Natural infection of Sitka spruce thinning stumps in Britain by spores of *Heterobasidion annosum* and long-term survival of the fungus

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Summary

On 48 sites throughout Scotland and north England freshly cut stumps were either inoculated with basidiospores of *H. annosum* or allowed to become infected naturally. Sites were in first rotation plantations growing in high rainfall or low rainfall areas on either peat or mineral soils. After 2 years, infection varied greatly between sites. It was generally much higher following inoculation, but otherwise little of the variation can be explained. Overall, only 10.5 per cent of stumps became infected naturally; colonies were generally small and almost half the stumps contained only homokaryons. Colonization took place principally in the heartwood but stumps sampled 8 years after inoculation showed that in contrast to previous findings this did not prejudice long-term survival. The cross-sectional area of each stump occupied by *H. annosum* at two years was also not a good predictor of survival. There was a marked decline in survival of *H. annosum* in stumps sampled after 8 years, which implied a corresponding decline in the overall level of incidence to only 2.8 per cent across all sites. Even in those stumps in which the fungus survived, it failed to colonize two-thirds of the available roots. These results reinforce the conclusions of earlier work that there is a low risk of serious disease on peat soils in the uplands. It is suggested that for Sitka spruce stumps on wet sites, the risk of successful colonization is so low, particularly on peaty soils, that *H. annosum* may not become permanently established in stands on these site types, even if ambient spore loads increase. By contrast, on mineral soils in low rainfall areas, the risk of a build-up of inoculum in unprotected thinning stumps and the transfer of infection to residual trees in the stand is much higher.

Introduction

In Britain, Sitka spruce (*Picea sitchensis* (Bong.) Carr.) constitutes 40 per cent of the conifer high forest and is the principal species grown in commercial conifer plantations (Locke, 1987). It is highly susceptible to decay by *Heterobasidion annosum* (Fr.) Bref. and losses can exceed 20 per cent of standing volume (Pratt, 1979a, b). The use of models has shown that losses are principally dependent on two factors: the soil on which the trees are growing and the incidence of stump infection by spores (Pratt *et al.*, 1989; Redfern *et al.*, 1994).

In contrast to Scots pine (*Pinus sylvestris* L.) and Corsican pine (*P. nigra* var. *maritima* (Ait). Melville), which were extensively studied by Rishbeth (1951, 1957), Meredith (1959, 1960) and Greig (1962), little information is available on the incidence of natural infection by spores in Sitka spruce stumps. Inoculation experiments with basidiospores have shown that they are less susceptible than those of lodgepole pine (*P. contorta* Douglas ex Loud.), one of the other pines most commonly grown in Britain (Redfern, 1982). In other inoculation experiments, control treatments have been exposed to inoculum from natural sources. The levels of infection reported in these experiments, which were carried out in Scotland, were 1.7 per cent (Redfern, 1993), 13.3 per cent (Redfern *et al*., 1997) and 8.3 per cent (Woods *et al*., 2000), and are much lower than those reported for pines in England and Wales (Rishbeth, 1951, 1957: Meredith, 1959, 1960; Greig, 1962). The discrepancy between these figures for natural infection and the much greater potential for infection in Sitka spruce revealed by inoculation (Redfern, 1993; Redfern *et al*., 1997), suggests that infection may be limited, at least to some extent, by a general lack of inoculum. In studies elsewhere, 19.6 per cent and 23.6 per cent of Sitka spruce stumps sampled in Canada were infected naturally (Morrison *et al*., 1986; Morrison and Johnson, 1999), and at a site in England, where there was a high ambient spore load, infection was as high as 42 per cent (Greig, 1962).

In upland Britain, the overwhelming majority of plantations were established on land with no previous forest history, and most were not thinned before stump protection was introduced in about 1960. In these circumstances there has been little opportunity for stump infection, either by spores or by root contact with inoculum in the ground. Basidiocarps are scarce and ambient spore loads are probably low. Elsewhere, for example in east Scotland, where there is a longer forest history, spore loads may be higher (Gladman and Low, 1963). Locally, in the vicinity of infected stands, ambient spore loads may be greatly exceeded since there is a sharp gradient in spore dispersal from basidiocarps (Kallio, 1970; Moykkynen *et al*., 1997).

In addition to the availability of inoculum, stump infection in a particular species is influenced by factors affecting the success of establishment of mycelia in the wood (Redfern and Stenlid, 1998). In Sitka spruce in Britain, wood moisture content is an important factor determining the success of infection, and the frequency and extent of stump colonization may be lower under the high rainfall conditions of west Scotland than in the drier east (Redfern, 1993).

