Treenet Proceedings of the 5th National Street Tree Symposium: 2nd and 3rd September 2004
ISBN 0-9775084-4-7

Craig Hallam provided the following research paper as an adjunct to his presentation on the **Assessment of Risk Associated with Decay in Trees**:

DEVELOPMENT OF DECAY IN THE SAPWOOD OF TREES WOUNDED BY THE USE OF DECAY-DETECTING DEVICES

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SUMMARY

The effects of drilling holes with the IML-Resistograph and an increment borer were examined in London plane and ash trees naturally infected with *Inonotus hispidus*. Eight months after inflicting the wounds on the lower stem, trees were felled and dissected. Micro-organisms were isolated in pure culture and identified from the decayed wood, host-fungus interface, discoloured wood and the sapwood. In both hosts the extent of discoloration and decay within the sapwood and also the frequency of isolation of *I. hispidus* from these altered zones were greater after wounding with the IML-Resistograph than with the increment borer. These differences may be related to micro-environmental conditions. Tightly packed wood shavings are retained within the IML-Resistograph drill holes, whereas the holes created with the increment borer remain open, allowing ingress of air into the sapwood. Such alterations in growth conditions seem to be unfavourable for growth of *Inonotus hispidus*, which reacted by forming mycelial plugs that firmly sealed each of the increment borer holes. As mycelial plugs were never observed in wounds induced with the IML-Resistograph, it appears possible that pre-existing conditions are not greatly modified and that the fungus can therefore grow out into the adjacent sapwood more readily. Additional laboratory studies showed that cross-infection via drills contaminated with fungal propagules appears unlikely.

INTRODUCTION

A frequent objection to invasive decay-detecting devices is that their use may cause long-term harm to trees. Critics point out that such devices penetrate and therefore breach reaction zones, which surround and delimit pre-existing zones of decay. This may, at least in theory, provide a channel for the outgrowth of a decay fungus into wood that was previously protected by the defences of the tree (LONSDALE, 1999). Also drill wounds could become external courts for infection by decay fungi, perhaps from infective particles carried on drilling equipment which has not been sterilised following previous use.

Concern about the potentially harmful effects of using invasive devices dates back to the introduction of the increment borer in dendrochronology and forest mensuration many years ago. These applications have, however, mainly involved decay-free trees, and so any concern has been mitigated. (MEYER & HAYWARD, 1936; DUJESIEFKEN *et al.*, 1999, KOWOHL *et al.*, 2001). There is more cause for concern when reaction zones are breached in trees that are already affected by decay (SCHWARZE & FINK, 1994; SCHWARZE *et al.*, 1995b; SCHWARZE *et al.*, 2004), but there has been an assumption that modern devices, such as the IML-Resistograph, that create relatively small-diameter holes are less damaging than the increment borer (RINN, 1994). There has, however, been little attempt to test the validity of this assumption.

When comparing the effects of inflicting wounds with different diagnostic devices, not only the size but also other features of the wounds should be considered. For example, the IML-Resistograph creates a hole that is both relatively narrow and is also packed with wood shavings. The conditions within such a hole, particularly with regard to aeration and moisture, are likely to influence colonisation by fungi and bacteria. There has been hardly any attempt to isolate and identify the full range of fungi colonising wounds that penetrate pre-existing decay columns, but data from such studies would help to elucidate the origins of fungal colonisation; i.e. (a) from pre-existing columns of decay, (b) from the external environment or (c) via contaminated drilling equipment.

Data from laboratory tests under controlled conditions can assist in the interpretation of findings obtained from naturally infected trees. This approach was therefore adopted in the present study of alterations associated with wounds inflicted through the use of different diagnostic devices. Thus, the study includes both macroscopic and microscopic evaluation of alterations within the wound area, together with fungal identification (KERSTEN, 2001). On this basis, we address here the following issues: (1) the extent to which the different types of wounding can promote the development of pre-existing decay or its extension into previously healthy sapwood; (2) whether the different wound types can serve as infection courts for wood-decay fungi and (3) whether wood-decay fungi can become established due to transfer via contaminated devices.

MATERIAL AND METHODS

Experiment No. 1:

In the initial phase of the investigation, wounds were inflicted on three living trees, each of ash and plane, using both the above-mentioned diagnostic devices: an IML-Resistograph M 300 (IML GmbH, Wiesloch, Germany) and an increment borer (Suunto Oy, Vantaa, Finland). These trees were selected as having been naturally colonised by the decay fungus *Inonotus hispidus* (Bull.: Fr.) Karst. and thus containing reaction zones within their sapwood. This host-fungus combination had previously been described in detail (SCHWARZE *et al.*, 1995a; SCHWARZE & FINK, 1997, SCHWARZE & BAUM, 2000) and therefore formed an ideal basis for further study. In August 1998 multiple wounds, consisting of radial drill holes or boreholes, were inflicted on the trees using the two devices. The holes were initiated on the side of the stem opposite axially orientated bark necroses associated with the fungal infection.

On each tree, wounds of both types were created at two different heights, approx. 0.6 to 1.0 m apart. This axial distance was provided so as to avoid the coalescence of any resulting columns of discoloration within the sapwood. For the same reason, the wounds at each height were spaced tangentially, taking account of the diameters of the holes created by the two devices (3 mm in the case of the IML-Resistograph and more in the case of the increment borer, which removes a core of 5 mm diameter and has a cutting head of approx. 9 mm diameter). Compression of the wood may occur across an even greater diameter. Successive drillings were made without sterilising the diagnostic devices, so as to represent normal practice in the use of these devices. In total, 20 drillings were made on the test trees, of which 14 were on ash.

