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Toxicity of Fungicides *in vitro* to *Cylindrocarpon destructans*

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Abstract : As part of a study on the ability of fungicides to control disappearing root rot of ginseng (*Panax quinquefolius*) caused by *Cylindrocarpon destructans*, 15 fungicides were screened for toxicity to the fungus *in vitro*. Highly toxic fungicides were Benlate (benomyl), Thiram (thiram), and Orbit (propiconazole). EC50 values (mg a.i./L) were less than 1 and EC95 values were less than 10. Crown (carbathiin and thiabendazole), ASC-66835 (fluazinam), and UBI-2584 (tebuconazole) were moderately toxic, with EC50 values in the range 1-10 and EC95 values in the range 32-45. Weakly toxic fungicides (EC50 in the range 20-80, EC95 in the range 35-140) included UBI-2643 (thiabendazole), UBI-2565 (cyproconazole), and Vitaflo-280 (carbathiin and thiram). Anvil (hexaconazole), Vitaflo-250 (carbathiin), UBI-2383 (triadimenol), Daconil (chlorothalonil), CGA-173506 (fludioxonil), and CGA-169374 (difenoconazole) were considered nontoxic to *C. destructans* (EC50 1.29->600, EC95>500). Relations between proportional inhibition of growth and concentration of fungicide were linear on arithmetic plots (Benlate, UBI-2643, UBI-2565, Vitaflo-280) or logarithmic plots (all other fungicides). Based on toxicity *in vitro* and formulation, it is recommended that Benlate, Orbit, and ASC-66835 be tested as soil drenches, and Benlate, Thiram, UBI-2584, and Crown be tested as seed treatments for controlling disappearing root rot.

Key words : Ginseng, *Panax quinquefolius*, disappearing root rot, *Cylindrocarpon destructans*, fungicide.

Introduction

The fungus *Cylindrocarpon destructans* (Zinssmeister) Scholten causes disappearing root rot of ginseng (*Panax quinquefolius* L.) and is one of several soilborne pathogens that seriously limit production of the crop in Ontario and elsewhere.[1,2,3,4,5,6,7] Ginseng generally requires six years of growth before yielding high quality roots, yet many growers harvest after three years as a result of large crop losses caused by *C. destructans*, *Rhizoctonia solani*, and *Phytophthora cactorum*.[3,4,5,8]

Fungicides are registered in Canada to control diseases on ginseng caused by *R. solani*, *Botrytis cinerea*, *Alternaria panax*, and species of *Phytophthora* and *Pythium* but not by *C. destructans*.[9] Furthermore, the toxicity of fungicides to *C. destructans* is not known, possibly because the fungus has been considered a minor pathogen of conifers in nurseries and forests or a secondary invader of numerous plants.[10,11,12]

Fungicides can be evaluated *in vitro* by inhibition of germination of spores or by inhibition of growth of mycelium.[13] Both tests are evaluated by generating a dose-response curve from which the effective concentrations required to inhibit germination or growth by 50% (EC50) or 95% (EC95) are estimated. Chemicals with high toxicity *in vitro* might then be tested for efficacy in controlling disease under field or greenhouse conditions. The objective of this study was to determine the toxicity of 15 fungicides to *C. destructans* under laboratory conditions. This information could be used to select products to be

tested as soil drenches and seed treatments to control disappearing root rot ginseng.

Materials and Methods

Fifteen fungicides were selected (Table 1) because they were commercially available in Canada but not registered for use on ginseng, or because they were experimental fungicides with potential for registration in Canada. The products containing benomyl, chlorothalonil, fluazinam or propiconazole as active ingredients were formulated for use as foliar sprays or soil drenches. The remaining products were formulated for application to seed, and contained dye to colour the seed.

