1997 Project Abstract
For the Period Ending June 30, 1999
This project was supported by the Environment and Natural Resources Trust Fund

Title: **Crop Management to Minimize Pesticide Inputs in Minnesota**
Project Manager: Linda L. Kinkel
Organization: University of Minnesota
Department of Plant Pathology
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1991 Upper Buford Circle
Saint Paul, MN 55108

Legal Citation: ML 1997 (Chap. 216), Sec (15) subd. (7b). CROP MANAGEMENT TO MINIMIZE PESTICIDE INPUTS 300,000
This appropriation is from the trsut fund to the University of Minnesota to develop non-pesticide management strategies for pest control crops.

Appropriation Amount: $300,000

Statement of Objectives: The objectives of this research were to investigate the utility of novel, non-pesticideal management strategies, including organic mulching systems, crop rotation, green manures, animal grazing, and manipulation of local non-crop vegetation, in providing significant pest control for diverse crops throughout Minnesota. Target pests included weeds, soil-borne microbial plant pathogens, nematodes, and leaf-spotting fungi. Research was conducted on potato, strawberry, wild rice, and soybean.

Overall Project Results: In total, results from this work have yielded substantial insight into the development of non-pesticidal management strategies for significant agricultural pests. While the organic mulching approaches investigated here proved to have significant detrimental effects on strawberry plants, the prospects for managing diseases of wild rice via manipulation of dike vegetation appear to be quite good. Further, at least 3 different plant species, when planted as green manure crops, show strong potential for providing an enhancement to natural soil suppression via the enrichment of indigenous pathogen antagonists. The ability of this enrichment to translate to effective disease control is currently under investigation. Finally, screening of over 2000 bacterial isolates yielded no bacteria that were consistently capable of inhibiting soybean cyst nematodes.

Project Results Use and Dissemination: Project results have been disseminated via talks at national scientific meetings, grower meetings, and through the scientific and technical literature. Results are being used in the development of practical, non-pesticidal pest management strategies, and as a basis for obtaining additional funding support from commodity groups, private groups, and state and federal agencies.

July 1 1999

LCMR Final Work Program Update Report

I. Project Title: Crop Management to Minimize Pesticide Inputs in Minnesota Proposal C-2

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Total Biennial Project Budget:

$LCMR	$300,000
- $LCMR amount spent	$300,000
$LCMR Balance	$0

A. Legal Citation: ML 1997, (Chap. 216), Sec. (15), subd. (7b). CROP MANAGEMENT TO MINIMIZE PESTICIDE INPUTS 300,000

This appropriation is from the trust fund to the University of Minnesota to develop non-pesticide management strategies for pest control for crops.

II. Project Summary and Results

The utility of novel, non-pesticidal management strategies, including organic mulching systems, crop rotation, green manures, animal grazing, and manipulation of local non-crop vegetation, in providing significant pest control will be determined for diverse crops throughout Minnesota. Target pests include weeds, soil-borne microbial plant pathogens, nematodes, and leaf-spotting fungi. Research will be conducted on potato, soybean, wild rice, and strawberry, crops important throughout Minnesota. The proposed studies are non-inoculative, in that they do not rely upon introduction of microbial agents into the environment to achieve pest control. Rather, this work relies primarily upon cultural manipulation of the cropping system to provide control of plant pests. Results from this work will provide the basis for controlling significant plant pests on Minnesota crops with minimal use of pesticides.

III. Progress Summary.

A. Potato

Result 1: Development of cultural strategies for maintaining or enhancing the naturally-occurring disease suppressiveness of Minnesota soils. Soil samples from a series of different crop rotation plots have been collected for processing to determine the differences in microbial antagonist populations and disease suppressiveness under different planting histories. Twelve different plant species (alfalfa, oats, barley, soybean, sudangrass, corn, green beans, buckwheat, mustard, fallow, canola and rye) were planted into suppressive and conducive soils in the greenhouse (10 pots per plant species) in two separate experiments. Soil samples were collected from each pot prior to planting. Plants were maintained for 12 weeks, at which time the second set of soil samples was collected from each pot and all aboveground plant matter was incorporated into the soil. Soil was kept moist for an additional 6 weeks, at which time the final soil sample was collected. Soil samples were assayed for total microbial populations, and for antagonist population densities. Three of the plant species showed the ability to significantly enhance pathogen-suppressive populations in the soil after 12 weeks. Additional analyses quantified the total antibiotic activity of pathogen-suppressive populations in all of the soil samples. Effects of green manure crops on population densities of antibiotic-producing organisms are still under investigation; studies will be completed by September 1999.

Result 2: Identification of cultural methods for enhancing populations of pathogen antagonists in the soil. Field plots at the Sand Plain Experiment Station (Becker, MN) were established to study the influence of small grains, potatoes, green beans, sweet corn, and fallow on antagonist populations in the field. Soil samples were collected and assayed as described above in all plots at planting and at harvest. Aboveground biomass was removed from all plots, as standard for agricultural production with these crops, and plots were subsequently split into 4 different green manure treatments and planted with sorghum (sudangrass), oats, buckwheat, or fallow in late August. Aboveground biomass of all crops overwintered on the plots, and was incorporated into the soil the following spring prior to planting. Additional soil samples were collected prior to planting, and the entire plot was planted to potatoes in spring, 1999. Disease levels (potato scab) on potato will be evaluated at harvest in fall, 1999 to determine the correlation between antagonist population levels determined in soil assays and disease severity. These results will be critical to determining the influence of green manure crops on disease in the field.