Two trees of each species were felled after approx. eight months, while one of each species was retained for long-term study. After the positions of the drill holes had been established, the trees were dissected so as to measure all the zones of associated discoloration. For the isolation of fungi from these zones, wood chips were extracted with forceps in the open air and also under sterile conditions. The wood chips were extracted from points within four

designated zones along the length of each drill hole (Fig. 1). Ten chips were extracted from each of the four zones, amounting to 40 chips from each hole and approx. 800 in total.

The wood chips were transferred to Petri plates consisting of 2% malt extract agar for the isolation of deuteromycetes, or with the addition of 0.2% thiabendazole for the selective isolation of basidiomycetes. The Petri dishes were sealed with Parafilm, labelled and then kept in the dark at 25□C. After eight weeks, all basidiomycetes were transferred to pure culture on malt extract agar for identification using morphological characters and enzyme tests (Fig. 2) (STALPERS, 1978). Deuteromyctes and ascomycetes were identified using the key of BARNETT & HUNTER (1988).

Experiment No. 2

An IML-Resistograph and an increment borer were each used to create two series of holes (30 replicates in each series) in the stems of four trees, giving a total of 120 holes. The trees, as shown below, were selected as having been naturally colonised by decay fungi; either *I. hispidus* or *Fomes fomentarius* (L.:Fr.):Fr.

IML-Resistograph:

Series 1: *Inonotus hispidus* on *Fraxinus excelsior* (ash)
Series 2. *Fomes fomentarius* on *Fagus sylvatica* L. (beech)

Increment borer

Series 1: *Inonotus hispidus* on *Malus* sp. (apple)
Series 2: *Fomes fomentarius* on *Fagus sylvatica*

All holes were drilled deeply enough to ensure that the IML-Resistograph probe or the auger of the increment borer penetrated the zone of decay. After each drilling, the needle probe of the IML-Resistograph was inserted into a growth medium and rotated so as to transfer any adhering particles of potentially infective material from the apical 7 cm (approx.) of the probe. In the case of the increment borer, the tip of the auger was inserted vertically into the medium to a depth of approx. 1 cm, so as to transfer particles from its inner surface. Subsequently, the external thread of the auger was rotated while in horizontal contact with the surface of the growth medium, so that any particles were transferred from a length of approx 7 cm, as with the IML-Resistograph probe. A selective growth medium was used exclusively as the substrate in this study, so as to guard against contamination. The cultivation and identification of pure cultures was done as described above.

RESULTS

Discoloration of the sapwood

Discoloration (Figs. 3 -5) developed within the wood in every case within the eight-month period following the infliction of wounds with the diagnostic devices. As found in other studies, (MEYER & HAYWARD 1936; BODDY & RAYNER, 1983) the extent of discoloration was in most instances greater above the wound than below; this can be explained by the upward withdrawal of water columns under transpirational tension.

The extent of the discoloration differed considerably between treatment-combinations. In the case of the increment borer holes, the length of the discoloured zones was approx. one third greater in ash than in plane. There was no significant difference between the tree

species in the case of IML-Resistograph holes. In both species, the holes created with the IML-Resistograph gave rise to longer zones of discoloration than those created with the increment borer (Figs. 6-7). An explanation for this should be considered in the context that oxidative changes following the ingress of air into wounded tissue are the main initial cause of discoloration, rather than colonisation by micro organisms (SHIGO, 1967; BODDY & RAYNER, 1983). It is possible, on this basis, that the use of the IML-Resistograph induced more discoloration because it generated a higher temperature during drilling. It is, however, necessary not to exclude the possibility that the observed differences might have been associated with microbial colonisation.

In order to gain some idea of the relationship between alterations of the wood and the presence and spread of fungi, it was necessary to analyse the fungal isolation data by separately recording the fungi found within different zones along the length of the drill hole (Fig. 1). These were (a) the decayed wood, (b) discoloured sapwood, (c) sound sapwood and (d) the reaction zone. From these four selected zones, 27 different fungal species were isolated and identified.

Drill wounds as avenues for fungal infection and development

Of the two kinds of wound, the open and larger increment borer holes obviously provided a better avenue for colonisation by fungi with airborne spores. This was evident from comparing the isolation of deuteromycetes from the discoloured sapwood around the two types of hole; this showed a higher frequency in the case of the increment borer holes (Fig. 8). These fungi included various members of genera such as *Trichoderma*, *Fusarium*, *Alternaria*, *Botrytis* etc. (Fig. 8), which commonly occur saprotrophytically in wood or in soil (BARNETT & HUNTER, 1998). Deuteromycetes include many moulds, whose spores are ubiquitously present in the air and which can colonise a wide range of substrates. Since wood decay in living trees is almost exclusively caused by basidiomycetes, the deuteromycetes were treated as a functional group for the data analysis, rather than as individual species.

The mycelia that develop from the germinated spores of deuteromycete fungi often grow very rapidly under favourable conditions by degrading readily available carbohydrates. Since they generally lack the ability to degrade woody cell walls, they do not, however, usually cause any significant loss of mechanical properties in wood. In this context it was interesting to observe that many such fungi were isolated from wood damaged by using the increment borer, whereas the same wood showed little evidence of outgrowth by *I. hispidus*. It is possible that some of the colonising deuteromycetes, such as *Trichoderma* spp., may have suppressed the growth of wood decay fungi through antagonism (SMITH *et al.*, 1981).

