Biocontrol of Decay or Pathogenic Fungi in Wood and Trees

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Abstract

Two types of biocontrol preparations are marketed for the treatment of wood and trees against harmful fungi: *Peniophora gigantea* spore tablets and *Trichoderma* pellets or wettable powder. Preparations containing *Trichoderma* spp. propagules required nearly 50 years of development to reach routine commercial stage. Both preparations are cost effective and are safe for the consumer, the worker and the environment. This report reviews the current situation, obstacles hindering use of biological fungicides in wood and trees and discuss opportunities for such use.

Introduction

This paper reviews the obstacles hindering the use of biocontrol fungicides in wood and trees and discusses expanding opportunities for such use.

Trichoderma will be dealt with mostly because of the extensive study of this organism as a biocontrol agent and its current commercial use in some countries (Ricard 1979).

Biocontrol in wood started with the treatment of pine stumps using *Peniophora gigantea* spores to prevent colonization by *Heterobasidion annosum* and the subsequent spread of the pathogen to adjacent pine trees (Rishbeth 1963). The British Forestry Commission applied this treatment extensively before registration was required for such preparations. *Peniophora,* however, is limited to protecting pine stumps. For Douglas fir or Norway spruce stumps other biocontrol fungi have to be considered, especially *Trichoderma* spp. (Nelson 1985, Kallio and Hallaksela 1979).

The biocontrol potential of *Trichoderma lignorum* against *Rhizoctonia solani* was reported by Weindling (1932), shortly after the antagonistic properties of Penicillium spp. were observed. However, nearly one-half century elapsed before significant commercialization materialized, and not against the pathogen involved originally.

Current commercial *Trichoderma* preparations are mainly available as a wettable powder and as pellets both containing propagules of *Trichoderma* spp. ATCC 20475 and 20476 (American Type Culture Collection) or IMI 206 039 and 206 040 (Commonwealth Mycological Institute) called T.75 and T-76 in this report. The powder is mixed with water and sprayed or painted on wood. Pellets are inserted in wood of live trees, in stumps, or in structural timbers such as poles, piling or curbing. Both are produced in Toreboda, Sweden, by Bio Innovation AB (BINAB TM)[1].

Routinely, *Trichoderma* mycofungicidal preparations are used for the treatment of fruit trees against silver leaf disease caused by *Chondrostereum purpureum.* Several other applications are approaching commercial use for greenhouse and field crops. At least six countries have registered Trichoderma preparations for biocontrol purposes. These countries are, in chronological order of their registery use: France, United Kingdom, Belgium, Switzerland, Sweden and Chile. In the United States, registration applications have been pending for the past 10 years with the Environmental Protection Agency (EPA). Although registration has not been granted, the EPA has issued an experimental use permit for genetically engineered *Trichoderma* strains (Harmen 1987). The USDA Animal and Plant Health Inspection Service has issued import permits to commercial and federal organisations in the United States for commercial *Trichoderma* preparations manufactured and registered in Sweden. However, those permits are for laboratory studies only. The State of Wisconsin has authorized field tests with the *Trichoderma* preparations mentioned above in 1987.

Grafted plum (cultivar Sta. Isabel) at Buin, Chile, treated in December 1986. (J. Poblete).

Footnote

1 *This publication reports research involving pesticides. It does not contain recommendations for their use, nor does it imply that the uses discussed here have been registered. All uses of pesticides must be registered by appropriate State and/or Federal agencies before they can be recommended.*

Currently Used Mycofungicidal Preparations

Control of silver leaf disease by *Trichoderma* - a case study

The largest, single commercial use of *Trichoderma* preparations for biocontrol purposes is on fruit trees to prevent and cure silver leaf caused by *Chondstereum purpureum*. Its use for this purpose was demonstrated by Grosclaude (1970), Corke (1974), Dubos and Ricard (1974). This use is discussed in detail below because it illustrates several important considerations when applying biocontrol agents as compared with applying toxic or synthetic chemical fungicides.

