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TITLE: A bioassay for studying biotic and abiotic interactions in the root rot syndrome of sweet corn.

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SUMMARY OF WORK

- ◆ Amount of root rot and plant biomass was proportional to the amount of soilborne inoculum when naturally infested soil was diluted with steam-pasteurized field soil.
- ◆ *Pythium arrhenomanes*, *Phoma* and an unidentified fungus, all isolated from diseased roots, caused significant root rot. Amount of root rot was proportional to amount of inoculum.
- ◆ *P. arrhenomanes* caused symptoms of root pruning and root tip necrosis. *Phoma* spp. and the unidentified fungus caused extensive radicle and nodal root necrosis. *Fusarium graminearum* caused pre-and post-emergent damping-off and limited root necrosis. *Trichoderma* spp. caused mesocotyl and radicle root necrosis. *F. oxysporum* caused limited root necrosis.
- ◆ Root rot was significantly greater and plant yield less in artificially infested soil (mixture of *P. arrhenomanes*, *F. oxysporum* and *graminearum*, *Phoma* and the unidentified fungus) compared to pasteurized soil (control).
- ◆ Relationship between kind of herbicide (Atrazine, Dual, Dual II, Frontier, Eradicane, RoundUp, none) and amount of inoculum on severity of root rot was significant in one field soil. Severity of root rot was less with Dual and Atrazine at their low rates than with Dual II, Frontier at the high rate, and Eradicane at the low rate in 10% naturally infested soil. In 100% naturally infested field soil, the no herbicide control, Frontier at the low rate, and RoundUp at both rates had significantly less root rot than Dual at the high rate and Dual II at the low rate.
- ◆ In a second field soil, the relationship between herbicides and amount of inoculum and herbicides alone on root rot severity was not significant ($P=0.19, 0.16$). However, root rot tended to be greater in all herbicide treatments compared to the control. In addition, root rot was generally greater with higher versus lower rates of each herbicide.

◆ INTRODUCTION

The root rot syndrome of sweet corn in the Willamette Valley involves several biotic agents. *Pythium arrhenomanes*, *Fusarium oxysporum* and *graminearum*, *Phoma* spp., *Trichoderma* spp., and an unknown fungus, all isolated from diseased roots, have been shown to cause root rot in greenhouse pathogenicity studies. Such pathogenicity studies are the first step in determining causality in disease systems. Another core principle of plant pathology is the inoculum dose/disease response relationship. This illustrates that disease is proportional to the amount of inoculum and supports the pathogenicity of an organism. Abiotic factors may also play a role in disease severity. In a greenhouse study *P. arrhenomanes* significantly reduced seedling biomass in all four herbicide treatments but not in the no herbicide control. In a field study, severity of root rot significantly differed among herbicides tested. To understand the single and interacting effects of these different factors a bioassay can be useful. This approach also allows rapid screening of potential management strategies.

The development of an appropriate bioassay must be based on the natural system of interest. In the field, root rot of sweet corn is characterized by lesions occurring as early as 3 weeks post emergence on the radicle, or primary root, followed by scattered lesions developing on nodal roots. The mesocotyl, or subcrown internode, may or may not become necrotic. A disease rating system was developed which evaluates each of these components of the root system (radicle, nodal roots, mesocotyl) (Table 1).

Table 1. Disease rating system for the three components of the root system, for a total of 10 possible points.

Mesocotyl		Radicle		Nodal root system	
0	Healthy	0	Healthy	0	Healthy
1	Lesion present	1	Lesion present	1	5-10% necrotic
2	100% necrotic	2	10-50% necrotic	2	11-25% necrotic
		3	50-99% necrotic	3	26-50% necrotic
		4	100% necrotic	4	>51% necrotic

This system is appropriate for evaluating root rot of seedlings and of mild to moderately diseased rootballs of mature plants. For plants grown full season which exhibit severe root rot the rating system can be expanded to evaluate the entire rootball.

Using this rating system, experiments were conducted in 2001 to examine: 1) the inoculum dose/disease response relationship in naturally infested field soil, 2) the inoculum dose/disease response relationship of known individual root rot pathogens, 3) the effect of a pathogen mixture on root rot severity, and 4) the relationship between amount of inoculum and kind of herbicide on root rot severity in naturally infested soil.

MATERIALS AND METHODS

Inoculum dose/disease response relationship.