In one of the plane trees, the decay fungus *Pseudotrametes gibbosa* (Pers.: Fr.) Fr. was detected in nearly 28 % of samples taken from the reaction zone near the wounds made with the IML-Resistograph (Fig. 9). A possible explanation for this is that the fungus was already locally present in the tree, perhaps having entered via old wounds. *Trametes gibbosa* is found mainly as a saprotroph in beech stumps, but is occasionally also found on the stems of living trees (KREISEL, 1961). *Polyporus squamosus* (Huds.) Fr. was detected within five of ten increment borer holes, but within only one of eight IML-Resistograph drill holes. Long-term studies would be needed to show whether this fungus, which is classified as a wound parasite (GRAFF, 1936), might also have a long-term colonising strategy. It seems possible that it might have an endophytic phase, as it was, surprisingly, isolated also from the healthy sapwood of the ash; proof of this would require incubation of the wood under controlled conditions (CHAPELA, 1989). *Aurantioporus fissilis* (Berg & Curt.) Jahn was another basidiomycete detected in the discoloured ash sapwood.

Fungal outgrowth from decay columns via drilling wounds

As expected, *I. hispidus* was found in a high proportion of samples taken from the decay columns and from the associated reaction zones and transition zones adjacent to the sapwood (Figs. 9 -10). This fungus was also isolated from the discoloured zones where the wounds traversed the sapwood, clearly indicating that it was able to grow out from the decay columns into the injured sapwood. The frequency of isolation from these discoloured zones was considerably higher in ash than in plane and, in both tree species, it was also higher in the IML-Resistograph drill holes than in the increment borer holes. The percentage isolation values for the IML-Resistograph drill holes and the increment borer holes in ash were respectively as follows: 71% and 25%, while the corresponding values in plane were only 6% and 2%. In two of the increment borer holes, the fungus was not isolated from the discoloured sapwood at all, and there was only an extremely slight development of the fungus in a further two such increment borer holes. It was therefore clear that the IML-Resistograph drill holes provided better conditions for the outgrowth of the fungus from pre-existing decay columns than the increment borer holes.

A further surprising difference was the formation of mycelial plugs by *I. hispidus* within increment borer holes, as shown in the example in Fig. 5, but not in the IML-Resistograph drill holes. The outer end of each plug developed more or less exactly at the outer edge of the pre-existing domain occupied by the fungus. Plug formation by an already established fungal colony may confer advantages in the maintenance of the existing micro-environment, as it will clearly limit gas exchange and drying, and also in protecting against entry by microbial competitors. It is remarkable that these plugs developed only in the increment borer holes, especially considering that such a hole might be expected to provide a decay fungus with an easy avenue for radial outgrowth from its established domain. On the other hand, the change in micro-environmental conditions created by the hole may be so great as to stimulate a homeostatic response by the fungus. In this context, the increment borer hole (Fig. 5) is a more open channel, which must have a stronger influence on the wood micro-environment than the IML-Resistograph channel which is not only narrower but also blocked with shavings (Fig. 11). Within the heart- or ripewood of trees wood decay fungi such as *Inonotus hispidus* grow under very low oxygen and very high carbon dioxide conditions. Studies by JENSEN (1969) show that in decayed heartwood of oak, oxygen concentrations of approx. 2% exist, whereas carbon dioxide concentrations of >20 % prevail. Wood decay fungi appear to have adapted to these high carbon dioxide conditions (BODDY & RAYNER, 1983) and any alterations in the prevailing conditions may have an adverse effect on their growth and development. This may explain why the increment borer hole promotes the development of a mycelial plug, which limits the radial outgrowth of the fungus, whereas the IML-Resistograph drill hole allows the fungus to grow out radially without stimulating plug formation.

Cross infection of decay fungi via contaminated drilling instruments

The results indicate that the fear of transferring fungal propagules from one tree to another is unfounded. No evidence was found for transfer of the decay fungi *I. hispidus* or *F. fomentarius* via any of the 120 drillings made in the present study. There was, however, quite a high frequency of other fungi, mainly deuteromycetes, isolated from the surface of the increment borer after its use in trees containing decay columns. The corresponding frequency of isolation was much lower in the case of the IML-Resistograph probe, perhaps because its penetration led to stronger heating and perhaps also because it provided less surface area for

the adhesion of microbial propagules than the thread of the increment borer. It seems unlikely that wood-decay fungi would be transferred in this way.

CONCLUSIONS

When interpreting any of the above results, it is important to consider that only two of many possible host-fungus combinations were examined here. Although some additional results were obtained in a short-term pilot study, this did not include enough replication for statistical analysis. Despite these limitations, the results can begin to replace a great deal of vague assumptions and hypotheses with facts, which help to provide a firm foundation for further, long-term investigations, now under way. Moreover, the light-microscope data strongly support the results of other studies (KERSTEN, 2001; SCHWARZE & BAUM, 2000, SCHWARZE & FERNER, 2003).

From a practical standpoint, the results should not be taken to mean that the above diagnostic devices should not be used; rather they demonstrate the need to consider carefully when their use is really necessary and when they may need to be used with particular care and responsibility, taking account of any available information about the particular host-fungus combination. In this context, the compartmentalising ability of the tree and the aggressiveness of the fungus (determined by inoculum potential and colonisation strategy) are of crucial importance (Schwarze et al., 2004). Not only do these factors determine the dynamics of decay within the tree, they will also interact with a third important component, i.e. the breaching of reaction zones by the use of drilling equipment. It must be acknowledged that a small breach of a reaction zone, as caused for example by the use of the IML-Resistograph, may lead to a correspondingly minor amount of wood discoloration and of fungal outgrowth. On the other hand, there is a need to take account not only of the size of a particular type of wound as a potential avenue for fungal outgrowth but also of its micro-environmental effects on the established fungus. These may be crucial in influencing any potential spread of decay and its compartmentalisation in the long term.

