PROGRESS REPORT TO THE OREGON PROCESSED VEGETABLE COMMISSION, 2000

TITLE: Pathogenicity of *Fusarium* isolates on sweet corn and effect of form of nitrogen on root rot development.

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PROJECT FUNDING: 11,700

SUMMARY OF WORK

- *Fusarium* accounted for 17% and 48% of fungi isolated from symptomatic corn roots grown in naturally infested field soil at 95% and 2 wk post-emergence, respectively. *Pythium, Trichoderma, Phoma* spp. and several unkown fungi were isolated in varying numbers.
- Some *Fusarium oxysporum* and *solani* isolates significantly reduced seedling height and dry weight in greenhouse pathogenicity tests.
- Some *Fusarum* isolates (18%) and non-*Fusarium* isolates (25%) caused necrosis of radicles in a germling assay. Three of the 6 non-*Fusarium* isolates were *Trichoderma* spp.
- Benomyl significantly increased seedling height and/or dry weight when applied alone or with metalaxyl as a soil drench to naturally infested field soil in a greenhouse study, but had no effect on isolation of *Pythium* or *Fusarium* from symptomatic roots.
- Metalaxyl effectively reduced isolation of *Pythium* from diseased roots in one of two field soils but had no effect on root necrosis in either soil.
- Ammonium versus nitrate form of nitrogen fertilizer had no effect on root necrosis or plant fresh weight in a greenhouse trial.
- Root rot severity was greater with Eradicane or Dual than with Frontier in a strip-till system.
- Strip versus conventional tillage had no effect on severity of root rot.

INTRODUCTION

It is clear that a biotic agent is the primary cause of root rot of sweet corn; however the identity of the organism or organisms involved has been difficult to determine. While *Fusarium oxysporum* and *solani* and *Pythium arrenhomanes* are

consistantly associated with diseased roots and *Pythium arrhenomanes* has been shown to be pathogenic on sweet corn seedlings in the greenhouse, other studies suggest neither fungus alone is the cause of this disease. In addition, the effect of various cultural practices on root rot development and severity is largely unkown. Experiments were conducted in 2000 to determine: 1) pathogenicity of *Fusarium* isolates and other fungi on sweet corn seedlings, 2) effect of soil treatments (fungicides and herbicides) on severity of root necrosis of corn grown in naturally infested field soil, and 3) effect of cultural practices (form of nitrogen and type of tillage) on suppression of root rot.

MATERIALS and METHODS

Fungal Isolations

To examine the possible role of other fungi in the root rot complex, isolations were made from roots of corn plants grown in the greenhouse in naturally infested field soil. Soil was collected from two fields with a history of root rot disease and placed in 550 ml soil tubes and then planted with surface disinfested sweet corn seeds, cv Golden Jubilee. Five plants per field soil were sampled at each of three times (95% emergence, 1 wk and 2 wks post-emergence). At each sample date rootballs were washed under running distilled water and 1-cm root pieces were surface disinfested before plating on agar media (water agar + streptomycin, cornmeal agar + streptomycin, or Nash-Snyder). Colonies growing from root pieces were transferred to pure culture and preliminary identifications were made.

Pathogenicity Tests

Seedling assay. Twenty-four *Fusarium oxysporum* and *solani* isolates collected from symptomatic corn roots in 1999 were examined in two greenhouse trials for pathogenicity on corn seedlings. Field soil was collected and steam pasteurized at 90C for 1 hr on each of two consecutive days. A cornmeal/sand inoculum mixture of each isolate was added to half of the pasteurized soil before being placed into soil tubes. The other half was nonifested and served as the control. Treatments were replicated five times. Surface disinfested sweet corn seeds, cv Golden Jubilee, were planted into the tubes. At 3 wk (trial 1) or 5 wk (trial 2) plants were harvested. Height was recorded and rootballs were washed and visually evaluated for disease. Ten 1-cm root pieces per plant were placed on Nash-Snyder medium for the isolation of fusaria. Shoots and roots were oven dried at 60C before weighing.

