TITLE:W-147 Managing Plant-Microbe Interactions in Soil to Promote Sustainable Agriculture

PROJECT NUMBER: W-147 (Continuation) (REVISED 4/1/98) **OFFICIAL**

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DURATION: October 1, 1998-September 30, 2003.

STATEMENT OF THE PROBLEM:

The use of microbes to control plant disease and enhance crop production is desirable for the following reasons: 1) chemical pesticides are being severely restricted; 2) the public is demanding reduced pesticide use; 3) resistance to biocontrol organisms is unlikely to develop; 4) biocontrol organisms are selective in their mode of action;5) biocontrol organisms have little effect on other beneficial organisms; 6) biocontrol organisms are biodegradable; 7) biocontrol organisms pose little danger to humans or animals; and, 8) many biocontrol methods improve the soil and enhance the sustainability of agriculture. While a number of biocontrol agents are now available commercially, problems with production, storage, delivery, reliability, efficacy, establishment and understanding mechanisms of action have prevented most biocontrol products for plant disease control from becoming established in mainstream agriculture. We believe that tremendous progress has been made in the field of biological control of plant disease in the past five years and the W-147 project has been a major contributor to this progress. Because the results of our efforts are just coming to fruition , we feel it is prudent to continue the W-147 project for another five years in an effort to improve and further stimulate the use of biocontrol organisms in agriculture.

JUSTIFICATION:

Soilborne plant pathogens are responsible for many acute and chronic diseases of crop plants that can result in severe losses for growers. Economic losses to soilborne pathogens are estimated at 50-75% of the attainable yield for many crops. Yield failures resulting from acute diseases such as vascular wilts, take-all of cereals, *Phymatotrichum* root rot, *Verticillium* and *Phytophthora* may be even more severe and have destroyed entire agriculture industries. About 90% of the 2000 major diseases of the principle crops in the US are caused by soilborne plant pathogens (Lewis and Papavizas, 1991). It has been estimated that the monetary losses to soilborne diseases is in excess of $4 billion /year (Lumsden et al., 1995).

While many soilborne diseases have been controlled, in part, by use of chemical pesticides, alternatives to the use of chemicals would be of value. Plant associated microbes used as biocontrol agents can play a role in reducing losses to such diseases, thus assuring a more sustainable agriculture and the long term ability of our land to produce food. Even when we were able to use pesticides freely, soilborne diseases were often ineffectually controlled and resulted in crop losses of $4 billion/year. Because of the difficulty in accessing the soilborne organisms in their chemically and physically diverse soil habitat, these organisms are notoriously difficult to control using traditional methods. Soilborne diseases cause by *Phytophthora, Verticillium and Fusarium* remain major problems after more than 100 years of study. Soilborne pathogens are well adapted to soil conditions, and once established are very difficult to eliminate by any known method of

control. Chemical control, when available, is often too expensive to be economically practical.

Although breeding for plant resistance to soilborne diseases holds much promise, the broad host range and large genetic variability of many soilborne pathogens makes plant resistance an incomplete method for disease control.

Biological control ~~is~~ therefore one of the best options for management of soilborne diseases at a time when chemical alternatives are drastically being reduced (Baker and Cook, 1974; Cook, 1993; 1990; Cook and Baker, 1983; Jacobsen and Backman, 1993; Lewis and Papavizas, 1991; Weller, 1983; Whipps, 1992) for the following reasons. Biocontrol of soilborne pathogens are: 1) non-polluting and biodegradable, 2) relatively harmless to humans, 3) difficult for pathogens to develop resistance against, 4)selective in their mode of action, 5) unlikely to harm other beneficial microorganisms, and 6)contribute toward improved soil conditions and the sustainability of agriculture.

Great strides have been made recently in the field of biocontrol of soilborne plant pathogens. Progress and success has been documented in the review by Cook (1990). Much of this success is due to activities of the members of W-147 (Cook 1990, Becker and Schwinn, 1993, Schroth and Becker, 1990, Weller, 1983). Today the EPA lists more than 24 commercial biocontrol agents which are registered and commercially available in North America. Nearly all of them have been registered during the past five years. Interest and enthusiasm about biocontrol have never been greater. A recent analysis of articles published in 1996 in Phytopathology, the premier plant disease journal in the US, shows that nearly 20% of the articles dealt with biocontrol. It appears that the W-147 regional project is both very timely and successful.

However, there remains a great deal left to be done in the field of biocontrol. Despite the promise, popular concept and public acceptance of biocontrol, few of the commercial biocontrol products are being used successfully in mainstream agriculture (Becker and Schwinn, 1993, Jacobsen and Backman, 1993, Whipps, 1992). Many researchers believe we have yet to find the best biocontrol agents, since they may inhabit remote areas where some of our soilborne pathogens arose. There are still major problems with cost, production, quality control, storage, stability, delivery , establishment, compatibility with agricultural practices, reliability, efficacy, slow effectivity, narrow host range and understanding of the life cycles and modes of action of biocontrol agents (Becker and Schwinn, 1993; Jacobsen and Backman; Lewis and Papavizas, 1991; Whipps, 1992). Powell (1991) summarizes the current status of plant biocontrol agents when he says," the real problem for biological control is to deliver an active agent to the site where it is required and keep it there while activity is required." We are yet unable to do that efficiently with most of our current biocontrol agents. Clearly there is much to be done in order to improve biocontrol agents so that they will become major factors in the control of soilborne diseases. Biocontrol agents isolated by participants of W-147 at ARS, CA-B, CA-D, CA-R, CO, ID, WA, IL, NM, MT, AK and NY have the ability to suppress a wide variety of plant pathogens that cause serious diseases of food, fiber and ornamental crops. The need for "high quality" biocontrol agents has never been more critical because of the pending loss of fungicides and fumigants upon which agriculture has been dependent for the last 50 years. Consider the billion-dollar-a-year commercial strawberry industry in California which relies exclusively on soil fumigation with a combination of methyl

bromide and chloropicrin at about 250 lb/acre for disease, insect and weed control. The mandated 75% reduction in the use of methyl bromide by 2001 will leave this industry vulnerable to soilborne pests and pathogens.