Chondrostereum purpureum is an ubiquitous basidiomycete adapted to a wide range of woody substrates in live trees and in logs or stumps. It has received considerable attention because of the damage it causes in valuable fruit trees, including the slow-growing long-lasting perry-pear trees popular in Southern England. It is also interesting as a systemic plant pathogen. The *C. purpureum* colony growing in wood of stem, root or major limb releases metabolites in the sap stream. These metabolites are not known to affect the tree adversely until they reach the leaves. In the presence of *C. purpureum* metabolites the leaf epidermis separates from the mesophyll. Air enters the vacant space, altering the usual light reflection of the leaf and giving it instead a metallic sheen - hence the name of silver leaf for the disease. Once gross symptoms have developed, trees die, sometimes in one year, sometimes after several seasons. In intensively managed orchards, trees developing gross silver leaf symptoms are replaced the same year.

Infection of the trees takes place when *C. purpureum* spores come in contact with unprotected wood, most often on pruning wounds. Root infection can occur also as with *Heterobasidion annosum* or *Ceratocystis ulmi*. In susceptible varieties, the pruning wound route can be used to demonstrate the infection mechanism. Grow silver leaf symptoms can be observed within a few weeks after dropping *C. purpureum* spores on a fresh wound shortly before bud break.

With obvious symptoms to provide controls, the preventive, effect of *Trichoderma* prepartions is clearly shown (Grosclaude 1970). This evidence has not been challenged - which is unusual where biocontrol agents are involved. For curative treatment, various preparations were inserted in the trunks treated by Dubos and Ricard (1974). Further work on curative treatment was performed at the Long Ashton Research Station of the University of Bristol. After seven years of field trials, comprehensive reports were published (Corke 1978) and commercial use started of *Trichoderma* in the United Kingdom with the approval of the Pesticide Safety Precautions Scheme authorities. The commercial use of *Trichoderma* pellets for curative treatment and wettable powder for preventive treatment of fruit trees and eucalyptus against *C. purpureum* has spread officially tu other countries since, particularly where intensive orchard management prevails.

Table 1. Cost, including product and labour, of curative treatment per plant with *Trichoderma* pellets, considering an average dosage.

Crop	Average yield/tree (kg)	Cost (kg of fruit)*	Cost (%)
Peach	76	1.78	2.34
Plum	55	5.68	10.33
Grapevine	40	2.86	7.15

** The cost is expressed as the equivalent of the wholesale value of one kg of fruit.*

Cost Effectiveness Once the efficacy and safety of the biocontrol approach to silver leaf treatment were demonstrated, comprehensive cost effectiveness studies were made over a period of 4 years. These results were summarized by J. Poblete at the recent Second International *Tricchoderma/Gliocladium* Workshop at the University of Salford, United Kingdom (April 1967) (tables 1 and 2). Comparison of *Trichoderma* and chemical treatment (Captafol, 1.5% + paint) for wound protection in Japanese plums (*Prunus salicina*) showed the *Trichoderma* treatment to be much more effective than the chemical treatment after four growing seasons in Chile.

Safety, Standard LD50 toxicity tests (acute and chronic) allergenicity tests (dermal and anaphylactic shock induction) showed no adverse effect from *Trichoderma* preparations tested. Detailed records of massive exposure to humans from T-75 and T-76 without harmful effect, have been kept as suggested by the World Health Organization (1974). One unique feature of these *Trichoderma* preparations as compared with classical chemical fungicides is their safety. This has been demonstrated by routine feeding of *Trichoderma* preparations to cattle.

Trichoderma enzyme extracts to enhance cellulose digestion of rough forage in ruminants, espcially cattle, was started in Japan in the 1960's (Toyama 1969). At least two Japanese firms manufactured *Trichoderma* preparations for that purpose. This indicated that cattle were not affected adversely by *Trichoderma* extracts. No adverse effect has been observed from feeding of BINAB's *Trichoderma* preparations to cattle in various forms ranging from plain-active ingredient to finished product, especially pellets. This practice was started in 1981 on a Scottish Highland cattle helfer, which has produced five normal calves since.

