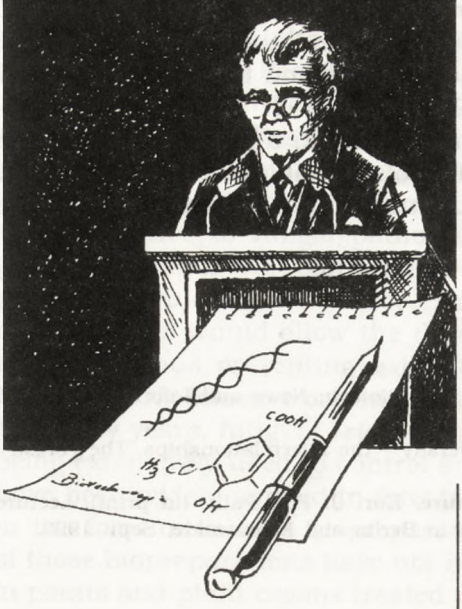
Konferencje



IUFRO

Konferencja "The role of biological control in fungal diseases of broadleaved trees"

Possibilities of *Trichoderma* for control of tree diseases – 10 years of experience and further perspectives

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1. Introduction

Over 10 years, a great number of experiments and studies have been conducted with *Trichoderma* at the Government Research Station for Phytopathology in Merelbeke (Belgium). The cooperation program with the Institute of Dendrology in Kórnik (Poland) during the last years has increased the possibilities of experiments and provided good perspectives for the continuation of this research program in the near future.

Not only the application of *Trichoderma* in horticulture, which gives very promising results, but also using *Trichoderma* to control diseases of trees presents good possibilities.

Studies and experiments were carried out for culturing and applying *Trichoderma* to trees for control of the several fungal pathogens. Preparations and models for the application of the fungus have been developed and applied both to decorative and forest trees. The follow up of the evolution needs of course several years and is still going on both in Belgium and in Poland.

2. Material and methods

2.1. The *Trichoderma* preparations

Several strains of *Trichoderma* isolated from different trees were used in the experiments. Starting in 1980 with the Binab strain isolated from Scots pine by J. Ricard for the control of Dutch Elm Disease (DED), we later isolated *Trichoderma* strains from oak, beech, and Scots pine. Also, strains of *Trichoderma harzianum* applicable in horticulture were involved.

Trichoderma is a well-known antagonist to several trees parasitical fungi. *In vitro* the effects on lots of *Basidiomycetes* and *Hyphomycetes* have been demonstrated: *Armillaria* spp., *Heterobasidion annosum* and other *Polyporaceae*. Other following fungi: *Verticillium*, *Fusarium*, *Pythium*, *Rhizoctonia*, *Sclerotinia* and *Ceratocystis* spp. may also be controlled by *Trichoderma*.

Through culturing *Trichoderma* on weath brans or on dried sugar beet pulp, we obtained several forms of preparations. The most concentrated form consisted of pure conidies, collected from the dried occupied sugar beet pulp, at the moment of grinding. The conidial spores concentrations of about $2\text{-}3 \cdot 10^9$ were obtained. This preparation was destinated to be injected into the trees.

To implant the pellets into the trees, fluid flowable culture of *Trichoderma* was mixed with organic supports and additives to assure a solid preparation in the form of small sticks.

2.2. The application techniques

Beside the use of an appropriate *Trichoderma* strain, the application technique can determine the efficient method of treatment. Several methods of inoculation were applied to each pathogen.

For example, in the vascular tree diseases *Trichoderma* must be entered into the tree and established in the wood tissues. Distribution of the conidies into the vessels is only possible before their germination. The appropriate preparations are the pure conidies combined with some glucosides as a carbon source. The installation of *Trichoderma* into the tissues produces the necessary glucanases to prevent the development of the pathogenical fungi and passes with the sapstream through the vessels all over the tree. This biological concept is clearly demonstrated by the attacks of *Chondrostereum purpureum*. Presence of the fungus somewhere in the tissues of the tree results in the production of diastases transported to the leaves, causing silver leaf disease.

Several techniques of introducing *Trichoderma* conidies into the trees have been tested. The simplest technique which we used was the injection of conidies of *Trichoderma* with a hypodermic injection pistol used in medicine. It allows to inject by shots 1cc of solution with pressure through adapted needles directly into the vascular tissues. Suspentions of *Trichoderma* conidies at concentrations of about $2 \cdot 10^8$ can be applied in this way.

Since the injection does no harm to the tree, the operation of inserting a needle into the bark could be easily done. The number of injection points can be enlarged, assuring a good distribution around the trunk. The last development of an apparatus with battery power supply makes it suitable to be used in the forest too.