Stock cultures were derived from single spores of *C. destructans* isolate DRS265 (CCFC 007989, Centre for Land and Biological Resources Research, Agriculture and Agri-Food Canada, Central Experimental Farm, Ottawa, Ontario). Subcultures were grown in 9-cm-diameter petri dishes containing Spezieller Nährstoffarmer (special nutrient-poor) agar with yeast extract, referred to hereafter as SNAY, for 10 days at 20℃ in the dark to allow colonies to reach a diameter of 8 cm.[14] Fungitoxicity to *C. destructans* was assessed by a dilution series method based on the protocol of Barak and Edgington (1984).[15]

The maximum tested rate was derived from the highest rate recommended in technical information sheets for experimental fungicides, or from maximum rates stated on labels of registered fungicides. Dilution series were prepared in sterilized distilled water (or 95% ethanol for Benlate) and 1 mL of each fungicide dilution was added to 100 mL of molten SNAY in 200-mL medicine bottles. Controls consisted of SNAY with or without added water (or 95% ethanol). Each 100-mL batch of medium was poured into six 9-cm-diameter petri dishes, and allowed to cool and solidify for 12 hours. One 10-day-old culture of *C. destructans* was used per fungicide. A number 2 cork borer was used to cut out 4-mm-diameter plugs from the leading edge of the colony. The colonies were large enough that one culture provided enough plugs for one experiment. The plug was placed in the centre of the petri dish, mycelial side down. The cultures were incubated at 20℃ in the dark. Colony diameter was measured every 24 hours along the same marked diagonal until the control colonies had reached the margins of the dish.

Colony diameters measured on day 5 were used to calculate proportional inhibition of growth compared to the control. Mean colony diameter was calculated from the six dishes per fungicide concentration. Proportional inhibition of growth, calculated as 1-(colony diameter of treatment/colony diameter of control) was regressed against the concentration of active ingredient in the fungicide product. For Crown and Vitaflo-280, the calculation was based on both active ingredients. EC50 and EC95 values were calculated from the regression equation $Y=b_0+b_1X$, where Y is the proportional inhibition of growth, X is the concentration of active ingredient, and b_0 and b_1 are constants.[16,17] The software for regression an- alysis was obtained from website www.minitab, version 11.13a, 1996, Minitab Inc., 3081 Enterprise Drive, State College, PA 16801-3008. The units for EC50 and EC95 in the following text are mg a.i./L. The tests were conducted three times and each test was considered a replication. The slopes of the replicates were compared using the general linear test for homogeneity of regression coefficients of two or more regression lines.[18]

Results

The general linear test for comparing regression lines showed that the regressions were the same for each replication within each fungicide tested except for CGA-169374, where the calculated F was greater than the tabulated value. Thus, adjustment of the lines for the three replicates to a common regression line was appropriate at P=0.05 for all fungicides except CGA-169374. Since this product was not toxic to *C. destructans*, further analysis of its regression lines was not attempted.