Soil was collected from two fields with a history of root rot and a portion of it was steam pasteurized at 90C for 1 hr on each of two consecutive days. Naturally infested soil was diluted with the steam pasteurized soil to 0, 1, 10, 33, 50, or 100% and placed in 550 ml

soil tubes. Treatments were arranged on a greenhouse bench in a complete randomized design and replicated 10 times. Surface disinfested sweet corn seeds, cv Golden Jubilee, were planted into the tubes. Tubes were kept moist and fertilized once weekly with a water soluble plant food at the label rate. Emergence was taken and seed and seedling decay was recorded for each treatment. At 6 wks plants were harvested and rootballs were evaluated for rot. Shoots and roots were oven dried at 60C and weighed.

Pathogenicity studies.

A pathogenicity test was conducted to examine the inoculum dose/disease response relationship of *P. arrhenomanes*, *Phoma* spp., *F. oxysporum* and *graminearum*, *Trichoderma* and an unidentified fungus, all isolated from diseased corn roots. A Chehalis sandy loam was collected and steam pasteurized at 90C for 1 hr on each of two consecutive days. A cornmeal/sand inoculum mixture of each pathogen was added at 4 rates (1x, 2x, 10x, 100x) and placed in 550 ml soil tubes. Pasteurized soil served as the control. Treatments were arranged on a greenhouse bench in a complete randomized design and replicated 10 times. Surface disinfested sweet corn seeds, cv Golden Jubilee, were planted into the tubes. Tubes were kept moist and fertilized once weekly with a water soluble plant food at the label rate.

At 6 wks plants were harvested and rootballs were evaluated for rot. Lesions on roots were surface disinfested and plated onto water agar for recovery of the pathogens. Shoots and roots were oven dried at 60C and weighed.

Pathogen mixture.

Soil from a field with a history of root rot was collected and two-thirds of it steam-pasteurized at 90C for 1 hr on each of two consecutive days. A pathogen mixture (*P. arrhenomanes*, *Phoma*, *F. oxysporum* and *graminearum*, and the unidentified fungus) was added to half the pasteurized soil. Soil for all treatments (naturally infested, artificially infested, uninfested) was placed in 20 L pots and treatments were arranged in the OSU Greenhouse courtyard in a complete randomized design and replicated 10 times. Surface disinfested sweet corn seeds, cv Golden Jubilee, were planted two per pot. Pots were kept moist and fertilized once weekly with a water soluble plant food at the label rate. Emergence was recorded and at two weeks pots were thinned to one plant.

At 14 wks ears were harvested and weighed. Plants were cut at the soil surface, oven dried at 60C and weighed. Rootballs were washed and evaluated for rot. Five 1-cm symptomatic root pieces per five plants per treatment were surface disinfested and plated on water agar for isolation of pathogens.

Herbicide study.

Soil was collected from two fields with a history of root rot and a portion of the soil was steam pasteurized at 90C for one hour on each of two consecutive days. Naturally infested soil and steam pasteurized soil were mixed to obtain four percentages of naturally infested soil (0, 1, 10, 100%) and placed in 550 ml soil tubes. Surface disinfested sweet corn seeds, cv Golden Jubilee, were planted into the tubes. Herbicides (Atrazine, Dual, Dual II, Frontier, Eradicane, RoundUp) were applied in water at two rates (Table 2). A water only treatment served as the control. Treatments were arranged on a greenhouse bench in a

complete randomized design and replicated five times. At 6 wks plants were harvested and roots evaluated for rot.

Table 2. Rates of herbicides.

	lbs ai/A	
	low	high
Atrazine	1	2
Dual Magnum	0.65	1.3
Dual II Magnum	0.65	1.3
Frontier	0.6	1.2
RoundUp	0.88	1.76
Eradicane	1.47	2.95

ANALYSIS

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

RESULTS

Inoculum dose/disease response relationship.

The percent of seed and seedling decay increased significantly as amount of inoculum increased for both field soils ($P \leq 0.05$) (Figure 1).