Since 1975 when Dutch Elm Disease was treated by injection of chemicals, several injection apparatus have been developped, by pressure or by baxter. It is possible to introduce *Trichoderma* conidies into the tree with both systems, allowing to enter bigger volumes of spores in adapted with nutrient solutions.

The introduction of the *Trichoderma* pellets to the diseased trees was developed by Ricard (Binab.) for control of DED and silver leaf disease. The pellets consist of organic support occupied by *Trichoderma* strains which were introduced to the drilled holes into the trunk. Moistened by the sap, *Trichoderma* develops and conidies with glucanases may be transported through the vessels.

By enlarging the holes up to 15 mm width and depth of about 8 cm, the pellets 10 cc of a suspension of *Trichoderma* conidies at 10^8 concentration can be added, assuring an immediate start of occupation by the fungus of a larger number of cut vessels. With the motorised drill, the installation of pellets with *Trichoderma* is easily done and can be applied in the forest. The choise of adapted *Trichoderma* strains is neccessery.

Although *Trichoderma* installation on trees is recommended for increasing the resistance of the tree and preferably done as prevention, it is also possible to cure attacked trees in so far that the extension of the disease will be limited and recovery process of the tree can start after the treatment.

This kind of intervention is also recommended in the case of attacks by: *Verticillium*, *Ceratocystis* spp., *Chondrostereum*, *Armillaria* spp., and *Heterobasidium annosum*. The availability of *Trichoderma* as a glucanase producer is most effective against the *Basidiomycetes* fungi containing lots of glucan in their mycelium structures.

Treatment of pruning wounds on fruit trees with *Trichoderma* has been successful in France for 20 years now. Combined with implants of *Trichoderma*, silver leaf disease is controled entirely. To control *Ceratocystis* spp. on oaks, implants combined with conidial suspensions were applied.

Interesting results were obtained by Ricard, who used implants for wood protection against all kinds of lignovorous fungi. Since the *Trichoderma* strain used by Ricard was isolated from resistant Scots pine, the use of implants on Scots pine against *Heterobasidion annosum* can be recommended.

Armillaria spp. attacks on all kinds of trees are still an unsolved problem. Although lots of studies have been done on the morphological and physiological behaviour of the fungus, control measures are rarely mentioned. Since *Armillaria* attacks normaly start by the occupation of the roots by the rhizomorphs, intervention in the case of this fungus is difficult. Also the rhizomorphs develop widely in the rooting area of the tree and even pass

to the roots of neighbouring trees. *Armillaria* has proven to be lignovorous, killing the bark and the wood of roots and of the trunk, and very often accompanied by several other lignovorous parasites. Both *in vitro* and *in vivo Trichoderma* strains can destruct and kill the *Armillaria* rhizomorphs, so that the presence of *Trichoderma* offers protection against the expansion of the pathogen rhizomorphs.

Different techniques of soil inoculation were applied. Around ornamental trees in city parks and private gardens the soil was removed by a hard water stream, so that the rhizomorphs at the base of the tree were denuded.

Spraying with fluid *Trichoderma* at concentration 10^7 C.F.U. assures an occupation of all the rhizomorphs with *Trichoderma* and results in their destruction. By replacing the removed soil using a suppresive substrate provided by *Trichoderma*, it is possible to assure further protection.

Since we observed the presence of rhizomorphs around the whole rooting area of the tree, treatment by soil injection completes the intervention. For this purpose, an organic nutrient solution provided with *Trichoderma* has been developed to be injected by ± 5 l at every 1 sqm of the surface.

This intervention also stimulates the recovery of the whole tree, increasing its resistance. Also in the case of oak decline problems, soil injection with this appropriate nutrient solution can be recommended. Recent developments of *Trichoderma* applications include the use of the fluid preparation combined with some adhesive products and nutrients to cover stumps of Scots pine to prevent extension of the *Heterobasidion annosum*. Trials were started successfully and results will be obtained in several years.

3. Experiments and results

On fruit trees. Silver leaf disease caused by *Chondrostereum purpureum* becomes generally spread on fruit trees, even on appletrees and peartrees. Successful application of *Trichoderma* conidies on pruning wounds prevents the spread of the fungus. In 1980 we installed *Trichoderma* pellets on about 1500 attacked trees, which resulted in a complete recovery of the most treated trees.

On poplar. Beside leaf bark and root fungal diseases, attacks by *Dothichiza populea* and *Armillaria mellea* on young poplar trees after planting cause a lot of damage. *Dothichiza* is known as a typical parasite on weakened trees. Latent infections from nursery develop after planting. Treatment of young plants with a spray of *Trichoderma* conidies before planting gives good protection against this fungus.