B. Strawberry

Result 1: Develop recommendations for the use of corn gluten meal (CGM) as a natural herbicide for strawberry production systems in Minnesota. Field plots using varying rates of corn gluten meal, with and without incorporation and with and without fertilizer, were established during the 1997 and 1998 growing seasons. Weed control was poor in CGM plots compared to the hand weeded controls. Yield obtained from the 1997 planting in 1998 showed greatly reduced production on all

CGM plots compared to the hand weeded controls. Strawberry plant stand was also greatly reduced in CGM plots. Based upon these results, CGM is not recommended for further use as a natural herbicide on strawberries.

Result 2: Determine the potential of recycled paper sludge in reducing weed seed germination in the establishment of strawberry plantings. Three greenhouse studies on the use of recycled paper sludge have been performed. Paper sludge has been mixed at four different rates (0, 10, 20, or 30 ton/acre) with field soil, and strawberry plants or sweet corn or tomato seed were planted into each mixture. Each treatment was also evaluated with and without fertilizer. Weed counts and plant growth parameters were measured in each experiment. In all three experiments, paper sludge reduced both weed number and growth. However, the paper sludge also reduced the plant growth and development rate of corn, tomato, and strawberry plants. The addition of fertilizer reduced the weed control effects of the paper sludge. Data suggest that the paper sludge binds up nitrogen in the soil thus reducing all forms of plant growth and development. Additional aspects of this research may be pursued with Superior Paper Company.

Results 3: Determine the potential of using angora goats for weed control and renovation in a strawberry management system. Field plots have been established, and angora goats were used to weed and renovate a 3-year old strawberry variety planting. The goats grazed equally on the strawberry plants and on the weeds, causing significant damage to the strawberry plants. yields collected during the 1998 season following the 1997 grazing season showed a significant reduction in plant stand, plant vigor, and total fruit yield on the goat treated plots compared to conventionally treated plots. Based on these results, use of angora goats for renovation and weed control in established strawberries is not recommended.

C. Wild Rice

Result 1: Determination of the primary overwintering location for major pathogens of wild rice. The disease formerly called FBS is two different diseases now called FBS (*B. oryzae*) and SB (*B. sorokiniana*). Each causal organism survives in a different environment, infects wild rice at different stages of plant development, and causes different severity levels of disease.

Bipolaris oryzae is an excellent pathogen but a poor saprophyte that survives poorly, primarily in two general sites, a) infested residue that remains dry or relatively dry from growing season to season and b) grasses, primarily reed canary grass and timothy, that grow on field dikes. *Bipolaris sorokiniana* is an excellent saprophyte but a poor pathogen that survives primarily in several grasses on field dikes. *Bipolaris oryzae* infects reed canary grass, a wet-site plant, when the grass is stressed by dry conditions. However, the fungus infects timothy, a dry site plant, when it is stressed by wet conditions. *Bipolaris sorokiniana* infects several different grasses under a wide range of conditions.

Previously, the large number of leaf spots on the floating leaves of rice were thought to be caused by *B. oryzae*. My research indicates these leaf spots are caused by another fungus called *Nakatea sigmoidea*, the causal organism of stem canker. Stem canker can become a serious disease under some circumstances. Circumstantial evidence suggests the incidence and severity of this disease becomes greater on floating leaves that are mechanically thinned during the spring.

Scab or Fusarium head blight has been found to be a common disease of both cultivated and natural wild rice. Furthermore, it was found that several *Fusarium* spp. are implicated in the cause of the disease. Some fungi had not been reported on small grains before. Mycotoxins were discovered in the fungus cultures but not in the grain itself. The yields of cultivated wild rice can be considerably reduced by the disease. However, it was found that most infected kernels will shatter and not be harvested. The causal organisms are rarely found on processed grain in the grocery store.

Other previously reported diseases caused by bacteria and other fungi such as *Colletotrichum* sp. and *Phoma* sp. may be a larger factor in the disease syndrome than previously thought and account for yield loss. Phoma leaf spot has been discovered as a previously unreported disease of cultivated wild rice. The *Phoma* sp. overwinters profusely on reed canary grass and other grasses such as timothy commonly found on dikes. Additionally Phoma leaf spot appears early in the physiologic development of the plant and may account for yield loss to disease.

Bipolaris spp. are seedborne on cultivated wild rice with the fungi found primarily in the external tissues and rarely in the caryopses. The most common site of *Bipolaris* infection is the awn. Seedborne *Bipolaris* spp. is related to incidence of fungal brown spot and spot blotch. However, seedborne *Bipolaris* spp. are not a factor in perpetuating disease as fungi do not survive when seed is immersed in water.

Result 2: Reduction in primary inoculum arising from overwintering sites through cultural methods. *Bipolaris oryzae* does not infect wild rice until the boot stage of plant development. Disease then becomes severe, a factor possibly related to plant stress caused by draining water from field at this time and lack of plant nutrients, especially nitrogen. Disease then progresses rapidly until maturity when plants may be killed. About 50% of lesions on the flag leaves at maturity are caused by *B. oryzae*. Conversely, *B. sorokiniana* infects wild rice beginning at the floating leaf stage of plant development. Disease progresses more slowly and is not as severe as that caused by *B. oryzae*. Preliminary results indicate that *B. oryzae* is airborne but not a large distance from it's overwintering site. Therefore, most inoculum that causes disease is likely of local origin.

Result 3: Reduced yield losses from disease by the reduction in overwintering inoculum. Fungicide applications were sometimes found to be ineffectual because they were applied before plants were infected by *B. oryzae*, that is at the boot stage or later. I suggest, that fungicides are more effective when applied later in the growing season.

Soil fertility, particularly nitrogen, is related to the incidence and severity of fungal brown spot and spot blotch. This interaction of soil fertility, fungicide use and disease incidence and severity will be studied further in cooperation with Dr. Paul Bloom.

