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INTERNAL SPREAD OF FUNGI INOCULATED INTO HARDWOOD STUMPS

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SUMMARY

Freshly cut stumps of beech (*Fagus sylvatica*), birch (*Betula pendula*) and oak (*Quercus robur*) were inoculated with wooden plugs permeated by mycelium of wood-decaying basidiomycetes and treated with 40 % (w/v) aqueous ammonium sulphamate solution (AMS), 1·5 % (w/v) 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in paraffin, or water. Successful establishment frequently occurred in water-treated stumps but internal spread was usually limited. AMS prevented establishment of certain fungi (e.g. *Daedaleopsis confragosa* in birch and oak), but greatly stimulated internal spread of others especially in wood adjacent to the bark. Here some fungi (e.g. *Hypholoma fasciculare*, *Phlebia merismoides*) were favoured because of their capacity for subcortical mycelial growth. 2,4,5-T stimulated colonization in beech, but not birch or oak.

Colonization patterns varied for different fungus-tree species combinations, e.g. *Chondrostereum purpureum* spread faster initially than any other fungus tested in beech and birch, and was not stimulated by AMS treatment in the former. Longitudinal spread of fungi such as *Bjerkandera adusta*, *Coriolus versicolor* and *Stereum hirsutum* in birch was considerably less after 6 months than in oak, but subsequently accelerated, whilst spread in oak ceased.

INTRODUCTION

Stumps of broad-leaved trees are frequently important sources of infection for *Armillaria mellea* (Vahl ex Fr.) Kummer. Rishbeth (1972, 1976) has recently attempted to evaluate the feasibility of stump-inoculation with other fungi for biological control of this and other fungal pathogens. Chemical treatments used to prevent stump regrowth were shown markedly to influence fungal colonization, e.g. *Bjerkandera adusta* (Willd. ex Fr.) Karst., *Coriolus versicolor* (L. ex Fr.) Quél. and *Phlebia merismoides* Fr. became established more readily in stumps treated with 40% (w/v) aqueous ammonium sulphamate solution (AMS) than those treated with 1·5% (w/v) 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) as its butyl ester in diesel oil, or left untreated, where *Chondrostereum purpureum* (Pers. ex Fr.) Pouz. was often favoured. These experiments suggested that a more detailed study of the fungal ecology of hardwood stumps should be undertaken. The experiments now described were designed to provide information about internal patterns of spread of decay fungi in stumps.

MATERIALS AND METHODS

Two series of experiments were carried out on freshly cut stumps of beech (*Fagus sylvatica* L.), birch (*Betula pendula* Roth) and oak (*Quercus robur* L.) in Forestry Commission plantations at various sites in the Breckland of East Anglia (details in Rayner, 1977a). Series 1 was set up mostly during November–December 1972, but

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with some experiments on beech and oak, including all 2,4,5-T treatments, during February to April 1973. Series 2 was set up during November 1973.

The inoculation method involved inserting wooden plugs, thoroughly permeated with mycelium of selected fungi, into the cut surface or side of stumps so that the starting point for fungal spread was known. This was also considered a more reliable method of inoculation than possibly more natural means of spores or mycelial fragments (c.f. Rishbeth, 1976) which might be more susceptible to adverse effects (e.g. competition and desiccation) at the cut surface.

Eighteen species were used: *Bjerkandera adusta*; *Chondrostereum purpureum*; *Coriolus versicolor*; *Daedalea quercina* L. ex Fr.; *Daedaleopsis confragosa* (Bolt. ex Fr.) Schroet.; *Fistulina hepatica* Bull. ex Fr.; *Ganoderma adspersum* (Schulz) Donk; *Heterobasidion annosum* (Fr.) Bref.; *Hymenochaete rubiginosa* (Dicks.) Lev.; *Hypoholoma fasciculare* (Huds. ex Fr.) Kummer; *Laetiporus sulphureus* (Bull. ex Fr.) Murr.; *Mycena galericulata* (Scop. ex Fr.) S. F. Gray; *Phlebia merismoides*; *Piptoporus betulinus* (Bull. ex Fr.) Karst.; *Pleurotus ostreatus* (Jacq. ex Fr.) Kummer; *Pseudotrametes gibbosa* (Pers.) Bond. & Sing.; *Stereum gausapatum* (Fr.) Fr.; and *Stereum hirsutum* (Willd. ex Fr.) S. F. Gray.

Preparation of inocula

Small discs of poplar wood (approx. 1 cm diam., 5 mm thick) and short rods ('dowels') 6·5 cm long cut from lengths of 1 cm diam. dowelling were soaked in 10% (w/v) malt extract solution for 5 h or more and then autoclaved (15 min at 120 °C). They were then transferred to petri dishes containing pure cultures of selected fungi on 3% malt agar, and incubated at 24 to 26 °C until thoroughly permeated with mycelium (6 to 20 days) after which they were stored at 5 °C until required.

