The Domestic Dry Rot Fungus, *Serpula lacrymans*, its natural origins and biological control

John W. Palfreyman

Dry Rot Research Group, University of Abertay Dundee, Bell Street, Dundee, Scotland

1. Introduction

The dry rot fungus, *Serpula lacrymans*, is one of the most important wood decay fungi in the built environment causing many hundreds of millions of pounds of damage each year in many countries around the world. This Basidiomycete is particularly common in countries of northern Europe especially where bad maintenance, particularly of old properties, and inappropriate design or alteration may result in water ingress followed by timber decay caused by the fungus. Notably, however, *S.lacrymans* is very rarely found outside the built environment in Europe, with there being only one published report of its occurrence in Europe in its presumed natural environment, the forest floor (Kotlaba, 1992). Reports of the fungus from other parts of the world are also limited, although there is now good evidence that *S.lacrymans* resides in regions of the Himalayan foothills; however, it does not appear to be prevalemt in that natural environment (Bagchee, 1954; White *et al*, 1997). Unlike other fungi discussed in this volume *S.lacrymans* cannot be considered to be a tropical fungus, since high tropical temperatures would be lethal to this temperature sensitive organism (White *et al* (1995); Cartwright and Findlay (1958); Segmuller and Walchli (1981)). Our interest is in the development of new treatments to counteract the organism and to determine, if possible, how it has developed such a widespread distribution in the built environment from, it would appear, a sparse distribution in the wild.

2. *Serpula lacrymans* – an important degrader of timber in the built environment

The dry rot fungus holds a special fascination and notoriety amongst the general public in many parts of the world, especially in Northern Europe, because of its very high destructive potential in buildings (see monographs and reviews by Hennebert *et al* (1990); Jennings and Bravery, 1991; Singh, 1994; Bech-Andesen (1995): Palfreyman *et al* 1995). The fungus develops in poorly ventilated spaces with elevated moisture levels (>20% moisture content). Though the principal nutrient source for the organism is wood, it very effectively colonises non-woody building materials notably plaster, brick and stone. From such materials the organism extracts calcium, which is probably used to neutralise oxalic acid, and iron which is a cofactor in a number of degradative processes (Bech-Andersen, 1985; Palfreyman *et al* 1996). *S.lacrymans* is a Basidiomycete and a member of the Coniophoraceae. It is one of only two common members of the *Serpula* genus, the other being *Serpula himantioides*. Unlike *S.lacrymans*, *S.himantioides* is found relatively commonly in the wild. Why *S.lacrymans* is almost completely restricted to the built environment is not known though the organisms extreme environmental sensitivity may explain its rarity in its natural habitat. (Alternatively it may just be that in the wild the organism fruits only sporadically and so its presence is rarely reported.)

Features of the organism that make it such a good coloniser within the built environment include the highly efficient transport system which allows transport of water, nitrogen, iron, etc via specialised rhizomorhphs and effective solubilisation system which allows extraction of metal ions from stone and plaster work. *S.lacrymans* is a typical brown rot fungus utilising non-enzymatic mechanisms to modify lignin and initiate the depolymerisation of cellulose. Hydrolytic cellulases and oxidative enzymes are then fully degrade and metabolise the cellulose. Apart from slight changes degradation of lignin does not occur. Because of various myths associated with the destructive nature of *S.lacrymans,* treatment has

traditionally itslef been harsh and destructive and indeed may cause more damage than the fungus itself.

3.   The search for the natural habitat of *S.lacrymans*

3.1   Background

Reports of *S.lacrymans* in the wild were, until recently, limited to sightings of basidiocarps, with no isolates available for analysis. Two reports are generally considered to be reliable, one from Bagchee (1954) which describes *S.lacrymans* at two sites in Himachal Pradesh in the Himalayan foothills of India (specifically at Narkanda and Pulga), the second from Cooke (1957) identified *S.lacrymans* on the slopes of Mount Shasta in Northern California in the USA. A third unconfirmed report suggests that *S.lacrymans* is found in the Sumava Mountains in the Czech Republic (Kotlaba, 1992).