The longevity of *H. annosum* in conifer stumps varies from as little as 5 years for pines in the southern United States up to 63 years for *Larix decidua* Miller (Low and Gladman, 1960; Ross, 1973). For Sitka spruce the greatest longevity recorded is 30 years (Greig and Pratt, 1976), but in this case infection may not have arisen directly from spores. In spite of this record, inoculation experiments have suggested that survival in Sitka spruce is poor. On wet sites *H. annosum*, at least initially, is confined largely to the heartwood, and in many stumps the extent of colonization is low, often comprising only isolated colonies < 1 cm^2 in area (Redfern, 1993). The potential of such small colonies for further development is unknown. Morrison and Redfern (1994) found that only those stumps in which *H. annosum* had been present in the sapwood 2 years after inoculation contained the fungus 6 years later. There was no precise threshold of sapwood area colonized which allowed *H. annosum* to survive, and some small colonies expanded greatly, but in general small colonies died.

The information available suggests that spore infection of Sitka spruce stumps in northern Britain is at present limited by the availability of inoculum, particularly in the west. However even if spore loads increase, the conditions for successful establishment and long-term survival may be more circumscribed than has previously been realised. As already mentioned, information on natural infection in Sitka spruce has hitherto been obtained only from the control treatments of inoculation experiments on a few sites. This paper describes a large-scale experiment in which Sitka spruce stumps on a wide range of sites throughout Scotland and northern England were either exposed to natural infection or inoculated with basidiospores. Sites were chosen in either high rainfall or low rainfall areas and on well-drained or poorly-drained soils. Infection and long-term survival were assessed by sampling stumps 2 years and 8 years after cutting.

Materials and methods

Site selection

In total 48 sites were chosen in first rotation, unthinned stands of Sitka spruce growing either on peat or on mineral soil. The crops were chosen to provide stumps of approximately 20 cm diameter but as a consequence of differences in site fertility they varied in age from 19 to 28 years old. Half of the sites on each soil type were in forests in which the average annual rainfall was less than 1600 mm (dry sites) while on the remaining sites rainfall exceeded 1600 mm annually (wet sites) (Anon., 1977). Site locations are shown in Figure 1. Peat soils were defined as soils in which the peat (organic) layer exceeded 10 cm in thickness. They were largely deep peats (with a peat depth exceeding 45 cm) and peaty-gleys (Pyatt, 1970). On mineral soils peat was generally absent and if present was less than 5 cm thick. These soils were mainly brown earths and surface-water gleys. In the upland forests of Scotland and northern England soils often occur as a mosaic of different types due to changes in topography. It was thus possible to pair the two soil types in the same forest (and rainfall area), sites often being separated by little more than 1 km. Separation exceeded 5 km in only one instance.

Design of experiment and statistical analysis

For logistic reasons inoculations were carried out on four occasions: in spring and autumn 1989 and in spring and autumn 1990. Pairs of plots from each rainfall sub-set were allocated at random to the four inoculation times. Each soil/rainfall combination was therefore represented three times at each inoculation time.

On each site 50 trees were felled to provide stumps between 10 cm and 30 cm in diameter and approximately 50 cm high. Trees with basal wounds caused by deer were excluded. Stumps were in one or more rows depending on the uniformity of the soil. A minimum of two rows of trees were left between adjacent rows of stumps. Two adjacent stumps within a row comprised a block, and within a block the inoculation and control treatments were allocated at random. On those sites that were inoculated in 1989, 100 trees were felled, and blocks consisted of four stumps in order to test the effectiveness of urea as a stump treatment chemical on two of them. This aspect of the work is reported separately (Pratt and Redfern, 2001).

Results were subjected to analysis of variance where appropriate, after transformation of percentages by arcsine. A large number of stumps failed to become infected when exposed to natural inoculum alone. For each site, incidence was calculated as the number of infected stumps expressed as a percentage of the total number of stumps. The area occupied by *H. annosum* was expressed as a percentage of the total area of those stumps that became infected. Where no stumps became infected on a site, both incidence and area were recorded as zero and not treated as missing values. In the case of stumps for which information was available from two levels (Figure 2), level was included as a split-plot factor.

Of the 2400 stumps in the experiment, the results for four were excluded from analysis due to pre-existing infection, leaving totals for each combination of soil and rainfall that varied from 298 to 300. In some stumps, *H. annosum* was present as only a single colony occupying an area smaller than it was possible to outline with a sharp indelible pencil (about 1 mm^2). These colonies were marked with a dot and given a notional area of 0.01 cm^2 for analysis.

Inoculation

One day after felling and approximately 1 h before inoculation stumps were recut to a height of 20 cm. In order to reduce the risk of contamination, all of the stumps to be inoculated with spores were recut and inoculated before the control stumps.