Germling assay. A germling assay was conducted to screen isolates of *Fusarium* and other fungi isolated from diseased corn roots in 2000 for pathogenicity on sweet corn. Three surface disinfested sweet corn seeds, cv Golden Jubilee, were placed in petri plates on sterile moist filter paper. Seeds were incubated at 32C until germinated. After radical emergence the radicle of each seed was either slit 2mm or not wounded. One 2mm plug of each isolate was placed on the radical at the point of emergence and site of wounding on all germlings for both wound treatments. Non-inoculated germlings served as the control. Plates were incubated at room temperature for 6 days. Germlings were evaluated for browning and/or necrosis of roots and reduction of fine roots compared to the control. A total of 34 *Fusarium* and 24 of other fungi were tested.

Soil Fungicide Tests

Agar medium assay. An agar medium assay was conducted to screen fungicides for activity against 10 isolates of *Fusarium*. Two fungicides with known activity against the fusaria, benomyl and fludioxonil, were added at four rates (0, 10, 50, or 100 ppm) to two types of agar (water or potato dextrose). Treatments were replicated five times. Radial growth was recorded over time. A similar test was conducted with metalaxyl on growth of *Pythium* and *Fusarium*.

Soil assay. A soil fungicide drench study was conducted in the greenhouse to examine the effect of metalaxyl and benomyl on seedling growth and root rot development. Naturally infested soil was collected from two fields with a history of corn root rot and placed into 550 ml soil tubes. Surface-disinfested seeds of sweet corn, cv Golden Jubilee, were planted in each. One of three fungicide treatments (benomyl, metalaxyl, or benomyl + metalaxyl) was applied as a drench to each tube. Treatments were replicated 10 times. Plants were kept watered and fertilized weekly with a water soluble plant food (15-30-15). At 1 mo height was recorded and plants were harvested. Roots were washed, evaluated for disease symptoms, and ten 1-cm long root pieces per plant were plated on both water agar and Nash-Snyder agar media for the isolation of *Pythium* and *Fusarium*, respectively. Roots and shoots were dried at 60C for 2 days, then weighed.

Form of Nitrogen Study

A study was conducted in the greenhouse to examine the effect of ammonium versus nitrate form of nitrogen fertilizer on sweet corn growth and root rot development. Naturally infested soil was collected from two fields with a history of disease and placed in 10L pots. Annual ryegrass was sown and grown for 2 mo to take up available nitrogen. After removing the ryegrass one of two fertilizer treatments (urea, 46-0-0 or urea + DCD, 46-0-0) was applied at 50 lb N/a and mixed into a band in the center of the pot. One sweet corn seed, cv Golden Jubilee, was planted in the center of each pot. Treatments were arranged in a randomized block design and replicated 10 times. Plants were kept watered, weeded, and P and K was applied in a water soluble plant food (0-10-10) every 3 wk. Nitrogen fertilizer (urea or urea + DCD) was added every 3 wk to each pot at 50 lb N/a and watered in. Soil was sampled at planting and every 3 wk until harvest and tested for ammonium and nitrate nitrogen.

At 13 wk plants were cut at the soil surface and weighed. Rootballs were washed and evaluated for disease symptoms and weighed.

Collaborative Studies

***Fusarium* pathogenicity assay**. A study examining the pathogenicity of several isolates of *Fusarium oxysporum* was done in collaboration with Dr. Cynthia Ocamb. Treatments were soil (field 1 or field 2), inoculum (- or +), and water (low or high). Treatments were replicated five times. Soil from two fields was collected and steam pasteurized at 90C for 1 hour on each of two consecutive days. Inoculum was mixed into half of the pasteurized soil at a rate of 4g/L. The infested and noninfested soils were placed in 550 ml soil tubes and tubes were placed on a greenhouse bench. Sweet corn

seeds, cv Golden Jubilee, were surface disinfested in 10% bleach for 5 min and rinsed in distilled water before planting and watering. Tubes in the low water treatment were allowed to dry out between watering and tubes in the high water treatment were kept moist throughout the study. All plants were fertilized weekly with a water soluble 15-30-15 at the recommended rate. At 5 wk plants were harvested. Plant height was recorded, rootballs were washed and evaluated for disease symptoms and shoots and roots were oven dried at 60C and weighed.