Preliminary evidence suggests disease severity can be lessened without the use of fungicides through proper plant nutrition and delaying draining of water from fields. Finally, it appears that most diseases of wild rice may be managed by a combination of 1) water management such as flooding to destroy inoculum in infested residue and delayed draining to minimize plant stress, 2) proper fertility, especially nitrogen, 3) the effect of other diseases, such as *Phoma* leaf spot, *Colletotrichum* leaf spot and stem canker caused by *Nakatea sigmoidea* and how they may be managed is not well understood at this time.

D. Soybean

Result 1: Determination of the potential for naturally-occurring soil bacteria associated with different crop plant species to interfere with the soybean cyst nematode life cycle. Roots with rhizosphere soil of soybean, corn, alfalfa, oats, wheat, sorghum, barley, potato, hairy vetch, rye, sugar beet, flax, and squash were collected from fields in Waseca and Lamberton during the growing season in 1998. In addition, soil samples collected from counties across southern Minnesota in 1997 were used to grow soybean, corn, and oats in the growth room. A total of 84 soil samples were used to grow soybean, 48 soil samples were used to grow corn, and 48 soil samples were used to grow oats. Bacteria were isolated from the roots and rhizosphere soil by plating bacterial suspension from the roots and rhizosphere soil on three culture media. Spore-forming bacteria were also isolated by heating the bacterial suspension and plating it on the culture media. A total of 1,722 bacterial isolates were isolated and purified.

In vitro test for effect of the bacteria on hatching of SCN: The isolated bacteria were first cultured on a solid medium for 1 day and then incubated in sterile water on a shaker of 1 day. The filtrates of the bacterial culture in the water were used in the test. Soybean cyst nematode eggs were collected from soybean roots in a field. The eggs were stored in an antibiotic solution in a refrigerator until used. The nematode eggs were incubated in the bacterial filtrates and percentage of eggs hatched was determined after an incubation period of 14 days. The preliminary screening test was finished in September 1998. Some of the bacteria induced hatching and some inhibited hatching. One hundred isolates that induced hatching in the preliminary screening test were selected and tested again with more replicates to assure the results. The repeating test will be completed by the end of 1998.

In vitro test for nematicidal effect on juveniles: Same preparations of culture filtrates for the above hatching test were used in this study. The nematode second-stage juveniles hatched within 1 day were used. About 50 juveniles were placed in each well for 24-well tissue culture plates contained bacterial filtrates. Viability of the juveniles after incubation for 24 and 48 hours were determined. Two hundred isolates that showed some level of toxic activity were screened from the 1,772 isolates. Repeated testing of the 200 isolates suggests that toxic activity may not be consistent among trials. Further investigation of the factors influencing toxic activities of bacteria towards nematodes are under investigation.

IV. Outline of Project Results.

In total, the result of this research will be the development of enhanced strategies for *drainage of water from fields can all contribute to reduction in yield losses due to plant disease. In particular, water management (flooding) can be used to destroy inoculum in infested residue, and delayed draining will also minimize plant stress (indirectly enhancing plant vigor).*

D. Soybean

Result 1: Determination of the potential for naturally-occurring soil bacteria associated with different crop plant species to interfere with the soybean cyst nematode life cycle. Budget: $100,000.

Despite an aggressive screening of more than 2000 strains, bacteria showing strong promise for field control of soybean cyst nematode have not been isolated. Research (funded by other groups) continues on this objective.

V. Dissemination

Results from this work will be disseminated through a variety of sources, including, but not limited to: presentations to grower's groups throughout the state, presentations at grower field days, publication in grower's newletters, publication in newletters of the MN Agricultural Experiment Stations (e.g. North Central Experiment Station Newsletter), publication in scientific journals, and presentation at scientific conferences.

VI. Context

A. *Significance:* Plant pests exact a significant economic and environmental toll to the state of Minnesota. Economically, they cost in terms of lost revenues due to reduced crop yields and as a consequence of the need for often expensive pesticidal inputs. Environmentally, pesticides represent a significant concern to consumers both on food and in the environment, and the need to destroy plant disease inoculum can force farmers to implement intensive tillage strategies that contribute to the loss of the valuable soil resource. These problems are common to all regions and crops in the state of Minnesota. At the present time, pest management relies extensively on two major strategies: host plant resistance, and pesticide use. Unfortunately, disease resistance is often not an option for many plant pests, and pesticide use is less than ideal for the obvious reasons. We propose to quantify the potential for a series of novel non-pesticidal and non-resistance-based crop management strategies to provide pest control in a diverse collection of crops throughout the state of Minnesota. Recent studies on the microbiology of the soil and the influence of tillage practices on disease development suggests substantial promise for reducing pest pressure by manipulation of the soil environment and surrounding non-crop vegetation through relatively simple, low-cost management practices. Our work will provide the basis for the development of effective and low-cost strategies for minimizing plant pests in a number of the major and important crops grown throughout Minnesota (soybean, wild rice, strawberry, and potato). Our group represents agriculture throughout the state, and our collaboration offers a unique opportunity to investigate parallel questions in these very diverse cropping systems.

B. Time. The proposed research will be largely completed within the budgeted time period. However, additional data from field plots established with the LCMR money is likely to be collected following the completion of the budget period. Additionally, further grant support will be sought from other sources (in particular from programs funded by the U.S.D.A.) to permit further investigation of issues related to the proposed work.

C. Budget Context 1992-1996:

a. LCMR Budget History: $0

b. Non-LCMR Budget History: None specifically related to these objectives; however, over $500,000 in funding on related work to reduce the impacts of disease on these crops has been obtained from the USDA Competitive Grants Program, Minnesota AURI, the Wild Rice Grower's Council, the UM Parker-Sanders Research Fund, the NorthStar Research Foundation Innovation Fund, the Area II Potato Growers, and the Minnesota Berry Grower's Association. Additionally, as noted above, further research support is being actively sought from outside sources (especially the U.S.D.A.) to permit extension of the research beyond the 1999 biennium.