Basidiospores were collected in the manner and at the location described by Redfern *et al.* (1997). Immediately before inoculation, sufficient spores were washed into 1 litre sterile water to form a cloudy suspension. Stumps were inoculated by applying either the spore suspension or sterile water dropwise from a 20 ml sterile plastic syringe. The absorbancy of stump surfaces varied greatly, so that where necessary even coverage was ensured by spreading the suspension with the aid of a small, sterilized paintbrush. The effects of rainfall during or after inoculation were avoided by placing cones, constructed from stiff but

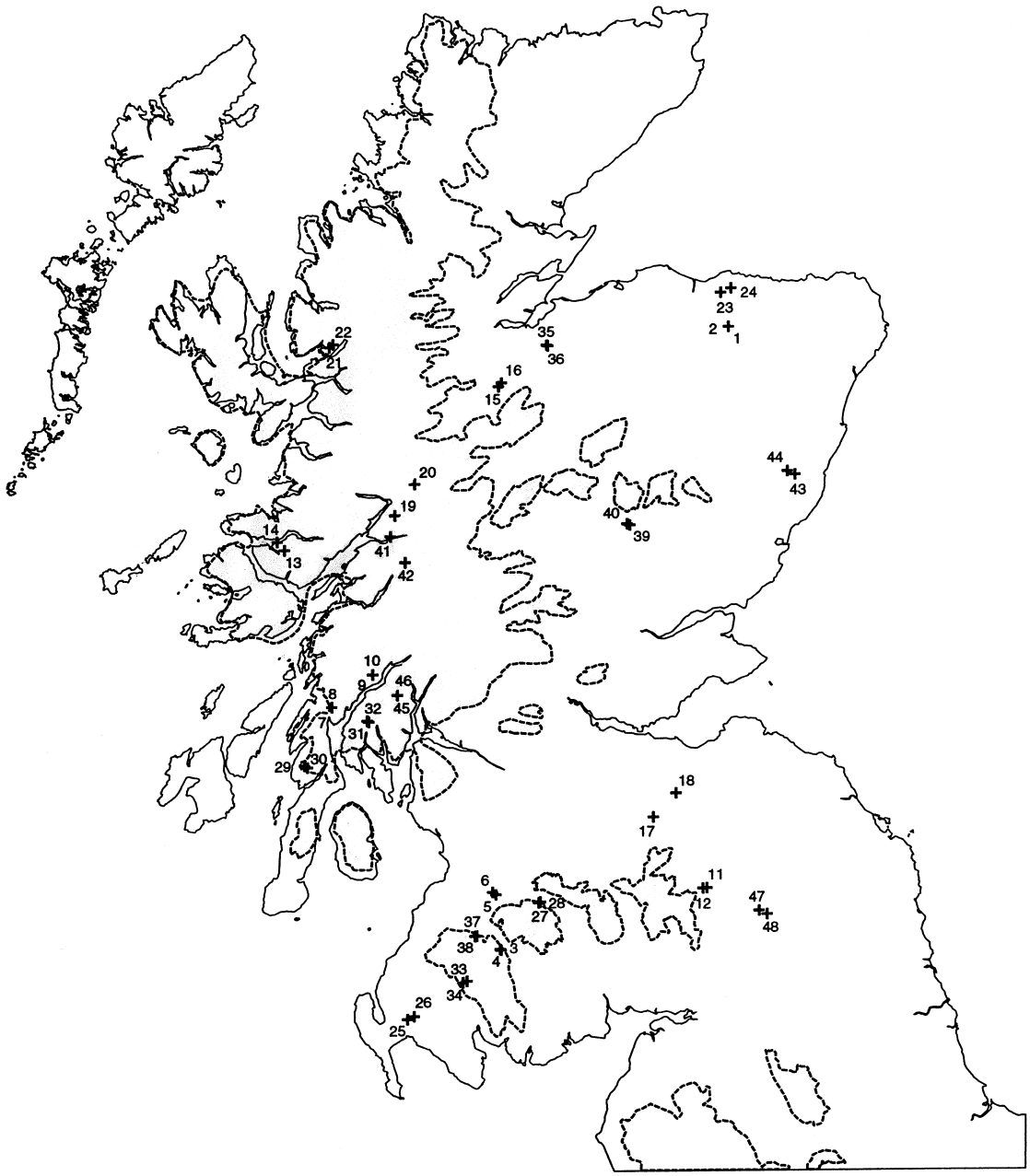

Figure 1. Locations of experiment sites. Even numbers indicate sites with peat soil and odd numbers indicate sites with mineral soil. The dotted line represents the 1600 mm rainfall isohyet.

