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# Antagonistic Interactions Between Pathogenic and Saprophytic Fungi Isolated from Plant Roots*

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**Abstract**

Antagonistic interactions between the fungi associated with crown and root rots of raspberry plants, red clover and subterranean clover have been studied. Both at 18 and 28°C *Trichoderma hamatum* inhibited *Phytophthora cinnamomi* isolates, *P. clandestina*, *Fusarium sambucinum*, *F. graminearum*, *F. trichothecioides*, *Alternaria alternata* and *Alternaria* spp.. *Penicillium variabile* inhibited the same fungi as *T. hamatum* only at 18°C but not at 28°C. At 18°C *Epicoccum purpurascens* slightly inhibited *F. culmorum* and *Trichothecium roseum* slightly inhibited *P. cinnamomi* isolates. In growth-chamber studies *P. cinnamomi* isolates induced large necrosis of the cane cortices. Necrotic lesions were visible as early as on the 3rd and 7th day after inoculation. Wilting and dying off of the whole canes was observed on the 16th day after inoculation. When canes and cane segments were inoculated with *T. hamatum* after previously inoculation with *P. cinnamomi* disease severity was lower. In our trials *T. hamatum* had antagonistic activity against *P. cinnamomi* isolated from and applied on raspberry plants.

## Introduction

During the last few years both plant pathologists and commercial companies have shown significant interest in the antagonistic potential of *Trichoderma* spp. Baker and Cook (1982) and Cook and Baker (1983) have dedicated books to biological control, in which they summarize some of the key publications about *Trichoderma* during the last 50 years.

The objective of our study was to verify antagonistic interaction between fungi isolated from diseased roots of plants and by expedious method to assume antagonistic effect on serial plant material.

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## Material and Methods

We tested following isolates from diseased root and cane cortex of raspberry plants (Rubus idaeus L.): *Phytophthora cinnamomi* Rands, *Fusarium oxysporum* Schlecht., *F. lateritium* Nees, *Fusarium* ssp. "*roseum*" – pathogens; *Trichoderma hamatum* (Bon.) Bain and *Penicillium variabile* Sapp. – saprophytes. From discolorated crown and root section of red clover (Trifolium pratense L.) *F. oxysporum* Schlecht., *F. solani* (Mart.) Sacc., *F. avenaceum* (Fr.) Sacc., *F. culmorum* (W.G. Smith) Sacc., *F. moniliforme* Sheldon, *F. graminearum* Schwabe, *F. trichothecioides* Wollenw. in Jamieson and Wollenw., *Alternaria alternata* (Fr.) Keissler, *Alternaria* spp. Nees ex Fr. -pathogens; *Epicoccum purpurascens* Ehrenb. ex Schlecht. *Trichothecium roseum* Link – saprophytes were tested. From roots of subterranean clover (*Trifolium subterraneum* L.) *Phytophthora clandestina* Taylor, Pascoe and Grenhalgh (obtained from Barbetti-Western Australia).

*In vitro studies.* Interactions were studied in dual culture, single fungi served as controls. The surface of potato dextrose agar plates and special medium for *Phytophthora* isolates was covered with 1 ml of water spore suspension and mass of mycelium of pathogenic fungi. After drying up the surface, four holes were cut out in the agar. Into them 0.5 ml suspension of saprophytic fungi were applied. Inoculated plates were incubated at 18 and $28 \pm 1$°C. Zones of inhibition were measured in mm after 3, 4 and 7 days for *Phytophthora* and 5, 10 and 15 days of incubation for other isolates. Each treatment in four replicates.

*Growth chamber studies.* Canes and cane segments of raspberry plants were inoculated with *Phytophthora* and *Fusarium* isolates. Inoculum (agar discs of fungi) was applied on artificially wounded canes and on cane segments. For each isolate tested and each treatment 10 canes and 10 segments were used. After inoculation spores of potentially antagonistic fungi ($10^6$ spores $ml^{-1}$) were sprayed on the canes and on the segments.

Plant materials were placed separately at $18 \pm 1$°C at 80% relative humidity and 18 hours daylight. On the 3rd, 7th, 16th and 23rd day after inoculation disease severity rating from 0 to 5 was evaluated (0 = without necrotic lesions, 5 = cane or segment completely necrotized).