Field inoculations

At each site trees reasonably uniform in size and shape (normally 15 to 25 cm diam. near the base) were felled with a power saw. To avoid splitting and facilitate cutting a horizontal surface a first cut was made at approx. 0·5 m, followed by a second 8 to 15 cm above the ground. After removal of sawdust from the surface, a single fungus was inoculated into each stump by drilling holes of approx. 1 cm diam. and 5 mm deep in a straight line across the cut surface and inserting a small disc plug into each. Similarly dowels were inserted into holes 6·5 cm deep drilled either vertically from the cut surface, or radially from the bark towards the centre of the stump. Control stumps not inoculated with fungi were either left intact, or perspex or polythene plugs inserted. The number of inoculations made per stump varied in series 1 experiments but for series 2, five small discs, one vertical and one lateral dowel were used. *B. adusta*, *C. versicolor* and *Stereum hirsutum* were inoculated into all three tree species, but the others into only one or two. Occasionally deliberately inappropriate hosts were used. The stumps were then treated with 40% (w/v) aqueous AMS solution, 1·5% (w/v) 2,4,5-T in paraffin or simply with distilled water. AMS and water were applied to the cut surface until run-off, whilst 2,4,5-T was painted onto the whole stump surface. For series 1 experiments on beech and birch AMS was the only chemical treatment tested, whilst for oak and all series 2 experiments both AMS and 2,4,5-T were used. Finally the sealing compound 'Synthraprufe' was applied to the cut surface of half (series 1) or three quarters (series 2) of the stumps in an attempt to retard natural colonization.

In general, for each tree species either eight stumps (one chemical treatment) or 12 stumps (two chemical treatments) were set up for each fungus: of these four or eight were chemically treated, and four water-treated.

Sampling methods and assessment of colonization

Direct observation. At approx. 2 month intervals the following features of the stumps were noted: changes in colour, presence and intensity of decay, presence of fungal fruiting or sporing structures and presence and condition of shoot regrowth.

Destructive methods. Destructive sampling was done 2 to $2\frac{1}{2}$ years after inoculation for series 1, after 6 months for half of series 2 and after 12 months for the remainder. Either the upper portions of stumps were cut off with a power saw or the stumps were extracted with as much of the root system intact as possible. All 6- and 12-month-old stumps and 20 to 30% of series 1 stumps were extracted.

Samples thus obtained were then sectioned both longitudinally and transversely on a band saw. The position and type of decayed or discoloured zones were noted, the latter outlined with an indelible pencil, and estimates of decay intensity made. The samples were then re-assembled, polythene being inserted between some sections to prevent cross-contamination, wrapped in moist newspaper and incubated individually in large polythene bags (10°C for 10 to 14 days) until recognizable mycelium of the inoculated fungus was present. Measurements were made of the radial and longitudinal extent of zones *throughout which* aerial mycelium of the inoculated fungus was produced. Other fungi present at this time and after a further period of incubation at approx. 20°C were recorded (Rayner, 1977a, b). The results were checked by isolation on to 3% malt agar, or by incubating surface-sterilized sections of wood approx. 2 cm thick in deep Petri dishes containing sterile moist filter paper.

RESULTS

Establishment and persistence of inoculated fungi

The success of fungal establishment was ascertained by recording the number of inocula (including both discs and dowels) from which a given fungus had spread into adjacent tissues: this was then expressed as a percentage of the total number of inocula used. Analysis was complicated in some instances by replacement by other fungi. Where despite some replacement the fungus could still be detected, it was recorded as present but where despite evidence that it had grown from the inoculum subsequently to be entirely replaced, it was recorded as absent. Representative results are given in Table 1.

With beech and birch the degree of establishment was similar at different inoculation positions, but with oak inoculations into the heartwood were seldom successful, so that no instances of 100% establishment appear in Table 1. Some colonization of heartwood, following inoculation with dowels, occurred with *Coriolus versicolor*, *Phlebia merismoides*, *Stereum gausapatum* and *S. hirsutum*.

The effect of chemical treatment on establishment varied. Whilst for certain fungi, such as *C. versicolor* in all three tree species and *P. merismoides* in oak, treatment with AMS had no obvious effect on establishment or persistence, many others became either better established or persisted for longer in water- or 2,4,5-T-treated stumps, e.g. *Daedaleopsis confragosa* in birch and *Pseudotrametes gibbosa* in beech. With other fungi, such as *Berkandera adusta* in all three tree species and *Hypholoma fasciculare*

and *Chondrostereum purpureum* in birch and beech, whilst AMS had no effect on establishment, it was associated with failure to persist, often due to replacement by fungi colonizing naturally. Two of the more important natural colonizers were *Phlebia merismoides* and *Phanerochaete velutina* (DC ex Pers.) Parmasto, both of which readily replaced *C. purpureum* and *B. adusta*. Natural colonization by *P. merismoides* was considerably enhanced by AMS treatment (Rayner, 1977b).