Ability of Trichoderma-based biocontrol agent to perform as a systemic. the precise mechanism by which T-75 and T-76 control silver leaf is not known, although progress is being made. However, it has been shown repeatedly that *Trichoderma* pellets inserted In the trunk of a fruit tree will control symptoms of the disease and improve fruit yield. Whether or not that improvement is the results of mycoparasitism on the *C. purpureum* colonies or inactivation of toxic metabolites released in the sapstream is not known. N15 studies have shown that labelled materials inserted in the trunk of young elms about 5m tall were distributed upwards very quickly and extensively. Downward translocation also takes place, although less readily (Wikstrom 1984). Detailed information on the mode of action of *Trichoderma* spp. and strains is given under a separate heading below,

Table 2. Control after one growing season, obtained with the use of *Trichoderma* ATCC 20475 and 20476

Crop	Without symptoms (%)	Mild (%)	Medium (%)	Severe (%)
Plums (1)	73.75	12.5	5.06	8.69
Peach (1)	34.43	10.49	19.52	35.34
Plums (2)	100.00	0	0	0
Peach (2)	100.00	0	0	0

(1) Plants inoculated with medium to severe symptoms
(2) Early detection and inoculation

Durability of Treatment Treatment of fruit trees against *C. purpureum* lasts at least 4 years in intensively managed orchards. Seven of the first group of *Prunus mariana* GF-8-1 treated in Southwest France by Grosclaude in 1969 were transported to Sweden and replanted in Sigtuna near Stockholm, across a road from a hedge heavily infected with *C. purpureum.* Fruiting bodies and dead stems in the hedge were readily noticeable at all times. Because of their vigorous growth the GE-8-1 were pruned every year in late winter. None showed extensive silver leaf symptoms. One tree trunk displayed a small sporophore of *C. purpureum* on the heartwood of a large well-healed wound, The foliage showed no silvering, the leaves were abundant and growth was comparable to that of the other trees. In contrast, in elms the efficacy of treatment with T-75 and T-76 pellets did not last over two years in areas of intense *Ceratocystis ulmi* infection.

Registration All developed countries require a sales permit for chemical pesticides and distributors of biocontrol agents usually are eager to have a permit system because of product liability. National governments also are interested, in principle, in biocontrol agents.

Generally registration authorities consider two aspects: safety and efficacy. Safety was the primary concern in the first two registrations granted in France and the United Kingdom succesively. Both countries were concerned about acute oral toxicity, chronic toxicity (90 days) and allergenicity.

In France, the main concern expressed was about the maximum temperature for growth of the *Trichoderma* strains. It was believed that cultures unable to grow at normal human body temperature (37°C) were not likely to cause human pathogenecity. Not that mesophilic cultures would automatically be harmful, but they would need greater scrutiny in quality assurance tests. Neither T-75 nor T-76 grow over 32°C on agar plates and not much over 25°C under production conditions. The British authorities were more concerned with the human exposure record. They requested a record of employees who came in contact with BINAB's preparations and the length of time they were in contact with the preparations. The employee with the longest exposure record was requested to provide blood for *Trichoderma* antibody tests. None were found.

Extensive environmental consideratiom were not raised because of the nearly universal distribution of *Trichoderma* spp. in soils and woods in the temperate zones as well as its quick recycling by many bacteria. The latter feature can be a distinct problem during manufacturing operations.

