Progress Report to the North American Strawberry Growers Association
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Title: INTERACTION AND BIOLOGICAL CONTROL OF THE STRAWBERRY BLACK ROOT ROT PATHOGENS *PYTHIUM* AND *RHIZOCTONIA*

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Rationale

Black root rot (BRR) is a widespread disease of perennial matted-row strawberries, and becomes an increasing problem as plantings age (Maas, 1998). BRR primarily affects perennial and feeder roots, rather than the plant crown (Wing et al, 1994). Roots affected by BRR have one or more of the following symptoms: 1) much smaller root systems than normal, 2) main roots are spotted with dark patches or zones, 3) most feeder roots are lacking, 4) all or part of the main root is dead and blackened throughout. Affected plants are less vigorous and produce few runners (Funt et al, 1997). Severely infected plants may die (Funt et al, 1997). Various fungi, nematodes and abiotic factors have been implicated in BRR development (LaMondia and Martin, 1989; Wing et al., 1995). The pathogens most frequently isolated from affected roots are *Pythium* and *Rhizoctonia* spp. (especially *P. ultimum* and *R. fragariae*), and the root lesion nematode, *Pratylenchus penetrans.* Other fungi, such as *Idriella lunata* and a gray sterile fungus have also been associated with BRR symptoms (Maas, 1998). The composition of the pathogen complex is influenced by soil type and environmental conditions. *Pythium* spp. reportedly predominate in sandy soils and *Rhizoctonia* spp. in clay soils (Maas, 1998). Lower temperatures tend to favor *Pythium* (Wing et al., 1994). Nematodes also play an important role in BRR, as these organisms restrict root growth by feeding directly on roots and make them more susceptible to root rotting fungi. Isolations from strawberry sites in Michigan have yielded *Pythium* and *Rhizoctonia* spp., *Idriella lunata*, and low levels of *P. penetrans* in the past. While the interaction of the lesion nematode with *Rhizoctonia* in black root rot development has been elucidated (LaMondia and Martin, 1989), the interaction between *Pythium* and *Rhizoctonia* has not. In a study on peas, inoculation with *Rhizoctonia solani* (a relative of *R. fragariae*) and *Pythium ultimum* resulted in decreased disease over either pathogen applied alone, suggesting competition between these pathogens (Xi et al., 1995). It is also possible that when both are present, they are synergistic and cause more disease than either would alone, as was suggested in a study on root rot of Alstroemeria (Chang et al., 1994). An understanding of this interaction may help to elucidate the disease development process when both fungi are present at a site, and whether control measures need to be targeted at both fungi.

In annual strawberry production systems, methyl bromide is always used for the control of BRR and other soilborne diseases, such as Verticillium wilt, whereas in perennial systems, methyl bromide fumigation is less ubiquitous (Maas, 1998). Since methyl bromide was found to be a potent ozone depletor, production is scheduled to be phased out in 2001 and use by 2005 (Ristaino & Thomas, 1997). Biological control alternatives have been tested for the control of soilborne diseases of many crops. Experiments with inoculated potted plants in Michigan showed promising results with various biocontrol agents, particularly *Trichoderma* species (Schilder and Olatinwo, unpublished). One commercial product, RootShield, is labeled for use in strawberries. While the label lists protection against multiple soilborne pathogens, including *Pythium* and *Rhizoctonia*, it is not know to what extent it protects against *Pythium* spp. and *Rhizoctonia fragariae* prevalent in strawberry. Variable results obtained with RootShield in potted-plant experiments with different soil inoculations (naturally infested vs. inoculated) suggest that RootShield is perhaps more effective against one pathogen than the other (Schilder and Olatinwo, unpublished). Adding a registered fungicide (Quadris) that is labeled in strawberries for control of *Rhizoctonia* as a drench may enhance activity of the biocontrol agent. On the other hand, it needs to be confirmed that Quadris does not reduce the activity of RootShield by killing the *Trichoderma* fungus or is in itself damaging the strawberry roots. The activity of RootShield in the absence of disease also needs to be studied as it may boost growth or may actually be in some way detrimental to strawberry roots.