	July 95-June 97	July 97-June 99	July 99-June 01
LCMR	**$0**	**$300,000**	**?**
Other State	**$0**	**$0**	
Non-State Match	**$0**	**$0**	
Match In Kind	**$0**	**$0**	
TOTAL	**$0**	**$300,000**	**?**

Personnel	**$205,910[a]**
Equipment	**$26,000[b]**
Acquisition	**$0**
Development	**$0**
Other (Supplies, Travel, and Publication costs)	**$68,090**
Total	**$300,000**

[a]Personnel budget will support the complete salaries for two post-doctoral scientists (with a possible alternative of one post-doctoral scientist and one M.S.-level technician), as well as for both seasonal and year-round technical and undergraduate student labor support.

VII. Cooperation

Dr. Senyu Chen
University of Minnesota
Southern Experiment Station
Waseca, MN 56093
LCMR: $0 salary dollars
55% time to LCMR project effort

Dr. Robert Nyvall
University of Minnesota
North Central Experiment Station
Grand Rapids, Minnesota 55744
LCMR $0 salary dollars
50% time to LCMR project effort

Dr. David Wildung
University of Minnesota
North Central Experiment Station
Grand Rapids, Minnesota 55744
LCMR $0 salary dollars
35% time to LCMR project effort

VII. Location

Work on this proposal will take place in areas: A, P, V, Q, I, F, N, G, J

IX. Reporting Requirements: Periodic workprogram progress reports will be submitted not later than November 1, 1997; March 15, 1998, and November 1, 1998. A final workprogram report and associated products will be submitted by June 30, 1999, or by the completion date as set in the appropriation.

1. *Potatoes (Linda Kinkel)*

I. Abstract. Soil contains many naturally-occurring pathogen antagonists that can have a significant influence on plant health and yield. These soil-borne microbial populations are significantly influenced by crop management strategies, especially crop rotational sequence and debris management. However, there is very limited information on the use of such management strategies for manipulation antagonist populations with the goal of reducing plant disease. Additionally, the influence of crop management on the ability of a soil to suppress plant disease is not well understood. The goals of this project are to investigate the manner in which crop plant species and debris management can influence disease suppressiveness and population densities of naturally-occurring pathogen antagonists in the soil.

II. Background. The soil supports a tremendous diversity of micro-organisms, including plant pathogens, pathogen antagonists, plant symbionts, and saprophytes. These microbes can significantly influence plant health and the yield of agricultural crops. Of particular interest to plant pathologists are the naturally-occurring pathogen antagonists. This group has been shown to have the potential to significantly reduce levels of plant disease through resource competition, production of antibiotics lethal to pathogens, and parasitism or predation of pathogens in soil. In addition, indigenous pathogen antagonists appear to be critical to the functioning of naturally-occurring disease suppressive soils in a number of different systems.

In northern Minnesota, a soil naturally suppressive to the potato scab pathogen *Streptomyces scabies* developed over about 25 years of continuous potato production. This soil has been shown to have significantly greater densities (colony-forming units per gram of soil) of antibiotic-producing antagonists (especially non-pathogenic *Streptomyces* strains) of the pathogen than nearby non-disease suppressive soils. Soil densities of antibiotic-producing *Streptomyces* strains and the development of natural disease suppressiveness both appear to be a function of the specific crop management strategies, including crop rotation sequence and debris management, employed. However, there have been few attempts to rigorously investigate how crop management specifically influences either the buildup of antagonistic *Streptomyces* strains or the enhancement or maintenance of the disease suppressiveness of a soil. Such information is necessary for the development of environmentally sound and pesticide-free strategies for minimizing soil-borne plant diseases.

III. Methodology

A. Development of cultural strategies for maintaining or enhancing the naturally-occurring disease suppressiveness of Minnesota soils. Four different crop rotation series will be initiated in a randomized complete block design on a naturally-occurring disease suppressive soil located at the North Central Experiment Station at Grand Rapids, MN (Figure 1). Potatoes were grown throughout the suppressive soil plot in 1996. In 1997, crop series initiated for 1997 and 1998 are proposed to be: potato-potato; alfalfa-potato; corn-potato; and small grain-potato. Plots within each block will be separated by a 5-foot bare earth buffer maintained by cultivation and/or herbicides. Blocks will be separated from one another by a 10-foot bare earth buffer maintained as described above. Crops will be maintained using standard agronomic practices, to the extent possible in small-scale field plots. Soil samples will be taken from each plot prior to planting and at harvest in 1997, and prior to planting at at harvest in 1998. Microbial populations in the soil will be studied in objective B, below. Potato scab disease levels (percent tuber surface area infected and number of lesions per tuber) will be quantified in all plots just after tuber initiation (June) and at harvest (1998) in the second year of the study. Comparison of the disease levels in plots exposed to different crop rotational sequences will provide information on the effects of the rotation crops on the scab suppressiveness of the soil. Further studies on the effects of the rotation crops on the microbial community within the suppressive soil (below) will provide insight into the mechanisms by which crop rotations may alter disease suppressiveness.

B. Identification of cultural methods for enhancing populations of pathogen antagonists in the soil. The effects of cultural methods and cropping systems on pathogen antagonists in the soil may be investigated in 3 different systems. First, as described above, soil samples from the different field plot rotation schemes will be collected to permit an investigation of potential shifts in the density of pathogen antagonists in response to different crops. Because soil samples will be collected both at harvest, and subsequently at planting in the next growing season, the potential for specific rotation crops to influence antagonist populations both as a function of root growth or following breakdown of crop debris in the soil will be determined. Details of soil sample processing are described below.