Biocontrol may with great input from researchers provide a safe, environmentally sound alternative to methyl bromide and other valuable agricultural chemicals which may be lost in the future. The solutions to these problems require a large research input. No one person or single entity is capable of solving these problems. Much of the necessary research will require the interactive and cooperative efforts of a team. Cooperation between and among states, agencies, disciplines and basic and applied scientists is required. It is therefore logical to continue and encourage the successful interactions (which are documented in the PROCEDURES section) of the W-147 regional project for another five years.

RELATED CURRENT AND PREVIOUS WORK:

There are a number of reviews on the use of biocontrol organisms to control soilborne plant pathogens (Baker and Cook, 1974; Becker and Schwinn, 1993; Cook, 1993; 1990; Cook and Baker, 1983; Jacobsen and Backman, 1993; Lewis and Papavizas, 1991; Powell, 1991; Weller, 1988; Whipps, 1992). All of these reviews agree that there is great potential for the use of biocontrol agents to control soilborne diseases, but that this potential has not yet been realized and the bulk of commercial agriculture does not yet use biocontrol agents for the control of soilborne diseases.

The EPA currently lists more than 24 biocontrol agents which are registered and available for commercial use. A list of 44 biocontrol agents which have reduced disease are listed by Cook and Baker (1983). However, few of these biocontrol agents are currently being used in mainstream agriculture. As a result most biocontrol workers are searching for new and better biocontrol agents. Most of these efforts involve isolating and selecting biocontrol agents on growth media and testing in the laboratory or the greenhouse. Few are looking for biocontrol agents in foreign lands where pathogens may have evolved or isolating slow growing or non-cultivable biocontrol agents using the pathogen as bait (Lewis and Papavizas,1991). With recent estimates of more than 100,000 species of fungi, many of which have not yet been named, we have barely scratched the surface in our hunt for biocontrol agents.

Unless we understand the mechanisms of disease control used by biocontrol agents, we cannot hope to efficiently control soilborne diseases. Mechanisms by which biocontrol organisms reduce disease include: 1) producing plant growth enhancing chemicals, 2) inducing resistance mechanisms in plants, 3) competition for nutrients or space, 4) antibiotic and toxin production, 5) siderophore production, 6) biosurfactant production. and 7) mycoparasitism (Jacobsen and Backman,1993). Several of these mechanisms were identified only recently by members of the W-147 project (Pierson, 1997; Raaijmakers and Weller, 1998; Thomashow and Weller, 1988; Stanghellini and Miller, 1997; Wood. et al, 1997), which indicates there are probably many more mechanisms waiting to be discovered. Furthermore, although the mechanisms are known for some biocontrol agents, these agents do not control disease efficiently. This suggests that we do not yet understand the effects of nutrients, environment and growth stage on the control mechanisms. For instance Pierson and Pierson, (1996) have recently shown that

environmental factors and other organisms will regulate the amount of phenazine antibiotic produced by the biocontrol bacterium, *Pseudomonas aureofaciens*. This means that it is not enough to understand that phenazine production is the mechanism for biological control, but we must understand when, where and under what conditions this antibiotic is produced. These examples serve to illustrate the point that every biocontrol agent-plant pathogen-host crop system requires special insight on how best to utilize the biocontrol agent to maximize disease control. This maximization of biocontrol will also be different for different regions of the United States. Biocontrol of very few diseases can be done efficiently, and therefore much research is needed to understand the mechanisms involved in biocontrol.

Finally, biocontrol organisms must be commercially produced, formulated and applied under field conditions before efficacy can be demonstrated and the agricultural industries accept biocontrol as a major tool for reducing disease. For most biocontrol organisms, this has not been accomplished. Indeed, commercial development of biocontrol agents for disease control has lagged far behind that which has occurred in entomology for the control of insects. Commercial formulations and delivery systems for biocontrol agents have been reviewed by Lewis and Papavizas (1991). However, Cook, (1990) cautioned against this rigid approach and recommended that field utilization of biocontrol agents could be placed into three broad categories: 1) Maximizing the use of naturally occurring biocontrol through cropping sequences, mulching, composting and other cultural practices. Crop systems management for biocontrol of soilborne disease may be the most practical and successful method of biocontrol and could lead to the discovery of more biocontrol methods. Cook (1990) included the utilization of naturally occurring disease suppressive soils in this category. 2) The introduction of well-adapted or rhizosphere competent biocontrol agents via the methods described by Lewis and Papavizas (1991) with the hope that they would survive and reproduce. With the introductive approach, amendments, additives, habitat modifications, or partial sterilization of the soil may enhance the establishment of the biocontrol agent. This method has been the one most commonly attempted, but mostly with seed pathogens, seedlings or crops grown in potting media. It has yet to be practiced widely under field conditions with a wide variety of crops. 3) Inundative applications of biocontrol agents which could be applied many times every season, and which would function much like microbial pesticides. This technique is virtually untested, but it has many advantages since it does not require that the organisms establish or survive and they can be applied in large numbers in a condition when they are most effective. The technology to attempt this type of biocontrol has only become available in the last few years. Regardless of the methods used to produce, formulate and apply biocontrol agents, few have proved successful in a large scale. Improved methods and more research is required under field conditions if biocontrol is to be widely used on a commercial scale.