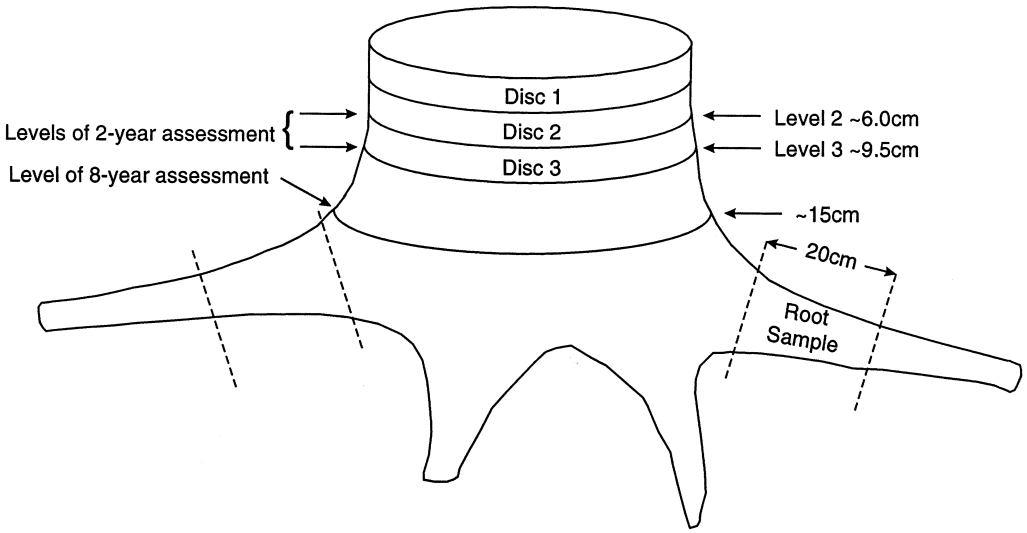

Figure 2. Position of root samples and discs cut from stumps, and depth below the stump surface (level) at which examinations were made.

flexible plastic sheeting, on the stumps after cutting. The cones, measuring approximately 50 cm wide and 50 cm high, were removed temporarily to allow inoculation and then attached more permanently by means of two staples inserted into the bark on opposite sides of the stumps. Since the diameter of the cones substantially exceeded that of the stumps this ensured that large air gaps were created at either side. The cones and staples were removed after 24 h. If necessary, stumps were sheltered from rain during inoculation by means of a large, self-supporting umbrella.

Spore suspensions were agitated at frequent intervals and on each site inoculation was completed within one day. As soon as possible after inoculation, and always on the same day, the viability of spores in each suspension was determined by plating six successive 10-fold dilutions onto triplicate plates containing selective medium (Kuhlman and Hendrix, 1962). The sterile water used in the control inoculations was plated onto triplicate plates without dilution.

With one exception, plots paired according to soil (see above) were inoculated on successive days; in the exceptional case there was an interval of 2 days.

Assessment

Stumps were first sampled from the paired sites 2 years after inoculation by successively cutting two or three discs, 2.5 cm thick, from the top (Figure 2). Two discs were cut from stumps on the 24 sites inoculated in 1989 and three from those on the 24 sites inoculated in 1990. The discs from each stump were put into a single plastic bag and transported to the laboratory. Discs were washed, drained, wrapped individually in dry newspaper and incubated in ventilated bins at 10–15°C for 7 days. After incubation, the lower surfaces of discs 2 and 3 were examined for the presence of conidiophores of *H. annosum* with the aid of a binocular microscope and colonies were outlined. The boundary between heartwood and sapwood was also outlined, its location being determined by viewing the disc with strong back-lighting against which the heartwood appeared opaque and contrasted with the translucent sapwood. The areas outlined on the discs were transferred

to tracing paper and the following measurements were made with a digitizer: area of heartwood, area of sapwood, and the area of both wood types occupied by *H. annosum*. Colonies that were too small to be outlined (i.e. < 1 mm^2) were marked with a dot.

In order to investigate the genetic composition of *H. annosum* in naturally infected stumps, isolations were made from most colonies that exceeded about 2 mm diameter on discs from both level 2 and level 3. This was done for 129 stumps from sites 15–48. Samples from which isolations were to be made were collected with an increment hammer, which was driven into the disc surface within outlined colonies. Most colonies were too small to provide more than one sample but several were taken from the more extensive colonies that were occasionally encountered. Each sample consisted of a cylindrical wood plug about 5 mm diameter and 2.5 cm long. The sides and ends of the plug were shaved off with a sterile scalpel, so that small chips could be cut from the interior for plating out. The increment hammer was rinsed in alcohol and dried between samples. To provide a comparison with colonies arising as the result of inoculation, on one site isolations were also made from eight inoculated stumps in which the pattern and extent of colonization were similar to those on the naturally infected stumps.

Initial isolation was on a medium devised by Kuhlman and Hendrix (1962). Colonies of *H. annosum* were subcultured after 10–14 days on to 1 per cent malt agar and examined for clamp connections by viewing through the reverse of the plates. Isolates with clamps were presumed to be heterokaryons. In order to distinguish homokaryons and heterokaryons lacking clamps (Stenlid and Rayner, 1991), clampless isolates were paired separately with two known homokaryons. In each case, two subcultures from the resulting colony were paired with each other and with the 'parental' isolates. If the reactions indicated that the first pairing had produced a single genet differing from both 'parents', this was taken to demonstrate that the clampless isolate was a true homokaryon. Several other outcomes are theoretically possible, all of which would suggest that the isolate tested may not have been a true homokaryon.

The phenomenon of 'somatic incompatibility' was used to distinguish different genets (Stenlid, 1985) and confrontations between isolates were carried out using Korhonen's (1978) method.

Upper discs (disc 1) were incubated in bins, but without wrapping in newspaper, for a further 14–21 days at 10–15°C to encourage fruiting or the formation of recognizable mycelium of fungi other than *H. annosum*.