Very often poplar plantations using rotation are badly infected with *Armillaria mellea*. Replying the plantholes with inoculated substrate assures protection against the *Armillaria* rhizomorphs, and also stimulates good root formation.

On beech. On ornamental beeches, the attacks of *Armillaria mellea* are entries for *Polyporus giganteus*, resulting in a dieback of the trees. Removing the soil at the base of the diseased tree to denude the attacked roots, makes it possible to reach the rhizomorphs of *Armillaria* by suspension of *Trichoderma* conidies. Also the mycelium of *Polyporus gigantia* in the wood can be treated in chemical or biological ways.

The denuded roots are covered with the suppresive substrate to prevent further development of *Armillaria*, and to stimulate the formation of young roots and mycorhizes fungi.

On elm. The explosion of Dutch Elm Disease in the last years gave rise to many experiments aimed at protection of these trees by chemical and biological methods.

The formation of thylloses excited by the *Ceratocystis* attacks cannot be limited by chemical intervention. The protection of the elm trees must be organised as prevention from *Ceratocystis* infections. Only on poorly attacked trees, the intervention with *Trichoderma* gave good results.

On lime and mapple. The fungus *Verticillium* causing a serious disease on lime and mapple trees is very susceptible to *Trichoderma* antagonism. Since the fungus starts its attacks from the roots, treatment at the base is most efficient. The treatment can be improved by the installation of *Trichoderma* pellets in the trunk.

On spruce. Several hundred of young plants of Norway spruce in the forest attacked by *Armillaria* have been treated by injection of *Trichoderma* conidies at the base of the tree. Although some success has been observed, it was not possible to save the already infected plants. The only cure so far seems to be the protection of plants surrounding the infected ones.

On pine. Ricard isolated a *Trichoderma* strain from resistant Scots pine in a Swedish forest in compromised areas. This strain was largely applied in several field experiments. A good antagonistic reaction to *Heterobasidion annosum* offers possibilities for intervention against this disease too.

4. Further perspectives

On oaks – since *Trichoderma* offers possibilities for D.E.D. caused by *Ceratocystis ulmi*, the same antagonistic effects can be expected to occur with regard to other *Ceratocystis* spp. too. In the oak decline process both *Ceratocystis* and *Armillaria* are involved. Intervention with *Trichoderma* can increase the resistance of compromised trees.

Recent cooperation programs with Polish and Czecho-Slovakia scientists have allowed us to install trial plots in Belgium, Poland and Czecho-Slovakia.

Trials on several hundred of oaks have been conducted very carefully and the development of the oak decline will follow up in the future. Several modes of application of *Trichoderma* are involved. Results will be estimated after several years of observations.

On plane trees – up to now, there has been no successful control of the attacks of plane trees by the fungus *Ceratocystis fimbriata* in Southern Europe. The scientists of these regions are ready to organise biological control systems with the use of *Trichoderma* preparates too.

On Scots pine – since Ricard isolated a good antagonistic strain of *Trichoderma* from Scots pine, this strain can be introduced for control of a disease caused by *Heterobasidion annosum*. By the propagation of the infection the porulations on the stumps after cutting trees are very dangerous. Treatment of the surface by an adapted *Trichoderma* preparate could prevent the propagation and the development of the pathogen.

Acknowledgement

This meeting marks the end of the 20 years of my scientific cooperation with the Institute of Dendrology in Kórnik, especially with Prof. Dr. R. Siwecki and his team. Since I am going to retire I hope, that the *Trichoderma* program will be continued in Kórnik. During all these years, an exchange of information and contacts between scientists was fruitful and agreeable, and I hope it will be continued in the future.

Możliwości *Trichodermy* w biologicznym zwalczaniu chorób drzew – 10 lat doświadczeń i przyszłe perspektywy

Streszczenie

Przez około 10 lat przeprowadzano zarówno w Belgii jak i w Polsce doświadczenia aplikacyjne i badania laboratoryjne nad zastosowaniem preparatów biologicznych na bazie grzyba *Trichoderma*. Doprowadziły one do opracowania skutecznych metod zwalczania niektórych groźnych chorób grzybowych drzew. Jednakże zastosowanie tych preparatów powoduje tylko zwiększenie odporności tkanek drzew, które nie są zaatakowane przez patogeniczne grzyby. *Trichoderma* nie może spowodować wyzdrowienia zaatakowanych już tkanek, ale może znacznie ograniczyć stopień ich uszkodzenia. Zastosowanie preparatów bezpośrednio do gleby i korzeni poprawia zdecydowanie zdrowotność chorych drzew.

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