Sensitivity to Copper and Phosphite of *Phytophthora* Species Associated with Ink Diseases of Chestnut

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Abstract

***Phytophthora cinnamomi* (23 isolates) and *P. cambivora* (3 isolates), isolated from chestnut showing ink disease symptoms or from soil near dead roots of chestnut trees were tested to copper and phosphite sensitivity. Isolates were characterized using an amended agar assay. EC_{50} values (copper (or phosphite) concentration inhibiting mycelial growth of the pathogen by 50%) for each isolate were calculated by plotting probit percentage inhibition against log_{10} active ingredient (a.i.) concentration. EC_{50} values of copper ranged from 0.043×10^{-3} to 2.025×10^{-3} M in *P. cinnamomi* and 1.970×10^{-3} to 4.603×10^{-3} M in *P. cambivora*. EC_{50} values of phosphite ranged from 2.99 to 172.39 µg ml^{-1} in *P. cinnamomi* and 47.23 to 237.25 µg ml^{-1} in *P. cambivora*. This study may contribute to access the potential utility of these a.i. for managing ink disease of chestnut and to use in the future as benchmark for baseline sensitivity.**

INTRODUCTION

Phytophthora cinnamomi and *P. cambivora* are soil born parasites, both associated with ink disease of chestnut. Chemical control of *Phytophthora* diseases is a common practice for agricultural crops (Erwin and Ribeiro, 1996; Guest et al., 1995). Copper and fosetil-Al are registered in Portugal for ink disease of chestnut. Resistance of some individuals of the pathogen to the chemicals is of special concern when chemical control is being planned. Some elements such as Cu^{2+} and Fe^{3+} were inhibitory to sporangial production and also to mycelial growth of *Phytophthora*. Cu^{2+} has been shown to be toxic to *Phytophthora* zoospores at 10^{-7} M and to mycelia at levels of 10^{-2}-10^{-3} M (Halsall, 1977; Smith, 1979). Phosphite, the anionic form of phosphonic acid (HPO_3^{2-}), which acts directly on the pathogen and indirectly in stimulating a host defence response, controls many plant diseases caused by *Phytophthora*, even at concentrations in planta that only partially inhibit pathogen growth in vitro (Guest and Bompeix, 1984; Guest and Grant, 1991; Wilkinson et al., 2001).

The purpose of this study was to determine the in vitro sensitivity to copper and phosphite of isolates of *Phytophthora* species, *P. cambivora* and *P. cinnamomi*, associated with ink disease of chestnut.

MATERIAL AND METHODS

The in vitro sensibility of 26 isolates of *Phytophthora* (Table 1) to copper concentration (1×10^{-4} M, 1×10^{-3} M, 2×10^{-3} M) and phosphite concentrations (5, 20 and 50 µg phosphite ml^{-1}) were evaluated by using an amended agar medium. *Phytophthora* isolates were grown in Petri dishes containing 15 ml of PDA (Potato Dextrose Agar, Difco) with copper addition ($CuSO_4.5H_2O$ – Riedel-de-Haën) or with phosphite addition (Phyto-Fos-K, A.M.C., Chemical S. Ltda, Sevilha – 33.7 g/100 ml). Phosphite was sterilized by passing it through a 0.22 µm Millipore filter (Millex-HA) and then added to the autoclaved PDA. The Petri plates were incubated in the dark at 24ºC and mycelial growth was measured 6 days after inoculation. Percentage of inhibition for each isolate at each copper or phosphite concentration was calculated as a percentage of growth in the absence of copper or phosphite. Each experiment contained three replicate plates for each isolate and chemical concentration. The EC_{50} value for each isolate was calculated by the

regression line of the probit-transformed percent inhibition plotted against log-transformed fungicide concentration. Data were analysed by General Linear Model (GLM). Independent variables were *Phytophthora* isolate and phosphite or copper concentration, and the dependent variable was the percentage of growth inhibition on the final day of the experiment.

RESULTS AND DISCUSSION

Copper concentration had a significant ($\alpha<0.05\%$) effect on inhibition of isolates grown on PDA. At low concentrations, mycelium grew normally from the inoculation point, but at higher concentrations mycelial growth was sparse and not uniform.

Growth inhibition becomes greater as copper concentration increases. The effect of the lower concentration of copper (1×10^{-4} M) varied between 34.53% of growth promotion (Pr 120) to 55.02% of growth inhibition (802) in *P. cinnamomi* isolates. *P. cambivora* isolates when exposed to the same copper concentration, showed 30.43% (Pr 135) of growth promotion and only 6.01% (Ar 101) of growth inhibition. Copper EC_{50} in *P. cinnamomi* varied between 0.043×10^{-3} M to 2.025×10^{-3} M (isolate 802 and Pr 125 respectively) (Table 1). At the higher copper concentration (2×10^{-3} M) studied 34.78% of *P. cinnamomi* isolates did not grow.

