Assessment of the biocontrol potential of a *Trichoderma viride* isolate

Part I: Establishment of field and fungal cellar trials

Heather L. Brown, Alan Bruce*

Scottish Institute for Wood Technology, School of Science and Engineering, University of Abertay, Dundee, Scotland, UK

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Abstract

A field trial has been set up to assess the biological control potential of a *Trichoderma viride* isolate, T60. This isolate had been shown in previous laboratory tests to be particularly effective against certain basidiomycete decay fungi. Wood was treated with T60 spores using vacuum pressure impregnation in a pilot preservation plant. Treated stakes were planted in the field site along with CCA-treated and untreated control samples. Replicate samples were also set up in an accelerated decay facility employed to give a comparison to the field trial results. This paper describes the setting up and monitoring of the field and fungal cellar trials, and presents results of moisture monitoring and sapstain assessment which indicate that *Trichoderma viride* isolate T60 has a marked effect on the rate of sapstain development under certain conditions. The paper also discusses the efficacy of pressure impregnation of spore suspensions for use as biological control agents © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Wood in ground contact is susceptible to a wide range of wood-decaying micro-organisms. As a result, timber intended for use in ground contact situations is generally treated using toxic chemicals such as copper-chrome-arsenic (CCA), which protect the wood against the effects of biodegradation. However, due to increasing awareness of the environmental impact of wood preservatives, and the introduction of more stringent legislation over operations at treatment sites and the disposal of preservative treated wood, there has, over the last 25 years, been an upsurge of research into the potential of biological control as an alternative technology. During this time, a number of authors (Ricard, 1970; Mankau, 1980; Cook and Baker, 1983; Mackauer et al., 1990; Nelson et al., 1995; Bruce, 1998) have reported on the uses of biological control agents in agriculture, forestry and forest products.

Many of the reported field trials in biocontrol of wood decay such as those carried out by (Bruce and King, 1986a, 1986b; Bruce et al., 1990) have dealt with remedial treatment of preservative-treated in-service timbers e.g. electricity distribution poles. Although preservatives such as creosote and CCA have been shown repeatedly to prolong the service period of ground contact timbers, premature failure of treated poles has nevertheless been observed. This has often been due primarily to insufficient penetration of the preservative, which can lead to internal decay in the unprotected centre regions of large-dimension ground contact timbers.

However, there have been relatively few field trials investigating the use of biocontrol agents such as *Tri-*
choderma sp. to protect untreated timber and the majority have been concerned with immediate post-harvest deterioration of timber. Schoeman et al. (1994) investigated the application of Trichoderma spores in chainsaw oil as a means of protecting freshly harvested wood against sapstain and basidiomycete decay organisms. Soil contact is commonly regarded as the most damaging environment in which wood can be placed in service. Field trials for the biocontrol of deterioration in timber in ground contact are therefore essential to establish the full credibility of biological control for wood protection. Little work however has been reported on this aspect of biocontrol of wood decay to date.

Trichoderma is currently the most extensively researched biocontrol fungus in the field of forest products protection, and has been shown on a number of occasions to provide a protective effect against certain wood decay fungi (Highley and Ricard, 1988; Bruce and Highley, 1991). Tucker et al. (1997) have shown that certain isolates of Trichoderma can protect wood against basidiomycete decay fungi. These results, using modified versions of American (AWPA M10-77, 1977) and European (EN 113, 1980) standard test methods as well as a soil burial test system, demonstrated that a Trichoderma viride isolate (T60), was totally effective in protecting wood from the decay action of selected basidiomycetes.

This paper describes a large-scale experiment adapted from European Standard EN 252 — “Field test method for determining the relative effectiveness of a wood preservative in ground contact” — assessing the biocontrol potential of Trichoderma viride isolate T60 under field conditions. This field trial and the associated accelerated decay test compare the biological control agent to a standard chemical agent.

2. Method

2.1. Wood preparation

The following parameters were selected from the EN 252 (1989) standard: wood type and size, reference preservative and burial pattern. One hundred and twenty Sitka spruce (Picea sitchensis (Bong).Carr) stakes and 120 Scots pine (Pinus sylvestris (L)) stakes (500 mm longitudinal × 50 mm radial × 25 mm tangential) were treated with a T60 spore suspension at a concentration of 10^6 spores ml^-1 then sectioned, and slivers of wood from various depths into the block were plated out onto 3% malt extract agar. Viability studies undertaken to determine the effectiveness of a wood preservative in ground contact at a concentration of 10^6 spores ml^-1 then initial vacuum — 600 mm Hg for 30 min followed by a 90 min pressure period at 12.8 kg/cm^2 then initial vacuum repeated. A spore suspension (8 × 10^6 spores/ml) in around 900 l of water pre-sterilised using purification tablets (Puritabs Maxi (sodium dichloro-s-triazine-trione 425 mg), Schering–Plough, Mildenhall, Suffolk, UK), was prepared from approximately 500 plates of T60 grown on 3% malt extract agar for four weeks. Viability studies undertaken to determine the effect of the water purification salt on the growth of T60 spores showed that viability was not compromised. Stakes were then pressure-impregnated with either fungal spores or a reference chemical preservative (Tanalith CCA 3%, Hicksons, UK). No final vacuum was drawn on the spore treatment cycle to avoid removal of any spores from the wood surface.