Herbicide study. A field study examining the effect of herbicides on root rot severity in a strip-tilled system was conducted in collaboration with Ed Peachey. Herbicide treatments were Round-Up, Eradicane, Frontier, Dual, and propane flaming. See Ed Peachey's report for herbicide timing and application procedures. At harvest three plants in each of four replicated plots per treatment were harvested. Rootballs were washed and visually evaluated for disease symptoms.

Tillage study. A field study examining the effect of strip- versus conventional tillage on root rot development was done in collaboration with Dr. John Luna, Mary Staben, and Dr. Cynthia Ocamb. Nine fields in the paired comparison trial (see report by Dr. Luna) were included in this study. Five plants per treatment (strip-till and conventional till) were randomly sampled in each field on three sample dates (5 and 9 wks post-planting and harvest). Rootballs were washed and visually rated for severity of disease symptoms.

ANALYSIS

Treatments were compared with analysis of variance and means were separated by Fischer's protected least significant difference.

RESULTS

Fungal Isolations

At emergence 29 fungal isolates (5 *Fusarium*, 1 *Pythium*, 6 *Trichoderma*, 3 *Phoma*, 1 *Penicillium*, 13 unknown) were isolated from the radicle. At 1 wk post-emergence, 5 *Fusarium*, 1 *Pythium*, 1 *Phoma*, and 5 unknowns of 3 types were isolated from radicles. At 2 wks post-emergence 19 *Fusarium*, 11 *Pythium*, 1 *Trichoderma*, 2 *Morteriella*, and 7 unknown isolates were isolated from lesions on radicles and other roots. All unknown fungi are currently being identified.

Pathogenicity Tests

Seedling assay. In the first greenhouse trial, none of the 8 *F. oxysporum* or 4 *F. solani* isolates significantly reduced plant height or dry weight (Table 1).

Table 1. Effect of *Fusarium* isolates on sweet corn height and dry weight, trial 1.

Isolate	Height (cm)	Dry weight (g)
1: Control	74.3	3.06
2: 9956-2b (*F. o.*)	72.8	2.86
3: 9980-8 (*F. o.*)	70.4	2.71
4: 9967-46 (*F. o.*)	69.6	2.82
5: 9966-4 (*F. o.*)	69.9	2.89
6: 9956-19 (*F. o.*)	69.7	2.73
7: 9986-7 (*F. o.*)	71.9	2.98
8: 9989-8 (*F. o.*)	69.2	2.65
9:9980-17 (*F. s.*)	70.1	2.94
10: 9956-1 (*F. s.*)	71.9	2.93
11: 9988-17 (*F. s.*)	73.6	2.86
12: 9958-11 (*F. s.*)	71.1	2.91
13: 9981-13 (*F. o.*)	74.4	3.09

In the second trial, 3 of 12 (25%) isolates of *F. oxysporum* significantly reduced both seedling height (16-25%) and dry weight (24-51%) compared to the non-infested control (Table 2).

Table 2. Effect of *Fusarium oxysporum* isolates on sweet corn height and dry weight, trial 2.

Isolate	Height (cm)	Dry weight (g)
1: Control	66.3	0.45
2: 9962-9	64.5	0.45
3: 9956-2	53.5*	0.34*
4: 9988-15	68.1	0.43
5: 9948-110	62.5	0.35
6: 9960-8	65.4	0.38
7: 9966-7	55.8*	0.30*
8: 9973-3	61.0	0.42
9: 9960-3	49.7*	0.22*
10: 9967-27	58.1	0.29*
11: 9962-2	62.7	0.37
12: 9962-12	64.8	0.40
13: 9958-6	58.0	0.29*

*$P \leq 0.05$ compared to control.