## Results

In "in vitro" studies at 18°C *T. hamatum* inhibited: *P. cinnamomi* isolates, *F. sambucinum*, *F. graminearum*, *F. trichothecioides*, *A. alternata* and *Alternaria* spp; *P. variabile* inhibited *P. cinnamomi* isolates, *P. clandestina*, *F. graminearum*, *F. trichothecioides*, *A. alternata Alternaria* spp.; *E. purpurascens* inhibited *F. culmorum*; *T. roseum* only slightly inhibited *P. cinnamomi* isolates. High significant effect was confirmed on the 4th day after inoculation (of incubation) in dual culture between *Trichoderma* and

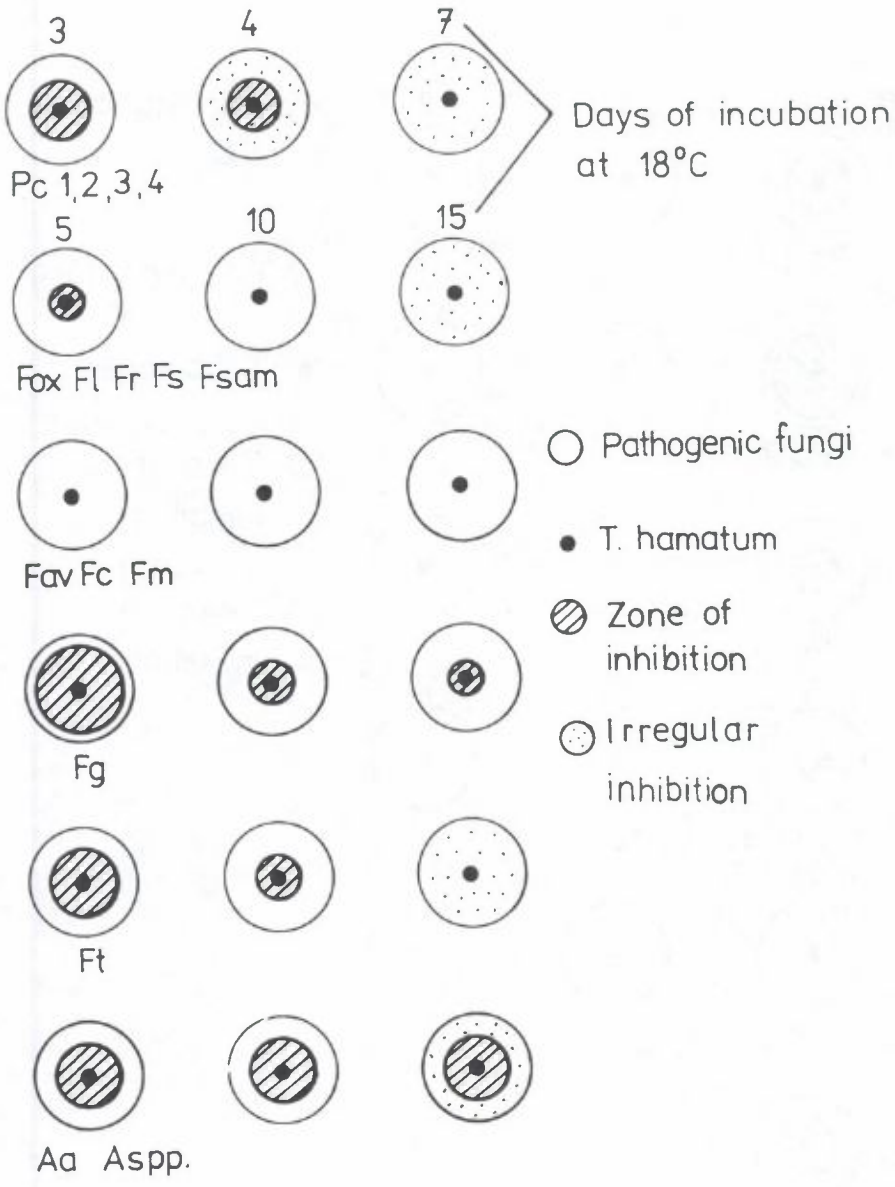


Figure 1. Interaction between *Trichoderma hamatum* – pathogenic fungi at 18°C. Pc 1, 2, 3, 4 = Phytophthora cinnamomi isolates, Fox = Fusarium oxysporum, Fl = F. lateritium, Fr = Fusarium spp. "roseum", Fs = F. solani, Fsam = F. sambucinum, Fav = F. avenaceum, Fc = F. culmorum, Fm = F. moniliforme, Fg = F. graminearum, Ft = F. trichothecioides, Aa = Alternaria alternata, A spp. = Alternaria spp.

