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Effect of Drenching Soil with Benomyl, Propiconazole and Fluazinam on Incidence of Disappearing Root Rot of Ginseng

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Abstract: Three fungicides, Orbit (propiconazole), Benlate (benomyl) and ASC-66835 (fluazinam), were tested as soil drenches to control disappearing root rot (DRR) of ginseng (*Panax quinquefolius*) in gardens artificially infested with *Cylindrocarpon destructans*. The incidence of DRR was low (0~3.5%) in uninfested plots and significantly higher in infested plots (2.6~19.9%). Significant reductions in the incidence of DRR were observed in 1-year-old and 2-year-old gardens that were treated and assessed for disease in the same year. Significant control was not obtained in 3-year-old gardens treated and assessed in the same year, or in 1-year-old or 2-year-old gardens assessed in the year following infestation. Disease incidence was significantly reduced by 49~77% by low and high rates of benomyl (45 and 1,250 mg a.i./L) and propiconazole (10 and 40 mg a.i./L) and by fluazinam at 150 mg a.i./L. These fungicides seem to be worthy of further investigation as soil drenches to control DRR of ginseng.

Key words: Ginseng, *Panax quinquefolius*, disappearing root rot, *Cylindrocarpon destructans*, disease control, soil drench, benomyl, propiconazole, fluazinam.

Introduction

Disappearing root rot is a major factor limiting production of ginseng worldwide.[1] A diseased root is worthless or extensively devalued. Thus, economic loss is directly related to incidence of diseased roots. Incidence of the disease increase in each successive year.[2] Thus it is important to limit the introduction and spread of the causal agent, *Cylindrocarpon destructans* (Zinssmeister) Scholten. Little information is available on the epidemiology and control of disappearing root rot, despite many reports on the pathogenicity and cultural requirements of the causal agent.[3-9] Fumigation of soil before planting may reduce populations of the fungus but is often ineffective in controlling the disease.[10] Brammall (1994) also noted that the disease is favoured by high plant densities and possibly by soil pH above 6.5.[10] There are no reports on the efficacy of fungicides for controlling disappearing root rot and no fungicides are registered for control of the disease. Benlate (benomyl), Orbit (propiconazole) and ASC-66835 (fluazinam) have been identified as highly toxic to *C. destructans in vitro*.[11] The objective of this study was to test the efficacy of these fungicides in controlling disappearing root rot of ginseng in the field.

Materials and Methods

1. Establishment of experiments

Eight experiments were established at the Southern Crop Protection and Food Research Centre of Agriculture and Agri-Food Canada, Delhi, Ontario (latitude 42° 52', longitude 80° 33', elevation 228 m). Variables examined were fungicidal product, concentration of fungicide, and age of garden when infested. Stratified ginseng (*Panax quinquefolius* L.) seed was precision seeded in raised beds in the fall. Beds were 1.65 m wide and contained 16 rows 10 cm apart sown at the rate of 250 seed/m^2. Oat straw mulch was applied to the beds after seeding to a depth of 10~15 cm. This mulch compacted to 5~7 cm by the following spring and to 4~6 cm by the second spring. Straw was added after two of ginseng growth to maintain a mulch depth of 4~6 cm during the third and fourth years. Garden age refers to years after sowing seed. Years 1, 2, and 3 refer to 1-year-old, 2-year-old, and 3-year-old gardens, respectively. Experiments were considered to be established when plots were first infested. Experiments 1, 2, and 3 tested fungicides in 1-year-old, 2-year-old and 3-year-old gardens, respectively. Each of these experiments was conducted in 1994 (1A, 2A, and 3A) and repeated in 1995 (1B, 2B, and 3B). Experiments 1A, 2A, and 3A were infested, drenched, and assessed in 1994 and experiments 1B, 2B, and 3B were infested, drenched, and assessed in 1995. In experiment 4, the garden was infested in year 1 (1994), drenched in years 1 and 2 (1995), and assessed in year 2. In experiment 5, the garden was infested in year 2 (1994), drenched in years 2 and 3 (1995), and assessed in year 3. These details are summarized in Table 1.

2. Inoculum

Four-year-old ginseng roots were harvested in early spring 1994 and 1995, washed, and dried. Cubes 1 cm×1 cm×1 cm cut from the roots were placed into 500-mL beakers at the rate of 200 cubes perbeaker. The beakers were covered with aluminium foil and autoclaved for 20 minutes at 121°C, then autoclaved again 48 h later. Cultures of *C. destructans* isolate DRS265, obtained from ginseng in Ontario, were grown at 20°C for 10 days on SNAY agar medium.[12] Conidia were washed with sterile distilled water from the surface of the cultures and the suspension was adjusted with the aid of a haemacytometer to contain 10^6 conidia per mL. Twenty mL of suspension was added to each beaker. The beakers were incubated at 20°C in the dark for 14 days and were shaken by hand every few days to ensure that the cubes remained separate and became uniformly colonized by the fungus.