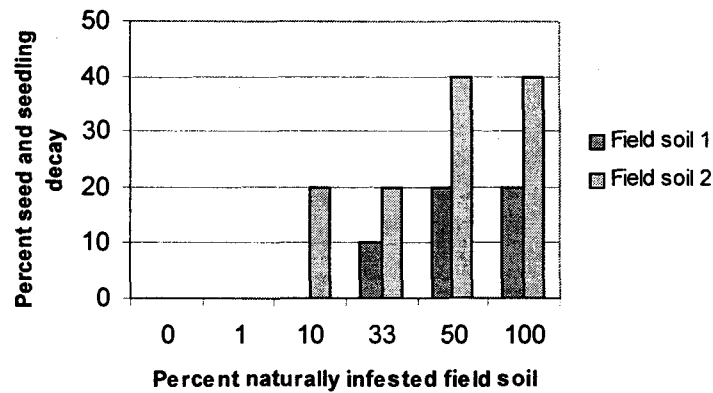


Fig. 1. Effect of soil inoculum on seed and seedling decay in two field soils.

The amount of root rot and plant biomass was proportional to the amount of inoculum for both field soils (Figs. 2 and 3). For both soils, all treatments containing natural inoculum had significantly greater root rot than the 0% (steam pasteurized control) ($P \leq 0.05$). Plant biomass was significantly less in the 100% infested soil compared to the steam pasteurized

control in field soil 1, whereas for field soil 2 biomass was significantly reduced in 33, 50 and 100% naturally infested soil ($P \leq 0.05$).

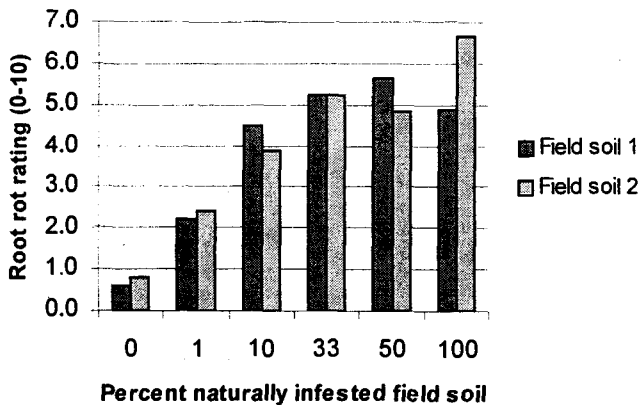


Fig. 2. Effect of soil inoculum on root rot in two field soils.

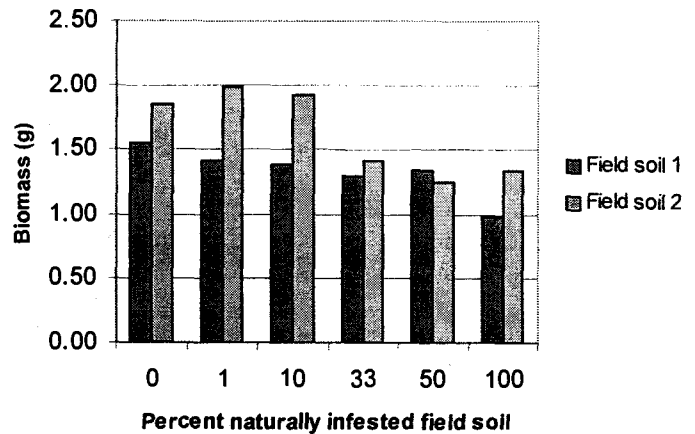


Fig. 3. Effect of soil inoculum on plant biomass in two field soils.

Pathogenicity studies.

With an increase in amount of inoculum of *P. arrhenomanes*, *Phoma*, and the unidentified fungus, the severity of root rot increased ($P \leq 0.05$) (Figs. 4-6). Amount of inoculum of *Trichoderma* and *F. oxysporum* and *graminearum* had no effect on severity of root rot (see Fig. 7 for *F. oxysporum*. Other data not shown). *P. arrhenomanes* caused symptoms of root pruning and root tip necrosis. *Phoma* and the unidentified fungus caused extensive radicle and nodal root necrosis. *F. graminearum* caused mesocotyl necrosis and *Trichoderma* caused mesocotyl and radicle necrosis. *F. oxysporum* caused very mild symptoms of root necrosis. All organisms were recovered from root lesions.

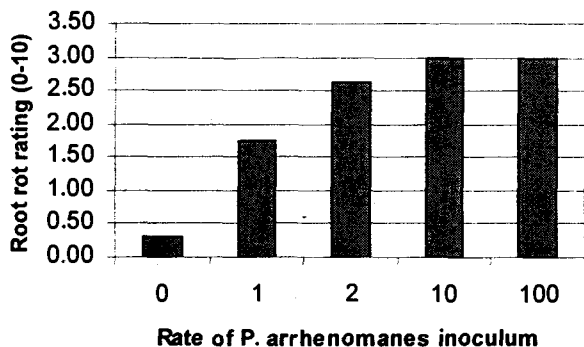


Fig. 4. Effect of amount of *P. arrhenomanes* on root rot.