In an attempt to clarify information regarding the 'biotope' for *S.lacrymans*, several expeditions have, in recent years, been undertaken to each of the three possible natural sites of the organism, and material in the form of basidiocarps has been returned to Europe for isolation purposes within our laboratories in Dundee, Scotland. Samples of both *S.lacrymans* and *S.himantioides*, as determined by the morphology of the basidiocarps, were collected to ensure that the subsequently used laboratory based techniques could effectively distinguish between the organisms. The Himalayan samples, on which most work has been done, were isolated from fruit bodies found on *Picea smithiana*, *Abies Pindrow* and *Taxus baccata*. Two of the samples were found in contact with the soil and in each case the fruit bodies were north or north-east facing and in relative shade or darkness. Associated basidiomycetes were *Heterobasidion annosum*, *Armillarea mellea* and *S.himantioides* (White *et al* 1997). The microenvironments of the *S.lacrymans* samples were not dissimilar to those found in infected buildings (relatively stable temperatures, high humidity, low air movement and shade). In the wild the organism appears to be a relatively sparse coloniser of forest wood and litter, though the mycelial form of the organism may of course be common but not apparent/ conspicuous for identification.   These findings are in marked contrast to the rampant nature of *S.lacrymans* in the built environment where it appears to have no effective competitors once it has established itself.

3.2   Isolation protocols

Two methods were used to prepare potential *S.lacrymans* isolates. In the first method axenic cultures were prepared by plating dissected portions of the hymenium of fresh basidiocarps on to 2% malt extract agar (MEA) containing filter-sterilised 0.4% benlate both with, or without, prior rinsing of the sample in 1% sodium hypochlorite (White *et al* 1997). Incubation was performed at 22°C and all promising growth was immediately subcultured. The second method was used to generate cultures from the basidiospores deposited from basidiocarps. Germination of basidiospores was promoted on acidified media – either 2% MEA containing 1% citric acid or 3.5% MEA containing 1% malic acid. Further details of these media can be found in White *et al* (1997) and Low (2000). As with the first method any promising growth was subcultured on to 2% MEA as it emerged.

3.3   Morphological charactertistics

Four isolates were obtained from the Himalayan basidiocarp material, one from material from the USA and two from the Czech Republic basidiocarps. The USA isolate showed stranding and yellowing as the culture aged but it also showed less dense mycelium and fewer aerial hyphae than the type strain of *S.lacrymans* FPRL 12C. So whilst the isolate resembled *S.lacrymans* in many ways differences were apparent. One of the Czech isolates showed pearly-white luxuriant mycelium, with prominent strands and mycelium that turned yellow then brown on ageing. These are all features typical of FPRL 12C. The other Czech isolate showed a less dense mycelium with no yellowing and less prominent stranding. This

isolate was subsequently shown to be *S.himantioides*.   Of the four Himalayan isolates three were typical of *S.lacrymans* and one was *S.himantioides* (White *et al* 1997; Low, 2000).

3.4   Physiological characteristics of the Himalayan *S.lacrymans* isolates

Five parameters of the isolates were investigated and compared with recent isolates from the built environment, viz. a) colony extension rates at the optimum temperature (22°C), b) the effect of temperature, water potential and pH on colony extension rate and appearance and c) the rate of timber decay. Measurement of colony extensions rates as an indicator of fungal activity is open to criticism as it does not necessarily reflect fungal biomass. Extension rates do, however, give a simple method for comparing responses to different environmental conditions. Information on the type of growth in each condition, i.e. whether diffuse or dense, proportion of aerial mycelium, was also recorded.

The highest radial colony extension rates for most of the *S.lacrymans* isolates was at 22°C, though one Himalayan isolate grew faster at 26°C. Without exception the extension rates of the building isolates were faster than those of the Himalayan isolates. At 4°C all the isolates maintained growth but that of the Himalayan isolates was noticeably more effuse than the building isolates. The Himalayan isolates in general were slightly less sensitive to temperature extremes although all isolates were sensitive to elevated temperatures and no growth was apparent with any of them at 37°C. At 32°C the Himalayan isolates grew better (White *et al* 1997).