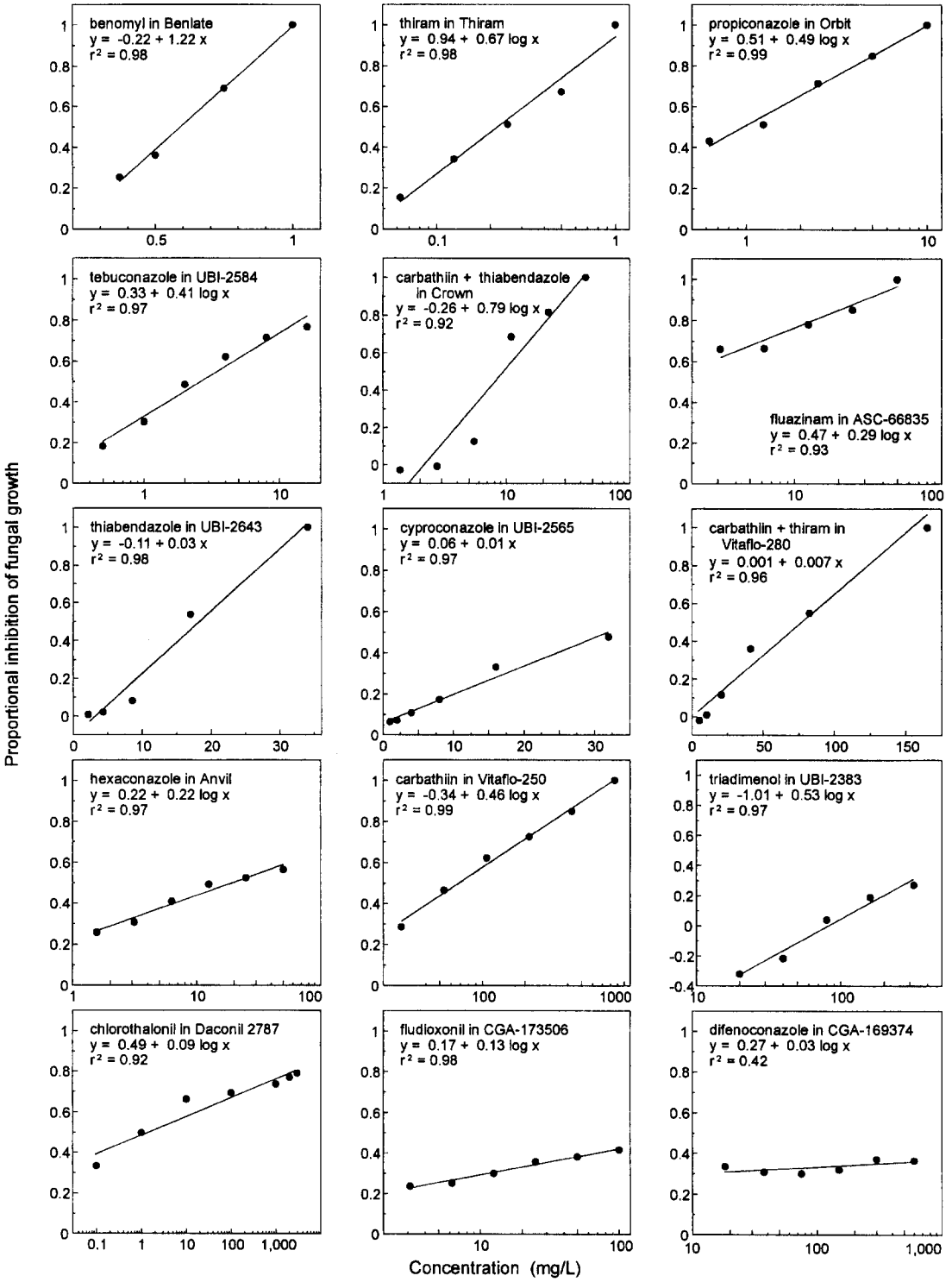

Fig. 1. Relation of concentration of fungicide (mg total active ingredient/L) in inhibition *in vitro* of colony growth of *C. destructans*. The equation of the line for each fungicide product was derived by linear regression analysis of data from three trials and six replications per fungicide concentration per trial.

Table 1. Active ingredients, concentration of active ingredients, chemical class, range tested, EC95 and EC50 of fungicidal products tested against *C. destructans in vitro*