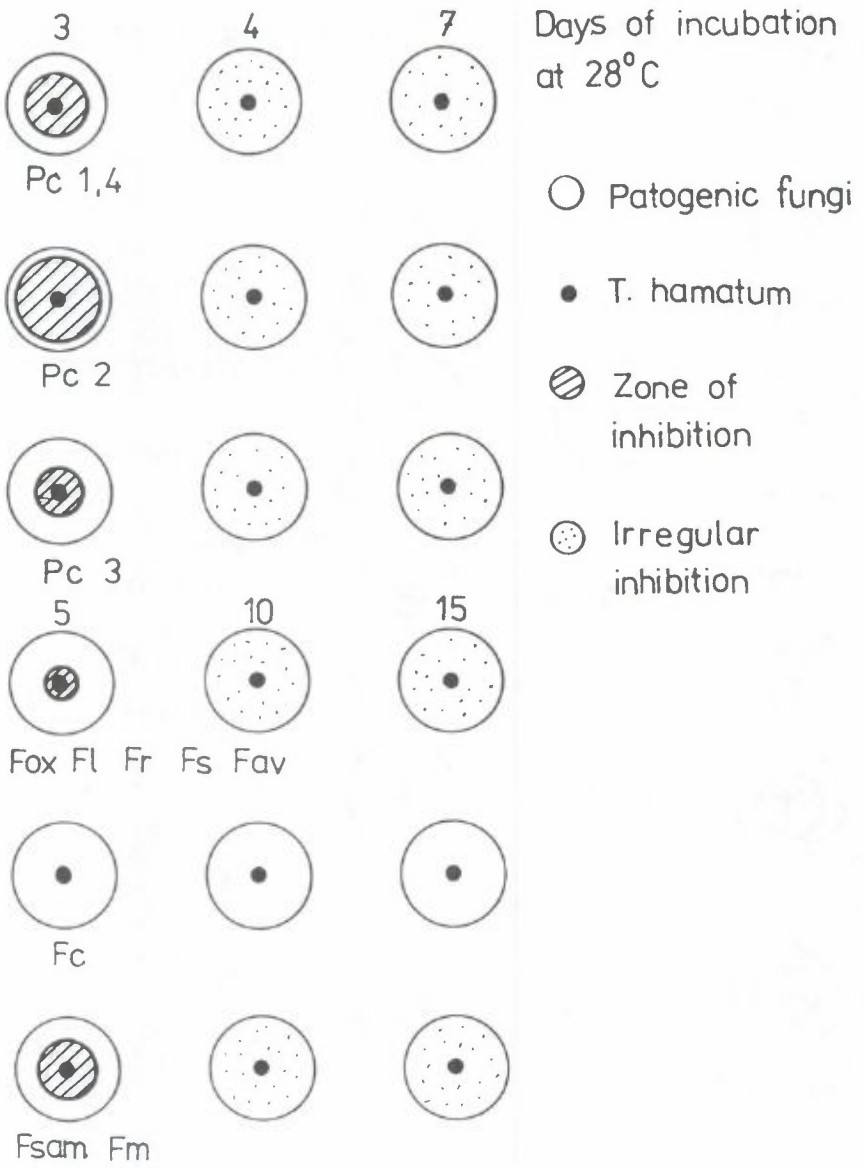


Figure 2. Interaction between *T. hamatum* – pathogenic fungi at 28°C. Pc 1, 2, 3, 4 = Phytophthora cinnamomi isolates, Fox = Fusarium oxysporum, Fl = F. lateritium, Fr = Fusarium spp. "roseum", Fs = F. solani, Fsam = F. sambucinum, Fav = F. avenaceum, Fc = F. culmorum, Fm = F. moniliforme.