In summary, research related to the objectives outlined above is in progress all over the world. This indicates that the objectives are valid and timely, and that the potential for success is great. In spite of this effort biological control agents have not resulted in great changes in agricultural methods or strategies. While similar strategies between W-147 and investigations on a worldwide scale are certainly being explored, the unique contribution of this project rests in its regional characteristics and unique cooperative efforts between researchers with similar research goals.

A comparison of the objectives of the regional projects NC-125 and S- 269 which are most closely related to the objectives proposed by W-147 is presented in Table 1.

Table 1. Comparison of Regional Projects whose objectives relate to biocontrol of plant pathogens in soil

W-147	NC-125	S-269
1. To identify and characterize plant microbe interactions that provide suppression of disease caused by soilborne pathogens.	1. Identify and develop microorganisms for intentional application as biocontrol agents of soilborne plant pathogens.	1. Selection and optimization of biological control agents, and evaluation of seed treatment and other application techniques, to enhance the biological control of diseases caused by soilborne plant pathogens.
2. To understand how environmental factors regulate microbial populations and the expression of genes responsible for disease control.	2. Manage the indigenous microflora to suppress diseases caused by soilborne plant pathogens.	2. Determination of the applicability and efficacy of biological control agents across different pathogens, crop species, and cultivars to select biological control agents for more effective disease control.
3. To develop and implement economic biological control systems to achieve sustainable agriculture.	3. Integrate biocontrol with existing crop management practices.	3. Implementation of management strategies including crop sequences, tillage, and other cultural practices to promote biological control with indigenous organisms.

Although it appears there is considerable overlap among objectives of the three projects, each project is using a different logical approach to attain their overall goals. It must be remembered that crops, pathogens, cropping systems and biocontrol agents differ radically between the three regions. Another project, NE 171, deals only with nematode parasites of plants.

Table 2 provides a comparison of the disease systems and hosts to be investigated by members of the three regional projects. Where there was duplication between W-147 and other regional projects, the specific research conducted on these disease systems is shown in Table 3. It is apparent even where there is overlap of host crop and pathogen, different research approaches and objectives differentiate the projects. Again, regional, soil, climate and farming practices also separate what appears to be similar projects.

OBJECTIVES:

1. To identify and characterize plant microbe interactions that provide suppression of diseases caused by soilborne plant pathogens.

2. To understand how biological and environmental factors regulate microbial populations and the expression of genes responsible for disease control.

3. To develop and implement economic biological control systems to achieve sustainable agriculture.

PROCEDURES:

The emphasis of W-147 is that direct collaboration is required among members in order to effect the demanding research required to obtain effective, economical and commercial biocontrol agents for the agricultural industries of the US. We require interactive collaboration among states, agencies, disciplines, and basic and applied scientists in order to achieve our goals. W-147 is committed to this cooperative research and an effort will be made throughout this section to illustrate the many research areas where cooperation is demanded and is occurring.

Communal cooperative threads which bind the entire W-147 project together into a unified effort include: 1) A communal biocontrol culture collection maintained by (AZ) which is shared and distributed among members; 2) joint testing of established biocontrol agents at several western locations to determine if efficacy is local or widespread; 3) intensive study of the microbial mechanisms of biocontrol, with experts of certain types of antagonism assisting other researchers with their biocontrol organisms and jointly focusing on certain biocontrol systems; and 4) placing greater emphasis on joint projects in the evaluation of delivery or crop treatment methods and/or enhancement of natural disease suppressiveness in soil-crop management systems. Research leaders, area of specialization and resources are listed in Table 12. The responsibilities of the states with respect to soilborne plant pathogens and objectives addressed are shown in Table 13.

Table 2. Comparative analyses of research on disease systems in the three Regional Projects

DISEASE SYSTEM	W-147	NC-125	S-269
Vegetables			
Southern blight			NC
Sclerotinia	AK		
Fusarium wilt	NY		
Potato early dying	CA-B, ID, OR		
Damping-off (various)-	MT		IN, FL, GA
Phytophthora	NM, AK		
Rhizoctonia diseases	NY, AK, CA-B, ID	OH	AL, GA, MD-ARS, SC
Pythium diseases	NY, OK, CA-B, MT, OR		LA, SC
Thielaviopsis	NY		
Macrophomina	NY		
Field crops			
Wheat			
Take-all	MT, AZ, WA-ARS	IL, IN	TN, IN, FL
Pythium-Rhizoctonia	WA-ARS		TN, LA, SC
Tan spot		KS, ND	
Soybean			
Pythium-Rhizoctonia		NE	TN, GA
Phytophthora	IL	NC	
Peanut			
Stem rot			VA
Sclerotinia			VA
Pythium	NM		VA
Cotton seedling disease			MS, VA, LA, IN, FL, SC, GA
Sugarbeet			
Aphanomyces		MN-Crookston	
Pythium		NE	
Rhizoctonia		NE, OH	
Corn			
Stalk rot		IA	
Pythium-Rhizoctonia	MT		GA
Rice seedling disease			LA
Ornamentals and turf			
Petunia - *Sclerotinia*	AK		
Phytophthora - *Pythium*	CA-R		SC, NJ
Bare patch		IL	
Diseases of perennials			MA
Nematode diseases	CA-R, NY		
Citrus and avocado			
Phytophthora	CA-R		
Strawberry			
Pythium - *Rhizoctonia*	ARS		
black root rot			MA
Walnut - *Agrobacterium*	CA-D		
Stone fruit tree diseases			TX

OBJECTIVE 1. To identify and characterize plant microbe interactions that provide suppression of diseases caused by soilborne pathogens.