Phytophthora species and isolates within a species vary in their sensitivity to phosphite. Coffey and Bower (1984) obtained EC_{50} values ranging from 5.2 in *P. cinnamomi* to 224 μg ml^{-1} in *P. infestans*. *P. cinnamomi* was considered to be one of the most sensitive species to phosphite with EC_{50} ranging from 5.9 to 11,9 μg ml^{-1}. Wilkinson et al. (2001) obtained phosphite EC_{50} values ranging 4 to 148 μg ml^{-1} on 66 *P. cinnamomi* isolates. The isolates of *P. cinnamomi* tested to phosphite, in this study, could be subjectively assigned to three groups: sensitive isolates were $EC_{50}<10$ μg ml^{-1}, intermediate isolates and, tolerant isolates were $EC_{50}>50$ μg ml^{-1}. Tolerant isolates (Pr 125, 805 and R108) are being tested over a large range of phosphite concentrations to determine consistent tolerance and EC_{50} values. The effect of lower phosphite concentration varied between 5.69% (Pr 125) of growth promotion up to 51.65% (802) of growth inhibition. The most sensitive isolates, at higher phosphite concentration were the isolate 806 and Pr 122 with 76.47% and 74.05% of growth inhibition, respectively.

EC_{50} values of *P. cambivora* were always higher than *P. cinnamomi* for copper and phosphite. Only three *P. cambivora* isolates were obtained in the studied chestnut orchards. The small number of studied isolates of *P. cambivora* did not allow the chemical characterization to be accomplished.

Results from in vitro tests can provide valid measurement for copper compounds which act directly on the pathogen. In vitro tests from phosphite, which has a more complex mode of action, may not provide a realistic and valid measure of tolerance and sensitivity and therefore alternative methods will be necessary to determine the sensitivity or tolerance of the isolates.

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Tables

Table 1. Isolates of *Phytophthora* species tested for sensitivity to copper and phosphite, and EC_{50} values for *Phytophthora* isolates.

Isolate	Isolated from	Location	Specie	Ec_{50} Phosphite ($\mu g\ ml^{-1}$)	Ec_{50} Copper (M)
Pr 112	Young chestnut tree	Paredes	*P. cinnamomi*	9.20	0.569×10^{-3}
Pr 115	Young chestnut tree	Paredes	*P. cinnamomi*	16.83	1.358×10^{-3}
Pr 120	Young chestnut tree	Paredes	*P. cinnamomi*	23.93	1.538×10^{-3}
Pr 122	Soil	Paredes	*P. cinnamomi*	8.09	0.561×10^{-3}
Pr 123	Soil	Paredes	*P. cinnamomi*	7.34	1.471×10^{-3}
Pr 124	Soil	Paredes	*P. cinnamomi*	19.53	0.808×10^{-3}
Pr 125	Soil	Paredes	*P. cinnamomi*	106.68	2.025×10^{-3}
Pr 128	Soil	Paredes	*P. cinnamomi*	16.10	0.678×10^{-3}
Pr 129	Soil	Paredes	*P. cinnamomi*	13.06	1.291×10^{-3}
Pr 130	Soil	Paredes	*P. cinnamomi*	13.18	0.446×10^{-3}
Pr 135	Soil	Paredes	*P. cambivora*	47.23	1.970×10^{-3}
801	Young chestnut tree	Rossas	*P. cinnamomi*	-	0.220×10^{-3}
802	Young chestnut tree	Rossas	*P. cinnamomi*	2.99	0.043×10^{-3}
803	Young chestnut tree	Rossas	*P. cinnamomi*	27.42	0.139×10^{-3}
804	Young chestnut tree	Rossas	*P. cinnamomi*	31.80	0.174×10^{-3}
805	Young chestnut tree	Rossas	*P. cinnamomi*	83.35	0.085×10^{-3}
806	Young chestnut tree	Rossas	*P. cinnamomi*	11.86	0.429×10^{-3}
807	Young chestnut tree	Rossas	*P. cinnamomi*	-	0.447×10^{-3}
808	Young chestnut tree	Rossas	*P. cinnamomi*	26.85	0.216×10^{-3}
809	Young chestnut tree	Rossas	*P. cinnamomi*	27.51	0.446×10^{-3}
810	Young chestnut tree	Rossas	*P. cinnamomi*	33.49	0.106×10^{-3}
R 105	Young chestnut tree	Rossas	*P. cinnamomi*	34.32	0.116×10^{-3}
R106	Young chestnut tree	Rossas	*P. cinnamomi*	9.54	0.211×10^{-3}
R108	Young chestnut tree	Rossas	*P. cinnamomi*	172.39	-
Ar 101	Old chestnut tree	Arufe	*P. cambivora*	237.25	-
Ar 104	Old chestnut tree	Arufe	*P. cambivora*	-	4.603×10^{-3}

- not calculated