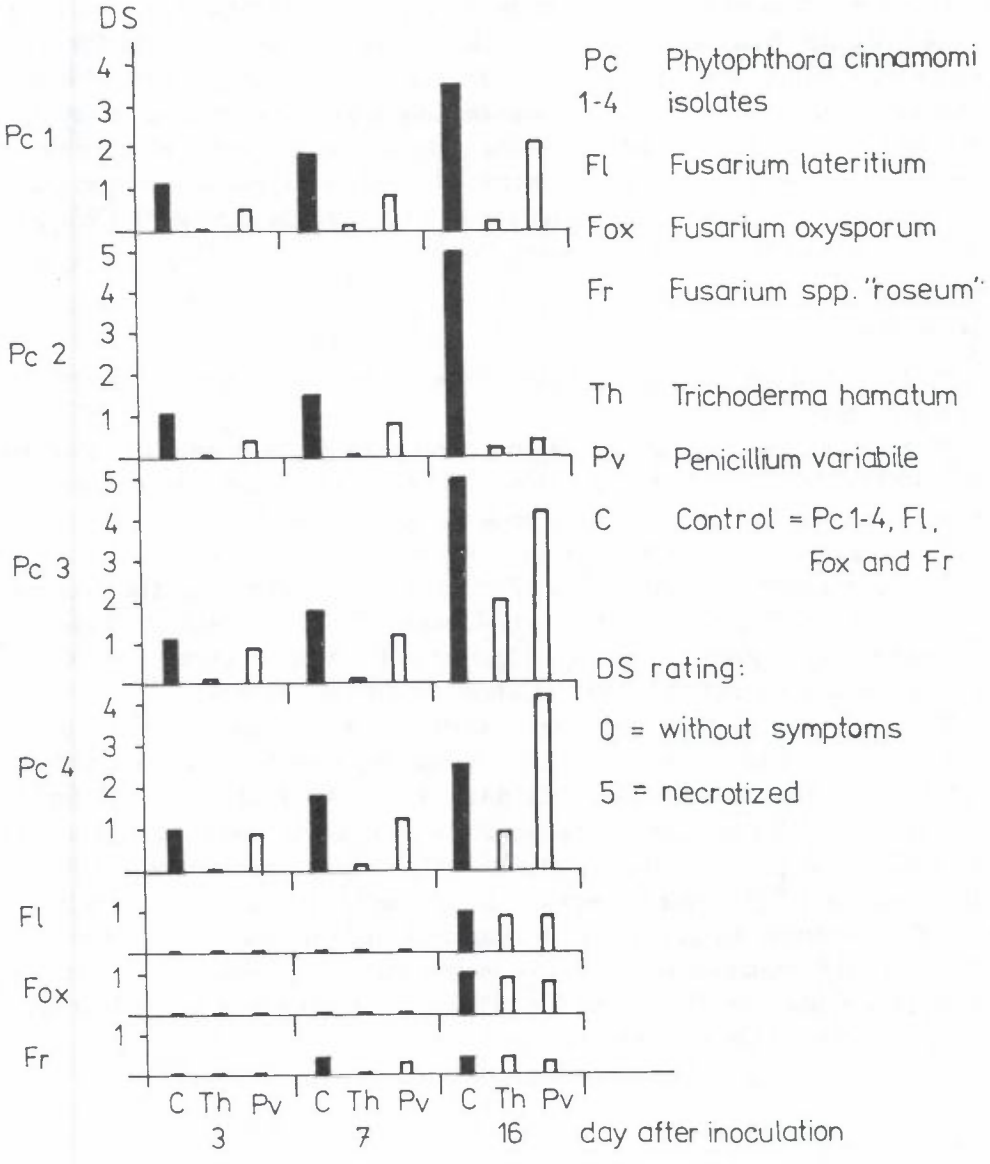


Figure 3. Disease severity (DS) after inoculation of raspberry canes with antagonistic and pathogenic fungi.

*Phytophthora* isolates (Fig. 1). At 28°C *T. hamatum inhibited P. cinnamomi* isolates only on the 3rd and 5th day. On next days this temperature inhibited the growth of *T. hamatum* isolates (irregular inhibition, Fig. 2).

In growth chamber studies Phytophthora isolates induced large necroses on the raspberry canes. Necrotic lesions were visible as early as on the 3rd to 7th day after inoculation. Wilting and dying off of the whole canes were observed on the 16th day after inoculation. Lesions caused by *Fusarium* isolates were small and they developed slowly. Similar results were obtained in the cane segments. Canes inoculated with *P. cinnamomi* and sequentially with *T. hamatum* displayed some necroses, smaller than after *P. cinnamomi* alone. Antagonistic activity of *P. variabile* against *Phytophthora* and *Fusarium* isolates was lower or none (Fig. 3).

**Discussion**

"*In vitro*" studies at 18°C demonstrated an effective interaction between saprophytic and pathogenic fungi.

*Phytophthora* and *Fusarium* genera are typical soilborne pathogens. However, as indicated in earlier studies these are able to incite diseases of above-ground parts of plants. That was the reason to apply antagonistic fungi on raspberry canes and or cane segments to control of *Phytophthora* and *Fusarium* pathogens.

The achievement of an antagonistic effect on above-ground parts of the plants to control of the pathogens is not clear yet. However, Dubos and Bulit (1981) applied *Trichoderma* spp. against *Botrytis* sp. in grapes and in vines, Tronsmo and Dennis (1977) in strawberry and Chet (1987) in wheat against *Septoria tritici.*

The antagonism of *Trichoderma* spp. against certain pathogens was found to be rather specialized on species-species level presumably based on enzymatic actions (Elad et al., 1980, Chet and Baker 1981, Sivan et al., 1984). Elad et al., (1982) found that enzymes of this antagonist penetrate and destroy mycelial walls of a pathogen. According to some authors the degradation of the pathogen mycelial walls might be attributed to $\beta$-1, 3-glucanase and extracellular chitinase of the antagonist (Davet 1983).

A close molecular interaction between antagonistic and pathogenic fungi is supposed. To explain the mechanism responsible for these interactions a number of experiments have been established. The application of bioagens in control of phytopathogenic fungi is supposed to be of increasing importance.

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