Since the beginning of this project biocontrol agents isolated by project participants at ARS, CA-B, CA-D, CA-R, CO, ID, WA, NM, MT, AK and NY have shown tremendous promise for the control of plant pathogens of greenhouse- and field-grown crops. The major impediment to widespread use of biocontrol systems continues to be inconsistent performance. The major thrust of this objective will be to identify biocontrol agents that will not only suppress disease, but, more importantly, provide a level of consistent disease control that is expected and required in commercial agriculture. Research from past W-147 projects has provided a firm foundation for implementing biological control. It is now time to utilize our expertise and select and develop new effective biocontrol agents.

To accomplish this objective, all states will continue to isolate and test individual and mixtures of microorganisms against diseases of local concern. This is necessary because often a single biocontrol agent will not provide control of a disease in all agroecosystems in which that pathogen occurs. Furthermore, the best agents against a pathogen often come from a local soil because the performance of biocontrol agents is greatly affected by biotic and abiotic factors.

All participants will continue to screen biocontrol agents against disease caused by *Pythium, Rhizoctonia,* and *Fusarium.* These pathogens are considered to be serious pathogens which are representative of the three main taxonomic groups of soilborne plant pathogens. In addition, intensive bi- and tri-state cooperative screening efforts will continue for antagonists of *Gaeumannomyces graminis* var. *tritici (ARS and MT), Phytophthora* (CA-R, IL and NM), and *Verticillium dahliae*(CA-B, ID and OR) because these pathogens are major yield limiting factors in some but not all participating states..

Each participant will contribute one to three "well studied" biocontrol agents to a " W-147 Core Collection" of strains which will be stored at -80 C in the laboratory of L. S. Pierson, University of Arizona. This collection will include fungi, bacteria and other organisms which suppress plant pathogens through a variety of different mechanisms. Once assembled, the core collection will be distributed to each participant, and all or parts of it will be tested in each state. We will also have extension researchers and county agents include these biocontrol agents in biocontrol demonstration plots. Each participant has identified at least one extension cooperator and these individuals will be encouraged to become full participants in the W-147 project. Strains will be added to the collection as new organisms become available. It is hoped this approach will facilitate identification of those "premier" biocontrol agents that perform on a broad range of hosts, in different soil types or environments, at low doses and on a variety of diseases. The core collection also will be made available to non-participants, and we believe that it will be especially valuable to researchers who are developing biological alternatives to the use of methyl bromide for disease control on fruit, vegetable and nut crops.

The search for new biocontrol agents will continue. Putative biocontrol agents will be selected from bulk soil, the rhizosphere, the spermosphere, inside plant tissue and pathogen propagules. Special attention will be given to slow-growing or non-cultivable microorganisms which may have been missed in the past because of the use of nutrient

agar as the substrate (CA-R). Suppressive soils such as the naturally-occurring Fusarium wilt suppressive soil from Salinas, CA or take-all decline soils in WA, OR and MT, which are induced by wheat or barley monoculture, will be assayed when possible (CA-B, CA-R, OR, ARS, MT, NY). Induced suppressive soils such as those which develop after mulching , plowing in crop residues or rotating crops will also provide a source of biocontrol agents (CA-B, CA-R, ID, OR, NY). These suppressive soils are known to contain a greater number of antagonists than non-suppressive (conducive) soils. D. Weller, USDA-ARS at Washington State, will develop a data base of suppressive soils and soils that have a long history of monoculture and so are potentially suppressive.

New biocontrol agents will also be pursued through foreign exploration. For example J. A. Menge (CA-R) will travel to New Guinea and surrounding areas to search for biocontrol agents of *Phytophthora cinnamomi* in areas where this fungus supposedly originated. This is a strategy currently employed successfully by entomologists to control introduced insect pests. D. Weller (ARS) will collect take-all suppressive soils from Great Britain and continental Europe.

The process of selecting candidate biocontrol agents will utilize methodology which includes: 1) testing for antagonism against a target pathogen or a variety of soilborne plant pathogens; 2) testing putative agents in a seedling assay in the laboratory, greenhouse or growth chamber; 3) testing strains that show promise in small field plots; and, 4) testing the most effective agents in large-scale field plots using commercial practices. Selection methods (i.e., type of media, environmental conditions, soil type, etc.) cannot be standardized because the antagonists and pathogens to be studied in this project vary. For example, results from ARS, CA-B, and NY indicate that suppression of damping off disease by *Pythium ultimum* can result from any of the three forms of microbial antagonism (antibiosis, hyperparisitism and competition) and that antagonists can be either bacteria or fungi.

Molecular genetic techniques will increasingly be used to facilitate the selection process. For example, biosynthetic loci for many metabolites known to be involved in biocontrol have been cloned and sequenced. Examples include phenazine-1-carboxylic acid, 2,4-diacetylphloroglucinol, pyrrolnitrin, pyoluteorin, rhamnolipids and hydrogen cyanide. Colony hybridization and PCR in combination with probes and primers specific for sequences within the loci encoding these metabolites can be used to rapidly select strains that contain these known biocontrol traits. L. S. Pierson (AZ) and D. Weller (ARS) will be responsible for providing information about the construction and use of these genetic tools.

Biological control of plant pathogens may be divided into three categories: 1) biological control of the plant pathogenic inoculum; 2) biological protection of plant surfaces; and 3) biological control of post infection, i. e. induced resistance or cross protection. This objective will continue the long history of cooperation that has occurred in this regional project since its inception. Tables 4, 5, and 6 below show a small sample of the extensive amount of on-going cooperation within the project based on the three categories of biological control.

Table 4. Biological Control Category 1: Biocontrol of pathogen inoculum.