In the case of the *I. hispidus* and ash, the combination of an aggressive fungus and a host tree with a weak compartmentalising ability may contra-indicate the use of any invasive diagnostic device from the standpoint of legal liability for risks to people and property.

Finally, it must be emphasised that any risks of harming trees by the use of invasive diagnostic methods are far less serious than the harm that is caused by the kinds of injury that typically allow the development of fungal decay; e.g. the severance of major branches and roots. If, through properly planned management, such injuries could be avoided, many of the decay-related problems that require detailed investigation would not occur in the first place.

Acknowledgements

We would like to thank the following persons for their helpful co-operation: Mr. P. Molzahn, staff member of the Amt für Grünflächen, Landwirtschaft und Forsten, der hessische Landeshauptstadt Wiesbaden for providing the test trees; Herrn Dipl. Forstwirt M. Bauer, of MB Baumdienste GmbH for providing labour and machinery for felling and sampling the test trees; Mr. Erich Hunger (IML) for his assurance of interest in the studies and for supplying the IML-Resistograph M 300. The authors also wish to thank Dr. D. Lonsdale for comments on the manuscript.

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FIGURE LEGENDS

Figure 1. Schematic diagram showing four areas from which isolations were obtained after wounding trees with the increment borer and the IML-Resistograph. Right (above). Mycelial isolations growing from infected wood samples placed on a selective growth media for basidiomycetes.

Figure 2. Reaction of enzymes to reagents and micro-morphological features of a pure culture of a *Polyporus squamosus* isolate. A: Positive reaction to -naphthol, indicating the presence of laccase. B: Positive reaction to naphthol, indicating the presence of laccase. B: Top: Positive reaction to pyrogallol & H_2O_2 indicating the presence of peroxidase. Right: Positive reaction to p-cresol indicating the presence of tyrosinase. Left. No reaction to KOH. C: Staghorn hyphae. D: Oidia.

Figure 3. Extensive discoloration in the sapwood of London plane after wounding with the IML-Resistograph. The drilling hole is firmly sealed with wood fragments. Sw = Sapwood; Rz = Reaction zone; Dw = decayed wood.

Figure 4. Longitudinal section of Common ash wounded with the increment borer. Note: Discoloration is strongly compartmentalized and restricted to areas in close proximity to the wound.

Figure 5. Longitudinal section of Common ash wounded with the increment borer. Note: The hole caused by the extraction of the increment corer is firmly sealed with a mycelial plug (arrow) of *Inonotus hispidus*.

Figure 6. Comparison of discoloration caused by the IML-Resistograph (RES) and increment borer (IB) in Common ash.

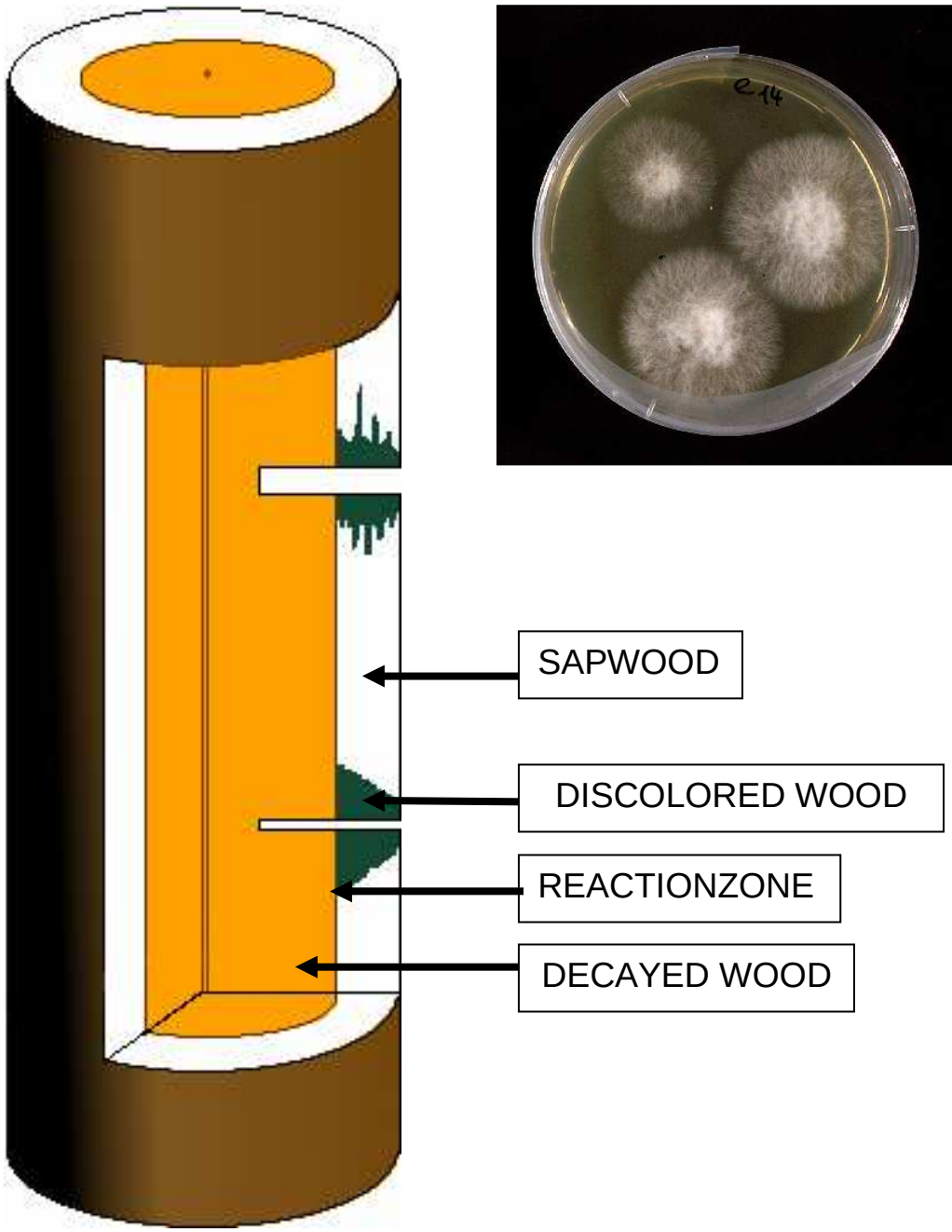
Figure 7. Comparison of discoloration caused by the IML-Resistograph (RES) and increment borer (IB) in London plane.