In the second approach, we will collect disease conducive soil from the North Central Experiment Station at Grand Rapids. The conducive soil is indistinguishable from the suppressive soil with respect to standard soil test variables, including nitrogen, phosphorus, potassium, pH, and organic matter. Because this soil has the potential to become `suppressive', as a function of significantly enriched populations of pathogen antagonists, it presents a good system for investigating how cultural methods can influence the rate or intensity at which antagonists may be enriched. A variety of different crop plants, including corn, soybean, alfalfa, sugarbeet, small grains (wheat, rye, oats), peas, and one or more crucifer crops, will be planted into conducive soil in the greenhouse. Plants will be grown either for a short season and incorporated into the soil as a green manure (treatment 1), or will be grown to maturity. For those crops grown to maturity, plants will be `harvested' in a manner analogous to standard agronomic practices, and above-ground debris will either be removed or incorporated into the soil (treatments 2 & 3). Controls will be non-inoculated soil. For each crop, soil samples (see details of processing, below) will be collected from each treatment immediately after harvest or incorporation of the inoculum, and again following an overwintering period.

As a final approach, in a related study rotation plots have been initiated on a disease conducive soil at the University of Minnesota Sand Plain Experiment Station at Becker, MN. These plots are being used to evaluate the manner in which an inoculated microbial biocontrol agent can be integrated with crop rotation to maximize control of potato scab. The design of the Becker plots will also permit investigation of the manner in which the crop rotation sequence influences the buildup of naturally-occurring antagonists in a disease conducive soil (in contrast to the shifts in the antagonist population in an already suppressive soil, as described in the first approach). Rotation sequences (1995-1996-1997-1998) within this study are: potato-potato-potato-potato; potato-potato-soybean-potato; potato-potato-corn-potato; and potato-potato-alfalfa-potato. Crops will be harvested at the end of the 1997 growing season using standard agronomic practices as far as possible. Soil samples will be collected from each plot at harvest in 1997 and prior to planting in 1998, and will be assayed as described below.

Antagonists against specific soil-borne plant pathogens in soil samples collected above will be quantified using an Andersen air spore sampler. Specifically, petri plates with 2% water agar will be spread with specific plant pathogens (primarily soil-borne pathogens, including *Streptomyces scabies*, *Verticillium dahliae*, *V. albo-atrum*, *Fusarium* spp., *Phytophthora* spp., or *Pythium* spp.). Immediately afterwards, a soil sample (approximately 0.2 g per sample) will be placed on the top layer of a 6-stage Andersen air spore sampler, and the petri plates that have been pre-inoculated with the pathogen will be placed on stage 6 of the sampler. Air will be pulled through the soil sample for about 20 seconds. The air will distribute microbes and small soil particles (up to 1 micron in diameter) over the surface of the pre-inoculated petri plate at 200 discrete locations. Petri plates will be incubated for 5-7 days, at which time the density of pathogen antagonists in the soil will be estimated based upon the presence of zones of inhibition of the pre-inoculated pathogens on the plates.

IV. Results and Products. Though the work will proceed in two distinct phases, the results from these objectives should be viewed in combination. In total, this work will provide specific information on the manner in which crop management strategies can influence the natural disease suppressiveness of a Minnesota disease suppressive soil, and can be used to enhance population densities of indigenous pathogen antagonists in Minnesota soils. Based upon this work, products may include recommended management strategies that rely upon specific crop rotation sequences, or suggestions for debris management or use of green manure crops. Subsequent research will focus on the efficacy of these recommendations for reducing levels of soil-borne disease in the absence of pesticide inputs.

V. Timetable

Summer, 1997: Initial evaluation of effects of rotation crops on soil suppressiveness.
Fall-Spring 1998: Evaluation of effects of crop plant and debris management on populations of soil-borne antagonists.
Summer, 1998: Further evaluation of effects of rotation crops on soil

suppressiveness.
Fall-Spring 1999: Quantification of the effects of crop plant species and debris management on population dynamics of specific antagonist populations in the soil.

VI. Budget

Personnel: $68,500
Other (Supplies, Travel, and Publication Costs): $30,500
TOTAL: $99,000

V. CV: See attached pages.

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2. Strawberries (David Wildung)

I. Abstract. Weed control is a major limiting factor in strawberry production. In recent years, strawberry herbicide choices have been reduced and there is a need for alternative weed control systems. This project will explore three potential alternative weed control systems for strawberries: 1) use of corn gluten meal, 2) use of recycled paper sludge, and 3) differential grazing with angora goats.

II. Background. There are about 1500 acres of commercial strawberries produced in Minnesota with a trend toward increased acreage. Nearly all of this acreage is marketed as a pick-your-own product and supports producers on both a full time and part-time basis. The most limiting factors for production in Minnesota are winter injury and weed control. in the past, weed control systems relied mostly on herbicide use. However, strawberry herbicide choices are becoming limited. Identifying potential weed control systems is highly desirable. Corn gluten meal, a waste by-product of ethanol production, has been shown to be an effective naturally source of nitrogen and to exhibit herbicidal characteristics by researchers at Iowa State University. Recycled paper sludge has been shown to reduce weed and crop seed germination in establishment studies of oats and alfalfa by agronomy researchers at the north Central Experiment Station (NCES), Grand Rapids, MN. Since strawberries are a transplanted crop, utilization of paper sludge could reduce early weed pressure on strawberry establishment. Angora goats have been utilized for several years in agronomic grazing research plots at NCES. In a preliminary grazing trial on strawberries in 1996, the goats differentially grazed the planting (initially eating weeds; later strawberry plants) and after four days renovated the planting. Based on this experience, it appears the goats could be used for rotational grazing for weeding during the growing season and for complete strawberry renovation when left for longer grazing periods.