Objectives

The specific objectives were to:

1) Study the interaction of *Pythium ultimum* and *Rhizoctonia fragariae* in disease development
2) Determine the efficacy of Quadris and Plantshield against *Pythium* and *Rhizoctonia*

Procedures

1) *Study the interaction of Pythium and Rhizoctonia isolates in disease development*

The trial was conducted in the greenhouse using potted plants of cv. Allstar, which is susceptible to black root rot. Runner plants obtained from mother plants from Nourse farms were used for the experiments. The runner plants had been rooted in sterile potting mix prior to the experiment. Steam-sterilized potting soil (sandy loam) was inoculated with fungal mycelium of *Pythium* spp. and *Rhizoctonia fragariae* at planting. These isolates were collected from strawberry fields in Michigan and selected based on prior screening for virulence on detached strawberry roots. More mycelium (100 mg compared to 20 mg) was used per pot in the second experiment because the runner plants had larger root systems. The fungal cultures were grown in liquid potato broth for two weeks, blotted dry between sterile filter paper, and weighed. The mycelium was blended in sterile distilled water. The isolates were applied alone and in combination. Untreated pots served as a control.

The trials were set up in a completely randomized design with nine replications (one pot per replication) in the first experiments and five replications in the second experiment. After 3 months, the plants were removed and the soil was washed off the roots in the first experiment. The second experiment is still in progress. However, the fifth replication was harvested and evaluated after 6 weeks of incubation. The percentage of the roots with lesions was visually estimated in both experiments. Dry root weight and shoot weight were determined by drying plant parts in an oven. Data from the first experiment were analyzed using the StatGraphics statistical program. Data from the second experiment could not yet be analyzed because they were not replicated.

2) *Determine the efficacy of Quadris and Plantshield against Pythium and Rhizoctonia*

The trial was conducted in the greenhouse using potted plants of cv. Allstar, which is susceptible to black root rot. Runner plants obtained from mother plants from Nourse farms were used for the experiments. The runner plants had been rooted in sterile potting mix prior to the experiment. Steam-sterilized potting soil (sandy loam) was inoculated with fungal mycelium of *Pythium* spp. and *Rhizoctonia fragariae* at planting. These isolates were collected from strawberry fields in Michigan and selected based on prior screening for virulence on detached strawberry roots. More mycelium (100 mg compared to 20 mg) was used per pot in the second experiment because the runner plants had larger root systems. The fungal cultures were grown in liquid potato broth for two weeks, blotted dry between sterile filter paper, and weighed. The mycelium was blended in sterile distilled water. The isolates were applied alone and in combination. Untreated pots served as a control. Each treatment or combination was also treated with the fungicide Quadris (Abound: azoxystrobin) or PlantShield (a biocontrol fungus: *Trichoderma harzianum*) as a drench one day after inoculation. Treatments were done at rates recommended on the label. The trials were set up in a completely randomized design with four replications per treatment. This experiment is still in progress. The percentage of the roots with lesions will be visually estimated and dry root weight and shoot weight determined. Data will be analyzed using the StatGraphics statistical program.

Results and discussion

1) Study the interaction of Pythium and Rhizoctonia isolates in disease development

In the first experiment, the plants that were treated with *Pythium* alone, *Rhizoctonia* alone, and a combination of *Pythium* and *Rhizoctonia* had significantly fewer healthy roots (and concomitantly more diseased roots) than the untreated plants (Table 1). The plants that received both *Pythium* and Rhizoctonia tended to have a higher percentage diseased roots than plants with either pathogen alone, but this difference turned out not to be statistically significant. It was noted that the untreated plants also appeared to have root lesions, although care was taken not to splash soil or water between plants. This may be due to incomplete steam sterilization of the potting soil mix.

Dry root weight was significantly lower than the untreated control in the *Rhizoctonia* and the combined-pathogen treatment. However, the root weight in the combined-pathogen treatment was significantly less than when treated with *Rhizoctonia* alone. There were no significant differences in the weight of the foliage and runners between the treatment, though a trend towards lower shoot weight was also evident.