Table 1. Results of inserting inocula into freshly cut stumps: occurrence of the fungi at intervals after inoculation

| Fungus | Tree | Period since inoculation (months) | % occurrence of growth from inocula† | | |
|---------------------------------|-------|-----------------------------------|--------------------------------------|-----------------|-----------------|
| | | | AMS-treated | 2,4,5-T-treated | Water-treated |
| <i>Bjerkandera adusta</i> | Oak | 6 | 79 | 71 | 71 |
| | | 12 | 36 | 43 | 71 |
| | | 26–29 | 0 ^r | 16 | 0 |
| <i>Chondrostereum purpureum</i> | Beech | 6 | 100 | 100 | 100 |
| | | 12 | 21 ^r | 79 | 100 |
| | | 30 | 0 ^r | — | 38 ^r |
| <i>Coriolus versicolor</i> | Birch | 6 | 100 | 100 | 100 |
| | | 12 | 93 | 86 | 93 |
| <i>Daedaleopsis confragosa</i> | Birch | 6, 12 | 11** | 32 | 46 |
| | | 26 | 0 | — | 75 |
| <i>Hypholoma fasciculare</i> | Beech | 6, 12 | 96 | 75 | 100 |
| | | 29 | 75 | — | 94 |
| <i>Phlebia merismoides</i> | Oak | 27 | 73 | — | 75 |
| <i>Pseudotrametes gibbosa</i> | Beech | 6, 12 | 4*** | 32 | 46 |
| | | 27 | 0 | — | 14 |
| <i>Stereum hirsutum</i> | Beech | 6 | 100 | 50 | 100 |
| | | 12 | 29 ^r | 36 | 50 |
| | | 30 | 13 | — | 19 |

r, Indicates replacement.

, * Differences between AMS and water treatments significant at $P < 0.01$ and 0.001 respectively.

† (No. inocula from which growth occurred into wood/total no. inocula) $\times 100$.

No establishment was detected in the following combinations: *Daedalea quercina* (oak), *Fistulina hepatica* (beech and oak), *Heterobasidion annosum* (birch and oak), *Hymenochaete rubiginosa* (oak) and *Mycena galericulata* (birch). Of those fungi inoculated into inappropriate hosts some did not grow, e.g. *Piptoporus betulinus* in beech and *Pseudotrametes gibbosa* in oak, but some others did become established. Thus, *P. gibbosa* caused substantial decay and formed sporophores in birch, its general absence from which may therefore be due to poor establishment rather than an intrinsic failure to grow in this wood. *Bjerkandera adusta* similarly caused decay and produced sporophores in oak (within 1 year) but failed to persist for long.

Longitudinal spread

Six to twelve months after inoculation. For selected species of fungi measurements of longitudinal spread from small discs after 6 and 12 months in beech, birch and oak stumps are given in Tables 2, 3 and 4 respectively. For beech and birch measurements are given for the zone of wood lying between the bark and centre (but not including the central column), but in oak for the sapwood only. The range between maximum and minimum values observed in each type of stump is given, ignoring

Table 2. *Longitudinal spread of fungi from inocula in beech stumps*

| Fungus | Treatment | Longitudinal spread (mm) after: | | | |
|---------------------------------|-----------|---------------------------------|------|-------------|------|
| | | 6 months | | 12 months | |
| | | Range | Mean | Range | Mean |
| <i>Bjerkandera adusta</i> | AMS | 70-190 | — | > 290-> 350 | — |
| | 2,4,5-T | 80-120 | 100 | > 200-> 250 | — |
| | Water | 16-68 | 38 | 20-70 | 42 |
| <i>Chondrostereum purpureum</i> | AMS | Up to 230 | — | Absent | — |
| | 2,4,5-T | 65-200 | 136 | > 150-> 300 | — |
| | Water | 140-250 | 198 | 100-> 300 | — |
| <i>Coriolus versicolor</i> | AMS | 70-160 | — | 115-> 240 | — |
| | 2,4,5-T | 90-250 | 175 | 75-> 260 | — |
| | Water | 30-90 | — | 35-70 | — |
| <i>Hypholoma fasciculare</i> | AMS | 60-130 | 105 | 120-180 | — |
| | 2,4,5-T | 35-120 | — | 65-90 | — |
| | Water | 20-80 | 55 | 15-70 | 41 |
| <i>Stereum hirsutum</i> | AMS | 80-200 | 138 | 110-210 | — |
| | 2,4,5-T | 75-90 | — | 70-135 | — |
| | Water | 25-43 | 34 | 20-80 | — |

unsuccessful inoculations. Means are given for beech and birch only when eight separate measurements were available, and for oak only when all inoculations into the sapwood had succeeded. To avoid complication measurements from the dowels are not given, but they generally fell within the range quoted for the discs. Accurate measurements in the central wood were rarely possible due to fusion of colonization zones with those spreading from lateral dowels. Results for beech after 6 months may be exaggerated since the overall dimensions of decayed or discoloured zones spreading from the inocula were recorded, rather than only those parts in which the inoculated fungus could be detected.

