Presumable mechanisms of antagonism involved in protection of standing oaks with *Trichoderma*

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1. Introduction

Over the last few decades, forest ecosystems have been severely stressed by many anthropogenic and climatic factors, including: air pollution, frost, drought, fluctuating water table. As a result, the vitality of the ecosystems has decreased and the sensitivity of trees to biotic harmfull agents has correspondingly increased (14). Similarly, a phenomenon called the "oak decline" also seems to be determined by the interaction of several abiotic and biotic factors weakening the trees (10). Repeated defoliations by insects and expansion of various more or less pathogenic fungi usually precede the death of a tree, very often caused by secondary organisms (7, 9, 11, 13).

To neutralize the activity of some biotic agents involved in the oak decline, chemical and biological methods of tree protection are elaborated (15,18). Till now several techniques have been developed, trying to introduce the propagules of *Trichoderma* into the living trees.

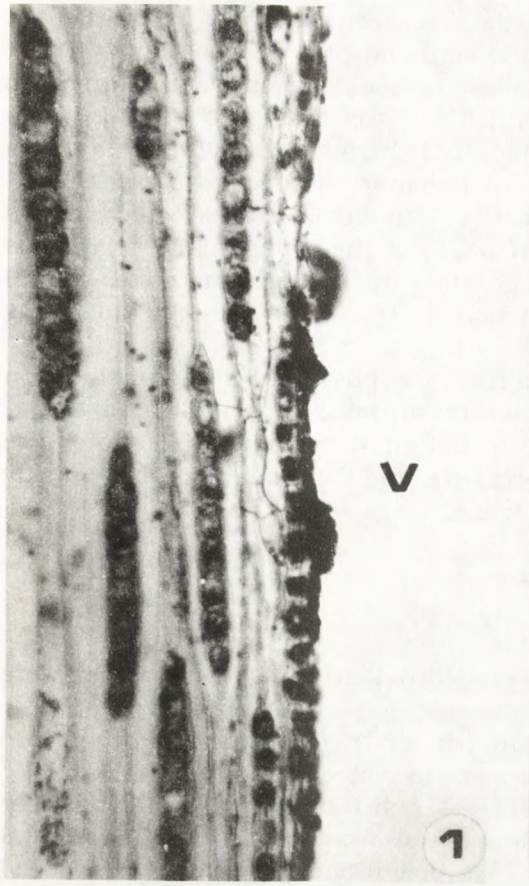
Interventions in the form of spores injection or insertion of pellets into the trunks were often used to control Dutch elm disease as well as several diseases of fruit trees (5, 6, 12, 17).

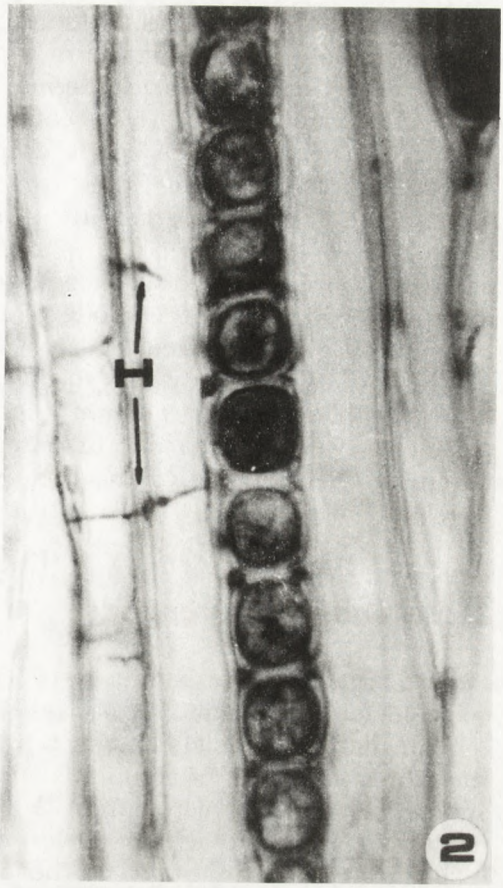
The success of intervention with *Trichoderma* in the form of spores suspension depends on good distribution of spores all over the tree at sufficient quantity and then on the establishment of the antagonist in the tree, so as to stop the development of parasite with sufficient amount of metabolites. Unfortunately, the hypothesis about the possibility of translocation of *Trichoderma* spores throughout the trunk, and then after establishing of the fungus, migration of its active products with the sap stream into the crown, has been criticized. The accumulated evidence suggests that *Trichoderma* is not able to establish itself in an elm and is no longer effective and presumably

Fig. 1. Penetration of hyphae from vessel (V) into tracheids and ray parenchyma of oak sapwood 24 hours after treatment. (Mag. 100x).