3. Infestation

In the year in which an experiment was established (either 1994 or 1995) plots were infested with colonized root cubes on 1 June. To infest a plot, one colonized root cube was placed in each of six holes and covered with soil. Holes were 3 cm deep and spaced 18 cm apart in two rows of three mid-way between the 5th and 6th plant rows from each short side of the plot. The central hole in each row was located 30 cm from the long side of the plot and 48 cm from the short side.

4. Fungicides

Three fungicides were tested: benomyl (Benlate 50WP, Dupont de Nemours, Mississauga, Ontario) at 45 and 1,250 mg a.i./m^2, propiconazole (Orbit 41.7%, Ciba-Geigy Canada, Cambridge, On-

Table 1. Age of ginseng gardens used in eight experiments and years when infested with *C. destructans*, treated with fungicide, and assessed for incidence of disappearing root rot

Experiment	Year plot infested	Year(s) plot treated with fungicide	Age of garden when experiment established (years)	Year disease assessed
1A	1994	1994	1	1994
1B	1995	1995	1	1995
2A	1994	1994	2	1994
2B	1995	1995	2	1995
3A	1994	1994	3	1994
3B	1995	1995	3	1995
4	1994	1994, 1995	1	1995
5	1994	1994, 1995	2	1995

tario) at 10 and 40 mg a.i./m^2, and fluazinam (ASC-66835 50WP, ISK Biosciences, London, Ontario) at 100 and 150 mg a.i./m^2. These fungicides were chosen because of their formulation for soil drenching and for their high toxicity to mycelial growth of *C. destructans in vitro.*[11] The high rates were based on industry assessments of rates that would be effective, practical, and reasonable in applications for registration. The low rate of propiconazole was set arbitrarily at one-quarter of the high rate. The high rates of benomyl and propiconazole, determined by industry expectations, differed considerably from one another. Therefore, because these fungicides had similar toxicities to *C. destructans in vitro*, it was decided to set the low rate of benomyl close to the high rate of propiconazole. Finally, since fluazinam was less toxic than the other two active ingredients *in vitro*, its low rate was set higher than the low rates used for the other two fungicides. Fungicides mixed in water were sprayed with a backpack sprayer at the rate of 0.5 L of fungicide mixture per plot two (15 June) and eight (27 July) weeks after plots were infested. An additional 0.34 L of water, referred to as a water drench, was sprayed onto plots immediately after the fungicide was applied. The total process of spraying plots with fungicide and then with additional water is referred to as a fungicidal drench. The fungicide and additional water were applied directly to the surface of the straw mulch. Experiments 1A, 1B, 2A, 2B, 3A, and 3B were treated with fungicide in the year of establishment only. Experiments 4 and 5 received two drenches of fungicide in the second year also. Three controls not receiving fungicide were used: (1) uninfested, (2) infested, and (3) infested and drenched with 0.34 L of water. The uninfested control enabled us to determine the background incidence of disappearing root rot. The infested control drenched with 0.34 L of water served as the check for the fungicide treatments, and, by comparison with the infested control not receiving the additional 0.34 L of water, enabled us to determine the effect of the water drench on disease.

5. Experimental design

Individual plots were 1.65 m wide and 0.6 m long (1 m^2) and spaced 0.15 m apart in the bed. Experiments 1A and 4 were accommodated in the same two beds. Two repetitions of each treatment and control were assigned randomly to each bed. Means of repetitions within a bed were considered as a replicated and experiments 1A and 4 were analyzed as a randomized complete block with two replicates. Experiments 1B, 3A, and 3B consisted of four replicates of treatments and controls randomized within a single bed and were analyzed as a completely random design. Experiments 2A, 2B, and 5 were arranged in four beds. Treatments and controls were randomized in each bed and the experiments were analyzed as a randomized complete block with four replicates. Following analysis of variance (ANOVA) and determination of the experimental coefficient of variation (CV), means were compared by the least significant difference (LSD) test at P=0.05. ANOVA and LSD tests were conducted on untransformed data (X), on $\sqrt{x}$, and on $\sqrt{(x+0.5)}$. The latter two transformations were recommended by Steel and Torrie (1980, p. 235) for percentage values in the range 0~20.[13] Data were analyzed using SAS/STAT User's Guide, Release 6.03 Edition, SAS Institute Inc., Cary, NC.