Figure 8. Isolation frequencies of *Inonotus hispidus, Polyporus squamosus* and Deuteromycetes in Common ash and London plane after wounding with the IML-Resistograph) and the increment borer.

Figure 9. Isolations of wood-degrading basidiomycetes from London plane after wounding with the increment borer (above) and the IML-Resistograph (below).

Figure 10. Isolations of wood-degrading basidiomycetes from Common ash after wounding with the increment borer (above) and the IML-Resistograph (below).

Figure 11. Tangential longitudinal section showing wood shavings within a drilling trace of the IML-Resistograph. Note the presence of cell fragments closely sealing the hole.



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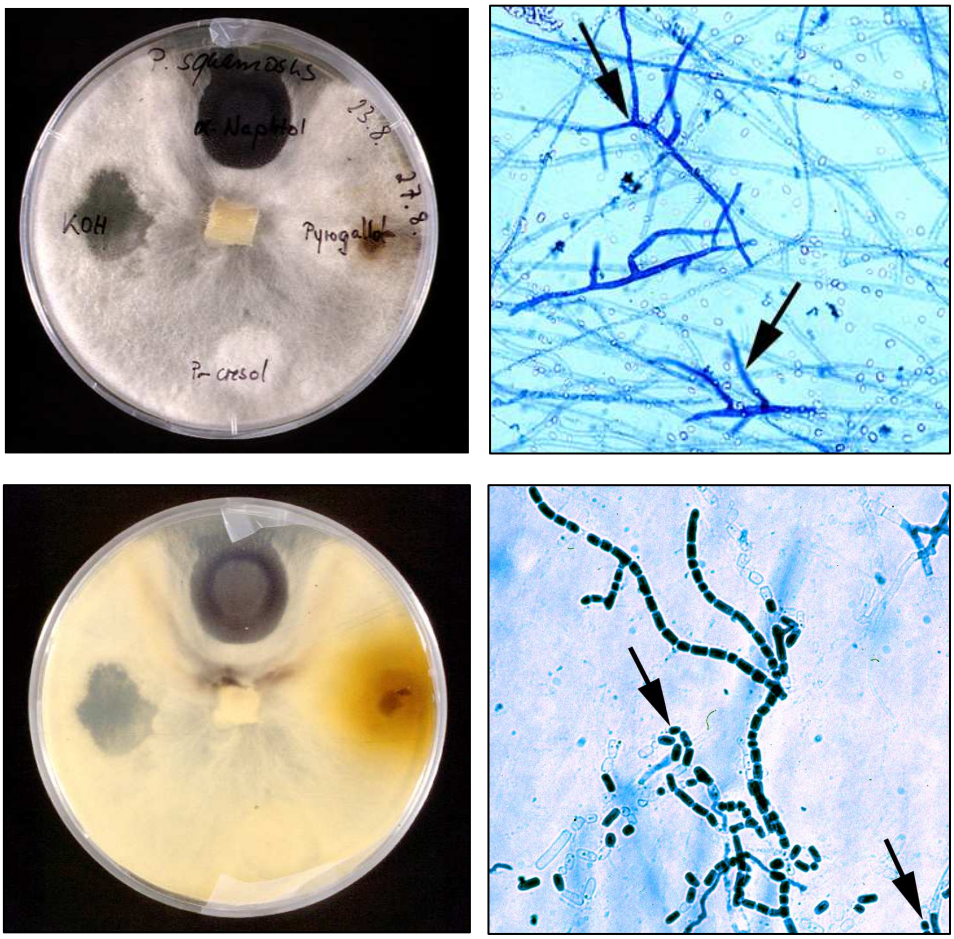