In spite of considerable variation, these results show several trends. One is that for the majority of fungi whose establishment was not markedly impaired by AMS (e.g. *B. adusta*, *C. versicolor*, *H. fasciculare*, *S. hirsutum* and *S. gausapatum*) such treatment led to considerably enhanced longitudinal spread in all tree species. 2,4,5-T had little effect, if any, on longitudinal spread in birch and oak, but in beech spread by *B. adusta* and *C. versicolor* was strongly stimulated.

Not all the results were amenable to statistical analysis, but analysis of variance of the mean longitudinal spread from small discs by each fungus in individual birch stumps after 6 months showed that the effect of chemical treatment (by implication AMS treatment) was significant at $P < 0.001$ for *H. fasciculare*, 0.01 for *C. versicolor* and 0.05 for *B. adusta* and *S. hirsutum*. However, the differences were much less

Table 3. *Longitudinal spread of fungi from inocula in birch stumps*

| Fungus | Treatment | Longitudinal spread (mm) after: | | | |
|---------------------------------|-----------|---------------------------------|------|-------------|------|
| | | 6 months | | 12 months | |
| | | Range | Mean | Range | Mean |
| <i>Bjerkandera adusta</i> | AMS | 75-100 | 92 | > 200-> 230 | — |
| | 2,4,5-T | 20-35 | 28 | 70-160 | 121 |
| | Water | 32-54 | 38 | 50-180 | 121 |
| <i>Chondrostereum purpureum</i> | AMS | 120-160 | 149 | 120-150 | — |
| | 2,4,5-T | 80-133 | 118 | > 240 | — |
| | Water | 60-100 | 75 | 230-240 | — |
| <i>Coriolus versicolor</i> | AMS | 75-100 | 83 | 110-> 220 | — |
| | 2,4,5-T | 10-40 | 25 | 40-200 | 112 |
| | Water | 30-50 | 42 | 70-160 | — |
| <i>Hypholoma fasciculare</i> | AMS | 75-90 | 82 | 115-180 | 140 |
| | 2,4,5-T | 24-34 | 27 | 70-120 | 80 |
| | Water | 30-50 | 37 | 100-120 | 106 |
| <i>Stereum hirsutum</i> | AMS | 60-75 | 64 | 100-200 | — |
| | 2,4,5-T | 20-45 | 30 | 90-130 | — |
| | Water | 30-55 | 40 | 80-100 | 87 |
| <i>Daedaleopsis confragosa</i> | AMS | Absent | — | Absent | — |
| | 2,4,5-T | Absent | — | (64) | — |
| | Water | 32-42 | — | 70-100 | — |

Figure in parentheses based on single measurement only. Also for Tables 4 and 5.

Table 4. *Longitudinal spread of fungi from inocula in the sapwood of oak stumps*

| Fungus | Treatment | Longitudinal spread (mm) after: | | | |
|--------------------------------|-----------|---------------------------------|------|-----------|------|
| | | 6 months | | 12 months | |
| | | Range | Mean | Range | Mean |
| <i>Bjerkandera adusta</i> | AMS | 128-190 | 159 | (130) | — |
| | 2,4,5-T | 48-57 | 52 | 45-95 | — |
| | Water | 60-85 | 68 | 50-90 | 68 |
| <i>Coriolus versicolor</i> | AMS | 195-225 | 211 | 145-225 | 195 |
| | 2,4,5-T | 40-71 | 60 | 80-200 | 135 |
| | Water | 45-75 | 60 | 55-115 | 75 |
| <i>Stereum hirsutum</i> | AMS | 170-220 | 198 | (205) | — |
| | 2,4,5-T | 70-93 | 86 | 90-105 | — |
| | Water | 70-95 | 77 | 85-90 | — |
| <i>Stereum gausapatum</i> | AMS | 140-170 | — | (190) | — |
| | 2,4,5-T | 53-105 | 79 | 90-115 | — |
| | Water | 60-90 | 75 | 70-125 | — |
| <i>Daedaleopsis confragosa</i> | AMS | 0 | — | 0 | — |
| | 2,4,5-T | 0 | — | 0 | — |
| | Water | 0 | — | 105-200 | — |

significant after 12 months (e.g. $P < 0.2$ for *H. fasciculare*), possibly partly because colonization had proceeded too far to be measurable in AMS-treated stumps (notably with *B. adusta*) but also because of competition. Thus in two AMS-treated stumps where unusually little spread of *C. versicolor* in one and *H. fasciculare* in the other had occurred, much dark brown stained wood was present occupied by *Coryne sarcoïdes* (Jacq. ex S. F. Gray) Tul., several yeasts and *Penicillia*, and a *Mucor* sp. (probably *M. plumbeus* Bon.). Analysis of variance of mean longitudinal spread in oak stumps by *B. adusta* and *S. hirsutum* after 6 months, and by *C. versicolor* after 6 and 12 months showed that differences observed between AMS- and water- or 2,4,5-T-treated stumps were significant at $P < 0.05$, 0.001 and 0.01 respectively.