Contributing State	Target pathogen	Biocontrol agent
CA-Riverside	*Heterodera schachtii*	*Bacillus* spp. suppressive soil
AK & NM	*Phytophthora capsici*	*Trichoderma atroviride*
AK	*Sclerotinia sclerotiorum*	*Trichoderma atroviride*
NY	*Meloidogyne hapla*	*Arthrobotrys dactyloides*
CA-Riverside	*Phytophthora cinnamomi*	*Phanaerochaete chrysorhiza* *Ceraceomyces tessulatus*
CA-Riverside	*Phytophthora parasitica* *P. citrophthora*	suppressive mulches with *Trichoderma harzianum* or *Gliocladium virens*
CA-Berkeley	*Cochliobolus sativus*	Fungistasis
CA-Berkeley & CA-Riverside	*Rhizoctonia solani*	*Actinomycetes*
CA-Davis	root-knot nematodes	*Monacrosporium cionopagum*

Table 6. Biological Control Category 3: Biological control of post infection, i.e. induced resistance or cross protection.

Contributing State	Pathogen	Biocontrol agent
ARS	*Gaeumannomyces graminis* var. *tritici*	*G. graminis* var. *graminis* avirulent *Gaeumannomyces graminis* var. *tritici*
MT	*Gaeumannomyces graminis* var. *tritici*	*Phialophora* sp.
CA-Berkely	Growth promotion in absence of pathogenic fungi	*Pythium oligandrum*
CA-Berkely & ID	*Verticillium dahliae*	*Fusarium* spp.
NY	*Rhizoctonia solani*	binucleate *Rhizoctonia*
AK	*Sclerotinia sclerotiorum*	*Trichoderma atroviride*

OBJECTIVE 2. To understand how biological and environmental factors regulate microbial populations and the expression of genes responsible for disease control.

Previous cooperation among W-147 has led to the identification of several new mechanisms of disease control. However, the largest single problem facing the use of biocontrol organisms is the inconsistent efficacy in the field. Both environmental and biological factors in the field affect both the populations of biocontrol agents and the expression of genes within the biocontrol agents which are responsible for disease control. In order to improve the consistency of biocontrol, the effect of biological and environmental factors on populations of biocontrol agents, on populations of pathogens, and on the genetic expression of the mechanisms responsible for disease must be understood. Members of W-147 are utilizing both genetic and environmental approaches to understand the causes of inconsistency and methods to overcome it. This objective continues to evolve as more specific techniques and knowledge are accumulated regarding various pathogens and biocontrol agents. This is a major reason to continue the work of W-147 since much of the work proposed in this section could not have been done during the past five years. The techniques used to do this work were simply not available until recently.

W-147 members have now established an impressive collection of potential biocontrol agents that utilize different antagonistic mechanisms against soilborne plant pathogens. AZ will serve as the repository for these organisms which include bacteria, fungi and actinomycetes currently being studied in the laboratories of each member (AK, AZ, CA-B, CA-D, CA-R, ID, MT, NM, NY, OR, WA). These strains will be utilized for a number of purposes in more than one of W-147's objectives.

Several members of W-147 are focusing on the genetic approach to solving problems with the consistency of biocontrol organisms. There is a major focus by OR, AZ and ARS-WA to understand the regulatory mechanisms utilized by biocontrol agents to control the expression of genes encoding products involved in disease suppression. For example, because antibiosis is involved in the efficacy of many bacterial biocontrol agents, researchers will be focusing on the regulation, biosynthesis and mutations of the pathways leading to antibiotic production. This work has already characterized the biosynthetic pathways for phenazine and phloroglucinol production. In addition phenazine antibiotic production in the fluorescent pseudomonad *Pseudomonas aureofaciens* is regulated in part by a quorum sensing system. This regulatory system is comprised of N-acyl-homoserine lactone (AHL) synthase (Phzl) which encodes for the production of the diffusable signal hexanoyl-homoserine lactone (HHL). A second protein (PhzR) serves as a transcriptional regulator that is believed to interact with HHL within the bacterial cell once it accumulates above a threshold concentration. Interaction of PhzR with HHL results in the binding of PhzR to DNA sequences, within the regulatory regions of specific genes, resulting in gene activation. Many plant-associated bacteria are now known to respond to AHL signals. An AHL-specific *P. aureofaciens* reporter has been constructed recently (AZ). This reporter is defective in Phzl, the gene coding for AHL synthase. This mutant cannot synthesize its endogenous HHL signal and therefore cannot activate the phenazine biosynthesis genes. The addition of sterile culture filtrates of wildtype *P. aureofaciens* results in the activation of the phenazine antibiotic genes. Another version of this mutant has been modified to express the reporter enzyme

β–galactosidase in the presence of exogenous HHL signal. These strains can now be used to detect AHL signals in a variety of biocontrol agents. The different strains of biocontrol agents from the W-147 collection will be tested for diffusable AHL using the mutants described above. The reporter strains will be spread onto an agar plate and samples of the test bacteria (and other organisms) spotted directly on top of the *P. aureofaciens* lawn. The production of AHL signals recognized by the *P. aureofaciens* PhzR protein will be evident by the restoration of the orange phenazine pigments. These assays will be further verified by extraction of the signals from cultures of the test organisms using acidified ethyl acetate. Followed by quantitative assays using the Phzl, β–galactosidase reporter. Further structural characterization of the AHL signals can be pursued via reverse phase thin layer chromatography and the GC-MS as deemed appropriate.

Once a subcollection of biocontrol agents utilizing various antagonistic mechanisms that positively communicate with *P. aureofaciens* is identified, mixtures of these organisms will be tested for enhanced biocontrol efficiency by several members of W-147 in different regions on various crops and against various plant pathogens (AK, AZ, CA-R, CA-B, ID, MT, NM, NY, OR, WA).