The effects of water potential on the isolates again showed differences between the groups. Under favourable conditions the building isolates grew faster, but at lower water potentials the Himalayan isolates rivalled the extension rates of the building isolates. Again the greater resiliance of the latter group to environmental extremes was exhibited.   The effects of pH were less marked resulting in similar growth rates for all the isolates.   All isolates showed remarkable tolerance to acidic, neutral and mildly alkaline conditions (pH range 3-8). In the field, the presence of alkaline minerals in masonry assists the fungus to continue the degradation of timber as the metallic ions apparently help to neutralise acid metabolites produced by *S.lacrymans* (Bech-Andersen, 1985; Palfreyman *et al*, 1996).

The wood decay capacity of the various isolates demonstrated some differences but there was no 'grouping' of isolates. This is despite the fact that one of them, the building isolate FPRL12C, has been in culture for more than 50 years, whereas the Himalayan isolates, and two of the control building isolates, were isolated only in the past 5 years. This remarkably stable timber degrading capacity is in marked contrast to many fungi which often temporarily lose this capacity during long term tissue culture. Maintenance of such organisms on woody based substrates is necessary to maintain activity, this is not necessary for *S.lacrymans*.

Overall the experiments indicated both similarities, and differences, between the isolates. As well as the two groups discussed a third group, containing just one isolate BF-050, showed differences from the other two. This isolate, originally from Australia, has been shown to have a protein profile from the other building isolates (Palfreyman and Vigrow, 1994) and other molecular techniques have now confirmed the uniqueness of BF-050 (see next section).

3.5   Molecular analysis of isolates

Both protein and nucleic acid techniques have been used to characterise *S.lacrymans* isolates. The use of the former method was instigated at a time when nucleic acid based techniques required sequence information. The advent of RAPD PCR systems and, more recently, the appearance of sequence information on *S.lacrymans* means that nucleic acid methods have now taken over, as in other areas of mycology.

3.5.1 Protein analysis

The identities of our original Himalayan isolates was confirmed by SDS-PAGE using the methods described by Palfreyman *et al* (1991). Banding patterns of the putative Himalayan

S.lacrymans isolates were identical to those of building isolates and putative Himalayan *S. himantioides* isolates were also confirmed as correct (White *et al* 1997). More recent protein studies on our 'wild' Czech *S.lacrymans* isolate has confirmed the identity of this organism (unpublished observations). To date all *S.lacrymans* profiles produced have been the same as FPRL12C with the exception of *S.lacrymans* BF-050 which showed differences between SDS-PAGE profiles for 'old' mycelium but not for 'new mycelium (i.e. material adjacent to the growing tips) and for antibody or lectin stained SDS-PAGE profiles (Palfreyman and Vigrow, 1994). Recent results indicate that the USA isolate of *S.lacrymans* showed similarities to FPRL12C but the profile was not identical.

3.5.2   Nucleic acid analysis

Nucleic acid analysis of the Himalayan isolates also confirmed their identity as *S.lacrymans* (White *et al*, 2000).   Both RAPD PCR and ITS based sequencing systems have been applied to the isolates producing essentially similar conclusions. Considering first the RAPD PCR results. Ten primers were tested for their ability to produce useful banding patterns with 10 building isolates of *S.lacrymans*, two Himalayan isolates and BF-050. Four of the primers produced useful patterns and each could distinguish between the *S.lacrymans* isolates and those of *S.himantioides*. Using the GelCompar image processing software (Applied Maths, Kortrijk, Belgium) it was possible to construct a composite banding pattern for each of the isolates using all four primers. A total of 1080 bands were used in the subsequent phylogenetic analysis based on the GelCompar results.

The results demonstrated no particular Himalayan subgroup or ecotype, this despite the physiological differences noted previously. The close identity between the Himalayan isolates and the building isolates was thus confirmed (White *et al*, 2000) . Isolate BF-050, previously identified as an anomalous isolate by SDS-PAGE, did, however, belong to a unique ecotype and further studies on this organism are underway to establish the nature of this organism.