The initial rate of spread by *Chondrostereum purpureum* in birch and beech was far greater than that of other fungi. In beech chemical treatment had little effect, but analysis of variance of the mean results from small discs in individual birch stumps after 6 months showed that spread was significantly greater in chemically treated stumps than those treated with water ($P < 0.05$). In 12 month samples spread from small discs into AMS-treated stumps was less than in those treated with water or 2,4,5-T, but this did not apply to colonization from dowels.

In water-treated stumps there was a tendency for greater spread in the inner wood (i.e. that furthest away from the bark), whilst for those treated with AMS spread was normally greatest in the outer wood (only marginally so in birch). The pattern of spread in 2,4,5-T-treated stumps was similar to that in water-treated ones for birch and oak, but in beech spread was greatest in the outer wood after 6 months, and in the outermost wood after 12 months. These patterns were most clear with *B. adusta*, *C. versicolor*, *H. fasciculare*, *S. gausapatum* and *S. hirsutum*, but were more obscure with *Chondrostereum purpureum*. With *Pseudotrametes gibbosa* in beech and *Daedaleopsis confragosa* in birch colonization of AMS-treated stumps was very restricted, occurring, if at all, from the innermost parts of dowels only. More substantial colonization occurred in 2,4,5-T- and water-treated stumps, but again mostly from dowels and in the inner wood.

In birch and chemically treated beech stumps, measurements after 12 months were substantially greater than after 6. However in water-treated beech and all oak stumps, results after 12 months did not differ significantly from those after 6, suggesting that spread rapidly declined or ceased following initially rapid growth. In oak this was associated with the development of brown discoloured zones resembling heartwood (often seeming to extend from it) surrounding decay columns. In beech, greyish, seemingly water-soaked zones developed around decay pockets.

Twenty-six to thirty months after inoculation. Fewer measurements are available after this period because fewer stumps could be extracted, colonization had often proceeded too far to be traceable and the inoculated fungus had sometimes been replaced by others. Nevertheless the results (details in Rayner, 1975) substantiated many observations made after 6 to 12 months.

In beech the clearest results were obtained with *B. adusta*. The fungus was no longer present in the upper portions of AMS-treated stumps, having been replaced by others including *Phlebia merismoides*, *Phanerochaete velutina* and a species of *Scytalidium* (probably *S. album* Klingström & Beyer). However old sporophores indicated its former presence in these upper portions and it was always detected lower down. As after 6 to 12 months, spread was greatest (200 to > 300 mm) in AMS-treated stumps and very limited in water-treated ones (35 to 120 mm) where it was maximal in the

inner wood. The decay zones in water-treated stumps were surrounded by hard greyish-brown discoloured wood and there was evidence of water-soaking. The most intensely colonized water-treated stumps bore much less callus and regrowth than the others. Results for *H. fasciculare* and *S. hirsutum* were less clear due to competition with *Xylaria hypoxylon* (L. ex Fr.) Grev. and *Hypoxylon serpens* (Pers. ex Fr.) Fr. *Pseudotrametes gibbosa*, *Ganoderma adspersum* and *Pleurotus ostreatus* were present only in water-treated stumps and in these had spread longitudinally to a maximum of 40 to 50 mm.

Colonization of 26-month-old birch stumps had often proceeded too far to be measurable, particularly with *B. adusta* and *C. purpureum* which were present at least 200 to 300 mm from the cut surface of all extracted stumps, regardless of treatment. As with beech, these fungi had been replaced in the upper portions of AMS-treated stumps by others, especially *Phlebia merismoides* and *Phanerochaete velutina*. Spread by *H. fasciculare* was erratic (90 to > 180 mm in AMS-treated stumps, 120 to > 200 mm in water treated ones). As after 6 to 12 months greater spread often occurred in the outermost wood of AMS-treated stumps but in more central wood for those treated with water where the outermost wood was often virtually uncolonized below approx. 70 mm.

In oak *P. merismoides* persisted best of the inoculated fungi, most others having been replaced in AMS-treated stumps, mainly by *P. merismoides* but also by *Hypoholoma fasciculare* and *Hypoxylon serpens*. As with other fungi after 6 to 12 months, *P. merismoides* penetrated considerably deeper into AMS-treated stumps than those treated with water. The trend for cessation of fungal spread noticed after 12 months was confirmed. Again heartwood-like zones surrounded the decay columns. It was also noticed that uncolonized portions of stumps, including those treated with AMS, often produced callus after incubation.

Horizontal spread

Table 5 lists the range of maximum values recorded for horizontal spread of some of the fungi from inocula of all types after 6 months.