Table 1. Effect of root pathogens alone and in combination on root health and plant weight (Experiment 1)

Treatment[a]	**% Healthy roots**	**% Diseased roots**	**Dry weight root (g)**	**Dry weight foliage + runners (g)**	**Total dry weight (g)**
Untreated	71.9 a	28.1 a	1.24 a	32.1	33.3
Pythium	44.1 b	55.9 b	0.94 ab	26.5	27.4
Rhizoctonia	46.7 b	53.3 b	0.88 b	29.4	30.2
Pyth + Rhiz	37.8 b	62.2 b	0.51 c	21.8	22.3
P-value	0.011	0.011	0.003	0.122 (NS)	0.101 (NS)

[a]Values are averages of 9 replications. Data were collected 3 months after planting.

In the second experiment (Table 2), similar trends were seen after 6 weeks, although no statistical analysis could be done because of a lack of replication at this stage. The other four reps will be harvested and evaluated after 3 months of incubation.

Table 2. Effect of root pathogens alone and in combination on root health and plant weight (experiment 2).

Treatment	**Total # of roots**	**# Healthy roots**	**# Roots with lesions at tip**	**# Roots with intercalary lesions**	**% Diseased roots**	**Dry weight root (g)**	**Dry weight foliage (g)**	**Total dry weight (g)**
Untreated	57	46	1	10	19.3	2.7	12.1	14.8
Pythium	46	38	0	8	17.4	2.9	10.9	13.8
Rhizoctonia	48	39	5	4	18.8	3.0	8.7	11.7
Pyth + Rhiz	36	25	7	4	30.6	2.3	10.4	12.7

[a]Values are from one replication. Data were collected 6 weeks after planting.

3) *Determine the efficacy of Quadris and Plantshield against Pythium and Rhizoctonia*

This experiment is still in progress. Due to the departure of the postdoctoral associate who was doing this research in the middle of the season, we were short-staffed and started the trial rather late. Results will be reported in the final report.

Conclusion

Pythium spp. and *Rhizoctonia fragariae* each increased the percentage of roots with lesions and reduced root weight (*Rhizoctonia* a little more than *Pythium*). Overall plant weight was not significantly reduced after three months but this difference might have been more pronounced if the experiment would have been continued for a longer period. A cocktail of both pathogens was worse than either pathogen alone, but not double that of the individual contributions. This means that the pathogens are not synergistic, i.e. the damage is not more than the damage of both pathogens added together. Instead the damage is additive, but not fully additive. An explanation could be that many of the same root sections are colonized by each fungus or that the pathogens actually compete with each other and are therefore less effective.

References cited

Chang, K.F., Barr., D.J.S., Hwang, S.F., and Mirza, M. 1994. The effect of interactions between Fusarium, Rhizoctonia, and Pythium on root and rhizome rot of Alstroemeria. Z. Pflanzenkr. Pflanzensch. 101: 460-466.

Funt, R., Ellis, M.A., and Welty, C (eds). 1997. Midwest Small Fruit Pest Management Handbook. Bulletin 861, Ohio University, Columbus.

Kurppa, S., and Vrain, T.C. 1989. Effects of *Pratylenchus penetrans* on the infection of strawberry roots by *Gnomonia comari*. Journal of Nematology 21: 511-516.

LaMondia, J.A. and Martin, S.B. 1989. The influence of *Pratylenchus penetrans* and temperature on black root rot of strawberry by binucleate *Rhizoctonia* spp. Plant Disease 73: 107-110.

Maas, J.L. (ed) 1998. Compendium of strawberry diseases, 2nd edition. APS Press, St. Paul, MN. 98 pp.

Ristaino, J.B., and Thomas, W. 1997. Agriculture, methyl bromide, and the ozone hole: can we fill the gaps? Plant Disease 81: 964-977.

Xi, K., Stephens, J.H.G., Hwang, S.F. 1995. Dynamics of pea seed infection by Pythium ultimum and Rhizoctonia solani: effects of inoculum density and temperature on seed rot and pre-emergence damping off. Can. J. Plant Pathol. 17: 19-24.