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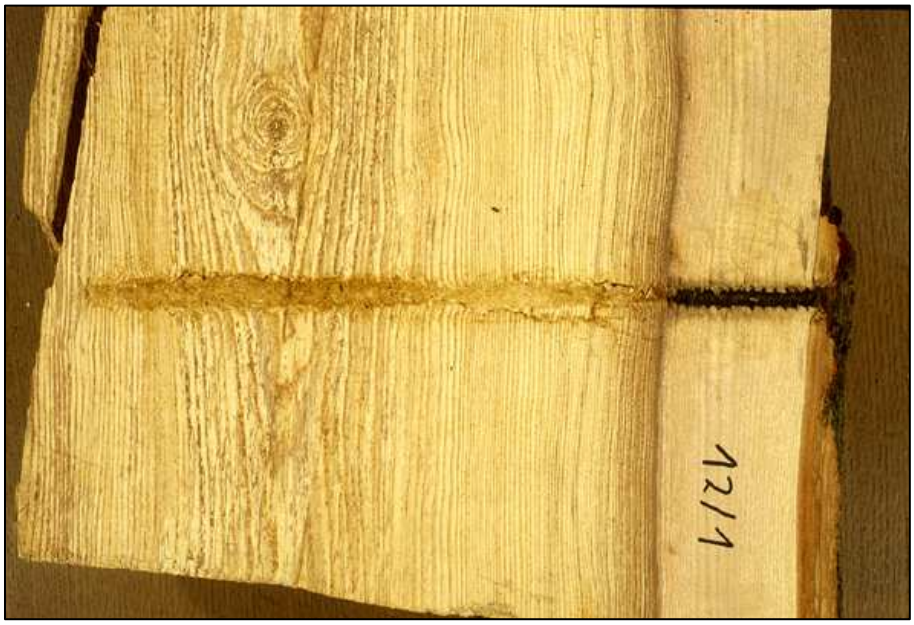

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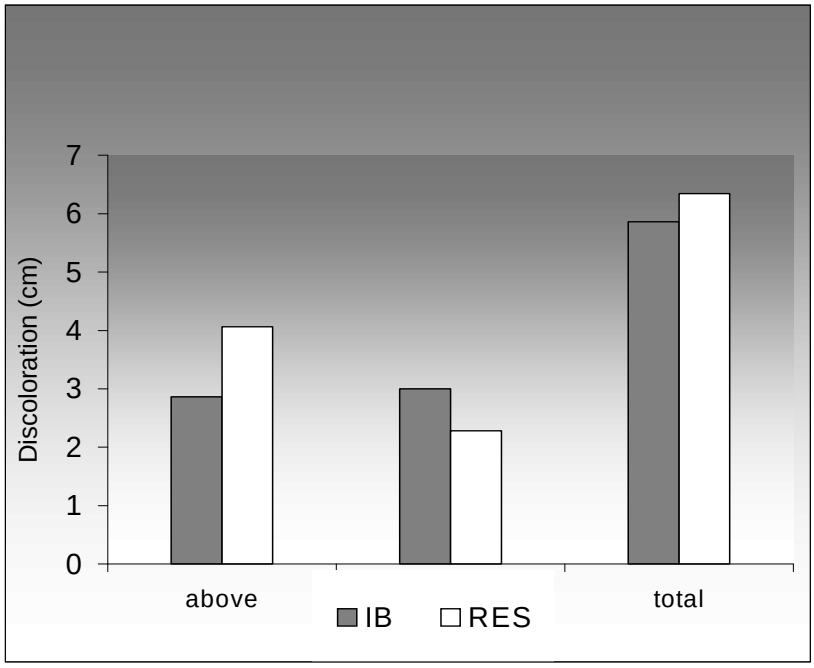

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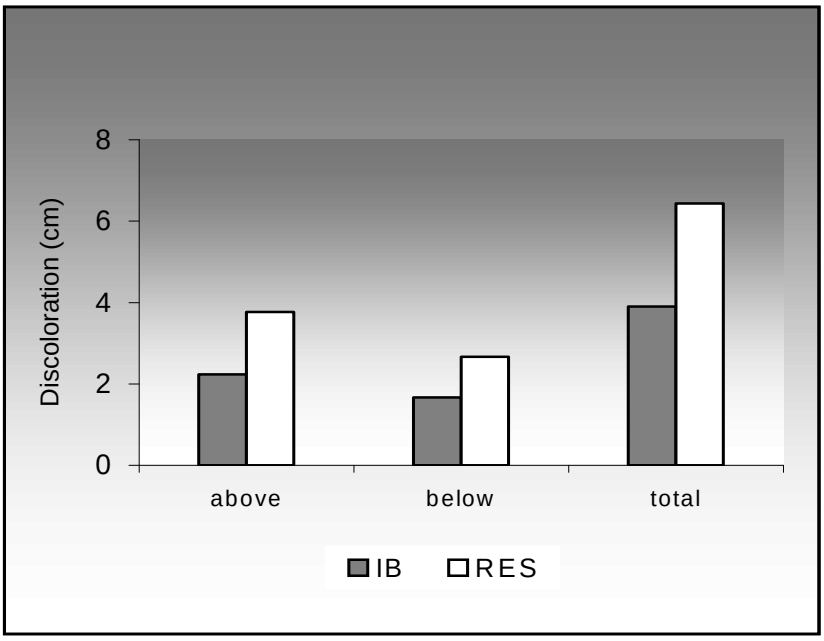