2.2. Pressure treatment

Preliminary experiments to assess the suitability of pressure impregnation for use with fungal spores and to determine their penetration into wood blocks were undertaken using a bench-top pressure impregnation system as described in (AWPA M10-77, 1977). Blocks measuring 100 mm longitudinal × 50 mm radial × 25 mm tangential were treated with a T60 spore suspension at a concentration of 10^6 spores ml^-1 then sectioned, and slivers of wood from various depths into the block were plated out onto 3% malt extract agar. Trichoderma growth established that the spores survived pressure treatment.

Having established the viability of spores following bench-top pressure impregnation, the appropriate pressure treatment cycle for use in the preservation plant (pilot model, Hicksons UK, Castleford, UK) was determined by “treating” both spruce and pine stakes using water under the recommended cycles for each wood type. This was undertaken to assess solution uptake and to determine the extent of stake-to-stake variations. The treatment cycle finally selected for both wood types was that recommended for pine for use in ground contact (BS 4072, 1987; BS 5589, 1989); initial vacuum — 600 mm Hg for 30 min followed by a 90 min pressure period at 12.8 kg/cm^2 then initial vacuum repeated. A spore suspension (8 × 10^6 spores/ml) in around 900 l of water pre-sterilised using purification tablets (Puritabs Maxi (sodium dichloro-s-triazine-trione 425 mg), Schering–Plough, Mildenhall, Suffolk, UK), was prepared from approximately 500 plates of T60 grown on 3% malt extract agar for four weeks. Viability studies undertaken to determine the effect of the water purification salt on the growth of T60 spores showed that viability was not compromised. Stakes were then pressure-impregnated with either fungal spores or a reference chemical preservative (Tanalith CCA 3%, Hicksons, UK). No final vacuum was drawn on the spore treatment cycle to avoid removal of any spores from the wood surface.

2.3. Soil burial

At the field site, T60-treated and CCA-treated stakes were buried to half their length in a random pattern of 10 stakes in eight rows, whilst the untreated controls
were planted in a separate plot with 10 stakes in four rows. In the fungal cellar, stakes were buried 10 in each tub (20 cm apart), with T60-treated and CCA-treated stakes again randomly mixed, and with untreated controls in separate tubs. The reason for separating the untreated controls was to reduce the risk of cross-contamination from the \textit{Trichoderma}-treated stakes.

### 2.4. Measurement of results

While the stakes were positioned in the field and fungal cellar, sapstain discoloration was visually assessed and moisture contents were measured using an electrical moisture meter every 4–6 weeks. This was done by selecting representative “sample” stakes (five from each category) which have been used on every occasion that moisture measurements were taken, to establish a “profile” of the moisture contents in the field trial and accelerated decay facility stakes. Sapstain was recorded for every stake using an arbitrary scale of 0–4 (see Table 1) based on percentage of stake surface covered with sapstain.

### 3. Results

#### 3.1. Field and cellar moisture contents

The results presented in Fig. 1 represent the mean moisture content of the stakes in both the field and the fungal cellar, measured at the groundline region using a moisture meter. These show that within a short time of planting the field stakes have reached fibre saturation point, and remained at or above that moisture content throughout the study. The cellar stakes have a higher overall moisture content. Fluctuation in the moisture content of the field stakes does not appear to be seasonal but rather is associated with individual rainfall events.

#### 3.2. Sapstain

The results shown in Table 1 represent the assessment of sapstain discoloration in field and fungal cellar stakes.

No sapstain was recorded in any of the CCA-treated stakes. Of the remaining treatment groups, there was less sapstain discoloration in the cellular stakes than in the field stakes, for both pine and spruce. The degree of discoloration was higher in the pine stakes than in the spruce, and the onset of sapstain in the untreated pine field samples was recorded earlier than in the T60-treated stakes. This effect was not observed in the spruce field stakes or the cellar stakes of either wood species.