ITS sequencing of *S.lacrymans* isolates from the built environment has previously been reported by Schmidt and Moreth (2000). Sequencing of the Himalayan isolates allowed CLUSTAL W analysis (Theodore *et al* 1995) which revealed the sequences to be identical to those of the building isolates (White *et al*, 2000). In addition 22 base pair differences in the ITS sequences of *S.lacrymans* and *S.himantioides* were revealed. Phylogenetic analysis using PHYLIP (Felsenstein, 1993) indicated the close relatedness of *S.lacrymans* and *S.himantioides* relative to the nearest related taxa in the sequence database (*Coprinus* and *Coniophora*). Recent studies in our group have confirmed that a) the Czech Republic isolate is closely related to the Himalayan and building isolates and b) that the USA isolate, whilst undoubtedly related to *S.lacrymans*, has notable differences, as determined by RAPD PCR (unpublished observations).

Nucleic acid data on the other *S.lacrymans* isolates indicates that both the USA and Czech isolates are related to the type strain (FPRL 12C) and recent PCR results, using a specific ITS sequence-based primer confirming this result. BF-050 did not react with this primer, once again confirming the unusual nature of this particular isolate.

4. Control of the Dry Rot Fungus in the Built Environment

Whilst searching for the natural origins of the dry rot fungus offers the possibility of determining a solution to an interesting puzzle (the unusual distribution of *S.lacrymans*) a further justification for this study lies with the need to develop better control methods for dry rot to use in the built environment, especially with regard to a reduction, or cessation, in the use of harsh chemical treatments. A better understanding of the biotope of *S.lacrymans* might help in the development of such methods. It is very apparent that the natural environment of the organism is not one that would be expected within a well maintained and designed building, viz. free moisture, high humidity, low ventilation. However, in other ways the built environment closely mimics the natural one, most notably the intimate mixture of organic and inorganic materials. Study of the incidence of dry rot in the built environment

indicates that development of *S.lacrymans* is not inevitable and it is always associated with poor design or maintenance of buildings. As such, the most effective method of treating dry rot is simply to ensure that the environmental conditions within the structure never become similar to those encountered in the native biotope of *S.lacrymans*.

Once the organism has developed, control methods are required and a variety of environmental control systems, either abiotic or biotic, which exploit the environmental sensitivity of the organism are under test in our laboratory.

4.1   Abiotic control

The decay of timber by *S.lacrymans* is crucially dependent upon the levels of humidity. In experiments with small wood blocks weight loss in the blocks was inhibited if relative humidity (RH) levels fell below 86%. Viability of the fungus was not, however, lost until lower RH levels (76%) had been reached. Below this value viability was rapidly lost and the fungus became noticeably shrivelled and discoloured. Control of growth and decay is also possible by subjecting decaying blocks to air flow. In a specially designed microcosm system air rates as low as 2.5 l/min^{-1} were sufficient to inhibit weight loss. However at lower rates (1.5 l/min^{-1}) the exploratory growth of *S.lacrymans* appeared to be stimulated as the fungus escaped stress by seeking a more appropriate growth environment (Low *et al*, 1999).

During these experiments the dry rot fungus was grown on an inert plastic surface, subsequent experiments involved incubation of the organism on tiles of inorganic building materials, notably a range of different sandstones containing a range of levels of specific metal ions. Whilst growth and decay capacity on the various minerals did not vary they were always higher than when the organism was grown on plastic alone. In the case of minerals rich in iron ions (e.g. brown 'Locharbriggs' sandstone) luxuriant growth of *S.lacrymans* mycelium was associated with rusty red/brown discoloration probably caused by translocation of ferric ions from the sandstone (Low, 2000; Low *et al,* 2000). Scanning electron microscopy (SEM) revealed the development of mycelial 'boiler plating' with crystals of calcium oxalate monohydrate (Whewellite) and calcium oxalate dihydrate (Weddelite). These results indicate that the fungus removed metallic ions from the underlying mineral, however no overt damage to the mineral surface was evident during the periods of inoculation used (Low *et al*, 2000). The absolute requirement of the dry rot fungus for certain divalent metal ions (Palfreyman *et al*, 1996) suggests that novel control methods could possibly be developed which exploit this feature. To date this has not been explored, however there is no doubt that certain building materials, for example the lath and plaster used in many historic constructions, are particularly favourable to the growth of the dry rot fungus.