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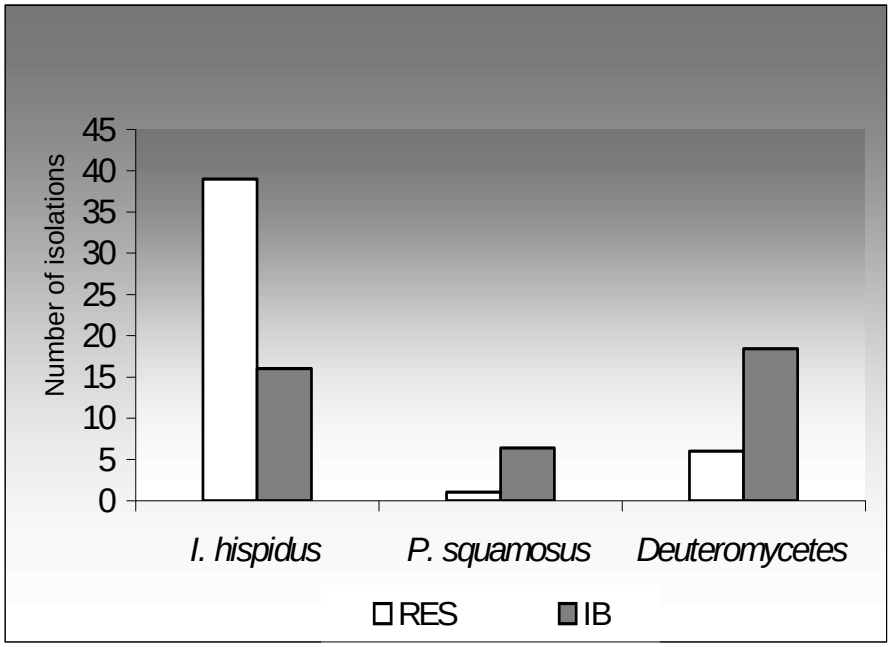

Figure 8. Isolation frequencies of *Inonotus hispidus*, *Polyporus squamosus* and Deuteromycetes in discoloured wood of Common ash and London plane after wounding with the IML-Resistograph (RES) and the increment borer (IB).

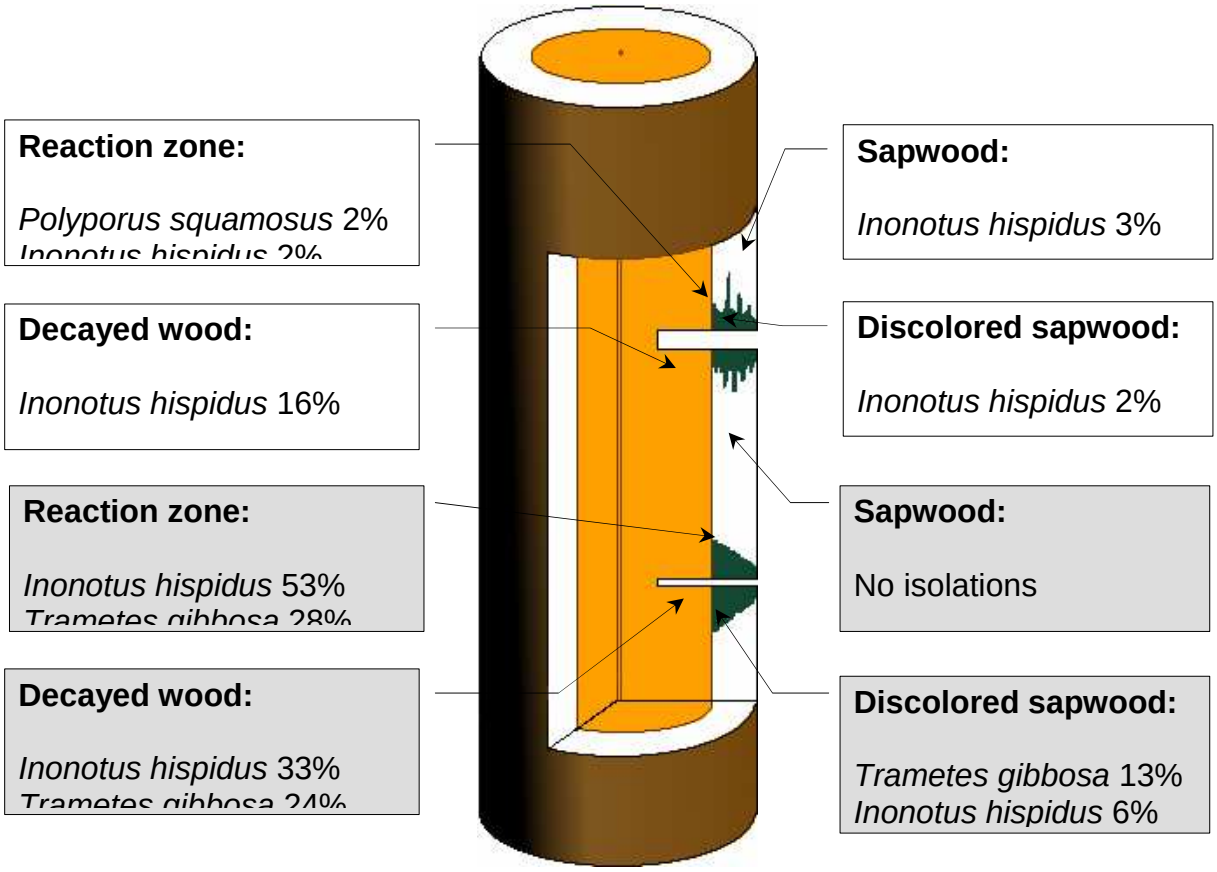

Figure 9. Isolations of wood-degrading basidiomycetes from London plane after wounding with the increment borer (above) and the IML-Resistograph (below). Results are showed as mean value and based on the percentage of individual wood chips.