Efficacy data from private sources were accepted by registration authorities in France, though that was changed after the temporary registration for *Trichoderma* preparations was issued in 1976 (Bally and Dubois 1979). French State Agricultural Institutions then demonstrated that *Trichoderma* preparations were unsatisfactory for the control of *Verticillium malthousei (=fungicola)* in casing soil of cultivated mushrooms *(Agaricus bisporus).* High yields required the removal of Excessive numbers of Agaricus sporophores at the pinhead stage (Oliver 1978),

In 1979 British authorities did not require officially efficacy data, only safety information for PSPS (Pesticides Safety Precautions Scheme). However no application for 'commercial clearance' was made for seven years until scientists at the Long Ashton Research Station of the University of Bristol had proven the efficacy of the preparations in field trials (Corke 1978). Registration rules in England have now changed making efficacy data from USDA-equivalent sources compulsory.

Belgium required efficacy data provided by a state research laboratory (Veldeman 1986). Swiss authorities requested information on results obtained elsewhere In Europe.

For a fee Swedish authorities made field tests primarily to show whether or not *Trichoderma* preparations were harmful to fruit trees. During the official trials no artificial inoculations were made with *Chondrostereum purpureum* and natural infection yielded no silver leaf symptoms in the control trees.

In the United States no attempt was made to obtain registration of *Trichoderma* preparations for treatment of silver leaf, only structural timber and tree wounds because of marketing considerations. No registration has been obtained even though standard laboratory tests show excellent efficacy against the main decay basidiomycetes in Douglas-fir and pine respectively *Antrodia carbonica* and *Lentinus lepideus* (Highley and Ricard 1987, Murmanis, et al 1987).

In Chile, in cooperation with state University experts, efficacy tests were made for three growing seasons mainly in private orchards. Registration was granted in 1987 (Poblete).

Funding This is a complex matter. A number of different approaches have been tried to finance the development of biocontrol agents. Failures have been more frequent than successes. State grants, private investors; venture capital organisations, high profit conglomerates, state-owned banks, end privately-owned banks have been involved in varying degrees.

An effective funding policy in terms of cost effectiveness has been offered by private banks in Sweden. The 'one small step at a time' with close monitoring of progress rather than the 'feast and famine' approach has been constructive in the commercialisation of *Trichoderma* preparations. Protracted British field trials were made by the Long Ashton Research Station of the University of Bristol at no cash cost to the manufacturer. On the other hand, Swedish official efficacy verification tests were invoiced at about $400 per year for evaluation of foliage condition. Labour and materials had to be provided by the manufacturer for initial inoculations. A yearly fee of about $1,600 is required for maintenance of the registration.

In certain European countries, more money has been invested in biocontrol-oriented companies, now defunct (Lisanski 1987), than in Sweden where BINAD is still in business. Administrators often overlook the fact that if 'time is money', the reverse is not true. For chemical pesticide development considerable time is known to be required, about 10 years between completion of research and beginning of marketing [Wellman 1977). No amount of money can eliminate this time lag. This was especially true for biocontrol agents during the years when registration was needed but before rules and regulations were established, or used.

Mode of Action In Nature, *Trichoderma* spp. are usually mycoparasites or saprophytes depending on strain and circumstances. How these activities are performed has been studied extensively but important questions remain. For example, occurrence of mycoparasitism against *Rhizoctonia solani* mainly and other basidiomycetes has been explored in great detail including recognition of the fungus to be attacked (Chet et al 1981), attachment (Chet et al 1985), and perforation of the cell wall (Elad et al 1983).

Lectins (Chet 1987) and exoenzymes (Hasegawa and Nordin 1969) are known to be involved in these events. However, it is not clear what the condition of the attacked basidiomycetes is when the *Trichoderma* hypha becomes attached to it. Volatiles have been known to be released by certain *Trichoderma* strains with lethal properties to other fungi (Deuuls and Webster 1971, Bruce et al 1984). Claydon (1987) isolated, identified and synthesized two such compounds: 6-n-pentyl-2H-pyran-2-one and 6-n-pentenyl-2H-pyran-2-one.