In beech, whilst the validity of results in chemically treated stumps was doubtful (see above), the patterns observed were very characteristic. In AMS-treated stumps the tangential component of spread increased outwards so that wedge-shaped zones of discolouration developed and this was repeated in 2,4,5,-T-treated stumps except that colonization of the outermost wood was strongly curtailed. Spread was very limited in water-treated stumps, usually being greater in the inner wood. These patterns were present with all but *Pseudotrametes gibbosa*, which occurred predominantly in the innermost wood of stumps treated with water or 2,4,5,-T. Similar results were found after 12 months except that the outermost portions of 2,4,5-T-treated stumps had become colonized. Little change had occurred in the horizontal dimensions of the colonized zones except with *H. fasciculare* which had spread extensively peripherally in AMS-treated stumps. This was associated with subcortical growth as mycelial sheets and fans and was most marked from lateral dowels passing through the cambial zone. Results after 26 to 30 months generally confirmed patterns found earlier but in a few water-treated stumps inoculated with *B. adusta* and *Pleurotus ostreatus* considerable spread had occurred, associated with absence of *Xylaria hypoxylon* (which restricted spread in other stumps) and/or sparse regrowth.

In birch after 6 months spread of *B. adusta*, *Chondrostereum purpureum*, *Coriolus*

versicolor, *H. fasciculare* and *S. hirsutum* was substantially greater in AMS-treated stumps than those treated with water or 2,4,5-T, and as with beech, wedge-shaped colonization zones were formed. Considerable discolouration occurred near the cut surface, probably mostly due to non-basidiomycetes, notably *Coryne sarcoides* (Rayner, 1977a). Spread in water- and 2,4,5-T-treated stumps was limited, usually being maximal in the inner wood. Measurements after 12 months were similar in AMS-treated stumps to those after 6, but substantially greater in stumps treated with water or 2,4,5-T, especially in the inner wood. Similar trends were found after 26 months. Here considerable subcortical spread by *H. fasciculare* had occurred, the entire perimeter sometimes being occupied by the fungus.

Table 5. Horizontal spread of fungi 6 months after inoculating stumps of various types

| Fungus | Treatment | Horizontal spread (mm) in: | | |
|---------------------------------|-----------|----------------------------|-------|-------|
| | | Beech | Birch | Oak |
| <i>Bjerkandera adusta</i> | AMS | 20 | 30–35 | 13–15 |
| | 2,4,5-T | 20–35 | 3–12 | 9–20 |
| | Water | 11–13 | 3–5 | (10) |
| <i>Chondrostereum purpureum</i> | AMS | 33–40 | 11–20 | — |
| | 2,4,5-T | 30 | 5–7 | — |
| | Water | 6 | 5–9 | — |
| <i>Coriolus versicolor</i> | AMS | 30 | 12–20 | 10–11 |
| | 2,4,5-T | 30–50 | 2–3 | 5–12 |
| | Water | 8–10 | 7–10 | 4–10 |
| <i>Hypholoma fasciculare</i> | AMS | 30–35 | 18–25 | — |
| | 2,4,5-T | 14 | 2–5 | — |
| | Water | 5–6 | 2–9 | — |
| <i>Stereum hirsutum</i> | AMS | 40–50 | 30 | 12–20 |
| | 2,4,5-T | (30) | 3–6 | 10–14 |
| | Water | 7–15 | 3–4 | 10 |
| <i>Daedaleopsis confragosa</i> | AMS | — | (10) | 0 |
| | 2,4,5-T | — | 1–3 | (7) |
| | Water | — | 3–8 | 0 |

Measurements in oak were restricted to the tangential component of spread in the sapwood. At first this was slightly greater in AMS-treated stumps, but after 12 months whilst results for most fungi were similar to those obtained after 6 (cf. longitudinal spread), those for *C. versicolor* were substantially greater in water- or 2,4,5-T-treated stumps. This trend was confirmed in similarly treated stumps after 27 months where very considerable spread by *C. versicolor* had occurred. *P. merismoides* was the only fungus showing considerably greater spread after 2 to 2½ years in AMS-treated stumps than in those treated with water: usually the entire sapwood of the former was occupied. It showed some capacity for subcortical mycelial spread as described for *H. fasciculare* in beech and birch. The following other trends were noted: spread was usually greatest in the outer sapwood of AMS-treated stumps, but in the inner sapwood of those treated with water or 2,4,5-T; sapwood immediately next to the heartwood was usually uncolonized; with 2 to 2½-year-old 2,4,5-T- or water-treated stumps inoculated with *C. versicolor* and *S. hirsutum*, spread was often greater near the cut surface than lower down, whilst in AMS-treated stumps spread usually increased with depth, a finding also noted in beech and birch.