III. Methodology

1. Develop recommendations for the use of corn gluten meal as a natural herbicide for strawberry production systems in Minnesota.
2. Determine the potential of recycled paper sludge in reducing weed seed germination in the establishment of strawberry plantings.
3. Determine the potential of using angora goats for weed control and

renovation in a strawberry management system.

Objective 1. Grower demonstration plantings and replicated rate studies will be conducted using protocol established by Dr. Gail Nonnecke from Iowa State University but modified to Minnesota conditions. Corn gluten meal will be compared to conventional growing systems. Grower members of the minnesota Berry Growers Association will be asked to participate in the demonstration plantings. Replicated rate studies will be conducted at NCES.

Objective 2: Greenhouse rate studies will be conducted to determine whether herbicidal activity exists and to determine if phytotoxic strawberry symptoms occur. Assuming these studies are successful replicated field studies will be conducted during strawberry establishment with paper sludge applied at planting and prior to mulching in the fall. Weed populations will be monitored and strawberry plant growth and production will be evaluated. While these studies are being conducted efforts will be made to identify any potential hazards or problems with application of recycled paper sludge to a food crop. All of these studies will be conducted at NCES.

Objective 3: Replicated studies will be conducted on an existing strawberry variety trial at NCES and will begin at renovation in July. Goats will be allowed to graze the planting until renovated and then periodically to weed the planting during the rest of the season. Grazing will be compared to conventional management. Since the planting contains several varieties it can be determined if grazing has potential and if varietal responses are similar.

IV. Results and Products. Positive results will provide several potential alternative weed control systems for strawberry growers. Data collected will include weed control ratings, weed type identification, strawberry plant growth (runner development, stand and vigor) and strawberry yield development (fruit yield, size, and earliness). Results will be presented at the Minnesota Fruit and Vegetable Convention, through their newsletter, the NCES Quarterly Newsletter, at grower field days and in appropriate scientific journals. Successful utilization of corn gluten meal would benefit the corn producers as an alternative product. Since much of the paper sludge is currently landfilled, successful utilization of this product would provide a good use for a product currently considered a waste product. For some producers, angora goats could not only provide mohair but also provide an organic alternative to strawberry production.

Comments regarding potential food safety concerns relating to applications of corn gluten meal or paper sludge: Researchers at Iowa State University have studied food safety concerns relating to corn gluten meal (CGM). The United States Environmental Protection Agency determined that CGM is of a character that it need not be regulated and will not pose unreasonable risks to public health or the environment; as such, it has been exempted from regulation under the FIFRA (Federal Insecticide, Fungicide, and Rodenticide Act) effective May 6, 1996.

Procedures followed in the proposed research will follow protocols established by researchers at Iowa State.

Prior to putting this project together, the Minnesota Pollution Control Agency was contacted about potential food safety concerns relating to paper sludge. To specifically address such concerns, collaborative efforts with MPCA personnel would be required since we lack the expertise to properly study this issue. The objective of the proposed work is to first evaluate under controlled conditions the potential for paper sludge to provide herbicidal activity at levels that are non-phytotoxic to strawberry. If paper sludge does not prove effective or economical for application as a herbicide, further work will not proceed. Because the food safety issue is costly to address, we will wait until evidence exists that paper sludge represents a potentially effective and economical approach for weed control in strawberry before aggressively pursuing food safety concerns in collaboration with MPCA, industry, or independent scientists.

V. Timetable

Summer 1997: Establishment studies on growers fields (Objective 1) and research plots (Objectives 1 & 2). Conventional renovation and grazing studies begin (Objective 3). Evaluate weed control and strawberry plant responses (all objectives).

Spring-Summer 1998: Evaluate yield responses and repeat all treatments at renovation (all objectives).

Spring-Fall 1999: Additional fruit yield data collected (all objectives).

VI. Budget

Personnel: $36,640
Other (Supplies, Travel, and Publication Costs): $4,860
TOTAL: $41,500

VII. CV: See attached pages.

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3. *Wild Rice (Robert Nyvall)*

I. Abstract. Cultivated wild rice is an important crop to growers in northern Minnesota. The plant diseases fungal brown spot and spot blotch reduce yields up 100 % in individual paddies. The present method of control is the prophylactic application of the fungicide propiconazol (Tilt) at $45.00/acre. It is proposed to research where the survival sites of the causal fungi are. The fungi are normally more vulnerable to management at these sites and less expensive agronomic practices may then be utilized to control destructive plant diseases. Satisfactory results will likely be obtained in 2 yrs and alternate control measures studied. The total cost to conduct this research for 2 yrs is $54,000. This will cover two thirds the cost of a full time laboratory technician and a part time summer help plus supplies, equipment and travel.

II. Background. Cultivated wild rice is grown on about 25,000 acres in Minnesota and is a major economic contributor to several northern Minnesota communities adding approximately $41.6 million to the state's economy. Approximately fifty Minnesota families earn most or a portion of their income from the growing of this crop.

A major contributor to the reduction of yields and the added cost of production is plant diseases. Plant diseases annually reduce yields from 10 % to 100% in individual fields. The most serious diseases are considered to be fungal brown spot caused by the fungus *Bipolaris oryzae* and spot blotch caused by *B. sorokiniana*. Preliminary research suggests spot blotch occurs early in the season and continues until harvest at a relatively uniform incidence. However, fungal brown spot does not occur until later in the growing season at approximately flowering time then increases rapidly in incidence and severity until harvest time. Plant health is affected by the destruction of photosynthetic leaf area and the production of the toxin ophiobilin by *B. oryzae*.

At present the only acceptable disease control is two applications of the fungicide propiconazole (Tilt) at the cost of $45.00 or more per acre resulting in a major production expense to the wild rice grower. The fungicide is applied as a prophylactic treatment on a chronological basis regardless of disease appearance.