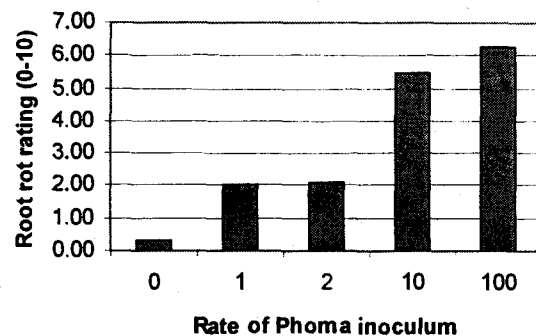


Fig. 5. Effect of amount of *Phoma* on root rot.

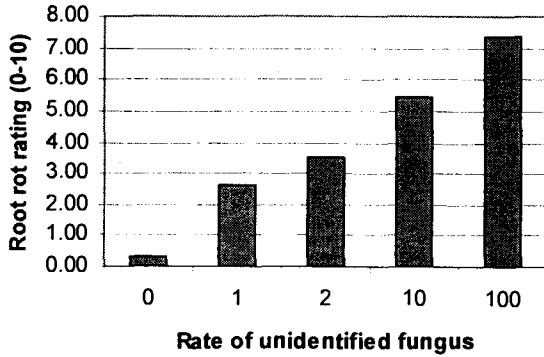


Fig. 6. Effect of amount of unidentified fungus on root rot.

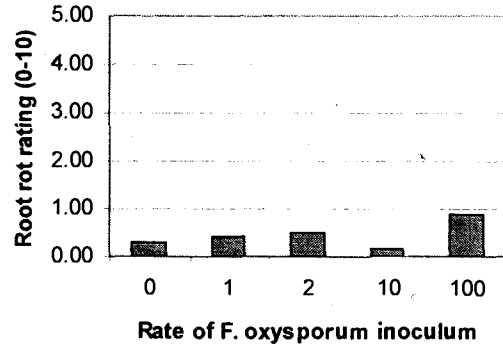


Fig. 7. Effect of amount of *F. oxysporum* on root rot.

Pathogen mixture.

At 1 wk post-planting, plants grown in artificially or naturally infested soil were at 15% emergence versus 35 for plants grown in pasteurized soil (Fig. 8). The rate of emergence was also slower in naturally infested soil compared to the pasteurized control.

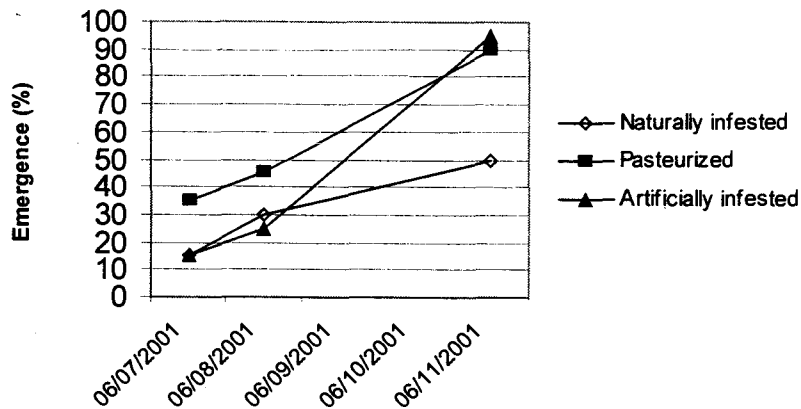


Fig. 8. Effect of inoculum (natural or artificial) on plant emergence.

At harvest, root rot was significantly greater and yield and plant biomass less in artificially and naturally infested soil compared to pasteurized soil (control) ($P \leq 0.05$) (Fig. 9-11).

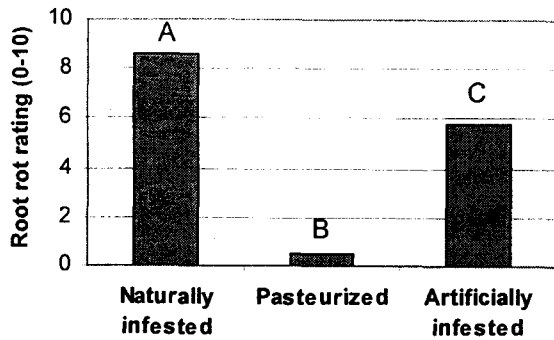


Fig. 9. Effect of natural and artificial inoculum on root rot.