### 4. Discussion

If biological control is to be considered a viable option in timber preservation it is necessary to investigate additional aspects such as formulation, efficacy of the delivery systems, and the influence of the organism on the microbial succession pattern within the test system as well as the protective effect. Traditional chemical treatments employ vacuum-pressure impregnation as a means of ensuring the maximum possible penetration and retention of chemical formulations in timber. For biological control to become an acceptable alternative timber preservation strategy it would be advantageous if delivery of biocontrol agents could follow standard industrial procedures as closely as possible. The use of fungal spores as biocontrol agents has already been investigated in the remedial treatment of \textit{Serpula lacrymans} by spraying infected wood with a \textit{Trichoderma harzianum} spore suspension (Score, 1998), and in the biocontrol of basidiomycete decay fungi, by treating wood blocks with a \textit{Trichoderma} spore suspension using a bench-top pressure impregnation system (Tucker et al., 1997). This study establishes, however, that a pilot preservation plant can be used to pressure impregnate larger dimension wood samples with a fungal spore suspension for biocontrol purposes. Spores retained viability following pressure treatment in this study, but the exact nature of the uptake and penetration of spores into the wood was not determined. A molecular detection system based on the polymerase chain reaction (PCR) is presently being developed to detect and quantify spores (Brown and Bruce, 1997). This system will be used in future studies to determine
the efficacy of pressure impregnation of fungal spores in spruce and pine stakes.

Moisture content and nutrient availability are vital factors in the decay of wood and wood products. Studies have shown that in order for decay fungi to colonise and decay wood, the moisture content must be above fibre saturation point, commonly regarded as 20–30% moisture content (Morrell and Gartner, 1998). The moisture contents of both test systems (field and fungal cellar) show that moisture retention within the stakes, as measured at the groundline region (see Fig. 1), are above the fibre saturation point and therefore suitable for decay. It can be seen that the fungal cellar stakes remain constantly above the fibre saturation point, and that the field samples reached fibre saturation point shortly after burial.

Sapstain is caused by the growth of darkly-pigmented fungal hyphae through the wood cell lumen, producing a blue-black discoloration of the wood. Sapstain organisms will preferentially colonise freshly felled wood, and as such the critical stages in controlling sapstain development are immediately post-felling and during the early service life of the timber before chemical treatment or pulping of the wood takes place.

Pre-treatment with *Trichoderma* spores had a marked effect on the rate of sapstain colonisation of Scots pine stakes in the field, when compared to the rate at which discoloration appeared in the corresponding controls. This effect was not apparent in the T60-treated Sitka spruce stakes in which the rate of sapstain development, though slower than pine, was similar to that in the untreated spruce controls. Pine has a higher proportion of soluble nutrients than spruce (Nayagam, 1987) and this may explain the increased rate of sapstain colonisation observed in the untreated pine stakes (see Table 1). Certain species of wood such as pine are particularly prone to attack by staining fungi, while spruce species are less susceptible (Breuil, 1998) and this may be due to their increased nutrient status. Staining fungi produce lipases to hydrolyse wood triglycerides into fatty acids and glycerol for use as nutrients for fungal growth (Breuil, 1998), in addition to utilising sugars and nitrogen. The observed biocontrol in the *Trichoderma*-treated pine stakes may be a result of competition for those nutrients between the inoculated organism and colonising fungi from the soil, thereby slowing the colonisation by sapstain fungi. Competition for nutrients is one of a number of ways in which *Trichoderma* spp. are thought to inhibit the growth of other wood-colonising fungi along with production of volatile organic compounds (Wheatley et al., 1997), lytic enzymes and soluble antibiotics. Another possible mechanism of action in biocontrol by *Trichoderma* is that proposed by Smith et al. (1981). These authors reported that the replacement of “pioneer” fungi by *Trichoderma harzianum* led to changes in the organic environment of the wood cell, which appeared to be less conducive to the development of decay fungi. Alternatively the apparent lack of protection in the spruce stakes may simply indicate a lower level of inoculum development. Since spruce is a more refractory species than pine fewer spores in the treatment solution may be deposited into the wood after pressure impregnation. Additionally, the nutrient status of spruce may mean that development of biomass will be reduced due to the lower nutrient availability for the biocontrol agent.

While this paper indicates that *Trichoderma viride* isolate T60 has reduced the level of sapstain in the field, the results of investigations against soft rot and basidiomycete decay are presented in Brown et al. (1999). As has been pointed out, the critical stages in the control of sapstain are those prior to the in-service use of timber. The ability of a biological control agent
to delay the onset of fungal discoloration of wood rather than completely prevent the occurrence of such staining is therefore relevant and this is demonstrated by the results presented in this paper.

References


European Standard EN 252, 1989. Field test method for determining the relative effectiveness of a wood preservative in ground contact. CEN, European Committee for Standardization.