Whereas mycoparasitism and saprophytism appear to be major characteristics of *Trichoderma spp* in nature, a variety of secondary metabolites have teen identified *in vitro* particularly antibiotics. In some cases controversy arose about the exact identify of the fungus studied in connection with certain antibiotics. *In vitro* activities have been comprehensively reviewed by Cook and Baker (1983), Papavizas (1985) and Tronsmo (1986)

Friom the biocontrol standpoint, a primary consideration is that not all Trichoderma strains perform in the same fashion. This applies to naturally occurring strains. In addition mutants (Abd-El Moity et al 1982, Ahmad and Baker 1987) and hybrids are being developed by genetic engineering (Harman et al 1987, stasz et al 1987). Furthermore, synergism takes place between certain strains (Highley and Ricard 1987), so that it is important in detailed research studies to refer to specific strains rather than *'Trichoderma harzianum'*.

The increasingly recognized diversity among *Trichoderma* strains is reflected by a recent classification scheme which proposes 63 species for the genus (Doi and Doi, 1986). Some years ago Bisby (1939) felt one species was all that should be included in the genus *Trichoderma.*

Strain differences concern morphology, optimum growth temperatures and range, release of exoenzymes or volatiles (for example, 'coconut' odour), the more recently studied chemical fungicide tolerance (Abd-El Moity et al 1982), and rhizosphere competence (Ahmad and Baker 1987).

Glucanases produced by *Trichoderma* spp. have received much attention and are used for pharmaceutical purposes. Chitinase has also been studied extensively. All naturally occurring Trichoderma strains form one or both types of exoenzymes. Since all fungi have chitin in their exoskeletal cell wall, all Trichoderma theoretically have the ability to parasitize other fungi. Whether or not parasitism occurs in a given situation depends mainly on respective adjustment of the main microorganisms involved to the particular econogical situation. For example, many *T. harzianum* strains parasitize Heterobasidion annosum at room temperature, but none seem to do it effectively in the woods (Kollio and Hallaksela 1979). When the minimum growth temperatures of the two fungi are considered, a difference clearly shows dark green conidia forming *T. harzianum* T-76 stops growing at about + 10°c whereas *H. annosum* and the white conidia forming *T. polysporum* (T-75 in particular) grow almost until freezing.

In another recently studied microenvironment, root tips, naturally occurring *T. harzianum* does not grow at the same rate as the root hair. Recently developed mutantsd of T. harzianum were found to be 'rhizosphere competent' and to provide lasting protection against certain soil borne pathogens (Ahmad and Baker 1987).

In the case of T-75 and T-76, Murmanis et al. (1987) found neither water soluble antibiotics nor exoenzymes in wood blocks that were overgrown by *Trichoderma* strains and protected against major brown-rot basidiomycetes. But abundant chlamydospore formation was observed. Relative dimensions of these chlamydospores and xylem vessels do not prelude systemic distribution of spores. Such phenomenon occurs in *Ceratocystis* in elms and seems to also apply to T-75 and T·76 spores in elms in a preliminary study with N15 tracers (Wikstrom 1984). The Murmanis et al. (1987) study showed, also on SEM microphotographs, that T·75 and T·76 spores became attached readily to hyphae of wood decay basidiomycetes, Thebe spores germinated on the surface of the basidiomycete hyphae and broke through its wall.

One question issued at the recent *Trichoderma* and *Gliocladium* workshop held in Salford, England, was whether or not successfully attacked basidiomycetes remained intact when invaded by T·75 and T·76 hyphae, Volatiles of a very distinct 'coconut' odour, for example, are known to occur in certain *Trichoderma* strains including T·76. Of the two volatile compounds recently identified by Claydon (1987), 6-n-pentyl-2H-pyran-2-one and 6-n-pentenyl-2H-pyran-2-one, the first showed potent inhibitory properties to a wide range of fungi'. Volatiles were shown also by Bruce et al (1987) to be active against *Lentinus lepideus.*

Obviously more insight into the mode of action of *Trichoderma* strains against harmful fungi would be extremely useful to improve the performance of the existing strains. However, It has not been a limiting factor to commercialisation altogether any more than it has been for penicillin which saved many human lives before its precise mode of action was understood. *Trichoderma* T·75 and T·76 have saved many thousands of plum, nectarine, peach, apple, pear and eucalyptus trees.