Germling assay. In the germling assay, 6 of the 34 (17.6%) *Fusarium* isolates caused extensive to severe necrosis of the radicle and finer roots. Wounding of germlings resulted in more severe necrosis compared to no wounding. Of the 24 non-*Fusarium* isolates, 6 (25%) caused severe necrosis along the radicle and reduced the number of fine roots. Of the 6 virulent isolates, three are *Trichoderma* spp. and the other three are currently being identified.

Soil Fungicide Tests

Agar assays. In the agar ammended assay, fludioxonil reduced the radial growth of *Fusarium* isolates on water agar 12-18% at 9 days. On potato dextrose agar growth was reduced 25-32%. Benomyl reduced the radial growth on water agar from 50-83% and on potato dexrose agar 2-65%, depending on the fungicide rate.

Metalaxyl had no effect on the radial growth of *Fusarium* whereas it effectively inhibited growth of *Pythium* 50-90% in water agar and 30-90% in potato dextrose agar.

Fungicide drench study. In the greenhouse fungicide drench study, the application of benomyl alone significantly increased plant height 6-12% in both field soils and plant dry weight 26% in field soil 1 (Table 3, Figs. 1 and 2). Metalaxyl alone significantly increased plant height 6% in both field soils but had no effect on dry weight. When applied together the fungicides significantly increased plant dry weight 20-23% in both soils and height 9% in soil 2.

Table 3. Effects of fungicide soil drench on sweet corn seedling growth.

Treatment	Height (cm)	Dryweight (g)
No fungicide		
Field soil 1	86.1	1.76
Field soil 2	85.3	1.78
Benomyl		
Field soil 1	96.1**	2.37**
Field soil 2	90.5*	2.0
Metalaxyl		
Field soil 1	90.8*	1.72
Field soil 2	89.6*	1.79
Benomyl + metalaxyl		
Field soil 1	90.5	2.16**
Field soil 2	93.2**	2.13*

**$P \leq 0.05$ compared to no fungicide treatment

*$P \leq 0.10$ compared to no fungicide treatment

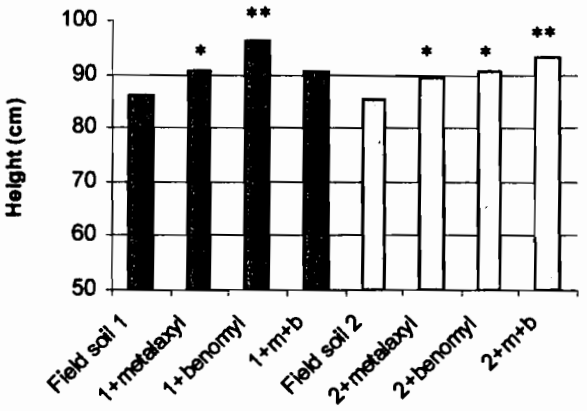

Figure 1: Effect of fungcides applied as a soil drench on seedling height of corn in two field soils (**$P \leq 0.05$, *$P \leq 0.10$ compared to no fungicide control).

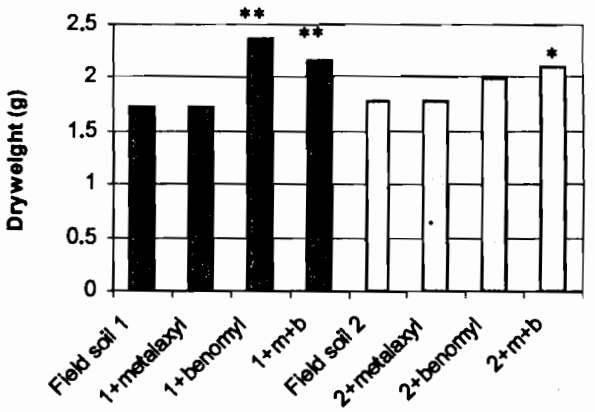

Figure 2: Effect of fungcides applied as a soil drench on seedling dry weight of corn in two field soils (**$P \leq 0.05$, *$P \leq 0.10$ compared to no fungicide control).