Eight years after cutting, naturally infected stumps on 16 sites (eight pairs on the two soil types) with the highest incidence of infection at 2 years were resampled. A 5 cm-thick disc was cut from the top of each stump in which *H. annosum* had been detected at 2 years and a 20 cm-long section was cut from each of the major roots, as near to the edge of the stump as practicable (Figure 2). There were generally 4–6 root sections from each stump. Samples were placed in individual plastic bags and transported to the laboratory. Stump discs were washed, incubated and examined for *H. annosum* as above. Root sections were washed and the ends recut to provide a 10 cm-long sample that was incubated and examined in the same way. To provide a comparison with earlier work, in which inoculated stumps were also examined after 2 years and 8 years (Morrison and Redfern, 1994), similar discs were also cut from inoculated stumps on three of the eight pairs of sites (i.e. six sites). No root sections were taken.

Results

Viability of inoculum

The mean number of viable spores in all 48 suspensions of inoculum which were used was 62×10^4 spores ml^{-1}, with a range of 7×10^4 to 180×10^4.

Incidence of H. annosum *after 2 years*

Results obtained from the level 2 samples are shown in Table 1. Among inoculated stumps the frequency of infection was significantly lower ($P = 0.04$) on peat soils (mean 71.5 per cent) than on mineral soils (mean 84.8 per cent), but there was no effect of rainfall. For stumps exposed to natural inoculum alone, the general level of infection was much lower than that following

Table 1: Frequency (per cent) of *H. annosum* in stumps inoculated with basidiospores or exposed to natural infection on various sites (n = 75)

	Site type and treatment							
	Low rainfall				High rainfall			
	Inoculated		Not inoculated		Inoculated		Not inoculated	
Innoculation date	Mineral	Peat	Mineral	Peat	Mineral	Peat	Mineral	Peat
Spring 1989	82.7	69.3	6.7	12.0	80.0	64.0	0.0	1.3
Autumn 1989	89.3	84.0	20.0	8.0	94.5	86.7	9.5	13.3
Spring 1990	82.7	72.0	28.0	13.3	89.3	76.0	6.7	9.3
Autumn 1990	64.0	52.0	21.6	14.7	96.0	68.0	5.3	0.0
Mean	79.7	69.3	19.1	12.0	89.9	73.7	5.4	6.0

inoculation; no soil effect was evident but infection was significantly lower (P = 0.008) on high rainfall sites (mean 5.7 per cent) than on drier sites (mean 15.5 per cent). There were no significant differences in stump size (area) between these two site types that could have affected incidence. However, this finding should be treated cautiously because no stumps were infected on 17 out of the total of 48 sites. There was no consistent effect of inoculation date and no interaction with soil or rainfall, but for some combinations of soil and rainfall there were large differences between dates, both with and without inoculation. There were also large differences between sites, even after inoculation. For example, the number of stumps infected after 2 years (out of 25) on the 12 low rainfall, mineral soil sites ranged from 0 to 14 for uninoculated stumps and from 6 to 25 for those that were inoculated. Thus, although inoculation increased the incidence of infection on all sites compared to the uninoculated controls, it was occasionally remarkably unsuccessful in establishing infection, with less than half the inoculated stumps becoming infected on four of the sites in the experiment as a whole.

Extent of H. annosum *in infected stumps after 2 years*

As is already implied by the higher incidence of infection among inoculated stumps compared with those exposed to natural infection, the extent of colonization was also greater (Table 2, and P< 0.001 from ANOVA). There were no significant soil or rainfall effects among inoculated stumps but for naturally infected stumps the proportion of the total stump area colonized was lower on wet sites than on dry sites (P = 0.001). However, again the number of zero values requires a cautious interpretation.

The extent of stump colonization was extremely variable both between and within sites. Natural infection is dealt with more fully below, but for inoculated stumps, the mean percentage stump area occupied varied from 0.04 to 5.1 per

Table 2: Proportion of cross-sectional area (in 2 per cent classes) of Sitka spruce stumps occupied by *H. annosum* after inoculation with basidiospores or following exposure to natural infection

	Number of stumps in each area class										
Area class (%)	2	4	6	8	10	12	14	16	18	20	22
Not inoculated	120	6	0	0	0	0	0	0	0	0	0
Inoculated	748	111	27	28	7	6	5	1	1	0	2

cent between sites, and on the site with the highest mean area, individual stump values ranged from 0.1 to 21.4 per cent.

Colonization pattern and colony characteristics

In inoculated stumps, colonization was much more frequent and extensive in the heartwood than in the sapwood (Table 3). Soil and rainfall effects reflected those already reported for the stump as a whole, i.e. the frequency of infection within both tissues was greater on mineral soils than on peat soils and there were no significant soil or rainfall effects on the extent of colonization. However there was a tendency for greater sapwood colonization on dry sites (i.e. mineral soils on low rainfall sites) than under wetter conditions and, by contrast, heartwood colonization tended to be greatest on high rainfall sites.