Experiments on small microcosms have now been replicated on full scale models of parts of buildings (Palfreyman *et al*, 2000). Again control of the dry rot fungus is crucially dependent upon humidity and air flow, although in larger models the buffering capacity of thick rubble walls means that decay potential can last for long periods of time in the absence of overt growth. In one specific model, which included 'safe havens' (i.e. areas isolated from the effects of, for example, aeration), rapid growth of the organism in these areas was stimulated during treatment by the moving air. In addition, fruit body formation occurred in the model, within a completely enclosed part of the construct. Subsequently a second fruit body developed outside the model whilst that in the interior rapidly withered. Presumably the organism could sense that the environment outside the model was more favourable for dispersal of spores.

Overall, these studies demonstrate that control of dry rot caused by *S.lacrymans* requires only that a specific set of environmental conditions which allow growth of the organism, be avoided in the built environment. However, in a building where dry rot is active, and where environmental conditions cannot be rapidly altered, e.g. because of the presence of wet masonry other control methods may be necessary. Use of natural biotic competitors of *S.lacrymans* may offer a solution in such situations.

4.2   Biotic control (biological control)

Biological control (biocontrol) can be defined as 'the suppression of a pest by means of the introduction, propagation and dissemination of the predators, parasites and diseases which attack it' (Bruce, 1997). Though the use of such systems is widespread in agriculture, the application of biocontrol for the protection of timber is limited (Bruce 1997). The use of biocontrol to combat *S.lacrymans* has been reported only in studies performed in Japan (by Doi *et al* 1992) and in those of our group (Score *et al* ,1994; Palfreyman *et al*, 1995; Score *et al*, 1998). Early laboratory studies demonstrated that a range of different species of the soil fungus *Trichoderma* were able to inhibit the growth of, and ultimately kill, *S.lacrymans* on a range of different media (Score *et al*, 1994). Significantly variations in the levels of nitrogen and iron in the basal medium used in these studies had considerable effects on the outcomes of interactions, generally the more stringent the nutrient conditions became, the less likely was effective killing of *S.lacrymans* by *Trichoderma,* though certain isolates of the latter organism were highly effective in all media tested. As might be expected, a range of results from *Trichoderma* killing *S.lacrymans* through to stalemate reactions and *S.lacrymans* killing of *Trichoderma* was also found.   To date all *S.lacrymans* isolates from the built environment have responded similarly to specific *Trichoderma* isolates. Tests on isolates from the Himalayas and the USA are currently underway.

Moving from agar to wood substrates demonstrated that pre-inoculation of the latter with various *Trichoderma* spp. could afford effective protection from attack by *S.lacrymans.* However when wood was precolonised with the decay fungus, *Trichoderma* spp. were unable to prevent further decay, as evidenced by continued weight loss.   In related experiments Palfreyman *et al* (1994) demonstrated that timber already partially decayed by *S.lacrymans* could be further degraded by *Trichoderma* spp. which by themselves are unable to degrade wood. Presumably the initial degradative action of *S.lacrymans* makes the cellulosic polymer, which is normally inaccessible to the *Trichoderma*, susceptible to the enzymes of such moulds. However *S.lacrymans* could never be re-isolated from blocks whether preinfected with this organism or *Trichoderma,* even in the presence of selective media. This result is consistent with the hypothesis that, even if decay is continuing, it is not caused by the basidiomycete.

More recent studies on natural substrates, i.e. wood, in a specifically designed microcosm (Score *et al* 1998) demonstrated that growth of *S.lacrymans* along wood sticks could be prevented by *Trichoderma* spp. However the former organism was not necessarily killed by the latter, and may be able to adopt survival strategies. Similarly in workshop sized model systems the interaction between *S.lacrymans* and control organisms is more ambiguous and, the *Trichoderma* spp. tested were unable to kill the dry rot fungus once it was well established within the woody substrate. *Trichoderma* could slow the growth of the dry rot fungus if the *S.lacrymans* was disadvantaged by the development of inappropriate environmental conditions (Low, 2000).