Fig. 2. Hyphae (H) crossing wood elements via pits on a way towards radial parenchyma (P). (Mag. 280x).

Fig. 3. Mycelium of *Trichoderma* 48 hours after inoculation. (Mag. 280x).


V
1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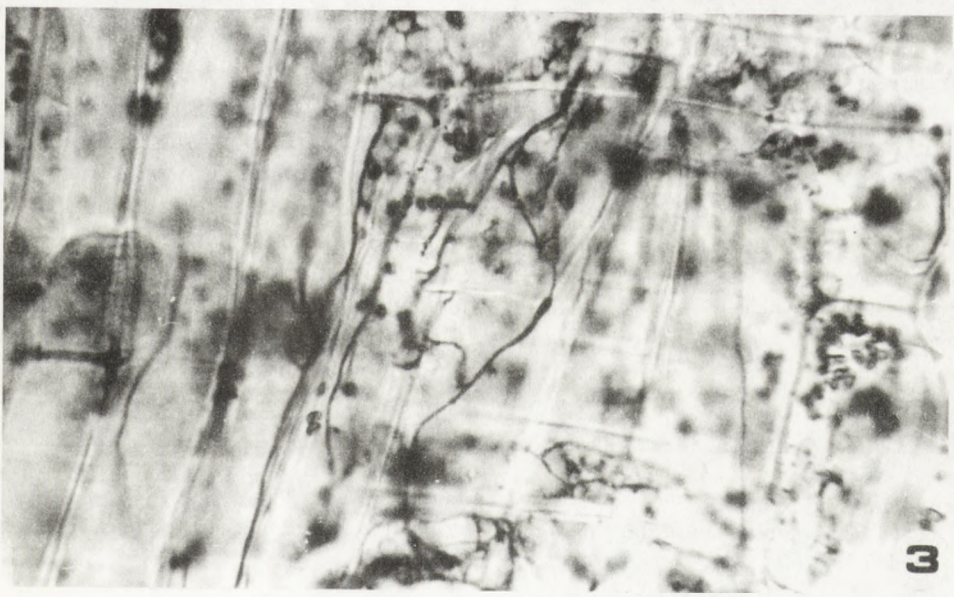
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dies when the food material of the pellet has been used up. Consequently, the data obtained from long-term experiments on protection of elms against *Ceratocystis ulmi* with *Trichoderma* allow to see preventive treatment as more effective than curative treatment. The latter seems to be more successful in the case when crown damage is light and heavy reinfection has not occured (5). However, this does not preclude the efforts to put the intervention with *Trichoderma* into practice. But before the selected species or isolates of *Trichoderma* have been used as the antagonistic organisms against the weak or real pathogens operating in the declining oaks, several questions concerning the mode of action of *Trichoderma* inside the living trees should be answered.

The aim of the study was to investigate the possibilities of translocation and germination of *Trichoderma* spores in sapwood of standing oaks. Experiments were carried out in order to determine whether the spores are taken by the sap due to the root pressure and how far and how quickly they can be distributed all over the trunk.

2. Materials and methods

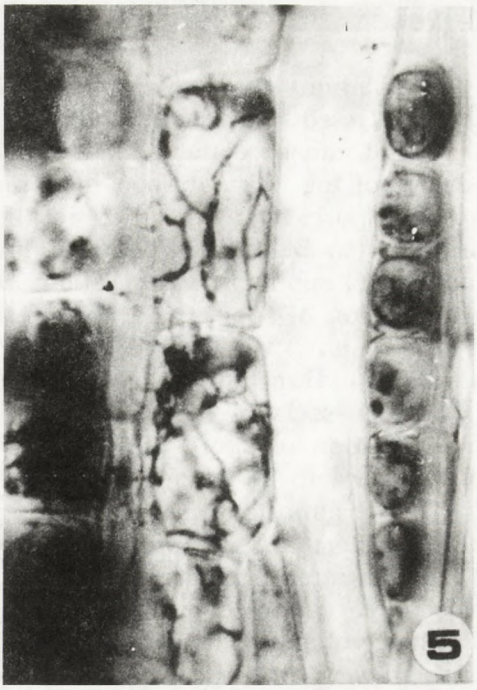
Two healthy, approximately 100-year-old oak trees (*Quercus robur*) were selected for treatment.