Naturally-infected stumps showed a broadly similar pattern, with colonization consisting principally of small colonies scattered in the heartwood (Figure 3a, b); infection of the sapwood was rare, either in the form of independent colonies or as extensions of heartwood colonies across the heartwood/sapwood boundary (Table 4). Seventy of the 126 stumps that became infected contained only a single colony. The remainder had multiple colonies that generally were widely separated, but which in some stumps were closely aggregated (Figure 3c). Colonies rarely exceeded 1 cm^2 in size and many consisted of a single clump of conidiophores. The largest single colony occupied 17.4 cm^2 (3.3 per cent of the stump area).

Effect of sampling depth on incidence and extent of colonization

This was studied on the 24 sites where three discs were cut from each stump. The lower surfaces of sample discs 2 and 3 were approximately 6 cm

Table 3: Frequency of *H. annosum* in sapwood or heartwood* of Sitka spruce stumps inoculated with basidiospores, and percentage of cross-sectional area† occupied

	Site type							
	Low rainfall				High rainfall			
	Frequency (%)		Area (%)		Frequency (%)		Area (%)	
Region of stump	Mineral	Peat	Mineral	Peat	Mineral	Peat	Mineral	Peat
Sapwood	9.3	4.7	1.8	1.4	5.7	2.7	0.4	1.3
Heartwood	79.3	69.0	2.9	2.5	89.3	73.3	5.8	4.0

* Includes stumps with *H. annosum* present in both sapwood and heartwood.
† For infected stumps only.

Table 4: Position and size of *H. annosum* colonies* in naturally infected Sitka spruce stumps

Number of stumps in category				
Position of colonies			Area of individual colonies	
Heartwood only	Sapwood only	Both	<1 cm^2†	>1 cm^2‡
115	6	5	112	14

* Excluding scattered clumps of conidiophores with negligible area.
† All colonies on stump <1 cm^2.
‡ At least one colony this size.