Fungicide	Active ingredient and Concentration	Chemical class	Range tested mg a.i./L	EC95 mg a.i./L	EC50 mg a.i./L
Benlate 50WP[1a)]	Benomyl 50 g/100 g	Benzimidazole	0.37~1.5	0.96	0.59
Thiram 75WP[1d)]	Thiram 7 g/100 g	Carbamate	0.06~4.0	1.03	0.22
Orbit 41.7WP[1b)]	Propiconazole 41.7 g/100 g	Triazole	0.63~20	7.91	0.95
UBI-2584[2d)]	Tebuconazole 8.33 g/L	Triazole	0.5~16	>16(32.53*)	2.60
Crown[2d)]	Carbathiin 92 g/L Thiabendazole 58 g/L	Oxathiin Benzimidazole	0.84~27†	34.01†	9.16†
ASC-66835 50WP[2c)]	Fluazinam 50 g/100 g	Pyradinamine	3.1~50	45.21	<3.1(1.27*)
UBI-2643[2d)]	Thiabendazole 348 g/100 g	Benzimidazole	2.1~34	>34(35.33*)	20.33
UBI-2565[2d)]	Cyproconazole 4.16 g/L	Triazole	1.0~32	>32(89.00*)	>32(44.00*)
Vitaflo-280[1d)]	Carbathiin 14.9 g/L Thiram 13.2 g/L	Oxathiin Carbamate	5.2~165†	135.57†	71.29†
Anvil[2c)]	Hexaconazole 50 g/L	Triazole	1.6~50	>50(2,081*)	18.74
Vitaflo-250[1d)]	Carbathiin 25.3 g/L	Oxathiin	26.9~860	637.2	67.01
UBI-2383[2d)]	Triadimenol 317 g/L	Triazole	20.0~320	>320(4,990*)	>320(706.50*)
Daconil 2787[2f)]	Chlorothalonil 40.4 g/L	Nitrile	1.0~3,000	>3,000(1.29×10^{5}*)	1.29
CGA-173506 25FS[2b)]	Fludioxonil 25 g/L	Cyanopyrrole	3.1~100	>100(1.00×10^{6}*)	>100(345.50*)
CGA-169374 150FS[2b)]	Difenoconazole 150 g/L	Triazole	18.3~600	>600(4.64×10^{22}*)	>600(4.64×10^{7}*)

[1)] Product commercially available in Canada, [2)] Product not registered in Canada. [a)] Du Pont de Nemours, Streetsville, Ontario, [b)] Ciba-Geigy Canada Ltd., Cambridge, Ontario, [c)] ISK Biotech, London, Ontario, [d)] Uniroyal Chemical Ltd., Elmira, Ontario, [e)] Zeneca Agro, Stoney Creek, Ontario, [f)] Fermenta ASC, London, Ontario, *Determined by extrapolation, † Toxicity refers to the combination of active ingredients.

Linear regressions derived from three replicate tests (Fig. 1) with high coefficients of determination (r^2=0.92~0.99) were obtained when fungicide concentration was expressed arithmetically (Benlate, UBI-2643, UBI-2565, and Vitaflo 280), or logarithmically (the remaining products, except for CGA-169374, for which logarithmic transformation produced an r^2 of 0.42). The generally strong coefficients of determination justify confidence in the EC50 and EC95 values lying within the range of tested concentrations.

Based on EC50 and EC95 values (Table 1), the products were divided into four groups, labeled highly toxic, moderately toxic, weakly toxic, and nontoxic.

1. Highly toxic

Benlate 50WP (benomyl), Thiram 75WP (thiram), and Orbit 41.7WP (propiconazole) were considered to be highly toxic to *C. destructans*. This group was characterized by EC50 values less than 1.0. EC95 values ranged from 0.96 for benomyl to 7.91 for propiconazole.

2. Moderately toxic

UBI-2584 (tebuconazole), Crown (carbathiin and thiabendazole), and ASC-66835 50WP (fluazinam) were moderately toxic to *C. destructans*. EC50 values were in the range 1-10 and EC95 values were in the range 32-45. The EC50 for fluazinam was extrapolated from the regression line, since the lowest concentration tested (3 mg a.i./L) inhibited growth by greater than 60%. The EC95 for tebuconazole was >16 and estimated as 32.53 by extrapolation.

3. Weakly toxic

UBI-2643 (thiabendazole), UBI-2565 (cyproconazole) and Vitaflo-280 (carbathiin and thiram) were weakly toxic to *C. destructans*. EC50 values ranged from 20.33 (thiabendazole) to 71.29 (carbathiin and thiram), and EC95 values ranged from 35.33 (thiabendazole) to 135.57 (carbathiin and thiram). The EC50 and EC95 values for cyproconazole were>32 and estimated by extrapolation to be 44.00 and 89.00, respectively.