There are a number of possible explanations for the discrepancy between the laboratory and 'workshop' experiments. For example, the former experiments were carried out in a sterile environment and at a fixed temperature, whereas in the latter experiments sterility was not maintained and temperatures fluctated. Furthermore, the workshop models were more representative of natural infections since genuine building materials were used. Compared to those in small-scale microcosms, fungal colonies were less restrained spatially and temporally; each colony therefore had the potential to exhibit more of its developmental capabilities, especially with regard to the regulation, maturation and ageing of the colony and to the allocation of its resources. Finally the workshop models were designed specifically to favour the dry rot fungus, whereas studies involving culture media will promote the growth of ruderal species such as *Trichoderma*. Furthermore the *Trichoderma* used in the experiments was isolated from a soil sample in the UK and may not necessarily be adapted to growing in the built environment. A possible strategy for improving the performance of the control agent is to look for natural competitors in the natural environment of the decay organism, i.e. in northern India or in the USA. Initial observations indicate that *Trichoderma* isolates from the USA are particularly effective at killing *S.lacrymans* in agar plate based systems. Whether or

not this efficiency is replicated in more realistic microcosms, and indeed in full scale models, remains to be seen.

Biological control studies have revealed other interesting features of *S.lacrymans*. Specifically the organism can be induced to produce a range of, putatively, defensive enzymes when cultured in the presence of *Trichoderma*. Thus, Score *et al* (1997) demonstrated production of various extracellular phenoloxidases during interactions, most notably the production of laccase, an enzyme normally associated with white-rot fungi. Various *Trichoderma* spp. could also be induced to produce laccase, again not an enzyme normally produced by these organisms. Additionally, melanisation of the mycelium of *S.lacrymans* seems to be an early response of the basidiomycete to attack by potential competitors both in agar culture and in microcosms (Score *et al* 1998; Low, 2000). Furthermore, formation of mycelial strands (cords) by *S.lacrymans* appeared to be a salient feature of its defensive response.

Effective protection of wood by *Trichoderma* crucially depends on effective colonisation by this antagonist (Bruce 1997), which itself depends upon nutrient resources and the presence of other, competing, organisms. In an attempt to understand, and eventually model, the interaction between *Trichoderma* and *S.lacrymans* in a range of substrates, a tessellated agar tile system for monitoring multiple interactions is under investigation. In this system, complex grids of up to 36 inoculated agar tiles can be established, with results to date demonstrating the effect that the spatial arrangement of interacting species has on the final interaction outcome (compare the 3x3 grids inoculated with *Trichoderma* either in the centre or in a corner tile). Other studies using this system (White *et al* 1998) have revealed that stochastic processes are important in the development of final interaction patterns, particularly when more than 2 species are present in the initial inoculation. Data from these tessellations can be incorporated into mathematical or computer models and a cellular automaton model has been shown to be able to predict outcomes of complex interactions under some circumstances (Bown *et al* 1998).

5. Conclusions

S.lacrymans remains a fascinating and enigmatic organism. Its origins are still obscure: for example was it once widely distributed around the world with current climatic changes forcing it to survive only in very restricted areas, or was its 'natural' distribution always limited, and have man's activities resulted in its introduction to the built environment within Europe? In addition, why is the organism so successful in the built environment? Is this due to lack of natural competitors or simply that a damp, badly maintained building in, for example, northern Europe offers a substitute environment for the forest floor in the Himalayan foothills. As more isolates of *S.lacrymans* become available, and more sophisticated techniques are applied to the study of their genomes, the answers to these questions should become available. Current evidence supports an emergence of the organism from its Himalayan home via the timber trade between India and the UK/ Europe, but the case is by no means proven as yet. However, our group continues to study the phylogeny and ecology of this important and particularly interesting Basidiomycete.

6. References

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