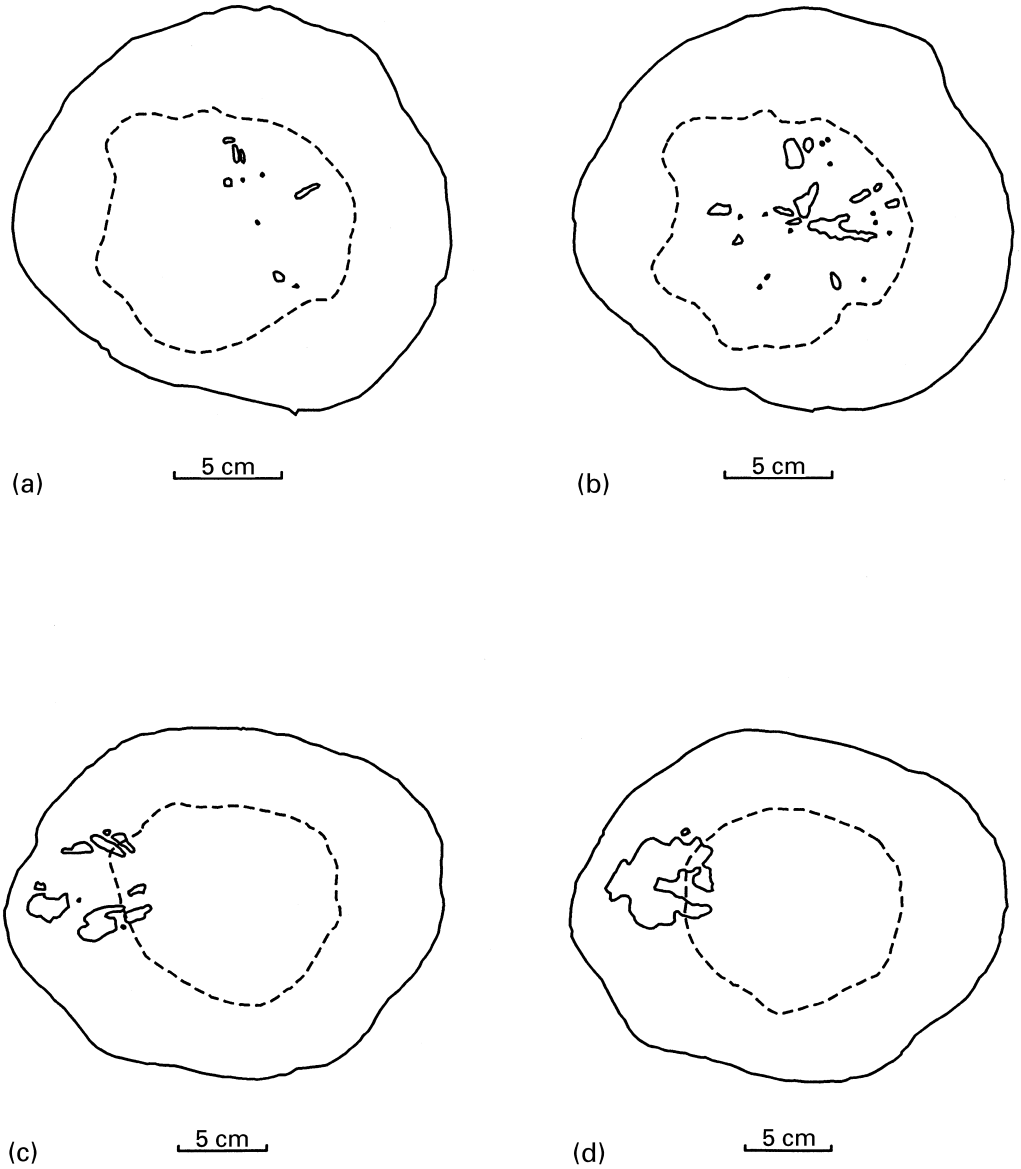

Figure 3. Typical patterns of colonization in two 2-year-old Sitka spruce stumps naturally infected by *H. annosum*. (a) and (b) Sections cut 6.0 cm and 9.5 cm, respectively, below the surface of stump 1. (c) and (d) Similar sections through stump 2, in which all colonized areas were occupied by the same genet. The position of the boundary between heartwood and sapwood is indicated by a dotted line.

(level 2) and 9.5 cm (level 3) below the stump surface, respectively (Figure 2). Insufficient data were available for naturally infected stumps to permit statistical analysis. Among inoculated stumps there was no significant difference in the incidence of *H. annosum* recorded at the two depths as a main effect, but there was a weak interaction with soil, rain and inoculation date

(P = 0.039): incidence was lower at level 2 than level 3 for some combinations of circumstance, but higher for others. In infected stumps *H. annosum* occupied a larger area at the lower level (P < 0.001; Figure 3).

Colonization by other wood-inhabiting fungi

Resinicium bicolor (Alb. et Schw. ex Fr.) Parm. was the most common colonist, occurring on 26.0 per cent of stumps. It was found principally in the sapwood, often in a position that suggested colonization by spores rather than invasion from the stump edge. This is supported by the observation that application of urea to stump surfaces as a protection against infection by *H. annosum* also reduced colonization by *R. bicolor* (Pratt and Redfern, 2001). *Melanotus proteus* (Kalchbr.) was present in 11.4 per cent of stumps, where it was recorded in both the heartwood and sapwood. *Hypholoma fasciculare* (Huds. ex Fr.) Kamm., *Sistotrema brinkmannii* (Bres.) J. Eriksss., *Ascocoryne* sp. and *Haematostereum sanguinolentum* (Alb. et Schw. ex Fr.) Pouzar were also recorded in small numbers of stumps. The incidence of *R. bicolor* and *M. proteus* varied greatly and there was no obvious relationship between the incidence of these fungi and the success of infection by *H. annosum*, but there were seasonal and site effects. *Melanotus proteus* was less common on stumps cut in spring 1990 than on those cut on the other three occasions (P = 0.04 and P = 0.06 for inoculated and uninoculated stumps, respectively). The incidence of *R. bicolor* was affected both by season and by rainfall. It was much more common on stumps cut in spring than on those cut in autumn (P = 0.001) and more common on wet sites than on dry sites (P = 0.001).

The genetic composition of H. annosum *colonies*

Of the 468 isolates obtained from naturally infected stumps, 244 had clamps and were classed as heterokaryons while the remaining 224 isolates lacked clamps. Of the latter, 195 were shown to be homokaryons, 16 grew too poorly to be tested and 13 gave results which could not be interpreted. In some of the clamped isolates, clamps were slow in forming and in others clamps were rare, but these were a minority and no example was found of a heterokaryon without clamps.

Fifty-eight of the 120 stumps from which identifiable isolates were obtained contained only homokaryons, and 32 of these stumps had only a single genet (Table 5). The maximum number of genets found on a stump was 18. There was great variation between sites, with heterokaryons predominating on some sites and homokaryons on others. By contrast, the eight inoculated stumps sampled from one site all contained several heterokaryotic genets, with only one homokaryotic colony.

Genetic analysis of colonies on 35 stumps revealed the following characteristics: (1) both homokaryons and heterokaryons penetrated to the lower surface of disc 3, i.e. to a depth of 9.5 cm; (2) the same genet was generally obtained from colonies in the same relative position to each other on discs 2 and 3; (3) the most extensive colonies consisted of one or more heterokaryons; (4) the largest heterokaryon was 19.8 cm^2 in area compared with the largest homokaryotic genet which occupied 2.1 cm^2. In addition, on three stumps, a large colony comprising a single genet on disc 3 was represented in the same relative position on the disc above by two or more smaller colonies each consisting of the same genet (Figure 3c, d).