4. Nontoxic

Six products were considered nontoxic to *C. destructans*. Daconil 2787 (chlorothalonil) presented a low EC50 of 1.29, but an EC95 greater than 3,000. Anvil (hexaconazole) also had a relatively low EC50

(18.74) but a high EC95 (>50, and estimated as 2,081 by extrapolation). UBI-2383 (triadimenol) was characterized by EC50 and EC95 values>320. Vitaflo-250 (carbathiin) exhibited an EC 50 of 67.01 and an EC95 of 637.2. EC50 and EC95 values were greater than 100 for CGA-173506 (fludioxonil) and greater than 600 for CGA-169374 (difenoconazole).

Discussion

This is the first report of toxicity of fungicides to *C. destructans*. Based on toxicity *in vitro*, several products appear to merit examination for efficacy against diseases caused by the fungus, including disappearing root rot of ginseng. These products include Benlate 50WP, Thiram 75WP, Orbit 41.7WP. ASC-66835 50WP, UBI-2584, and Crown. The preferred method of testing these products may depend on their formulation. Benlate 50WP, Orbit 41.7WP, and ASC-66835 50WP were formulated for use as foliar sprays or soil drenches, whereas the remaining highly toxic or moderately toxic products were developed as seed treatments.

The experimental technique used produced highly consistent results among replications, as judged by the analysis of slopes of regression lines for three replications of each fungicide test. However, it is recognized that the results refer to a particular set of conditions and may not reflect relative toxicity in other environments. There is some evidence for this in the data. For example, Crown was more toxic than its separaete components, carbathiin and thiabendazole. Toxicity of the product *in vitro* appears to depend on more than the toxicity of the ingredients assessed independently.

There was a moderate tendency for EC50 and EC95 values to have similar ranks. For example, the highly toxic group occupied the top three ranks judged by either EC50 or EC95 values. The moderately toxic group occupied ranks 4, 6 and 7 judged by EC50 and ranks 4, 5, and 7 judged by EC95. The weakly toxic group occupied ranks 9, 10, and 12 by EC50 and ranks 6, 8, and 9 by EC95. The nontoxic group occupied ranks 5, 8, 11, 13, 14, and 15 by EC50 and ranks 10, 11, 12, 13, 14, and 15 by EC95. Daconil 2787 was unusual in that it would have been judged moderately toxic to *C. destructans* by its EC50 (1.29) and nontoxic by its EC 95 (>3,000). Its low EC50 makes it worthy of testing for efficacy in the field.

Members of chemical families differed in toxicity. Among the benzimidazoles, benomyl showed greater activity than thiabendazole. Triazoles presented a wide range of toxicity, from the highly toxic propiconazole, through moderately toxic tebuconazole, and weakly toxic cyproconazole, to nontoxic hexaconazole, triadimenol, and difenoconazole.

Since this is the first report of the sensitivity of *C. destructans* to fungicides, the results cannot be compared directly with previous studies. However, it may be noted that sensitivity of the fungus to the benzimidazoles (benomyl and thiabendazole) is consistent with the fungitoxicity of this group to ther members of the Phialosporae, such as *Verticillium* and *Fusarium*, which typically show EC50 values for benomyl of less than 1 mg a.i./L.[19] Similarly, the weak toxicity of carbathiin to *C. destructans* matches the general weak activity of oxathiin compounds against the Phialosporae.[20]

Based on toxicity *in vitro* and formulation, it is recommended that Benlate 50WP, Orbit 41.7WP, and ASC-66835 be tested as soil drenches and Benlate 50WP, Thiram 75WP, UBI-2584, and Crown be tested as seed treatments for controlling disappearing root rot of ginseng.

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