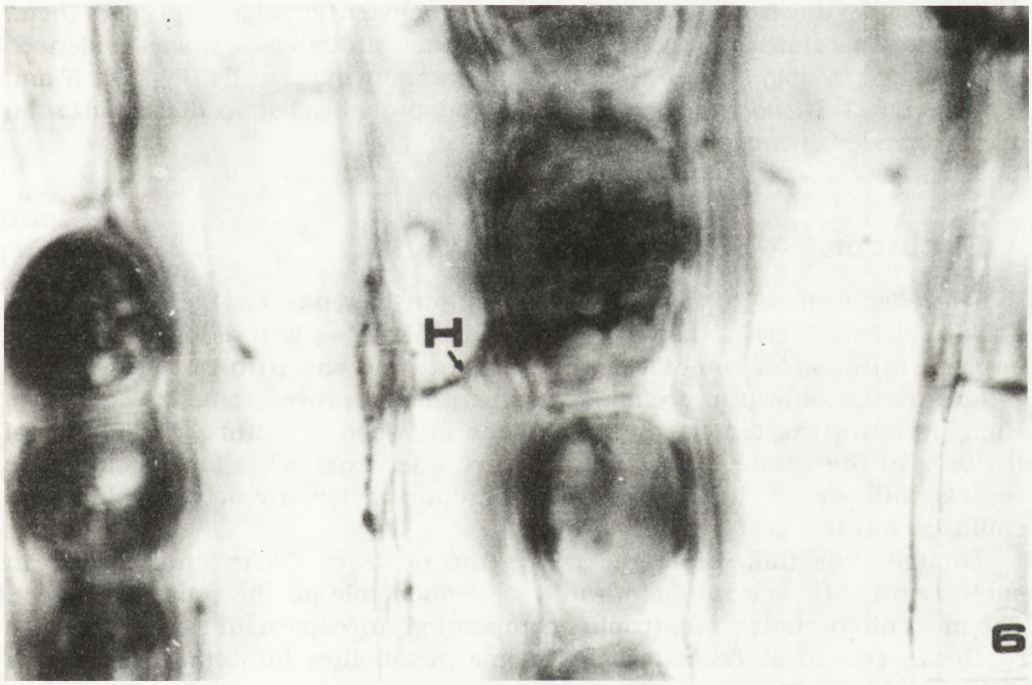
The method consists in the introduction of *Trichoderma harzianum* into the trunk in a form of conidial suspension in concentrations 0.7-1.5 x 10^{10} spores per ml, harvested from 2-week-old cultures growing on malt agar medium. The conidial suspension flowed out from bottles into horizontal holes drilled in the trunk at the breast height inside the white coloured sapwood. The use of bottles assured slow uptake of spores with the transpiration stream. In another experiment, the conidial suspensions were injected into the trunk of oak by pressure (2-4 at.).

The experiments were carried out in May 1991, on warm, sunny days when weather conditions favoured the process of transpiration. Detection of the spores along the length of the trunk after inoculation was performed by: incubation of wood pieces in wet chambers and subsequent observations of the development of the fungus colonies, reisolations of the fungus on MA medium or sterilized sweet beet pulp and by microscopic examination of wood slides made by hand and stained with aniline blue. Wood samples were collected from different levels of the trunk, under and below the point of inoculation and at different intervals, up to 9 days after treatment.

Fig. 4 and 5. Elements of sapwood occupied by the hyphae of *Trichoderma* 5 days after inoculation. (Mag. 280x).

Fig. 6. Necrotic reaction of the parenchymatic cells (P) invaded by the hyphae of *Trichoderma* (H). (Mag. 700x).




H

3. Results

Reisolations of *Trichoderma* on both MA medium and sugar beet pulp from collected samples of wood proved to be positive. On the basis of obtained results it can be concluded that translocation of *Trichoderma* spores inside vessels of the oak sapwood took place.

The spores were recorded in the sapwood at the level of 50 cm above and 80 cm below the point of inoculation and only 48 hours after the moment of spores injection. Microscopic examination of wood slides revealed germination of spores and mycelium development 24 hours after treatment (Figs. 1 and 3), and shed some light on the mode of colonization of oak sapwood by the hyphae of *Trichoderma.*

Wide vessels, a characteristic trait of oak, can play a positive role in translocation of spores. Also small size of *Trichoderma* spores gives good possibilities to be carried through the sap stream of oak tree.

The germination of spores and mycelium development ceased, in a great degree, the migration of spores through the vessels in a period later than 48 hours after treatment, and probably determined the dimensions of the wood area occupied by the fungus.

The hyphae of *Trichoderma* were found quite often inside the pits. Some of them were crossing the pits on their way towards adjoining wood elements (Figs. 2 and 4). These findings suggest the way of colonization of the oak sapwood by *Trichoderma*.