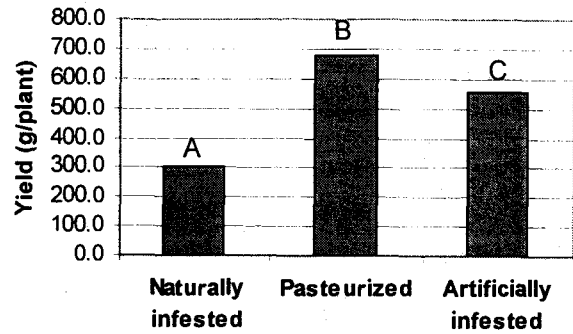


Fig. 10. Effect of natural and artificial inoculum on yield.

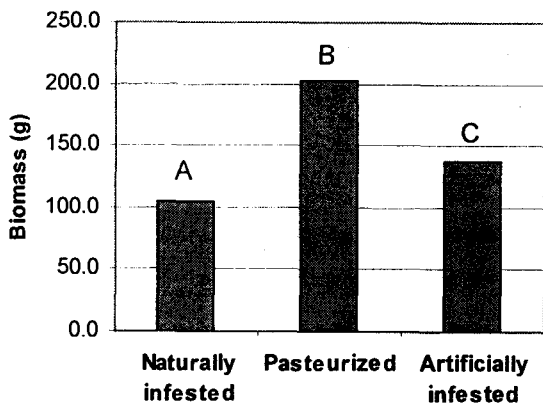


Fig. 11. Effect of natural and artificial inoculum on plant biomass.

Herbicide study.

The relationship between kind of herbicide and amount of inoculum on severity of root rot was significant for field soil 1 ($P \leq 0.01$). At 0 and 1% naturally infested soil, root rot did not differ among herbicides tested. However, as the amount of inoculum increased, severity of root rot differed significantly among herbicides ($P \leq 0.05$) (Figs. 12 and 13). Severity of root rot was less with Dual and Atrazine at their low rates than with Dual II, Frontier at the high rate, and Eradicane at the low rate in 10% naturally infested soil. In 100% naturally infested field soil, the no herbicide control, Frontier at the low rate, and RoundUp at both rates had significantly less root rot than Dual at the high rate and Dual II at the low rate. This is similar to a field study conducted in 2000 with Ed Peachey in which disease was significantly greater with Eradicane and Dual compared to Frontier.

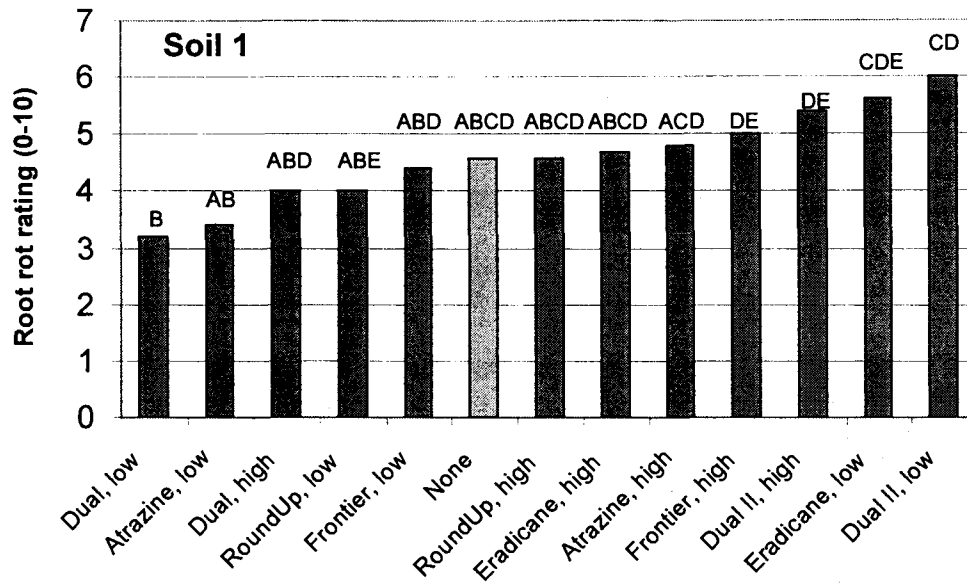


Fig. 12. Effect of herbicides on root rot in 10% naturally infested field soil.
Treatments with the same letter are not significantly different ($P \leq 0.05$).