In addition, several participants of W-147 (AZ, ARS-WA, OR) will continue to characterize the biosynthetic and regulatory pathways involved in antibiotic production. ARS-WA has identified a series of "premier" take-all biocontrol strains by characterizing suppressive soils with RAPD primers. They will construct transgenic strains of these bacteria that contain multiple antibiotic biosynthesis pathways. These strains may be more inhibitory to target pathogens than the wildtype biocontrol organisms. These transgenic strains will also be tested for cross communication with *P. aureofaciens*.

Many members of W-147 will pursue the mechanisms of biocontrol utilizing the environmental approach. Many participants have identified soils which are naturally suppressive to specific soilborne plant pathogens. Soils suppressive to *Fusarium* (NY), *Meloidogyne* (NY), *Pratylenchus* (NY), *Pythium* (CA-B, MT, NY), *Phytophthora* (CA-R, NM), *Rhizoctonia* (CA-B, ID, NY), *Thielaviopsis* (NY),*Gaeumannomyces* (WA-ARS), *Heterodera* (CA-R), and *Verticillium* (CA-B, ID) have been identified. Those soils suppressive against *Fusarium* (NY), *Meloidogyne*(NY), *Pratylenchus* (NY), *Pythium* (CA-B, MT, NY), *Rhizoctonia* (ID, NY), *Thielaviopsis* (NY) and *Verticillium* (ID, CA-B)and *Gaeumannomyces* (WA-ARS) are influenced strongly by cropping sequences, organic amendments and crop management. Those soils suppressive to *Phytophthora* (CA-R) are a result of mulching. Those soils suppressive against *Heterodera* (CA-R) appear to be naturally suppressive. The suppressive factors in all of these soils will be sought by treating the soil with pesticides, fumigants, steam or heat to identify fractions with the biocontrol factor. Isolations, baiting, and RAPD analysis will be utilized to try to identify the organisms responsible for biocontrol.

New technologies not available in past years will be utilized to identify antagonistic agents in the suppressive soils. For example, monoclonal antibodies specific for *Verticillium dahliae* (CA-B) are now available and can be used to obtain quantitative information on root colonization by *Verticillium* as influenced by the various environmental factors (CA-B, ID). The effect of cropping practices such as plowing in green cover crops of Sudan grass and corn or applying manganese on colonization of

potatoes by *Verticillium* will be assessed using the new technology. Roots of potato will be grown with various potential biocontrol organisms or mixtures of biocontrol organisms and the monoclonal antibody, immunohistochemical assays will determine if these organisms are responsible for the suppressiveness of the treatments.

Mulching avocados results in a soil suppressive to *Phytophthora cinnamomi.* It has been shown that cellulase and glucanase produced during the decomposition of the mulch damages the cellulose-glucan cell walls of *Phytophthora* (CA-R). Armed with this new information we intend to choose biocontrol organism-mulch combinations using assays for cellulase and gluconase. Mulches will be "bioenhanced" with a variety of potential biocontrol agents based on their ability to produce gluconase and cellulase and they will be tested for their ability to cause suppressiveness in avocado soils.

Finally, plant pathogens will be labeled with genetic markers and introduced into the soil (CA-R). Assays will be performed using a variety of genetic microbial probes to identify what possible biocontrol agents contain these markers. This is a new and novel way to determine which soil organisms are predators or parasites of soilborne plant pathogens even though these biocontrol agents may not be cultivable. Using this method, the organisms responsible for soil suppressiveness may be discovered.

In summary, the proposed research will focus on understanding the mechanisms of biocontrol of a collection of biocontrol agents identified and shared by members of W-147. When the interactions of these biocontrol agents between themselves, soilborne plant pathogens and host plants is well understood, the inconsistency of biocontrol organisms will also be understood. This objective requires cooperation between basic, applied , genetic, molecular , soil science, agronomy, mycology and bacteriology scientists such as we have in W-147. Table 7 documents the interactive, cooperative approach of this objective.

Table 7. Interaction between W-147 members working on Object 2.

Project	Cooperating States
Study of regulatory, biosynthesis and mutation of antibiotic pathogens	ARS, AZ, OR,
Communication among biocontrol organisms	AZ, AK, CA-B, CA-D, CA-R, ID, MT, NM, NY, OR, WA
Loss of biocontrol ability	AZ, CA-R, OR
Mechanisms of suppressive soils	CA-B, CA-R, IJ, NY, OR

OBJECTIVE 3. To develop and implement biological control systems to achieve sustainable agriculture.

All participants of W-147 are either directly or indirectly involved in this objective (AK, AZ, CA-B, CA-D, CA-R, ID,IL, MT, NM, NY, OR and WA). To date Objectives 1 and 2 have done much to lay the ground work for the implementation of the biological control of plant diseases and results are providing reasons for optimism. With the continuation of W-147, we can now utilize results developed previously to obtain practical and beneficial economic results for the grower.

During the next three years the W-147 project proposes to use all three of Cook's (1990) strategies for biocontrol to accomplish Objective 3 (Tables 8, 9, and 10): 1) the treatment of plant material and soil with biocontrol agents to reduce plant disease and maintain soil quality; 2) to encourage natural biological control with mulches, soil composts, and/or cropping practices to increase and support biocontrol agents; and, 3) the continuous application of biocontrol agents into irrigation water. For this last strategy, EcoSoils has perfected a field fermenter which is capable of producing 480 L of a concentrated biocontrol agent and injecting it automatically into irrigation water at every irrigation. This new and innovative technology warrants study because it overcomes many of the deficiencies of other biocontrol delivery systems ,since the continuous application system biocontrol agent need not colonize or survive for a long period to be effective. This system can be used to produce massive amounts of microbial antibiotic or surfactants which may act much like natural pesticides when applied in the irrigation water.