Immunizing Commensality When artificial inoculation of wood with related fungi was first proposed in Oregon, it met with considerable resistance for a number of reasons including the general impression that all fungi were bed in plant tissue. Even *Peniophora gigantea* damages the wood stumps it infects. That damage is not significant because stump wood is seldom used and *Peniophora* does not penetrate deeply enough to spread from the inoculated stump to adjacent trees.

Several mycoparasites will not grow in sound unsteamed wood. But if the wood is at the incipient decay stage, growth of the mycoparasite can take place without damage to the wood beyond what has been caused by the decay organism. Furthermore, if nutrients are added together with the propagules of the mycoparasites, for example in a pellet, growth will occur without damage to the wood (Ricard, 1981). Such fungi function as classical commensals, T·75 and T-76 perform in this fashion in wood and so does Scytalidium sp. ATCC 16675. When those commensals have grown into wood, it becomes protected against subsequent decay at least from *Lentinus lepideus* or *Antrodia carbonica.* This particular protective feature may last even after the commensal has been killed by steam (Ricard and Bollen 1968) or sterilizing gas (Bruce and King 1983). The protection is a form of immunization, hence the name of immunizing commensals or IC for such fungi.

In the silver leaf treatment, T·75 and T·76 perform as IC in a striking fashion, especially in susceptible plum trees which have grown for a short time after rejuvenation grafting. The new shoots from the scion sometimes display small sparse silvery leaves. Then the trunk is treated with *Trichoderma* pellets and the subsequent foliage on the new branches is normal. At blossom time, the same branch will show typical silver disease symptoms on the lower part of the new shoot and a normal condition on the upper part of the shoot. Reisolation attempts, made during the first few years of treatment on GF 8-1, readily yielded *Trichoderma* cultures from wood cores extractcd a few cm above the inoculation points.

Disease evidence is not as readily and clearly obtained from utility poles or forest trees, where gross disease symptoms cannot develop and disappear during the same growing season. For this reason treatment of silver leaf disease has been much easier to promote than pole treatment.

Other Uses

Grapevines Chlorotic vine leaf curl is a serious disease in vineyards of South America. It is caused by a basidiomycete whose precise identity is unclear, Recent trials with T·75 and T·76 were described as follows at the recent Salford meeting by J. Pobiere (1987).

'The first trials with *Trichoderma* to cure this disease started in 1984 with a set of 12 heavily infected grapevines treated with different dosages of *Trichoderma* pellets. Nowadays those plants are completely cured, and producing perfectly normal bunches. The higher the dosage, the quicker the recovery. Based on that experience, we have now started to inoculate diseased grapevines massively using these criteria. If the trunk is heavily diseased and most of the conductive tissue is dead, we are cutting the trunk, leaving just a small stump to allow the grapevines to regenerate a new plant, and of course, inoculating the stump. This is very impressive, since at the end of the season it is possible to have almost a completely developed plant that is also completely cured of the disease. Now, if the trunk or more precisely the conductive tissue is not heavily diseased we are inoculating along the whole length of the trunk, and by the next season it is possible to have a normally producing grapevine.'

Plum tree (cultivar Laroda) at Padre Hurtado, Chile, showing site of Trichoderma inoculation. (JPoblete).

Large Wounds Trees can get infected through open wounds in two ways: (1) by basidiomycetes, mainly in the heartwood (which is essentially dead tissue) and (2) by basidiomycetes and/or moulds in the sapwood where sap flows and reaches the cambium layer.

Damage from the infection of wounds varies drastically from one tree species or even variety to another and one fungus to another, ranging from deadly *Chondrostereum purpureum* or aggressive *Ceratocystis ulmi* to immunizing commensals IC mainly *Trichoderma* and *Scytalidium* strains. In most cases the safer approach is (1) to encourage callus formation to heat sapwood and protect the cambium layer and (2) implant IC in the heartwood to prevent development of decay basidiomycetes. For pine stumps it is best to destroy the wood superficially with *Peniophora gigantea* making that substrate unsuitable for other more damaging fungi.