6. Disease assessment

In September in the year in which the experiment was established (experiments 1A, 1B, 2A, 2B, 3A, and 3B) or in the following year (experiments 4 and 5), all plants were dug from the plots. Roots were rinsed in water and the number of plants with symptoms of disappearing root rot was determined. Disease incidence was calculated as the percentage of diseased plants.

Results

Analyses for untransformed values (Table 2) were essentially identical to those for transformed values (data not presented). Coefficients of variation ranged from 33.2% to 86.7%.

Table 2. Effect of fungicide drenches on incidence (%) of ginseng roots with symptoms of disappearing root rot

Treatment g a.i./m^2	Experiment							
	1A	1B	2A	2B	3A	3B	4	5
Benomyl 1,250	3.2de[§]	3.6abc	2.2de	1.7b	4.4ab	3.2ab	5.8ab	13.3a
Benomyl 45	3.7cde	5.0ab	4.8bcd	2.2b	6.1ab	5.8a	6.2ab	14.9a
Propiconazole 40	4.0cd	1.5cd	2.9de	2.5b	5.9ab	3.9ab	4.7ab	14.7a
Propiconazole 10	4.6bcd	5.7a	3.4bcd	2.5b	7.6a	6.7a	8.5a	16.8a
Fluazinam 150	7.2abc	3.0bcd	4.3cd	6.7a	–[†]	–	4.9ab	14.6a
Fluazinam 100	7.9ab	3.9abc	7.2abc	2.3b	–	–	4.9ab	15.3a
Infested	8.5a	2.9bcd	8.8ab	2.9b	7.6a	2.2ab	7.8a	12.8a
Infested, water drench	6.0abcd	4.4ab	9.4a	2.6b	5.9ab	5.3a	10.1a	19.9a
Uninfested	0.0e	0.6d	0.0e	0.7b	0.0b	0.3b	0.7b	3.5a
CV (%)	33.2	50.8	57.8	63.2	86.7	81.1	43.3	81.8

[§] Within a column, means followed by the same letter are not significantly different according to a protected LSD test at p=0.05.
[†] Not tested.

The incidence of disappearing root rot was low (0~3.5%) in uninfested plots and significantly higher in infested plots (2.2~12.8%). Adding a water drench two and eight weeks after infestation did not significantly alter the incidence of the disease in infested plots within an experiment (2.6 ~19.9%).

Significant reduction of disease incidence by fungicides compared to the infested waterdrenched control was observed in experiments 1B and 2A (Table 2). Fungicide treatments that significantly reduced disease were the high rate of propiconazole (experiments 1B and 2A), high rates of benomyl and fluazinam (experiment 2A), and low rates of benomyl and propiconazole (experiment 2A). In experiment 2A, no significant differences occurred among fungicide treatments that reduced disease compared to infested controls. The rank of efficacy of fungicide treatments in experiments 1B and 2A was variable but high rates of benomyl and propiconazole ranked consistently in the top three of the six fungicide treatments. The greatest degree of disease reduction was provided by the high rate of benomyl in experiment 2A (77%).

The mean number of plants per plot ranged from 57 in experiment 3A to 169 in experiment 1B and averaged 92 across all experiments. There was no relationship between plant stand and incidence of disease in infested check plots (data not shown).

Discussion

The procedure used for infesting plots was effective in increasing the incidence of disappearing root rot above background levels and to levels high enough to show efficacy of fungicide treatments. It was not necessary to place inoculum immediately adjacent to the plant in order to produce disease. The occurrence of disease above background levels indicated that the fungus moved through the soil at least 5 cm, the shortest distance from the inoculum site to the nearest root. The mechanism of this movement is not known. Possibilities include mycelial growth of the fungus and distribution of spores by rainfall, irrigation, or soil animals.

The infestation methods was simple and rapid. Among all experiments assessed in the year of infestation, the mean incidence of diseased roots in infested controls ranged from 2.2% to 9.4%. On average there were about 100 plants per plot. Given that there were six infested sites per plot, the average number of diseased plants per infested site may be roughly estimated to range from 0.37 to 1.6. It appears likely, therefore, that in future studies, it would be possible to increase the incidence of disappearing root rot by increasing the number of infested sites.

