# Integrated control of *Phytophthora cinnamomi* by *Trichoderma viride* and fungicides in rooting of *Ericaceae* plants

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## 1. Introduction

Root rot caused by *Phytophthora cinnamomi* Rands. is a serious soilborne disease of *Ericaceae* plants occurring especially during rooting of cuttings and microcuttings in a greenhouse. Chemical control of the pathogens if applied to the soil before planting is only partly effective (2,4 and 5). The use of *Trichoderma* spp as a biocontrol agent against *P. cinnamomi* seemed to be an interesting possibility (12). Data concerning possible antagonism between *Trichoderma* spp. and *P. cinnamomi* are scarce and sometimes controversial (1,10 and 13). The accumulated evidence suggests that antifungal metabolites produced by *Trichoderma* species (e.g. antibiotics) may well have a part to play in antagonism with several pathogens (6,8 and 9). The following account describes the examination of two species of *Trichoderma*: *T. viride* and *T. harzianum* for antibiotics activity. We also tested effects of preparations of *T. viride* and some fungicides on the root rot caused by *P. cinnamomi* on cuttings and microcuttings of *Ericaceae* plants in the greenhouse conditions.

Portion of the work has been published previously (3 and 4).

## 2. Materials and methods

*Trichoderma* isolates were obtained from M. Mańka (Agriculture University, Poznań, Poland) and O. Kamoen (Rijksstation voor Plantenziekten, Merelbeke, Belgium).

Production of non-volatile and volatile antibiotics active against *P. cinnamomi* was tested as described by Dennis and Webster (7 and 8), by an agar layer technique.

Samples for microscopic examination were taken from 5-day-old culture of *P. cinnamomi* treated with non-volatile antibiotics of *T. viride* 29.

For greenhouse experiments, strain *T. viride* 29 as the most active against *P. cinnamomi* was chosen. Mycelium of antagonistic fungus was cultivated

in a Roux bottles on a liquid medium (Malt Extract Broth-Difco) as described by Bojarczuk et al. (3) and added into the medium (peat + perlit, 3:1) where cuttings and microcuttings of *Ericaceae* plants were rooted. Fungicides, Captan or Benlate, were also included in the media in the concentration of 3g per 10 l and 4g per 10 l of the medium, respectively.

## 3. Results and discussion

Antibiotics produced by many isolates of *T. viride* and *T. harzianum* induced severe inhibition of mycelial growth of *P. cinnamomi*. The susceptibility of the pathogen to antibiotics produced by *T. viride* and *T. harzianum* revealed great variation (tab. 1). The most active strain *T. viride* 29 was selected for further experiments. Microscopic examination of the mycelium of *P. cinnamomi* affected by antibiotics produced by *T. viride* revealed obvious morphological differences as compared to the mycelium not treated (Fig.1 A, B). The hyphae were very often irregular, more branched and sometimes thicker than normal. Strong vacuolation and formation of transparent spore-like cells were also observed. All these symptoms were considered as a process

TABLE 1

GROWTH INHIBITION OF *PHYTOPHTHORA CINNAMOMI* BY NONVOLATILE AND VOLATILE FRACTIONS OF ANTIBIOTICS PRODUCED BY STRAINS *TRICHODERMA HARZIANUM* AND *T. VIRIDE* (AFTER 6 DAYS OF CULTURE), AS PERCENTAGE OF THE CONTROL

| Test organisms | Nonvolatile antibiotics | Volatile antibiotics |
|---|---|---|
| *T. viride* 29 | 12,9 | 83,3 |
| *T. viride* 23 | 14,6 | 88,3 |
| *T. viride* 24 | 45,9 | 96,6 |
| *T. viride* 30 | 75,0 | 98,3 |
| *T. viride* 34 | 75,0 | 88,3 |
| *T. viride* 31 | 79,1 | 91,6 |
| *T. harzianum* 27 | 20,8 | 80,0 |
| *T. harzianum* 33 | 37,5 | 70,0 |
| *T. harzianum* 28 | 41,6 | 90,0 |
| *T. harzianum* 26 | 87,5 | 85,0 |
| Control (without treatment) | 100,0 | 100,0 |