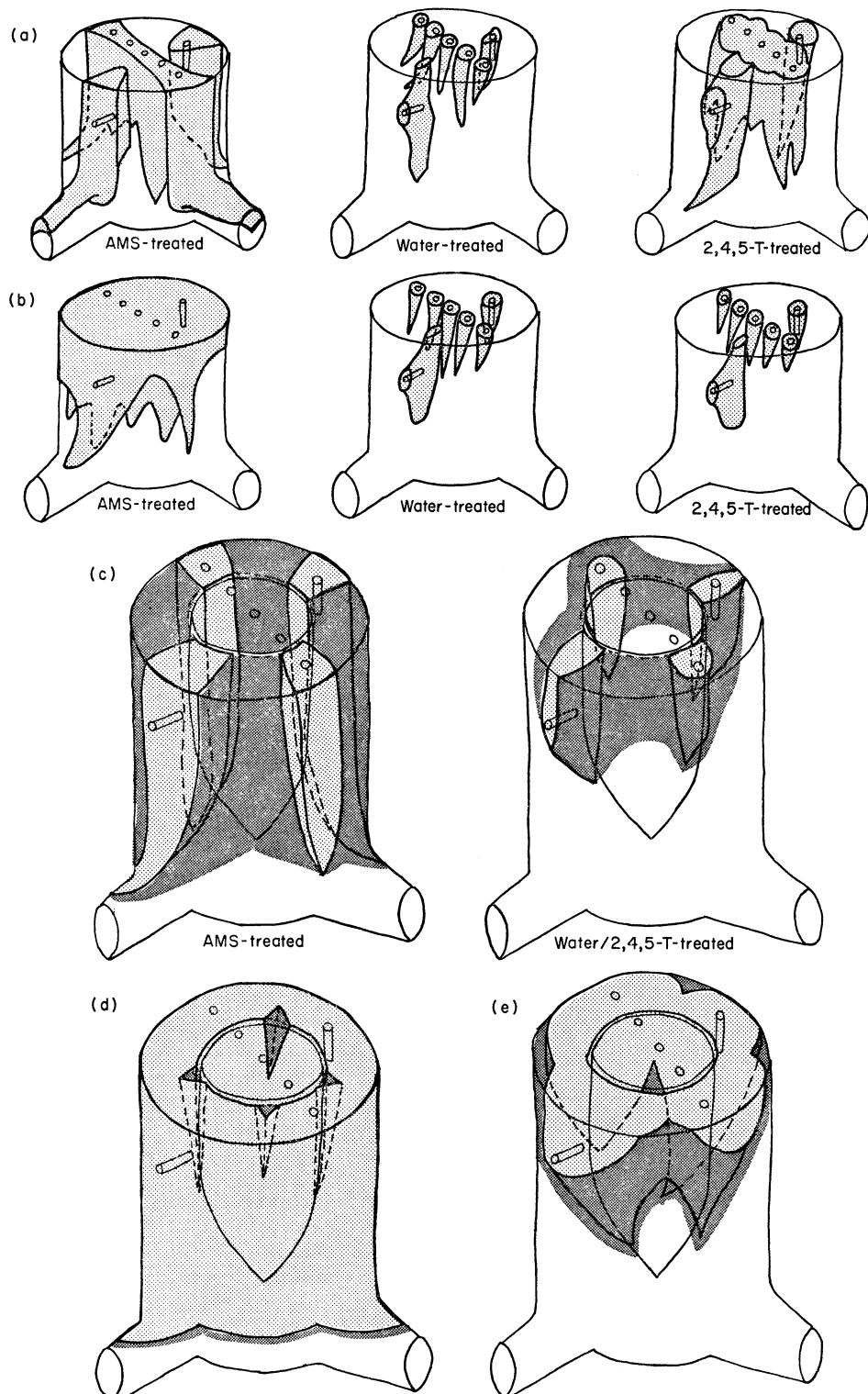


Fig. 1. For legend see opposite page.

Patterns of spread

The distribution of a fungus in a stump is the product of its capacity to spread both horizontally and longitudinally. As might be expected, longitudinal spread was generally greater for most of the fungi, which usually resulted in discrete columns of colonized wood spreading from the inocula. However this was by no means an invariable pattern. Idealized diagrams depicting patterns of spread in variously treated and inoculated stumps are given in Fig. 1.

DISCUSSION

Internal patterns of colonization shown by fungi inoculated into hardwood stumps have been demonstrated to be markedly influenced both by the tree species concerned, and by chemical treatments used to prevent regrowth. Many of these patterns were also shown by decay fungi colonizing naturally (Rayner, 1977b).

Treatment with 40% AMS usually stimulated longitudinal and horizontal spread, at least initially, particularly in the outer wood. With certain fungi (*Hypholoma fasciculare* in beech and birch, *Phlebia merismoides* in oak) this treatment led to extensive colonization of the outermost wood, resulting from subcortical mycelial growth. Natural colonization by *H. fasciculare* and *P. merismoides*, as well as by certain cord-forming fungi, e.g. *Phanerochaete velutina* and *Phallus impudicus* (L.) Pers., was similarly often associated with such growth, and favoured by AMS treatment (Rayner, 1977b). These findings may be important in relation to biological control of *Armillaria mellea* which is known to colonize stumps by subcortical mycelial growth following rhizomorph penetration of roots and basal portions (e.g. Redfern, 1968). If establishment of the pathogen is to be prevented it is important that any potential competitor should come into early contact with it, and this is most likely with fungi sharing its capacity for subcortical mycelial growth and ability to colonize roots. Otherwise competition may be avoided until a late stage.