As a rule, the hyphae entered the parenchymatic cells, occupied them, and after some time produced a reaction of their protoplasts. It was evidenced by a change in colour of contents of the parenchymatic cells (Figs. 4, 5 and 6). Activity of *Trichoderma* inside the wood probably led to disorganization of the invaded living protoplasts.

4. Discussion

The effectiveness of biological intervention depends on the antagonistic activity of *Trichoderma* species or selected isolates as well as on the method applied. Injection of conidial suspension into the oak trunk by pressure (2 - 4 at), as was shown in preliminary experiments, proved to be less effective. High pressure exerted harmful influence on wood structure. Formation of thyloses in the tissue injured by pressure was responsible for chocking the vessels with spores. In this way, a strong barrier for any active metabolites could be formed.

Though it is difficult to determine the mode of *Trichoderma* action in such treatments, several factors may be responsible for the protective effect. The most often quoted are: trophic competition, mycoparasitic action, release by the mycelium of *Trichoderma* of some metabolites inhibiting the growth of the pathogen (5, 16).

The antagonistic activity of *Trichoderma* ssp. against *Ceratocystis ulmi* in elms treated by Ricard, could not be explained only in terms of mycoparasitic action as the intermingling of antagonistic hyphae was rather rarely observed (12). However, species of *Trichoderma* produce several catabolic enzymes: cellulases, chitinases and glucanases involved in the antagonism against plantpathogenic fungi (2, 3, 8). Another explanation is based on a release of metabolites by mycelium of *Trichoderma* into the sap stream, and then their translocation, in the same way as toxic products of some parasites are distributed within the tree. There are three main types of known metabolites to be released by *Trichoderma* mycelium. Apart from the exoenzymes, mentioned above, these are: volatiles and in some cases antibiotics (1, 2, 5).

Though at this stage of the investigation it is difficult to answer the question what is the real cause of the cell death, two options should be taken into account. One involves the hypersensitive (non-host) response of the parenchyma to the presence of the hyphae. Injury of protoplast does not mean its utilization – the action recognized as typical of virulent pathogens. The other may be based on an assumption, that just like in the case of an action of a parasite the cell death is simply caused by the enzymatic activity of some strains or isolates of *Trichoderma*. Anyway, phenolic and/or toxic substances formed in the course of these two processes can play a role of a chemical barrier restricting the development of any pathogens and saprofites, including the introduced *Trichoderma*. The limited duration of the protective effect observed in elms after intervention with pellets was described by Ricard as the cause of exhaustion of nutrients provided with the pellets by the fungus (12).

5. Conclusions

Apparently quicker translocation of *Trichoderma* spores down than up the trunks after the treatment, allows to see this kind of intervention as especially preferable for supression of root attacks by *Armillaria* sp. and other root decaying fungi. Demonstration of the existance of propagules or active metabolites of *Trichoderma* in the upper parts of the tree after biological intervention needs long-term experiments. They should be carried out in order to determine the advisability of such treatment in the process of protection against pathogens operating in the crown. But in this case it should be remembered that defoliations observed after attacks of parasite in the crown disturb the process of transpiration and make migration of spores and metabolites of *Trichoderma* far less possible.

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Przypuszczalne mechanizmy antagonistycznego oddziaływania grzyba *Tichoderma* spp., jako czynnika ochronnego w drewnie rosnących dębów

Streszczenie

Po wprowadzeniu zarodników *Trichoderma* w formie wodnej zawiesiny do pni rosnących dębów zaobserwowano ich kiełkowanie oraz rozwój grzybni w drewnie bielastym. Obecność zarodników stwierdzono w naczyniach na poziomie 50 cm powyżej miejsca inokulacji i 80 cm poniżej tegoż miejsca już w 48 godzinach. Transport zarodników na dalsze odległości w obrębie pni, po 48 godzinach od momentu inokulacji, był hamowany na skutek rozwoju grzybni i kolonizowania bielu przez strzępki.

Wyniki uzyskane na podstawie obserwacji mikroskopowych wykazały, że strzępki grzyba *Trichoderma* rozrastają się w drewnie poprzez szparki. Po wniknięciu strzępek do komórek parenchymatycznych zaobserwowano nekrotyczne reakcje protoplastów gospodarza.

W celu ustalenia możliwości i ograniczeń stosowania biopreparatów wykorzystujących antagonistyczne oddziaływanie grzyba *Trichoderma* w stosunku do wielu szkodliwych mikroorganizmów, przedyskutowano prawdopodobne mechanizmy działania antagonisty w obrębie żywego drzewa.

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