An alternative disease management is proposed by which growers may be able to reduce the source of primary inoculum through agronomic practices. The source of primary inoculum is suspected to be the overwintering or survival site of the fungi and likely occurs somewhere in the vicinity of the wild rice paddy. If this site of survival or overwintering can be identified, the fungi may then be managed when they are most vulnerable to control at a lesser expense than application of expensive fungicides. At present, the overwintering or survival of these two fungi and their subsequent infection of cultivated wild rice is unknown or poorly understood. This research will focus on the sites of overwintering and survival of *B. oryzae* and *B. sorokiniana* and the reduction of primary inoculum from these sites.

III. Methodology. The research will involve two general phases. Each phase is representative of two theories of whether the fungus or fungi are disseminated to the cultivated wild rice or the wild rice grow through fungal inoculum that arises in place.

Phase 1. Dissemination of fungi to wild rice. Survey of grasses and vegetation on cultivated wild rice paddy dikes and subsequent determination of collateral hosts as a significant source of inoculum. A logical site for the overwintering of fungi is the grasses in the vicinity of the paddy. Preliminary work began this year with the systematic surveying of prevalent grasses at three different paddies, each paddy was located on a separate farm that was representative of three different cultivated wild rice growing areas. This work will continue for two more years to determine if any of the common dike grasses become infected. Determination of the presence of the fungus will be made utilizing special techniques to isolate each fungus. If a grass is determined to be a collateral host, research will be conducted to determine if the fungi can be disseminated in a large enough incidence to initiate an epiphytotic.

Phase 2. Wild rice growing through fungal inoculum. Examination of cultivated wild rice residue. Several plant diseases originate from primary inoculum on residue from the previous year. An agronomic practice of wild rice growers is to flood paddies either in the autumn or the spring. Preliminary research indicates that neither fungus survives in residue that is subjected to flooding. However, circumstantial evidence such as the uniform incidence of disease incidence across a paddy suggests that this may be the source of primary inoculum. Therefore, the manner in which residue responds to flooding, sporulation of fungi on the residue, and subsequent response of fungal spores will be studied. It is not known if small but uniform amounts of residue float on the water surface. When spores may be produced and how spores respond to flooding. Possible survival structures of spores will be studied and their survival during the winter. If inoculum is in place in the paddy, the wild rice plants may then grow through the inoculum in the paddy water and the aerial leaves become infected. Infected residue will be subjected to in situ conditions and sporulation and subsequent disposition of spores noted utilizing standard plant pathology methods and Chinn and Ledingham techniques. Wild rice plants will be grown in different residue regimes in water in greenhouse.

IV. Results and Products. This research is expected to identify the site or sites of the overwintering of *B. oryzae* and *B. sorokiniana*. Once the site(s) are known, research will commence on common agronomic methods to manage the disease and sufficiently reduce or eliminate the amount of fungicide utilized by growers. At this time the expected disease management practices will focus on what is determined to be the most important site(s) of overwintering. For example if grasses on the paddy dikes are found to be important in survival, mowing, burning, or reduced fungicide application directly to the dikes may be feasible. If residue is an important overwintering site, the management of residue will be studied. Factors such as burial of residue, reduction in the size of residue fragments, influence on time of paddy flooding, and vulnerability of fungus spores will be examined.

V. Timetable

It is expected that the sites of overwintering will be known within two years. Preliminary research has characterized the behavior of the diseases. As an example, it is known that fungal brown spot does not occur until late in the season then rapidly increases in incidence and severity. In contrast spot blotch is common early in the growing season but occurs at a constant low to moderate incidence and severity throughout the growing season. Preliminary research suggests that, initially, the diseases occur uniformly over a paddy. It is not known if this uniform distribution is due to dissemination of inoculum in the form of spores from collateral hosts or fungal inoculum somehow surviving within the paddy itself.

VI. Budget

Personnel: $39,200
Other (Supplies, Travel, and Publication Costs): $20,300
TOTAL: $59,500

VII. CV: See attached pages.

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4. Soybeans (Senyu Chen)

I. Abstract. The soybean cyst nematode is a major soybean yield suppressive factor in southern Minnesota. Using crop rotation and planting resistant cultivars are currently the only available tactics for management of the nematode. This study will explore natural biological factors that may be useful in an integrated management program. The results of the study will provide a better understanding of the effects of bacteria colonizing the rhizosphere of various crops common in southern Minnesota on the nematode life cycle and population development.

II. Background. The soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is economically the most important soilborne pathogen of soybean. The nematode has been in the southern United States since 1954. According to a recent survey, SCN caused an estimated annually loss of 48.6 million bushels ($267 million) during 1989-1991 in the north central region of the US. Since the first detection of the soybean cyst nematode in 1978 in Faribault county, the nematode has been reported in 32 counties in southern MN. Recognition of nematode damage by growers is problematic because in many cases the above-ground symptoms of nematode infection are often indistinct and non-specific. The infestation of SCN has had and will continue to have a significant impact on the soybean industry in MN.

Due to the high costs and environmental concerns associated with their use, nematicides are not a realistic alternative for SCN management. To date, the major tactics for management are crop rotation, planting resistant cultivars, or a combination of these tactics. These tactics, however, vary in efficacy. soybean yield losses caused by the nematode are directly related to the number of nematodes present at planting (initial population = Pi), and, in turn, Pi is dependent on the length of time since the previous suitable host as well as the survival rate of the nematode in the absence of a host. In southern regions of the US, planting 1-2 years of non-host crops may be sufficient to reduce the nematode population below the level causing significant crop damage. In the northern region, however, the nematode overwinter survival rates are generally higher, and the non-host crop rotation period needs to be longer to reduce nematode populations below the critical level. Even `resistant' soybean cultivars cannot yield successfully in fields with high nematode populations. In addition, a single resistant cultivar should not be used frequently in the same field because this can contribute to the development of new virulent races of SCN.