Although statistical comparisons were not possible, the data offer some insights into the effects of rainfall, age of garden, and duration of the experiment, on the incidence of disappearing root rot. The disease appeared to be favoured by high rainfall during the growing season. Disease incidence was higher in 1994 than in 1995 in comparable treatments, and rainfall was greater by 62 mm and 114 mm in June and July, respectively, in 1994 than in 1995. This supports conclusions by Kari and Ondieki (1972) and Matturi and Stenton (1964) that *C. destructans* is more active in cooler wetter soils.[14, 15] There was no indication from experiments 1A to 3B that age of garden when infested affected disease incidence in the year of infestation, which ranged in controls from 5.9% to 9.4% in 1994, and from 2.2% to 5.3% in 1995. There was some indication that disease incidence increased between year 1 and year 2. Disease incidence in controls for experiment 4 in 1995, after infestation of a 1-year-old garden in 1994, was slightly higher than in controls for experiment 1A, in which a 1-year-old garden was infested and assessed in 1994. In addition, disease incidence in controls in experiment 5 in 1995, after infestation of a 2-year-old garden in 1994, was considerably higher than in controls for experiment 2A, in which a 2-year-old garden was infested and assessed in 1994. The higher incidence of disease in the year after infestation tham in the year of infestation could be attributed to the greated time available for infection and symptom expression, a possible increase in the susceptibility of older roots to infection or symptom expression, and greater contact between roots in older gardens.

Fungicides reduced the incidence of disappearing root rot by 49% (low rate of benomyl in experiment 2A) to 77% (high rate of benomyl in experiment 2A), Therefore, the products examined appear to have potential to control the disease. Although the high variability in the experiments, indicated by the generally high coefficents of variation, indicates that the data should be interpreted with caution, the following discussion is based on the results of protected LSD comparisons.

Within experiments 1B and 2A, significant effects were shown by high fungicide rates four times and by low rates twice. Propiconazole was effective twice at the high rate and once at the low rate. Benomyl was effective once at the high rate and once at the low rate. Fluazinam was effective once, at the high rate. Within each fungicide, high rates consistently reduced disease more than low rates, although the differences were significant only for propiconazole in experiment 1B. High rates are therefore considered to be more effective than low rates, and benomyl and propiconazole appear to be the best candidates for further testing.

Efficacy could not be directly related to toxicity *in vitro* because drench rates were not in constant proportion to either EC50 or EC95. It would be expected that inherent toxicity of the fungicide to the pathogen would be a factor influencing fungicidal control of the disease. This is supported by results for fluazinam, which was the least toxic of the three fungicides *in vitro* and effective only once, at 150 g a.i./m^2, against the disease. Propiconazole at 40 g a.i./m^2 was as effective as benomyl at 45 g a.i./m^2 against the disease in one experiment, and more effective in another. The two fungicides had similar EC50 values but their EC95 values differed by an order of magnitude.[11] The relation of toxicity *in vitro* to efficacy in disease control is therefore not simple.

When gardens were infested in June and disease was assessed in September of the same year, disease was reduced in 1-year-old and 2-year-old gardens (experiments 1B and 2A) but not in 3-year-old gardens (experiments 3A and 3B). This suggests that the disease is more readily controlled by fungicide drenches in younger gardens. When the garden was infested in year 1 (experiment 4) or year 2 (experiment 5) and assessed one year later, fungicides were not effective, even though applied twice each year. This fits the conclusion that fungicide dreches control the disease less readily in older gardens. The reasons for this are not known. Perhaps rising levels and diversity of biological activity in the soil lead to a progressive increase in the ability of the soil to bind or decompose fungicides. Although age of the garden when infested did not appear to affect disease incidence, its effect on disease severity is not known. A second possibility, then, is that older plants are more susceptible to disappearing root rot, and therefore more difficult to protect with fungicides.

The volume of fungicidal drench used per plot (0.84 L/m^2) is equivalent to a drench rate of 8,400 L/ha. This is more than twice the rate (4,000 L/ha) recommended in Ontario for the application of quintozene as a soil drench to control *Rhizoctonia* crown rot of ginseng.[16] Furthermore, the volume of water we used would represent about 20% of the total water needed to raise the top 3 cm of soil from oven dry to field capacity. Since the drench was applied to well watered plots, and greatly exceeded the drench rate recommended for a registered, soil fungicide, it is likely that the fungicide applied penetrated the soil to the depth of the added inoculum. We conclude that disease control in these experiments was not unduly limited by penetration of fungicide to the inoculum.

This study shows that fungicide drenches have potential to reduce disappearing root rot. At the rates tested, propiconazole and benomyl were more effective than fluazinam. When disease was assessed in 1-year trials (experiments 1A, 1B, 2A, 2B, 3A, 3B) fungicides were more effective when applied to younger plants (on trial each with 1-year-old gardens and 2-year-old gardens, neither trial with 3-year-old gardens). Fungicide applied to 1-year-old gardens provided control in the year of application (experiment 1B) but not in the following year (experiment 4). Therefore, fungicides may be most effective when applied to young gardens. Further research is needed to determine factors affecting efficacy of fungicide drenches, and whether fungicide drenches are effective after symptoms of the disease appear. Since disappearing root rot initially has little effect on the shoot, sampling of roots would be necessary to detect the onset of the disease, and might become part of a system for determining the need for fungicidal drenches.

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