Rootballs exhibited extensive browning and scattered lesions in the no fungicide treatment in both soils. Metalaxyl alone had no effect on root necrosis. In contrast, when benomyl was applied alone or with metalaxyl, rootballs were generally healthier and had fewer scattered lesions than the no fungicide treatment.

Pythium spp. were consistantly isolated from roots in the no fungicide and benomyl alone treatments. With metalaxyl, *Pythium* spp. were isolated from 50% of plants in field soil 2 and none in soil 1. When both fungicides were added, *Pythium* spp. were isolated from 70% of plants in soil 2 and none in soil 1. Overall, metalaxyl controlled *Pythium* in field soil 1 but not in field soil 2. *Fusarium* spp. were isolated from all plants in all treatments.

Form of Nitrogen Study

Form of nitrogen fertilizer had no effect on root necrosis or plant fresh weight at harvest (13 wks). Necrosis of the rootball averaged 56-68% across fertilizer treatments.

Collaborative Studies

***Fusarium* pathogenicity study**. In the greenhouse study examining the pathogenicity of *F. oxysporum* isolates from Dr. Cynthia Ocamb, the inoculum effect differed significantly between soils ($P \leq 0.05$). In field soil 1, *F. oxysporum* significantly reduced seedling height 20-21% and dry weight 38-53% compared to the non-infested controls. In field soil 2, *F. oxysporum* significantly reduced seedling height 2-4% but not biomass.

Herbicide study. In a field study root necrosis varied signficantly among herbicide treatments in a strip-till system (Fig. 5). Disease was significantly more severe with Eradicane and Dual compared to Frontier. See Ed Peachey's report for other effects and correlations in this study.

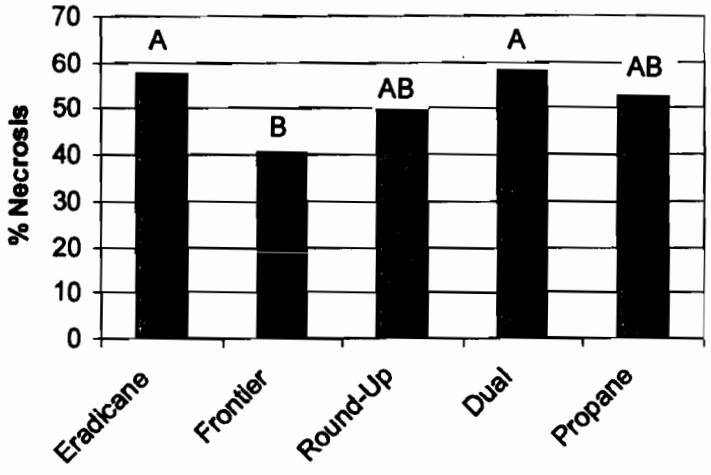

Figure 5: Effect of herbicides and propane flaming on root rot of sweet corn. Treatments with the same letter are not significantly different ($P \leq 0.05$).

Tillage study. In the field study examining the effect of strip- versus conventional tillage on severity of root rot, necrosis of the rootball did not differ significantly between treatments (see report by Luna and Staben for a comprehensive review of the study). Of the nine fields sampled at 5 wks post-planting, six were healthy and three showed initial stages of disease development. At 9 wks post-planting plants in all fields but one had symptoms of root rot. Data were collected for six of the fields at harvest (3 were dropped from the study, see report by Luna and Staben). Three fields were healthy at harvest (exhibited limited lesions and root system browning) and three were moderately diseased (percent necrosis of the rootball varied from 30-85).