Fig.1. Hyphae of *Phytophthora cinnamomi*; A. normal, B. affected by nonvolatile fraction of *Trichoderma viride*. Note greater branching and vacuolation of affected hyphae.

of mycelium degeneration influenced by the metabolites of *Trichoderma*. Such results appeared a promising background for using selected strains in biological control of *Ericaceae* plants against root rot disease during rooting cuttings and microcuttings in a greenhouse. The highest percentage of healthy and rooted cuttings of heath and heathers was obtained in the medium treated with *T. viride* and the fungicide Captan (3g per 10 l of medium), (tab. 2).

TABLE 2

EFFECT OF *TRICHODERMA VIRIDE* AND CAPTAN PREPARATION ON DISEASE INCIDENCE OF *PHYTOPHTHORA CINNAMOMI* DURING ROOTING OF *CALLUNA* AND *ERICA* CUTTINGS (CUTTINGS WERE PLANTED IN A MIXTURE OF A PEAT AND PERLITE, 3:1 IN A GREENHOUSE)

| Treatment | % of diseased cuttings | | |
|---|---|---|---|
| | *Calluna v.* "Aurea" | *Calluna v.* Mrs. R. Gray | *Erica* darleyensis |
| *T. viride* | 46 | 42 | 20 |
| Captan | 36 | 36 | 28 |
| *T. viride* + Captan | 28 | 28 | 21 |
| Control | 62 | 60 | 34 |

Microcuttings (from *in vitro* culture) of *Rhododendron* rooted in the highest proportion and formed the strongest rooting systems in the medium treated with the fungus *T. viride* and the fungicide, Benlate (4g per 10 l of medium), (tab. 3).

TABLE 3
EFFECT OF *TRICHODERMA VIRIDE* AND BENLATE PREPARATION ON ROOTING OF *RHODODENDRONS* MICROCUTTINGS (MICROCUTTINGS WERE PLANTED IN A MIXTURE OF PEAT AND PERLITE, 3:1 IN A GREENHOUSE)

| Treatment | % of rooted cuttings Cultivars | |
|---|---|---|
| | "Van der Hoop" | "Boursault" |
| *T. viride* | 100,0 | 95,0 |
| Benlate | 72,7 | 90,0 |
| *T. viride* + Benlate | 95,5 | 95,5 |
| Control | 71,8 | 70,0 |

Tolerance of *T. viride* to relatively high doses of fumigants has already been shown by Munnecke et al. (11) with suggestion that this phenomenon could be the basis for integrated control of pathogens. From presented results, it may be concluded that *T. viride* exibited some inhibitory properties towards *P. cinnamomi*. Disease control of root rot during rooting cuttings and miccrocuttings of *Ericaceae* plants may be succesful when fungicides (Captan, Benlate ) in a low dose are used along with the application of *Trichoderma*.

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## Zwalczanie zgnilizny korzeniowej roślin wrzosowatych przez zastosowanie grzyba *Trichoderma viride* w kombinacji z fungicydami

Streszczenie

Nielotne i lotne antybiotyki wytwarzane przez *Trichoderma viride* i *T. harzianum* hamują rozwój grzybni *Phytophthora cinnamomi*. Do ukorzeniania sadzonek i mikrosadzonek roślin wrzosowatych zastosowano w warunkach szklarniowych podłoże zawierające grzybnię *T. viride* oraz substancje grzybobójcze takie jak Kaptan lub Benlate. Stwierdzono, że sadzonki i mikrosadzonki najlepiej ukorzeniają się w kombinacji wymienionego grzyba antagonistycznego z małymi dawkami fungicydów.

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