Specialty Crops Research Program
Quarterly Report

Executive Summary

Report Period: Inception of project to March 31, 2004

Project Title: Investigation of Organic Seed Treatments for Spinach Disease Control

Principle Investigator: Steven T. Koike

Spinach is a very important leafy vegetable crop in California, with both acres and total crop values increasing tremendously over the past 8 years. Both conventional and organic spinach products are valued by consumers. Spinach contains high levels of nutrients and beneficial anti-oxidants and is increasing in popularity. Regulation changes in the organic production segment of the agricultural industry now prohibit the use of seed treated with synthetic fungicides. For the organic spinach industry, this change could signal the potential increase of damping-off disease caused by soil borne pathogens. Because spinach is very sensitive to these organisms, research is needed to investigate damping-off management for organic spinach.

The first objective of our project is to document the occurrence and severity of spinach damping-off disease as it develops in the organic industry. The second objective will be to test various chemical and biological substances that will be allowed in organic production systems. The third objective will be to extend our findings to the organic agricultural community.

In the research completed thus far, we conducted surveys among growers who produce organic spinach. They ranked damping-off of spinach as an important concern that could reduce final yields. At this time, field surveys did not indicate an observable, significant increase in the incidence of this disease, despite the fact that in 2003, spinach seed treated with conventional fungicides could no longer be planted. During these surveys, we collected diseased spinach and recovered *Pythium*, one of the main damping-off pathogens of spinach.

We repeated experiments in which spinach seed was treated with various biological and natural products. Such seed was placed on agar medium substrates and then exposed to *Pythium* cultures. Most treatments did not appear to inhibit the advance and growth of the organism; one exception was Mycostop, which visually inhibited *Pythium* growth near the seed line. Some of the treatments appeared to reduce the amount of *Pythium* growing directly on the seed itself. We planted treated seed into pots that were infested with *Pythium*. Thus far, none of the treatments significantly reduced disease caused by *Pythium*. This potted spinach experiment will be repeated, and improved assay techniques will be explored.

Steven Koike acknowledges his research collaborators Richard Smith (University of California Cooperative Extension, Monterey County) and Eric Brennan (USDA-ARS in Salinas), and thanks technicians Kat Kammeijer and Pati Ayala. Koike acknowledges the Specialty Crops Research Program and also thanks members of the spinach industry in California and the companies that provided the test materials used in this study.

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Objectives: The first objective of our project is to document the occurrence and severity of spinach damping-off disease as it develops in the organic industry. The second objective will be to test various chemical and biological substances that will be allowed in organic production systems. The third objective will be to extend our findings to the organic agricultural community.

Procedures and Methods:

Major activities: Objective 2. Repeat Petri dish tests

Various chemical and biological substances, all designed for use in organic agriculture, were obtained for these tests. Materials, nature of the product, and rates are listed in Table 1. Each material was measured and mixed to appropriate concentrations to treat 17 grams of spinach seed (cultivar: Spinnaker). Seed were treated with the materials for 5 minutes, placed on clean paper towels for 3 minutes, and then placed on top of sterile water agar media in standard 100 mm Petri plates. Ten seed were arranged in a line in each plate; 10 plates were prepared per treatment per *Pythium* isolate. Each plate is considered a replicate.

Table 1. Materials tested for *Pythium* inhibition study.

Product	Contents	Rate/1 lb of spinach seed
Kodiak	*Bacillus subtilis*	0.14 gram
L1277	experimental	0.35 ml
Mycostop	*Streptomyces* sp.	3.6 gram
Prestop	Glioclad. catenul.	3.6 gram
Promot	*Trichoderma*	30 ml (per gallon water)

Soilgard	*Gliocladium virens*	9.1 gram (per gallon water)
Sporan	plant oils	56 ml (per gallon water)
Yield Shield	*Bacillus pumilus*	0.35 gram
Control 1	untreated	---
Control 2	water only	---

After seed were arranged on the plates, a *Pythium*-colonized agar plug was placed on the edge of the test plate. Three *Pythium* isolates (03-10, 03-11, 03-12) were used in this experiment. Plates were incubated in the dark at 22-24 C and observed daily for *Pythium* growth and evidence of inhibition due to seed treatments.

Observations: In the first experiment, no chemical or biological seed treatment showed observable inhibitory effects on advancing *Pythium* mycelium in agar plate tests. All *Pythium* cultures grew evenly towards the treated seed, and treated seed were soon surrounded by the fungus. No significant differences in *Pythium* inhibition were observed between any of the treatments, nor between treatments and the two types of controls. We did note that for some treatments (Mycostop, Prestop), the active organism in the product colonized the outer seed surface; *Pythium* growth on these seeds appeared reduced compared to controls. Seeds treated with the plant oil product Sporan also appeared to support less fungal growth on the actual seed coat.

However, when the experiment was conducted a second time we observed slight differences in treatment effects (Table 2). Products Mycostop, Promot, Soilgard, and Sporan had no to limited fungal growth on the seed itself. When *Pythium* growth on the agar medium was evaluated, one product showed partial inhibition of fungal growth: Mycostop. Therefore, some limited efficacy against *Pythium* was visible in this second experiment.

Table 2. Results of *Pythium* inhibition study.

Product	Degree of seed colonization	Inhibition on medium
Kodiak	extensive to moderate	none
L1277	moderate to none	none
Mycostop	none	partial
Prestop	moderate	none
Promot	none	none
Soilgard	none	none
Sporan	slight to none	none
Yield Shield	partial	none
Control 1	partial to moderate	none
Control 2	partial to moderate	none

Major activities: Objective 2. Test products in potted spinach tests

To further test efficacy of these biological products, we established a greenhouse, potted spinach assay. Spinach seed was planted into 6-inch pots that were infested with *Pythium* isolates. The

inoculum was prepared by autoclaving rye seed in flasks, allowing the flasks to sit for 24 hours, then autoclaving them again for a second time. The rye seed medium was then inoculated with *Pythium* isolates and incubated for 15 days. This infected rye seed was then mixed into the potting mix used for the experiment. Seed treated with the various materials (Table 1) was planted into replicated pots and maintained in a greenhouse. Untreated seed controls and uninfested controls were also included.

Plant stand counts were evaluated 3 weeks after planting. Most treatments resulted in 0 to 13% germination and plant survival. Untreated seed had similar 0 to 13% germination rates. Uninoculated controls had 46 to 68% germination. Therefore, none of the biological treatments showed satisfactory efficacy in this trial. This experiment will be repeated, and also different greenhouse assay methods will be developed.

Major activities: Objective 3. Outreach and education efforts

Preliminary results have been presented to and discussed with clientele who grow organic vegetable crops, including growers on the central coast, pest control advisors, and participants at the Rural Development Center program.

Problems and issues

None at this time. Additional greenhouse assay tests need to be examined.

Budget Summary: The actual expenditures closely match the estimated expenditures as put forth in the proposal.

Documentation

Figure 1: Greenhouse test of biological materials. In each photo, two pots on the far left are spinach plantings that were uninoculated. The other pots contained *Pythium* inoculum, into which was planted spinach seed treated with different products.

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Figure 1. Examples of potted spinach plants. Uninoculated control plants are on the far left side of the photograph.