An integrated management strategy is necessary for successful control of SCN. Rotation of soybean with non-host crops and the use of SCN-resistant cultivars should represent one component of an effective management program. The purpose of this research is to explore natural biological factors that may be useful in an integrated management program. The focus of this study will be to determine the potential for naturally-occurring soil bacteria to interfere with the SCN life cycle.

Rhizobacteria which colonize roots in the presence of the indigenous soil microflora are important to keeping plants healthy. Some rhizobacteria provide the front-line defense for roots against attack by pathogens. One area of great possibilities for increasing plant yields and making dramatic change in agricultural practices involves manipulation of bacteria that protect plant roots from the many deleterious microbes, including nematodes, that occur in agricultural soils. Only limited research has been done of the effects of rhizobacteria on nematodes and little is known about the effects of these bacteria on the life cycle and population dynamics of SCN. Bacteria isolated from the root systems of some plants have been shown to have the ability to limit nematode penetration. Furthermore, it has been shown that some rhizobacteria, when applied to seeds or as root treatments, were able to reduce nematode infection in potato, cucumber, tomato, and sugarbeet. The potential for rhizobacteria from host or non-host plant species to influence the hatching, mortality, or host recognition ability of SCN has not been investigated. The proposed research on the rhizobacteria from diverse crop species will provide a better understanding of the potential for these microbes to effect the SCN life cycle and population dynamics in MN.

III. Research Methodology

Roots of soybean, corn, oats, peas, and alfalfa will be collected from fields at Waseca and Lamberton. In addition, soil samples will be collected from soybean fields in counties across southern Minnesota. A random collection of bacteria from roots and soil will be purified and stored for further evaluation as follows. Roots of plants will be cut into 1-cm pieces and placed in a 250 ml beaker containing 100 ml sterile 0.1 $MgSO_4$. The bacteria will be removed from roots by vigorous shaking for 3 h. Resulting bacterial suspensions will be plated in King's medium B (KMB), 10% tryptic soy agar (TSA), and potato dextrose agar (PDA) and incubated at 25 C. For isolation of spore-forming bacteria, a portion of each suspension will be heated to 90 C for 10 m. A minimum of 3 bacterial colonies will be randomly selected from each root on each medium, with the total collection estimated to be about 3,000 bacteria. Those bacterial strains that show promise in inhibiting SCN will be identified to species using Bergey's manual.

Bacterial strains will be grown on solid and subsequently in liquid media. Culture filtrates from each bacterial isolate will be evaluated in vitro for their effefcts on hatching of SCN eggs. Briefly, SCN eggs will be collected from soybean roots in the field. Eggs will be placed in wells of 24-well tissue culture plates. Bacterial filtrates will be added to the wells, with deionized water or $ZnCl_2$ as controls. After 14 d incubation at 24 C, percent egg hatch will be determined. Bacteria whose filtrates showed a significant negative effect on juvenile mortality or on the mobility of the juveniles will be further studied for their ability to inhibit recognition of soybean roots by SCN stage 2 juveniles.

Bacteria will also be evaluated for their nematicidal effects on SCN juveniles. Specifically, juvenile SCN will be hatched from eggs collected from soybean roots grown in the greenhouse. Juveniles will be incubated in 24-well tissue culture plates in the presence and in the absence of bacterial filtrates. Juvenile viability after incubation for 24 and 48 h will be determined for each bacterial filtrate solution.

All bacteria that influenced SCN egg hatching positively or negatively will be studied further in vivo greenhouse soil assays. Bacteria will be cultured in liquid medium, mixed 1:1 with 0.2% methyl cellulose, and used to coat soybean seeds prior to planting. Coated seeds will be planted in pots containing SCN-infested soil collected from the field. Non-treated seeds will serve as controls. Two weeks after planting the number of juveniles in soil and roots will be determined.

Bacteria that showed nematicidal effects on juveniles or that impair the abilities of juveniles to recognize soybean roots will also be studied further in in vivo greenhouse assays. Specifically, the impacts of these bacteria on penetration and reproduction of SCN on soybean when inoculated into soil will be quantified in greenhouse pot trials. Seeds will be coated with bacterial cultures as described above. Field soil will be mixed with sand in a 1:1 ratio, and placed in 10-cm diameter pots. Pots will be inoculated with SCN (10,000 eggs/100 cc soil), and planted with treated or nontreated (control) seeds. Soybean roots will be collected 1 wk after planting, and roots will be stained and examined microscopically to determine juvenile numbers. Additionally, roots will be collected after 40 d soybean growth, and numbers of SCN females on roots and plant growth will be quantified. That is, for all bacterial strains investigated, the effects of inoculated bacteria on plant height and weight will be quantified in the greenhouse assays. Finally, the ability of inoculated bacteria to colonize the soybean rhizosphere will be determined using spontaneous streptomycin resistant mutants of the bacteria.

IV. Results and Products. The result of this study will be a significantly enhanced understanding of the effects of common rhizosphere bacteria on the SCN life cycle and soybean growth. Naturally-occurring bacteria selected in this work will form the basis for further focused efforts on using cultural and inoculation strategies for controlling SCN.

V. Research Timetable

Summer and Fall, 1997: Soil and root samples will be collected from throughout southern MN.
Winter and Spring, 1997/1998: Isolation, screening, and in vitro studies of bacteria.
Spring 1998-Spring 1999: Greenhouse bioassay studies and identification of effective SCN-inhibiting bacteria.

VI. Budget

Personnel: $61,570
Equipment: $26,000 (see justification below)
<u>Other (Supplies, Travel, and Publication Costs): $12,430</u>
TOTAL: $100,000

VII. CV: See attached pages.