Colonization of water- and 2,4,5-T-treated stumps of birch and oak, and of water-treated beech stumps, was restricted by comparison with AMS-treated stumps and normally least in the outer wood. Treatment with 2,4,5-T accelerated spread of saprophytic species in beech, although the outermost wood was poorly colonized for the first 6 months after inoculation and a more substantial natural colonization by *A. mellea* subsequently occurred than in stumps treated with AMS. These observations add weight to the contention (Rishbeth, 1976) that AMS treatment may be effective in reducing outbreaks of disease due to *A. mellea*, and indicate that simultaneous inoculation with a subcortical saprophyte may be valuable.

Fig. 1. Idealized diagrams illustrating patterns of spread of fungi in hardwood stumps. (a) Beech 6 months after inoculation with *Bjerkandera adusta*, *Coriolus versicolor*, *Hypholoma fasciculare* and *Stereum hirsutum*. The same general patterns were present after 12 months, except that greater spread had occurred in chemically treated stumps, and those treated with 2,4,5-T were similar to those treated with AMS. (b) Birch 6 months after inoculation with *B. adusta*, *C. versicolor*, *H. fasciculare* and *S. hirsutum*. Not all the discoloured wood near the cut surface of AMS-treated stumps was occupied by the fungus inoculated. (c) Oak 6 months after inoculation with *B. adusta*, *C. versicolor*, *Stereum gausapatum* and *S. hirsutum*. (d) AMS-treated oak 27 months after inoculation with *Phlebia merismoides*. (e) Water- or 2,4,5-T-treated oak 26 months after inoculation with *C. versicolor*. Light stippling indicates zones of colonization spreading from inocula. In (c), (d) and (e) dark stippling indicates zones of dark discolouration surrounding colonization zones spreading from inocula (always markedly paler). The heartwood is shown as a tapering cylinder, free from colonization.

The differences between the effects of chemical treatments may partly be explained by their mode of action. AMS is translocated in the phloem and hence able to penetrate readily into stumps, killing living tissues and eliminating host resistance, and enhancing their nitrogen content. Increased fungal growth would therefore be expected. Since host resistance is likely to be most limiting in the outermost wood, its removal would be expected to result in greater fungal spread in this region relative to controls. In the cambial zone where death of cells leads eventually to the separation of the bark the consequent removal of mechanical impedance would favour subcortical mycelial growth of saprophytic fungi in the fashion observed. Extensive mycelial systems of many such fungi are frequently present in decaying logs and branches lacking living tissue. In contrast 2,4,5-T is not translocated, only killing tissues at the point of contact (hence the need to apply it to the whole stump surface) and would not increase nitrogen content. It was less effective in killing stumps, especially oak and birch in which healthy callus was often produced after incubation, even near the cut surface. Its failure to stimulate growth of saprophytic fungi in birch and oak is thus not surprising. Indeed it may often cause sufficient loss of host resistance to allow growth of *A. mellea* (Rishbeth, 1970), but insufficient to stimulate colonization by saprophytes. Its greater effectiveness in beech may simply be that in this thin-barked species, greater penetration into the cambial zone occurs than in thick-barked trees.

Several differences in patterns of colonization were found between tree species, e.g. longitudinal spread in birch was generally less after 6 months than in beech and oak, but continued during the following 6 months whilst that in water-treated beech stumps and all oak stumps ceased. Some such differences may be due to anatomical factors, e.g. ring-porous oak wood in which numerous large vessels occur might be expected to be more conducive to fungal spread than birch wood where vessels are smaller, and the wood is diffuse-porous. The latter possibly explains the initially more limited longitudinal spread in this species. Other differences may be related to variations in the ability of different species to remain alive and produce regrowth. In beech and oak, regrowth was extensive on water-treated stumps and fungal spread ceased 5 to 10 cm from the cut surface. Less intense regrowth was found on water-treated birch stumps and here considerable longitudinal spread eventually occurred. In both birch and beech AMS treatment killed all living tissues and was associated with extensive root-colonization, but in oak living tissues were still present in the basal regions, which were poorly colonized. Formation of heartwood-like zones in oak, and apparently water-soaked zones in beech may have restricted fungal spread.

The behaviour of *Chondrostereum purpureum* differed from that of other fungi. During the first 6 months after inoculation it spread much faster longitudinally and was the only fungus in beech to spread as fast in water-treated stumps as in chemically treated ones, and in birch to grow faster in those treated with 2,4,5-T. These findings partly confirm those of Rishbeth (1972, 1976) which indicated that colonization by this fungus was favoured on 2,4,5-T- or untreated stumps rather than those treated with AMS. The ability of this well-known parasite to infect living wood may explain its unusual behaviour. Observations by Rishbeth (unpublished), confirmed in the present study, suggest that this fungus is able to kill regrowth readily. It is an important early colonist of birch stumps (Rayner, 1977b) and its ability to kill tissues may permit subsequent colonization by fungi causing more extensive decay. This development would be favoured by its poor competitive ability and ease of replacement by other fungi (Rayner, 1975, 1978).

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