Comparison in different temperatures of growth rate of *Trichoderma* spp. and some fungi isolated from declining oak-trees

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1. Introduction

Observations of decline symptoms of oaks (*Quercus robur*) and lime-trees (*Tilia* sp.) were carried out in May 1991 in Belgium.

Dying of twigs in the upper, middle and bottom part of a crown, as well as necroses on twigs, branches and trunks were established on oak trees. In the case of lime-trees, only dying of twigs was observed.

This paper reports the results of fungi isolation from the above mentioned trees and an effect of *Trichoderma* spp. on most frequently occurring strains.

Trichoderma is a genus of fungi known to attack others. Over several years, therefore, a considerable interest has been shown in *Trichoderma* spp. as biological control agents, which still the spread of some fungi in nature (1,2,3,4).

2. Materials and methods

2.1. Isolation of fungi

Investigation was carried out in 30-60 year old oak stands, in the localities of La Garenne and Wortel. In the case of lime-trees, symptoms of dying twigs were observed in the village Warnant.

Inocula were taken from the living tissue above 2 cm before brown streaks, from living tissue at the point of contact with the necrotic spot and from more or less advanced necroses.

Inocula were transferred onto potato dextrose agar (PDA) and malt agar (MA). Samples of collected material were also placed into most Petri dishes.

2.2. Effect of *Trichoderma* spp.

Trichoderma harzianum Rifai (T) was obtained from R. Veldeman, Rijksstation voor Plantenziekten in Merelbeke, Belgium.

The other fungal cultures, *Ophiostoma querci* (02, 04B), *Diplodia mutila*, *Fusarium lateritium*, *Trichoderma harzianum* (T1) and *T. aureoviride* (T1A) were in most cases isolated from trees on which the studies were carried out.

Standard method was used in this experiment. Disks (5 mm) cut from the margins of vigorously growing cultures were placed at the same time on the surface of PDA plates opposite each other. *O. querci*, *D. mutila* and *F. lateritium* were also inoculated 24 hours before *Trichoderma* spp. In this case the *Trichoderma* isolates were placed about 2 cm away from the edge of the colony.

The plates were incubated in darkness at 3°, 10°, 15°, 20° and 25°C. Observations were carried out on the 7^{th} and 14^{th} day after inoculation.

3. Results

3.1. Isolated fungi

On oak trees 35 fungi strains were identified: *Alternaria tenuissima* (Kunze) Wilt, *Aureobasidium pullulans* (de Bary) Arnoud, *Coniothyrium quercinum* (Bonord.), *Diplodia mutila* F. Fr. apud Mont, *Fusarium solani* (Mart.) Sacc, *Fusicoccum quercus* Oudem, *Mucor* spp. Micheli, *Ophiostoma querci* (Georg.) Nannf., *Penicillium* spp. Link ex. Fr., *Trichoderma harzianum* Rifai, *T. aureoviride* Rifai.

The following species were isolated most frequently:

- *Diplodia mutila*; from branches
- *Fusicoccum quercus*; from twigs
- *Ophiostoma querci*; a) from sapwood of trunk; b) from living tissues above 2 cm before brown streaks (02 isolate); c) from border line between necrotic and healthy sapwood (04B isolate); d) from necrotic bark.
- *Trichoderma harzianum* and *T. aureoviride* from bark of base trunk.

Fusarium lateritium dominated among the fungi isolated from twigs of lime-trees.

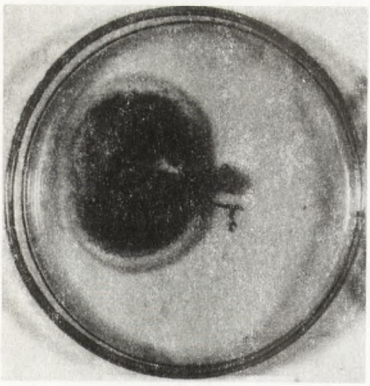
3. 2. Effects of *Trichoderma* spp. on *O. querci* (02, 04B isolates)

Slow growth rate of *T. harzianum* and *T. aureoviride* mycelium was observed at 3°, 10° and 15°C in darkness (Figs. 1A,B, 2A).

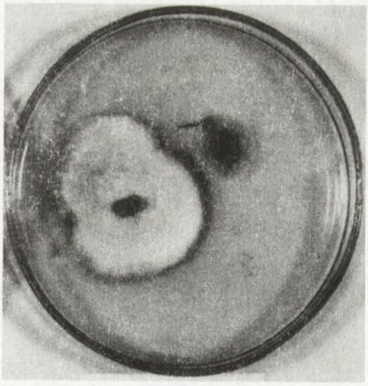
The expansive growth of *T. harzianum* colony overgrowing the *O. querci* colony was noticeable at 20°C (Fig. 2B).

At 25°C, between *T. harzianum* and *O. querci* colonies a narrow, gap line (about 5 mm in 02 isolate, about 1 mm in 04B isolate) with scant contacting each other mycelium was observed (Figs. 3A, B).

