign exploration to seek and study candidates for future importation.

Regions of the Old World that are climatically similar to Minnesota will be identified through initial reference to published climate atlases (e.g., Heinrich,1975), and more detailed analyses from a biological perspective will be done using the computer climate matching program CLIMEX (Sutherst and Maywald, 1985).

**A.2.c. Materials:** The University of Minnesota library, and affiliated lenders, will be the prime sources of literature, and we anticipate that available collections will be adequate. Host-parasite records will be managed with database and linked mapping software. CLIMEX will be obtained from colleagues in CSIRO, Australia.

**A.2.d. Budget:** $5,000 **Balance:** $-0-

**A.2.e. Timeline:**

| | 7/93 | 1/94 | 6/94 | 1/95 | 6/95 |
|---|---|---|---|---|---|
| Literature review | ---------- | | -------- | | |
| Correspondence | ---------- | | ---------- | | |
| Progress reports | | ---- | ---- | ---- | ---- |

**A.2.f. Status:** Final Status Report, September 1, 1995. We completed a literature review of the geographic distributions of dung-breeding flies in central Asia. We also completed a review of the climatic similarity between Minnesota and central Asia. Results indicate there is a large area in SE Russia and NW Kazakhstan that is likely to contain beneficial insects that will kill our face fly and horn fly, and that are likely to be climatically suited to establish after release in Minnesota.

**V. Evaluation:** Results of activity 1 will be evaluated in a scientific sense by the abilities of the survival and development models to predict overwintering success and timing of spring emergence by *M. zaraptor* in selected field locations. Further, the feasibility of winter augmentation will be judged from the extent of overwintering success of the released parasites. In a practical sense, success will be judged ultimately from the degree of producer adoption of the winter release approach to filth fly control. Results of activity 2 will be evaluated from the extent of guidance obtained for foreign exploration in the Old World, *i.e.*, whether or not specific areas of the Old World can be identified as having a high probability of containing parasites suitable for further study prior to future importation.

**VI. Context within field:** At present, there is considerable public interest in using biological control to manage filth flies around animal confinement facilities, as evidenced by public inquires and advertisements for "fly predators" in trade magazines. Vendors are marketing parasites to be released in an augmentative approach during summer months. However, there have been repeated failures in many parts of North America (CA, KS, NE, and NY) to demonstrate success with this approach. The winter augmentation strategy being developed in Activity 1 is designed to overcome the ecological and logistical factors that may be limiting the conventional summer augmentation approach. Activity 2 is a logical extension of recent research that has confirmed native natural enemies of face fly and horn fly are inadequate, leading to development of a plan for subsequent foreign exploration.

**VII. Benefits:** Successful augmentation with stingless wasps for house fly and stable fly control will simultaneously improve the quality of life by reducing fly annoyance to people, improve the comfort of livestock, and reduce use of organophosphate and pyrethroid insecticides around confined domestic livestock and people. If suitable natural enemies of face fly and horn fly can be located, then concrete plans can be made to obtain living material for further study in quarantine. Assuming new species are established in the pasture environment, then irritation of pastured cattle will be permanently reduced without insecticides.

**VIII. Dissemination:** Results will be presented for peer review in publications, and they will be incorporated into existing extension programs on livestock pest management. The climatematching portion of Activity 2 will be shared with other colleagues working on biological control in the north central region.

**IX. Time:** The proposed activities will not require additional funding beyond one biennium.

**X. Cooperation:** Cooperators for the Activity 1 are commercial boarding and riding stables in Dakota and Scott Counties. These enterprises are now contributing access and space for release of parasites and evaluation of results, and managers are helping us to collect field data on their respective premises. All have pledged continued support in the future. Cooperators for the second activity are parasite taxonomists in the USA (Drs. P. Marsh and E. Grissell), Canada (Dr. A. Campbell) and UK (Dr. I. Boucek). The authors of CLIMEX (Dr. R. Sutherst and G. Maywald) will make CLIMEX available and counsel us on installation and use of the program.

**XI. Reporting Requirements:** Semiannual status reports will be submitted not later than January 1, 1994, July 1, 1994, January 1, 1995 and a final status report by June 30, 1995.

**XII. References cited: (See Detailed Work Program)**

**Qualifications:**

Dr. Roger D. Moon, **Principal Investigator**
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 17 years research and teaching experience in entomology, with major emphasis in biological control and population dynamics of filth breeding flies. Current projects involve biological control of filth flies of animal confinement facilities, and natural control of dung breeding flies associated with pastured cattle. He teaches Livestock Entomology, and Sampling Biological Populations, and contributes to the team-taught courses in Veterinary Parasitology. Medical

Entomo and Insects and Society. His role in the proposed projects , to design the experiments, direct the activities of an Assistant Scientist (Valerie Cervenka), and to analyze the resulting data and write progress reports.



**OBJECTIVE 1**

**A. Title of Objective:** Integrating biological control of arthropods into commercial greenhouse production.

**A.1. Activity:** *Delphastus pusillus* - evaluation as a predator of the greenhouse whitefly (*Trialeurodes vaporariorum*) on fuchsia and tomato under production greenhouse conditions. *Encarsia formosa* - evaluation as a biological control agent for the greenhouse whitefly (*T. vaporariorum*) in commercially-grown alstromeria crops. **New Crop Biological Control Establishment** - implementing and establishing biological control as the principal source of integrated pest management for roses in newly constructed commercial greenhouses.

