Specialty Crops Research Program
Quarterly Report

Executive Summary

Report Period: Inception of project to December 31, 2003

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

Principle Investigator: Steven T. Koike

Spinach has become a very important leafy vegetable crop in California, with both planted 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 (figure 1). 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.

Initial tests consisted of treating spinach seed with various organically approved biological and natural products, placing seed on agar medium substrates, and exposing the seed to *Pythium* cultures. Thus far, the treatments did not appear to inhibit the advance and growth of the organism. However, some of the treatments appeared to reduce the amount of *Pythium* growing directly on the seed itself. These tests will be repeated. The next set of experiments will involve treating seed, planting the seed in pots containing *Pythium*, and evaluating how the spinach grows under controlled greenhouse conditions.

Steven Koike acknowledges his research collaborators Richard Smith (University of California Cooperative Extension, Monterey County) and Eric Brennan (USDA-ARS in Salinas). 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 1. Assess disease severity.

We made contact with organic spinach producers in the central coast area and discussed spinach damping-off with these growers. We also contacted pest control advisors who deal with organic production. All of these industry members agreed that damping-off continues to be a concern for spinach production. However, these interviews and our own field assessments did not indicate that spinach damping-off incidence had increased significantly during this year of planting fungicide-free seed. Further field assessments are planned next season to see if this disease is increasing in importance in organic systems.

During the assessment phase of the project, we collected symptomatic spinach from organic field. We conducted lab isolations and recovered mostly *Pythium* species from these diseased samples. Ten *Pythium* isolates were archived for use in the study. An occasional *Rhizoctonia* isolate was recovered.

We are now mass producing inocula of five of the *Pythium* isolates. The inocula will be used in potted spinach studies that will test efficacy of various organically acceptable products for potential use against spinach damping-off.

Observations: This small survey gave preliminary information that Pythium may be the main spinach damping-off organism found in the surveyed area. Initial responses do not indicate that a significant increase in spinach damping-off occurred in 2003, a year in which fungicide-treated seed was not used by the organic industry.

Major activities: Objective 2. Test various substances.

Various chemical and biological substances, all approved or 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 (figure 2); 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: 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 (figure 3). 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. These experiments will be repeated. We will also conduct potted spinach tests to further evaluate the treatments under greenhouse conditions and to assess any seed germination or phytotoxic effects.

Problems and issues:
Some of the *Pythium* cultures became contaminated. We are attempting to re-purify and recover these isolates. We have not identified an organic field plot in which spinach damping-off has been a consistent problem. We will continue to search for such a location for possible field tests.

Budget Summary: The actual expenditures closely match the estimated expenditures as put forth in the proposal. Extra time, and therefore extra labor, has been spent on trying to recover the contaminated *Pythium* cultures.

Documentation

Figure 1: Damping-off of spinach in a commercial field in the Salinas Valley.
Figure 2: Laboratory test set up. Treated spinach seed are placed on an agar substrate, then exposed to active colonies of *Pythium*.
Figure 3. Close up of laboratory test. Treated spinach seed apparently do not inhibit *Pythium* mycelial growth.

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Figure 1. Damping-off of spinach in a commercial field in the Salinas Valley.

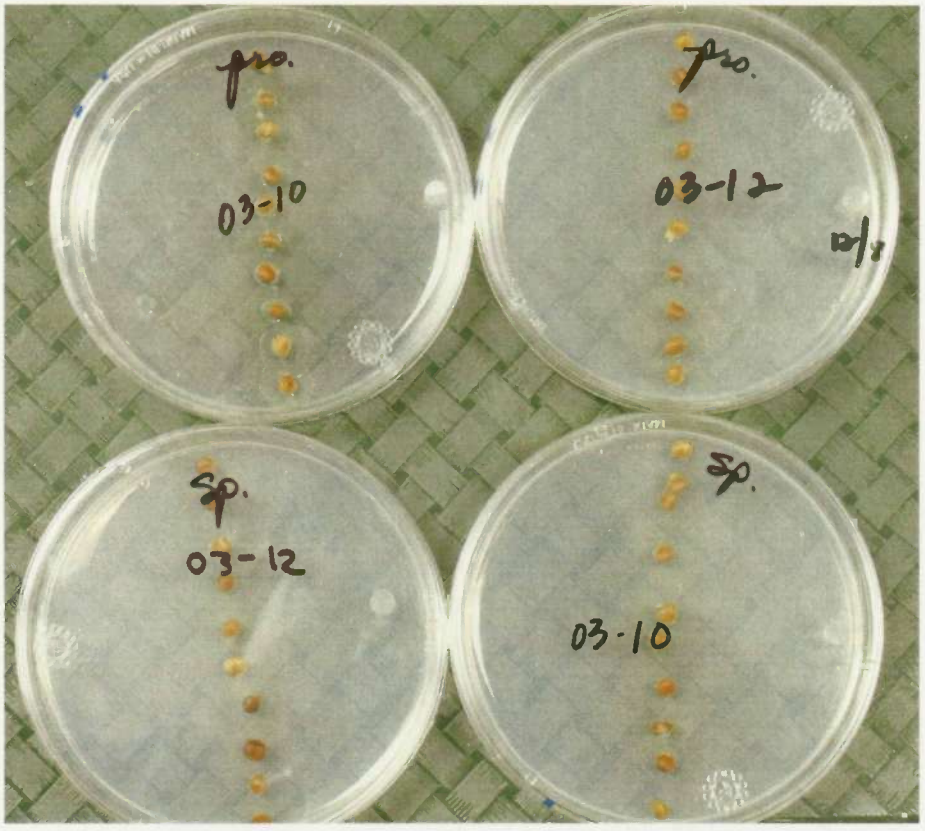

Figure 2. Treated spinach seed are placed on an agar surface, then exposed to *Pythium* colonies.

Figure 3. Treated spinach seed apparently do not inhibit *Pythium* mycelium.