Although target pathogens differ between states, crops, and soil conditions, members of this project are working cohesively toward the same goal of sustainable agriculture. Without exception, all states are cooperating with at least one other state associated with W-147 (Table 11). For specific examples of projects and methodology see Tables 8 9, and 10. During the last 5 years a dramatic shift has occurred from the use of biocontrol agents primarily as seed treatments, to the use of specific cropping practices to sustain biological control. In addition to effects with biocontrol agents, more general effects are also being achieved by the use of mulches that favor the build up of organisms and green manures and rotation crops that are shown to significantly affect the microflora in the soil to bring about biological control. We propose to maximize these cultural controls and maximize the biocontrol abilities of these crop management practices. We will search for new and better means of introducing and sustaining biocontrol agents in the field. As we learn more about managing plant diseases with biocontrol agents and with cropping practices, new crop management systems will unfold. Economic expertise will be utilized in each of the states from economists and business professionals, primarily with Cooperative Extension Service appointments, when such expertise is needed to bring about the practical implementation of the various biocontrol stategies.

Many practical, economical, and efficient alternatives are evolving for the replacement of pesticides. The examples are many (Tables 8, 9 and 10). However, more time and research is necessary before these alternatives are widely used in agriculture. At this time in history, it is crucial that this project not only be continued, but also be significantly encouraged if sustainable agriculture is to be achieved.

Table 8. Strategy 1: The treatment of plant material and soil with biocontrol agents to maintain soil quality and health.

Contributing states	Target pathogens	Biocontrol agents
AK	*Sclerotinia sclerotiorum* *Phytophthora capsici*	*Trichoderma atroviride*
CA-Berkeley, Davis	*Agrobacterium tumefaciens*	*A. radiobacter* K-84
CA-Riverside	*Heterodera schactii* *Phytophthora*	Unidentified soil microbes *Gliocladium virens* *Trichoderma harzianum* *Pseudomonas putida*
WA-ARS	*Rhizoctonia, Pythium* and Take-all	*Bacillus* sp. 324
MT	Take-all *Pythium* seed rot	*Phialophora* I-52 *Pseudomonas aureofaciens* AB254
NY	*Pythium, Rhizoctonia* *Fusarium, Thielaviopsis*	*Bacillus subtilis* *Gliocladium virens* Strain T22 of *T. harzianum*
CA-Berkeley	non-pathogen (growth regulator) *Rhizoctonia solani*	*Pythium oligandrum* *Actinomycetes*

Table 9. Strategy 2. To encourage natural biological control with mulches, soil composts, and/or cropping practices to increase and support biocontrol agents.

Contributing States	Target pathogen	Practices
CA-Riverside	Phytophthora spp	mulches + amendments (e.g. gypsum) + biocontrol agents
CA-Riverside	H. schactii	crop rotations for sustaining soil suppressiveness
NY	Pythium, Rhizoctonia, Fusarium Thielaviopsis, Meloidogyne hapla	green manures
MT	Pythium seed rot	green manures for sustainable agriculture
OR	Verticillium dahliae	green manures (sudangrass)
CA-Berkeley	Verticillium dahliae	green manures rotation crops
ID	Verticillium dahliae	green manures rotation crops

Table 10. Strategy 3. The continuous application of biocontrol agents into irrigation water.

Contributing States	Target pathogens	Method
CA-Riverside	Phytophthora spp	Field fermentation machines with capacity for growing and injecting biocontrol agents into irrigation water
CA-Riverside	Phytophthora and Pythium spp	Introduction of bacteria into irrigation water with capability of producing rhamnolipids for controlling zoospores

Table 11. A summary of cooperation between states according to strategy.

Strategy 1	Cooperation With
AK	NM
CA-Berkeley	CA-Riverside
CA-Davis	CA-Berkeley
CA-Riverside	CA-Berkeley
CA-Riverside	AZ, extended invitation to other members of W-147
WA-ARS	AZ
MT	AZ, NY
NY	MT
<u>Strategy 2</u>	
CA-Riverside	CA-Berkeley
CA-Berkeley	ID, OR
ID	CA-Berkeley, OR
OR	ID
<u>Strategy 3</u>	
CA-Riverside	AZ

EXPECTED OUTCOMES

If continued the W-147 participants will continue to write and publish, on the average, 55 scientific and public oriented articles, papers, book chapters and pamphlets per year on the subject of biocontrol of soilborne plant pathogens. These published works will be aimed at communicating with a variety of audiences including, scientists, growers, packers, agro-industry workers consumers and the general public. Oral presentations by W-147 members will reach an additional group of people. All W-147 projects have at least one extension worker associated with the project. It is the job of these extension workers to further publicize and distribute this information to the clientele for whom it is targeted. A database will be established on naturally occurring soils which are suppressive to one or more soilborne plant pathogens. The W-147 project will continue to foster and encourage close research cooperation between states, agencies, and areas of expertise which will more rapidly advance the progress toward the goal of providing practical, economic biocontrol systems which will benefit US agriculture.

A W-147 core collection of biocontrol agents will be established which will be available to researchers and the private sector. Several new patented biocontrol products aimed at controlling soilborne plant pathogens will result from the work. An entire new biocontrol industry is beginning to develop which is based on research like that provided by W-147. This new industry deals with the biocontrol of insect, weed and disease pests and will provide jobs for researchers, laboratory personnel, field workers, and applicators. Numerous new cultural management, crop rotation, and crop management systems which reduce soilborne diseases will be passed on to growers.