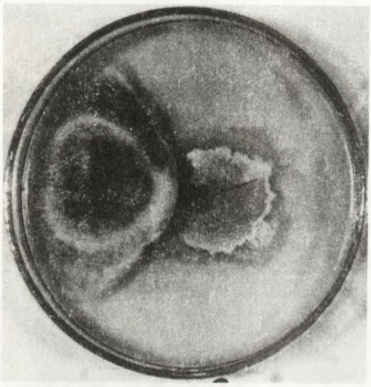
In the case of *T. aureoviride* no differences in growth rate were found between 20° and 25°C. In these temperatures, *T. aureoviride* mycelium spread faster than *O. querci* isolates, but between them a narrow, thin mycelial barrage line was observed (Figs. 4A, B).



1 3° C
0.4B T.



10° C
0.4B T.



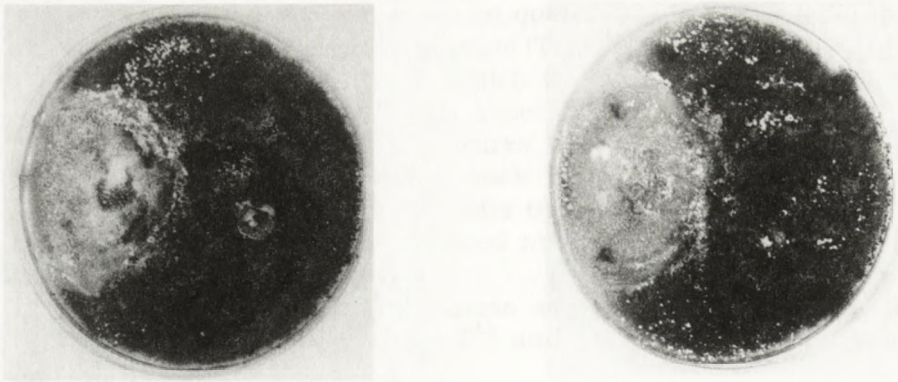
2 15° C
0.4B T.

20° C
0.4B T.

3 25° C
0.2B T.

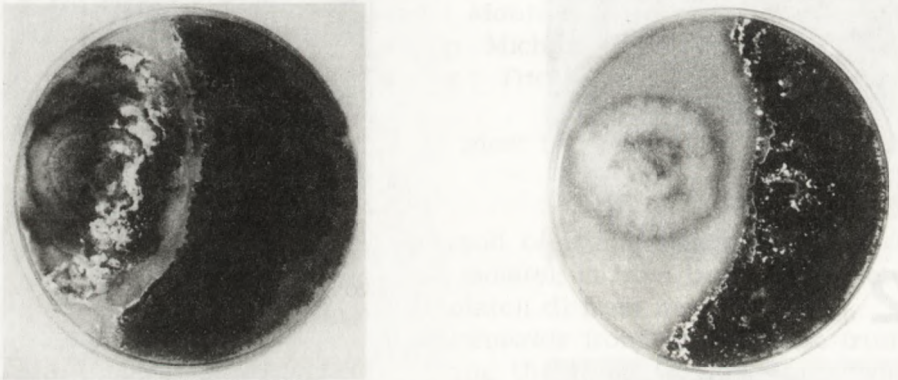
25° C
0.4B T.

Figs. 1,2 and 3. Effect of temperatures on the growth rate of *O. querci* (02, 04B) and *T. harzianum* (T).



4 04B T1A 02 T1A

Fig. 4. Growth of *T. aureoviride* (T_1A) and *O. querci* (04B, 02) colonies at 25°C.



5 D T1A F T1A

Fig. 5. Growth of *T. aureoviride* (T_1A) *D. mutila* with *F. lateritium* (F) at 25°C.

3.3. Effects of *Trichoderma* spp. on *D. mutila* and *F. lateritium*

Between *T. aureoviride* and *D. mutila* or *F. lateritium* no mycelium contact was observed at 20° and 25°C. On the contrary, the inhibition of growth of *Trichoderma* colony was visible (Figs. 5A, B).

T. harzianum growth rate was usually faster than those of *D. mutila* and *F. lateritium* at both 20° and 25°C. *Trichoderma* mycelium only partially covered the opposite fungi colony.

4. Conclusions

The obtained results lead to the following conclusions:

1. *D. mutila*, *F. quercus*, *O. querci*, *Trichoderma* spp. belonged to species most frequently occurring on oak trees with symptoms of decline in Belgium.

2. Apparent antagonistic activity of *Trichoderma harzianum* against *O. querci* was visible at 20°C in darkness despite the mycelial contact between two fungi at 25°C was observed.

Literature

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Porównanie w różnych temperaturach wzrostu szczepów *Trichoderma* spp. z grzybami *Diplodia mutila*, *Fusicoccum quercus* i *Ophiostoma querci*

Streszczenie

Z dębów (*Quercus robur*) rosnących w La Garenne i Wortel (Belgia), wykazujących symptomy zamierania najczęściej izolowano następujące gatunki grzybów: *Diplodia mutila*, *Fusicoccum quercus*, *Ophiostoma querci*, *Trichoderma harzianum* i *T. aureoviride*. Przeprowadzono wstępne obserwacje nad właściwościami antagonistycznymi szczepów *Trichoderma* w stosunku do wymienionych grzybów.

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