**A.1.a. Context within the Project:** *Delphastus pusillus* - *Delphastus pusillus* has proven to be an effective predator of *T. vaporariorum* in small scale, controlled greenhouse studies. However, little work has been done on the effectiveness of *D. pusillus* in uncontrolled, "natural" greenhouse settings. This work will aid in determining if *D. pusillus* is a viable control option for commercial greenhouse operators.

The focus of this activity will be on introducing and establishing 2 predacious mite species (*Neoseiulus cucumeris* and *N. barkeri*) to control thrips, and combining the efforts of lady beetles and a small parasitoid (*Aphidius matricariae*) to control aphids on roses. We will also evaluate reduction in chemical use and control costs, versus costs of biological control agent establishment to determine economical feasibility.

If found to be efficacious, these biological control agents can be incorporated into existing IPM programs for commercially grown greenhouse crops where production is the primary concern.

**A.1.b. Methods:** *Delphastus pusillus* - *Delphastus pusillus* will be evaluated on two crops, fuchsia and tomato. Each crop will be heavily infested with *T. vaporariorum*. Treatments will consist of one time, *D. pusillus* releases at rates of two and four beetles per plant, and an untreated check. Four replicates, consisting of 10 plants each will be utilized for each treatment.

ANOVA will be performed to determine if significant differences exist among the 3 treatments regarding *T. vaporariorum* numbers. *Delphastus pusillus* numbers will also be determined.

**New Crop Biological Control Establishment** - thirty-six rows of roses (18,000 plants) in two, newly constructed houses will be utilized. One yellow sticky trap will be placed in each row one week before agent release to determine initial thrips and aphid populations. In addition, leaves from several plants within each row will be selected and thrips and aphid populations will be counted.

**A.1.c. Materials:** *Delphastus pusillus* - approximately 120 plants of each crop (fuchsia and tomato) will be used. *Delphastus pusillus* numbers needed will be approximately 480. In addition, 24 yellow, sticky traps will be utilized on a weekly basis. *Encarsia formosa* - materials will consist of yellow sticky traps and *E. formosa* in numbers to be determined at a later date. **New Crop Biological Control Establishment** - materials will consist of 1,080,000 *N. cucumeris*/*N. barkeri*, 2000 *A. matricariae* and lady beetles as needed. Yellow sticky traps will be utilized weekly to monitor populations.

**A.1.d. Budget:** $54,000 **Balance:** $-0-

**A.1.e. Timeline:**

| | 7/93 | 1/94 | 6/94 | 1/95 | 6/95 |
|---|---|---|---|---|---|
| Predator/Parasitoid Introductions | ---------- | | | | |
| Evaluate Predator/Parasitoid Success | ---------- | | | | |
| Evaluate Data | ------- | | ------- | | ------ |
| Training/Presentations | ---- | | ---- | ---- | ---- |
| Reports | | ---- | ---- | ---- | ---- |

**A.1.f. Status:** Final Status Report, September 1, 1995. Pesticide residue sensitivity studies for *Delphastus pusillus* are finished. Data analysis is nearly complete and reports for publication are being prepared. Data shows strong indications of differences among various common greenhouse pesticides with respect to their effects on *D. pusillus*. Greenhouse growers will thus become more aware of not only the direct effects of pesticides on biological control agents, but also that pesticide residues are important as well when considering their use in biological control programs. This information has already been disseminated to several greenhouse growers when questions arise regarding biological control agent introductions.

The study regarding delivery methods for the thrips predator *Neoseiulus cucumeris* are also complete. Data is being analyzed and reports for publication being prepared. The data is suggesting differences in each mite delivery method and this should aid the biological supply industry in providing proper release information for this mite.

Successful introduction of biological control agents into commercial greenhouses has proved difficult. The grower's inability to accept in full, all adjustments that must be made in their pest management programs has hampered progress. However, this knowledge, combined with the successful research we have completed, will aid in making growers more aware of the changes and commitment that are necessary when implementing biological control programs.

**V. Evaluation:** In all studies, either positive or negative results would be considered successful. The biological control agents in these experiments will be tested under commercial circumstances which require that the crop condition and sale be the primary concern. If the biological control agents perform well and keep pest populations low, grower confidence in using them as permanent control solutions for greenhouse pest problems will increase. Negative results will produce documented evidence that will increase the knowledge base for biological control establishment and aid in future studies.

**VI. Context within field:** Biological control in greenhouses is receiving increased attention in the U.S. This project has the unique aspect of evaluating biological control agents under commercial circumstances as compared with many studies that approximate commercial greenhouse conditions.

**VII. Benefits:** Successful establishment of biological control agents would greatly reduce pesticide use. This would, in turn, aid in limiting insecticide resistance of greenhouse pests as well as promote better conditions for greenhouse workers. If agents are unsuccessful, this work will help to better determine constraints in the use of biological control agents under commercial conditions.

**VIII. Dissemination:** Results, as they become available, will be summarized and presented to professional greenhouse operators at several Minnesota Extension Service conferences including the Commercial Flower Growers Short Course and the Annual Bedding Plant Conferences. Research results will also be reported at annual meetings of the Entomological Society of America. Fact sheets will be produced and disseminated as needed.

**IX. Time:** Indications of success should be available in the two year span of the project.

**X. Cooperation:** Len Busch Roses, Plymouth, Minnesota, will be the primary cooperator. They will provide greenhouse space, maintain proper cultural conditions, and aid in monitoring pest and biological control agent populations.

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

**Qualifications**

Dr. Mark E. Ascerno, **Principal Investigator**
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

commer[illegible]perators. Dr. Ascerno is also Head of the Department of E[illegible]ogy and Director of the Dial U Insect and Plant Information Service at the University of Minnesota.

**Publications: (See Detailed Work Program)**