California Leafy Greens Research Board
Annual Report

Project Title

Characterizing damping-off and root rot pathogens of spinach in California: Year 2

Project Investigators

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Cooperating Personnel

Growers, pest control advisors, and other industry personnel in central coast California.

Abstract

Spinach growers and pest control advisors have been expressing increased concerns regarding soilborne disease issues. This project was therefore initiated to investigate the causes of damping-off and root rot problems of spinach in California's central coast region. 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; diseased roots had an overall light brown appearance. *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 on larger plants. These findings match the results of the UC Cooperative Extension (UCCE) diagnostic laboratory in Salinas, in which *Pythium* is the

organism most commonly recovered from very young spinach plants, and Fusarium is isolated most often from older plants. Work is still in progress. The Koike lab is testing selected isolates for pathogenicity to spinach, as well as evaluating various spinach cultivars for possible differences in susceptibility to *Pythium* and *Fusarium.* Gordon and Martin labs will be working on species identification on *Fusarium* and *Pythium* isolates, respectively, based on the results of the pathogenicity tests. Pathogenicity tests with *Pythium* have been inconsistent and resulted in low incidence of disease; modifications to these tests are currently being considered.

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 ultimately devise management strategies.

Objectives

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

2. Test spinach cultivars for possible differences in susceptibility.

Procedures

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

A. Collections. 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.

In 2013, random isolates were saved from the UC Cooperative Extension diagnostic lab in Salinas. Disease incidence appeared to be reduced in 2013 and fewer diseased spinach samples were received.

B. Pathogenicity to spinach: Experiments are currently underway to test isolates for pathogenicity to spinach and to document whether these isolates are primary pathogens of spinach. *Pythium* is being tested using the method described by Martin (In: Methods for Research on Soilborne Phytopathogenic Fungi). Inoculum is prepared by mixing autoclaved field soil, sterilized bean leaves that have been dried and macerated, and water. *Pythium*-colonized agar plugs are added to this mixture and allowed to incubate at room temperature for 7 days. After incubation, the mixture is dried in a sterile transfer hood. This mixture is used to inoculate spinach seeds or seedlings. Subsequent tests will determine the optimum amount of inoculum to be used so that various spinach cultivars can be compared for susceptibility or tolerance to *Pythium*.

Pathogenicity of the *Fusarium* isolates will be tested by preparing a conidial suspension in water and drenching the roots of potted spinach seedlings. The intent here is also to optimize the level of *Fusarium* so that pathogenicity of isolates can be established while also seeking to discern differences in cultivar susceptibility.

C. Identification of isolate species: Gordon and Martin labs have made preliminary observations regarding *Fusarium* and *Pythium* isolates, respectively. Observations have been made based on general morphology and colony appearance.

2. Test spinach cultivars for possible differences in susceptibility.

Work on this objective will be based on the pathogenicity tests once inoculation protocols have been determined and optimized for *Pythium* and *Fusarium*. This objective is therefore awaiting the inoculation results and will be reported to the CLGRB at a later date.

Results and Discussion

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

A. Collections. From the 12 spinach fields surveyed in 2012, 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. Overall disease incidence in 2013 was reduced compared with 2012. A formal survey was not conducted in 2013 but isolates were periodically saved from spinach root rot cases submitted to the UCCE diagnostic lab.

B. Pathogenicity to spinach: Experiments are in progress. Initial root drench inoculations have been started for the *Fusarium* pathogenicity tests but results are not available at the time of this report. For *Pythium*, a series of different inoculations have been completed and include some of the following parameters: age of plant (seeds placed in inoculum or newly emerged seedlings planted into inoculum); concentration of inoculum (diluted with additional sterile soil or non-diluted); placement of inoculum (planting spinach seed directly into inoculum or overlaying inoculum onto seed); incubation conditions (soil kept under water saturated conditions or not saturated). To date the various inoculation experiments have not resulted in consistent damping-off or root rot disease. Disease has developed but at low incidences; such inoculations have also not been repeatable. For example, trial one with spinach seed placed directly into undiluted inoculum resulted in 10% infection while trial two using the same method resulted in no disease). We are producing a new batch of *Pythium* inoculum and will be repeating these inoculations.

C. Identification of isolate species: Preliminary species characterization has been done on selected isolates. *Pythium ultimum* appears to be the most commonly recovered species in the *Pythium* set. For *Fusarium*, it appears that *Fusarium oxysporum* is the most common species. Further identifications (based on both molecular and morphological analyses) will be completed following pathogenicity tests. Species confirmations have not yet been completed for the few *Rhizoctonia* isolates in the collection. However, it is very likely that these are isolates of *Rhizoctonia solani*.

2. Test spinach cultivars for possible differences in susceptibility.

Work on this objective will be based on the pathogenicity tests once inoculation protocols have been determined and optimized for *Pythium* and *Fusarium*. This objective is therefore awaiting the inoculation results and will be reported to the CLGRB at a later date.

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