A comprehensive study was conducted at the British Forestry Commission by Mercer and Kirk (1984) comparing various chemical commercial compounds with *Trichoderma* preparations. Mercer found that *Trichoderma spp* out-performed chemical compounds, both in the acceleration of callus formation by the sapwood and in prevention of basidiomycete infection of the heartwood. The study is continuing (Lonsdale 1987). In practice, Trichoderma wettable powder can be diluted to paint consistency with drinking quality water and applied with a brush.

Spores of *Peniophora gigantea* are sprayed usually, but only for the prevention of *H. annosum* on pine stumps. *Annillaria mellea* and *Dothichiza popular. A. mellea* can cause severe losses in young plantations, old trees or intensely managed orchards or vineyards generally when plants are in a stress condition, while the second fungus causes poplar canker. Extensive studies were made in Belgium (Veldeman 1987) to improve the implantation rate of poplar cuttings or on older trees after the upper roots had been exposed partially by high pressure water spray, *Trichoderma* preparations were applied as a spray and/or pellets in an artificial planting substrate. Improved root formation resulted from these treatments in both cuttings and older trees.

Potential Uses

Poles in service (remedial treatment) Attempts at development of biocontrol agents for remedial treatment of poles in service started in the early 1960's (Ricard et al 1969). So far only two short-lived commercial episodes (one in England and one in Sweden) resulted from these attempts. Field trials are continuing in England and Scotland.

Remedial treatment for poles in service with biocontrol preparations become more of an issue in Europe with: (1) scarcity of readily and throughly creosoted poles, and (2) unpopularity of toxic chemicals. For many years large poles were made from Western red cedar or *Pinus sylvestris* (Scots pine alias Baltic redwood) and creosoted. The sapwood portion of these poles readily absorbed the preservative under proper pressure treatment. The heartwood was either relatively resistant to decay basidiomycetes or somewhat nonsignificant in the overall mechanical strength properties of the pole. That is because heartwood comprises a relatively small proportion at the center of the wood mass in comparison with the large peripheral sapwood band in pines.

When Douglas-fir started to replace Western red cedar, remedial treatment became more important. The sapwood layer in Douglas fir is rather thin and the heartwood is decayed readily by *Antrodia carbonica*. The same consideration applies to Noway spruce, though *Lentinus lepideus* is the more common decay basidiomycete in Western Europe.

Scytalidium sp. ATCC 16675 was first suggested as a biocontrol agent for the remedial treatment of Douglas fir poles in service (Ricard 1966). In Sweden in an attempt to increase the implantation rate of artificially inoculated IC., *Scytalidium* implantation was found to depend on other factors besides inoculum viability and compatibility with the substrate (Ricard et al 1969). Eventually pellets were developed combining nutrients and propagules with a much improved implantation rate (Ricard 1961).

In the meantime, registration became a new limiting factor to commercialisation. This form of official approval revolved around efficacy and safety in varying degrees of relative important, depending on the country. For the purposes of registration *Scytalidium* spp. presented disadvantages because of known occurrence of mild human pathogens in the genus (Campbell et el 1977, Peirls et al 1979, Dixon et al 1980, Dickinson et al 1983).

Trichoderma spp., on the other hand were not known to cause human health problems even after years of human exposure in laboratories and in Japan under industrial conditions (Toyama 1969), Development efforts were concentrated then on a psychrophilic *Trichoderma* strain isolated in Sweden (Ricard, 1970). This strain became T·75. It was found in a Norway spruce (*Picea abies*) immune to *H. annosum*, for unobious reasons, in an area of very active root rot. The exceptional condition of the spruce hosting T·75 was displayed by rings of paint applied every 4 years to the trunk of 100 spruce trees originally selected to determine the rate of growth of *H. annosum* from the ground up into the stems. On this particular spruce all the paint rings were at the same height while on the other trees 10 to 50cm separated the various rings. This indicated the highest level where *H. annosum* was isolated at the various sampling periods. The immune spruce yielded the *Trichoderma polysporum* T·75. This strain was tested in a number of poles in Sweden and found eventually to become implanted readily when provided with

nutrients and combined with another faster growing strain (T-76) for temperatures above + 10°C T-76 (Ricard 1976).