If agriculture continues to lose chemical pesticides and they are not replaced with alternative control measures such as biocontrol, the monetary loss to growers, shippers, packers, and food stores will far exceed the $4 billion per year we are currently losing to soilborne plant pathogens. Losses may reach $12 billion per year. Even if biocontrol products become widely used, we would expect that they would, on the average, not be as effective as current chemical pesticides. However, for certain intractable soilborne pathogens which have been difficult to control with pesticides, biocontrol might be more effective. In the long run biocontrol may be cheaper than pesticides especially if biocontrol agents establish and reproduce,so that fewer applications would be necessary. Since it will be more difficult for plant pathogens to develop resistance against biocontrol agents than chemical pesticides, both the biocontrol producers and the growers will benefit monetarily. Furthermore, since biocontrol agents active against soilborne pathogens are selective and do not harm other beneficial organisms such as insect biocontrol agents, Rhizobium or mycorrhizae, growers costs should be reduced.

However, the major benefits of biocontrol may be the indirect benefits to society. Since biocontrol agents are non-polluting, biodegradable, and harmless to humans and wildlife. they are superior to chemical pesticides in these respects. If biocontrol becomes widely adapted, fewer chemical pesticides will be used and enormous benefits and cost reductions to society will result from cleaner air and water, healthier environments and wildlife, healthier food for consumers and safer conditions for farm workers. Finally biocontrol will contribute to a more sustainable agricultural industry which will be able to better provide food and fiber to its consumers for the foreseeable future. These benefits will be priceless to future generations.

ORGANIZATION:

The W-147 regional research program will be administrated by a technical committee consisting of a project leader from each of the participating states. Officers of the committee will be the Chairman and Secretary. The Secretary will be elected each year and will advance to Chairman the following year. For 1997-98 the committee officers will be:

Chairman- J. G. Hancock

Secretary: J. A. Menge

Meetings will be called each year by the administrative advisor, and a local arrangements coordinator will be determined for each annual meeting. At those meetings research accomplishments will be reviewed and recommendations made for coordination and publication of results.

SIGNATURES:

REGIONAL PROJECT TITLE; Managing Plant-Microbe Interactions in Soil to Promote Sustainable Agriculture

5/15/98
ADMINISTRATIVE ADVISOR DATE

7/21/98
CHAIR, REGIONAL ASSOCIATION OF DIRECTORS DATE

9-8-98
ADMINISTRATOR, COOPERATIVE STATE RESEARCH, EDUCATION AND EXTENSION SERVICE DATE

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ATTACHMENTS:

Table 12: Project Leaders/Resources (with Appendix D attached)
Table 13: Project Objectives
Critical Review

Table 12. Regional project leaders

Resources State	Agency/ Institution	Principal Leader	Cooperators	Area of Specialization	SY	PY	TY
Alaska	University of Alaska	J.H. McBeath		Plant Path	0.3		
			Y. Huang			0.3	
Arizona	University of Arizona	L.S. Pierson		Plant Path	0.1		
			P. Figuli				0.1
			E. Pierson			0.5	
California	University of California Berkeley	J.G. Hancock		Plant Path	0.3		
			A.C. Magyrosy/ D. Schmidt			0.3	0.2
California	University of California Berkeley	O. Huisman		Plant Path	0.4		0.35
California	University of California Davis	L. Epstein		Plant Path	0.1		
California	ARS/USDA	F. Martin		Plant Path	0.2		0.5
California	University of California Riverside	J. A. Menge		Plant Path	0.33		
California	University of California Riverside	J. Borneman		Plant Path	0.15		0.05
			J. Hartin		0.15		
California	University of California Riverside	M. Stanghellini		Plant Path	0.15		
California	University of California Riverside	J.O. Becker	A. Westphal J. Darsow	Nematology	0.2	0.3	0.2
Idaho	University of Idaho	J.R. Davis			0.5		
			A.T. Schneider			0.5	
Illinois	University of Illinois	H. Wilkinson		Plant Path			
Montana	Montana State University	D.E. Mathre		Plant Path	0.3	0.5	0.5
Montana	Montana State University	N.W. Callan		Plant Path	1.0		
New York	NY SAES Geneva	G.S. Abawi			0.3		
			T.L. Widmer			0.5	
			N.A. Mitkowski			0.5	
			J.W. Ludwig				0.3
			J. Bosard				0.2
Oregon	Oregon State University	M.L. Powelson		Plant Path			
Oregon	ARS/USDA	J.E. Loper		Plant Path			
Washington	ARS/USDA	D.M. Weller		Molecular Biol	0.3		
			R.J. Cook		0.1		
			L.S. Thomashow		0.2		
			D.V. Mavrodi			0.2	
			K. Schroeder				0.3
CA	USDA/ARS	C. Bull		Plant Path	0.5		0.5

Table 13. Pathogens Studied by State in W-147 Region

Pathogen	State	Obj 1. (Identification)	Obj 2 (Mechanisms)	Obj 3 (Develop)
Fusarium	New York	X	X	X
Gaeumannomyces graminis	Washington	X	X	X
Heterodera	California	X	X	X
Meloidogyne	New York	X	X	X
Pratylenchus	New York	X	X	X
Pythium	California	X		X
	California	X		X
	Montana	X		X
	New York	X	X	X
	Oregon		X	
	Washington	X	X	X
Phytophthora	Alaska	X		X
	California	X		X
	California	X	X	X
	California	X		
Rhizoctonia solani	California	X		X
	California	X		X
	Idaho	X	X	X
	New York	X	X	X
	Washington	X	X	X
Thielaviopsis	New York	X	X	X
Sclerotinia	Alaska	X		X
Verticillium	California		X	X
	Idaho		X	X
	Oregon			X
Agrobacterium	California			X