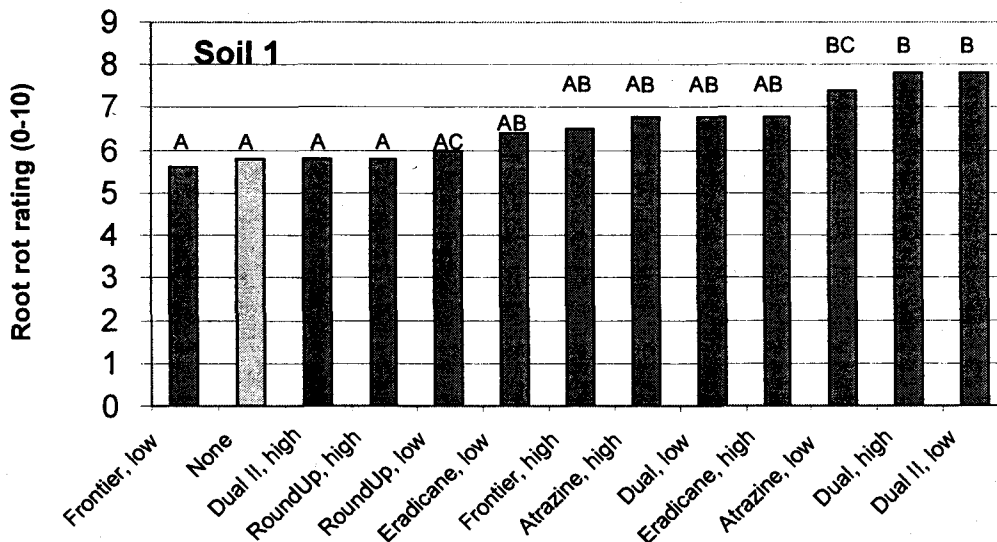


Fig. 13. Effect of herbicides on root rot in 100% naturally infested field soil.
Treatments with the same letter are not significantly different ($P \leq 0.05$).

In field soil 2, the relationship between herbicides and amount of inoculum and herbicides alone on root rot severity was not significant ($P=0.19, 0.16$). However, root rot tended to be greater in all herbicide treatments compared to the control (Fig. 14). In addition, root rot was generally greater with higher versus lower rates of each herbicide.

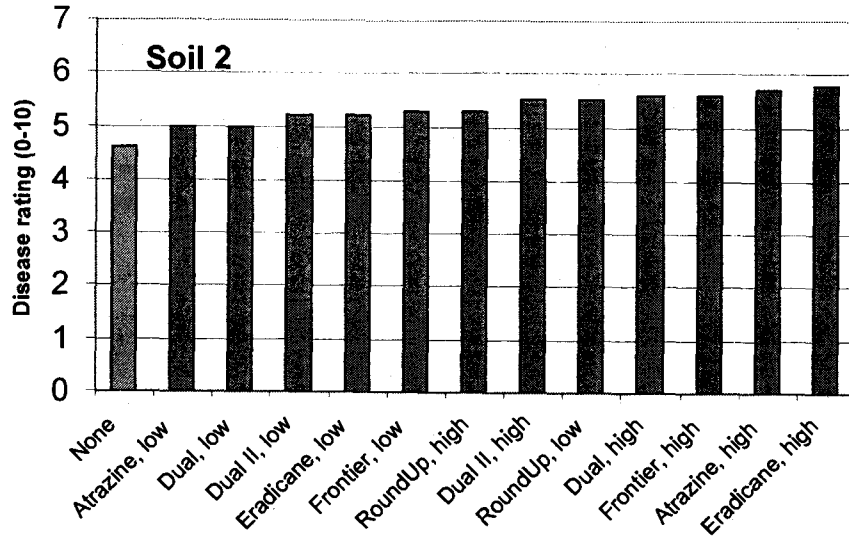


Fig. 14. Effect of herbicides on root rot.

In fields where disease has been severe (i.e. the amount of inoculum is high), kind and rate of herbicide can significantly exacerbate the severity of disease. In fields with low amounts of inoculum, the interaction between pathogen and kind and rate of herbicide is not significant.