Project Title

Characterizing damping-off and root rot pathogens of spinach in California

Project Investigators

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Abstract
Spinach acreage has significantly increased in California over the past years, along with a possible increase in soilborne diseases of the crop. A survey of diseased fields in the central coast was conducted to determine which pathogens are involved in this problem. *Pythium* was the soil organism most commonly isolated from diseased spinach roots; *Pythium* was associated with young spinach plants that had only cotyledons or first two true leaves developed. *Fusarium* was recovered with the second highest incidence and was associated with larger spinach plants that had dark brown to black taproot tips. *Rhizoctonia* was found third most frequently and was also linked to darkened taproot tips. Except for *Fusarium*, soilborne root rot pathogens have not been documented as seedborne in spinach. A limited collection and testing of commercial spinach seed failed to find *Pythium* and *Rhizoctonia*, though *Fusarium* isolates were commonly recovered.

Introduction
Spinach acreage has significantly increased in California over the past few years. Along with this trend, soilborne diseases of spinach appear to also be increasing. Soilborne problems can occur in several different ways. With pre-emergence damping-off, spinach seed and newly germinated seedlings are attacked and rotted prior to the above-ground emergence of the seedling. Secondly, post-emergence damping-off occurs when spinach plants successfully emerge from the soil but quickly develop symptoms consisting of yellowed leaves, general poor growth and stunting,

wilting, and eventual collapse and death of plants. Roots of infected plants appear water-soaked or brown to black in color. Finally, larger more mature spinach can also develop a root rot disease that results in discoloration of the main taproot and subsequent stunting, yellowing, and wilting of the spinach plant.

These different soilborne diseases can be caused by a number of fungi and fungus-like organisms such as *Aphanomyces, Fusarium, Phytophthora, Pythium*, and *Rhizoctonia*. However, care must be taken when diagnosing these soilborne diseases because symptoms resulting from abiotic problems, caused by factors such as overwatering and poor planting technique, can look similar to some symptoms caused by damping-off and root rot pathogens. In California, a formal and thorough investigation into the causes of spinach damping-off and root rot has not been done. To best devise management strategies, it is necessary to have precise information as to which organisms are causing these diseases in coastal California. The purpose of this project is to define the causal agents of spinach root rot, determine aspects of where and why disease occurs, and devise management strategies.

Objectives for Year One

1. Survey coastal regions for disease and characterize the causal agents of damping-off and root rot diseases of spinach.

2. Assay seeds for presence of soilborne pathogens.

Procedures

1. Survey coastal regions for disease and characterize the causal agents of damping-off and root rot diseases of spinach.

In the spring and summer of 2012, we collected diseased spinach plants from 12 fields throughout Monterey, Santa Cruz, and San Benito counties. Fields were selected based on the occurrence of wilting or collapsed foliage. 12 samples were collected from each field; each sample consisted of 8 to 10 diseased plants. At the UCCE-Monterey County diagnostic lab, samples were divided into two subgroups. For one group, symptomatic, discolored roots were rinsed in sterile distilled water to remove soil, blotted dry on paper towels, and placed onto petri plates containing water agar amended with 0.1% Tergitol NPX. These plates were incubated for 36 to 48 hours before examining them for fungal growth. *Pythium*- or *Rhizoctonia*-like growth was subcultured by excising hyphal tips and transferring them to fresh water agar plates. Representative isolates collected in this way were further purified and sent to the Martin lab for archiving and identification.

For the second group of diseased spinach plants, roots were rinsed in tap water to remove soil, soaked for 1 minute in a dilute bleach solution (0.1%), rinsed thoroughly with sterile distilled water, and blotted dry on paper towels. Small (5 to 10 mm long) pieces of symptomatic roots were then cut and placed onto petri plates containing acidified corn meal agar (LA-CMA). These plates were incubated for 2 to 3 days before examining them for fungal growth. Representative isolates were subcultured onto fresh LA-CMA plates and sent to the Gordon lab for archiving

and identification.

2. Assay seeds for presence of soilborne pathogens.

With the exception of the *Fusarium* pathogen, there is no clear evidence that spinach root rot pathogens are seedborne in spinach. Industry has expressed to us their interest in exploring this possibility. To address this question, we collected 11 samples of commercial spinach seeds taken from seed bags in field warehouses in the Salinas Valley. For each cultivar, approximately 300+ seeds were placed in a strainer and rinsed under running tap water for 15 minutes to remove or dilute chemical treatments made to the seeds. Seeds were then spread to dry for 2 hours on a paper towel. After drying, 100 seeds were distributed onto 15 petri plates containing a semi-selective medium (PARP) for *Pythium*/*Phytophthora* organisms. Likewise, 100 seeds were plated onto a semi-selective medium (FS) for *Fusarium* and 100 seeds plated onto a general purpose fungal medium (LA-CMA). All plates were incubated in the dark and were examined on a regular basis for fungal growth. This experiment was conducted two times.

Results and Discussion

1. Survey coastal regions for disease and characterize the causal agents of damping-off and root rot diseases of spinach.

From the 12 spinach fields, three groups of suspect pathogens were isolated. *Pythium* was recovered from eight of the 12 fields and approximately 150 isolates were collected. *Pythium* was associated with tan or brown spinach roots from various stages of spinach, though *Pythium* was commonly the only agent recovered from very young (cotyledons only, or 2 to 4 true leaves) seedlings. *Fusarium* was recovered from 10 of the 12 fields and roughly 115 isolates were saved. *Fusarium* was commonly associated with dark brown to black taproot tips on older (6 or more true leaves) plants and not on very young seedlings. A brown mycelial *Rhizoctonia* (presumably *R. solani*) was found in nine of the 12 fields and 55 isolates were saved. *Rhizoctonia* was also associated with older plants. Other reported spinach pathogens, such as *Aphanomyces* and *Phytophthora*, were not found in these surveys. Other fungi were also present in the isolation plates but apparently are all non-pathogenic soil fungi and included *Cladosporium*, *Penicillium*, and *Trichoderma*.

Subsequent work will include identifying the recovered isolates to species and testing the species to determine whether they are primary pathogens that cause root disease to spinach. We will also compare various spinach cultivars to determine if detectable differences exist regarding resistance or tolerance to these soilborne pathogens.

2. Assay seeds for presence of soilborne pathogens.

On the semi-selective PARP medium, no oomycete fungi (*Pythium*, *Phytophthora*) were recovered from any tested seed sample. *Rhizoctonia* also was absent from the seed assay tests.

Fusarium isolates were commonly observed on both the semi-selective (FS) medium and the general purpose LA-CMA. Future studies will examine whether these recovered *Fusarium* isolates are pathogenic, and whether they can be considered the same as the Fusarium wilt pathogen that infects spinach seed crops.

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