One hundred and sixty-six poles in Britain and Scotland in service were inoculated with pellets combining T-75, T-76 and *Scytalidium* sp. ATCC 16675. The work was conducted by Bruce and King with several publications reporting positive results (1986). The original poles inoculated in the United Kingdom are still under study (Bruce 1987).

New poles Pretreatment decay (PTDK) is receiving increasing attention (Taylor 1984, Lundstrom and Edlund 1987). Current energy costs have made air seasoning the more common drying practice, and preservative impregnation seldom raises the temperature high enough to destroy decay fungi in the heartwood. For these reasons, *Trichoderma* preparations have been considered as a means to eliminate or prevent heartrot. SEM evidence indicates that in a starvation situation T-75 and T-76 parasitize major brown. and white-rot fungi (Murmanis et al 1987). Unsteamed conifer heartwood is a starvation situation for *Trichoderma* spp. and mycoparasitism can be expected to take place, especially where *Antrodia carbonica* and *Lentinus lepideus* and concerned.

However moulds are known to slow down at least development of *Trichoderma* T-75 and T-76 in heartwood (Bruce and King 1986). Comprehensive studies of interactions between wood inhabiting moulds and *Trichoderma* spp. would be time-consuming and costly. For conifer species with thin sapwood, a feasible alternative may be to fumigate the new poles in the treatment cylinder before creosoting them or treating them with other surface chemical treatment. After fumigation and surface treatment, the heartwood should be free from fungi providing a substrate readily colonized by *Trichoderma* pellets.

Methyl bromide is effective against *Antrodia carbonica* (Ricard 1966) and is used currently for the treatment of oak logs for the eradication of *Ceratocystis fagacearum* (Liese et al 1981, MacDonald et al 1985), Methyl bromide and sulfuryl fluoride were tested recently against major wood decay basidiomycetes by King (1987) who found methyl bromide effective at the dosage used for the oak wilt fungus, i.e., C/T 4848. Heartwood penetration studies are underway at the Forest Products Laboratory, Madison, Wisconsin.

Marine piling and curbing timbers, One somewhat unique feature of piling with respect to decay is top rot. Once the piling is driven in place the excess length is cut off leaving untreated heartwood exposed. *Trichoderma* wettable powder preparations could be applied to such exposed wood. Implantation should take place provided suitable moisture prevails in the wood with subsequent protection against decay. Similarly, inoculation of curbing timbers with *Trichoderma* pellets should result in *Trichoderma* colonization.

Prospects

Rishbeth (1963) achieved the first breakthrough in biocontrol of pathogenic fungi in trees with *Peniophora gigantea* for the preventive treatment of pine stumps against infection by airborne spores of *Heterobasidion annosum*, a major fungal pathogen in forests.

Development of *Trichoderma* spp, as biocontrol agents should be accelerated by the recent formation of an international group. This group has met on two separate occasions during the past three years, first in Beltsville outside Washington D.C. and in 1987 at the University of Salford near Manchester in the United Kingdom. Another meeting is planned three years from now.

At the Salford meeting 46 participants from 13 countries presented and discussed 30 reports on topics ranging from genetic engineering in *Trichoderma* strains to large scale use of *Trichoderma* preparations in intensive fruit production operations. The results reported indicate that *Trichoderma* preparations are safe for the consumer, the worker and the environment; and they are cost effective. *Trichoderma* preparations have reached commercialisation in intensive orchard management. Other agricultural applications approaching the stage of commercial use include seed dressing and various greenhouse crops treatments.

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