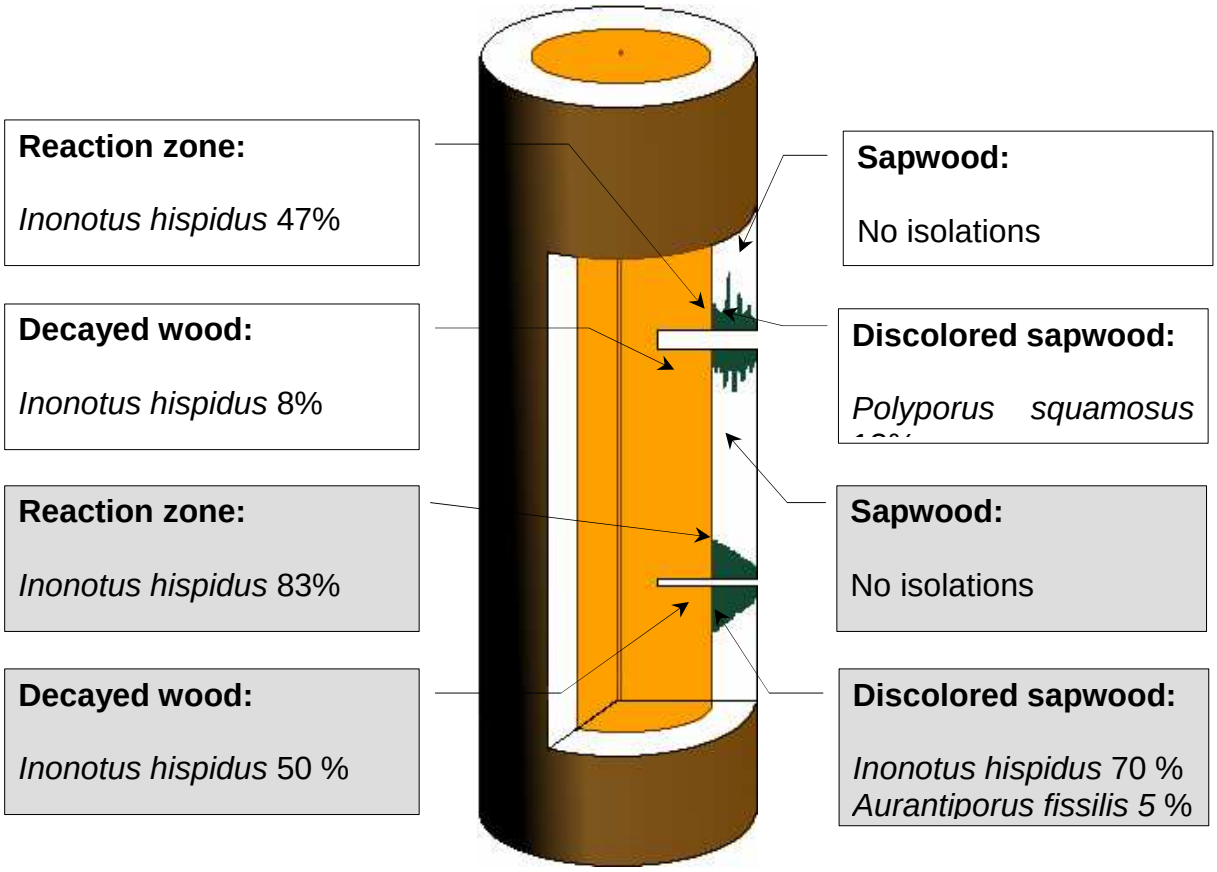

Figure 10. Isolations of wood-degrading basidiomycetes from Common ash after wounding with the increment borer (above) and the IML-Resistograph (below). Results are showed as mean value and based on the percentage of individual wood chips.

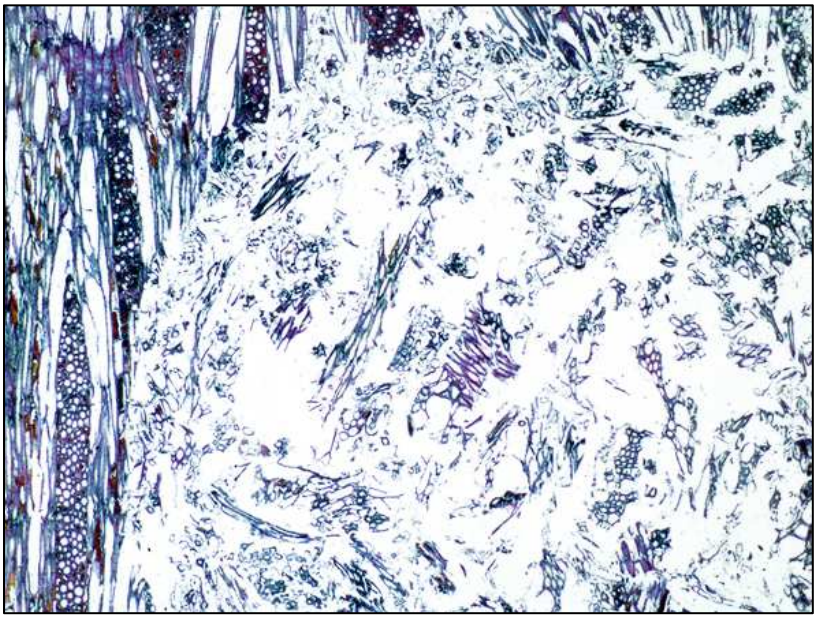

Figure 11. Tangential longitudinal section showing wood shavings within a drilling trace of the IML-Resistograph. Note the presence of cell fragments closely sealing the hole.