Table 5: Frequency of homokaryons and heterokaryons in Sitka spruce stumps naturally infected by *H. annosum* and the number of genets per stump

Number of genets per stump	Number of stumps											
	1	2	3	4	5	6	7	8	9	10	>10	Total
Homokaryons only	32	15	5	5	1							58
Heterokaryons only	18	7	0	2	1							28
Homo. + Hetero.	–	10	6	1	1	3	1	5	2	2	3	34

Survival in infected stumps after 8 years

Eight years after cutting, naturally infected stumps found to contain *H. annosum* at 2 years were resampled, so that survival at 8 years can be compared with the total area colonized and the genetic identity of colonies on these stumps at 2 years (Table 6). *Heterobasidion annosum* died in the majority of stumps which had only small colonies initially, but they comprised equally stumps containing heterokaryons and those with homokaryons. Survival was greater among the few large colonies sampled, all of which were heterokaryons.

Overall, *H. annosum* survived in only 26.3 per cent of stumps in which it had been recorded 6 years previously (Table 7). Because of the greater incidence of natural infection on low rainfall sites and mineral soils, these site types contributed the majority of stumps examined to determine long-term survival. Results also varied greatly between sites (e.g. sites 15 and 43; Table 7), but it is nevertheless clear that survival at 8 years was highest on dry mineral soils. In infected stumps *H. annosum* usually extended into the proximal portion of at least one root, but the number of roots invaded was only poorly related to the area colonized in each stump; the location of colonies in relation to the points at which roots originated was equally important. Out of a total of 148 roots available for infection on these stumps it was present in the proximal portion of only 36 per cent. In three stumps *H. annosum* was detected in roots but was no longer viable in the stump body.

Figure 4 shows that total colony area at 2 years was generally a poor predictor of the extent of colonization 6 years later. Whilst the largest colonies tended to survive and extend, and the majority of small colonies died, there were many exceptions. Eighteen per cent of 94 stumps which had a total colony area <0.5 cm^2 at 2 years were colonized to a greater extent at 8 years. In one case such a small colony gave rise to the largest colonized area recorded (Figure 5). By contrast, in two stumps much larger colonies (3.3 and 8.3 cm^2) failed to survive.

As already established, initial colonization, at 2 years, was almost exclusively confined to the heartwood. However, in those stumps in which colonization later increased substantially, *H. annosum* extended into the sapwood (Figure 5).

On average, survival among inoculated stumps (30.9 per cent) was no greater than among those that were infected naturally, despite more extensive initial colonization (Tables 2, 8). Between-site differences were similarly large and, as in the naturally infected stumps, whereas most small colonies tended to die, some gave rise to much larger ones. In one stump a colony of 134 cm^2 was present at year 8 whereas no infection had been detected at year 2. Although this stump had not been recorded as infected at year 2, it was fortuitously included among the sample of infected stumps (at 2 years) that were later re-sampled. While the explanation for the record at 8 years must be speculative, it seems likely to represent expansion of a 'dot' colony that was too small or too weak to be detected earlier.

Survival was exceptionally high among inoculated stumps on sites 35 and 36 (Table 8). This

Table 6: Relationship between survival of *H. annosum* in stumps 8 years after natural infection by spores and the extent of colonization and the genetic identity of colonies 6 years earlier

	Number of stumps			
Area colonized at 2 years:	<1 cm^2		>1 cm^2	
Genetic identity of colonies at 2 years:	Heterokaryons*	Homokaryons	Heterokaryons*	Homokaryons
Survival at 8 years				
+	16	5	6	0
–	29	33	3	0

* Heterokaryons only or heterokaryons with homokaryons.

Table 7: Survival of *H. annosum* in Sitka spruce stumps 2 years and 8 years after exposure to natural infection

Site			Numbers			
			Stumps* with *H. annosum*		Roots on stumps with *H. annosum* at 8 years	
Rainfall	Soil	Identification number	At 2 years	At 8 years	Total	Infected
Low	Mineral	1†	–	–	–	–
		15	13	8	34	8
		29	9	2	8	2
		35	15	9	50	20
		43	16	1	4	1
	Peat	2	6	2	8	7
		16	2	1	4	3
		30	4	1	5	1
		36	10	2	13	2
		44	11	1	4	2
High	Mineral	19	3	0	0	0
		21	4	0	0	0
		31	4	1	7	4
	Peat	20	6	1	5	3
		22	2	1	6	1
		32	9	0	0	0
	Totals		114	30	148	54

* Out of 25.
† Plot felled.

Table 8: Survival of *H. annosum* in Sitka spruce stumps 2 years and 8 years after inoculation with basidiospores

Site			Number of stumps* with *H. annosum*	
Rainfall	Soil	Identification number	At 2 years	At 8 years
Low	Mineral	15	23	1
		35	23	14
		43	25	1
	Peat	16	19	3
		36	24	17
		44	25	7
	Totals		139	43

* Out of 25.

was reflected in the extent of colonization. The mean proportion of each stump occupied by *H. annosum* was 35.7 and 19.4 per cent on the two sites, respectively. However, as in the naturally infected stumps, individual values varied greatly: out of 31 infected stumps on the two sites, colonization exceeded 50 per cent in seven stumps whereas in 10 others it was <5 per cent.

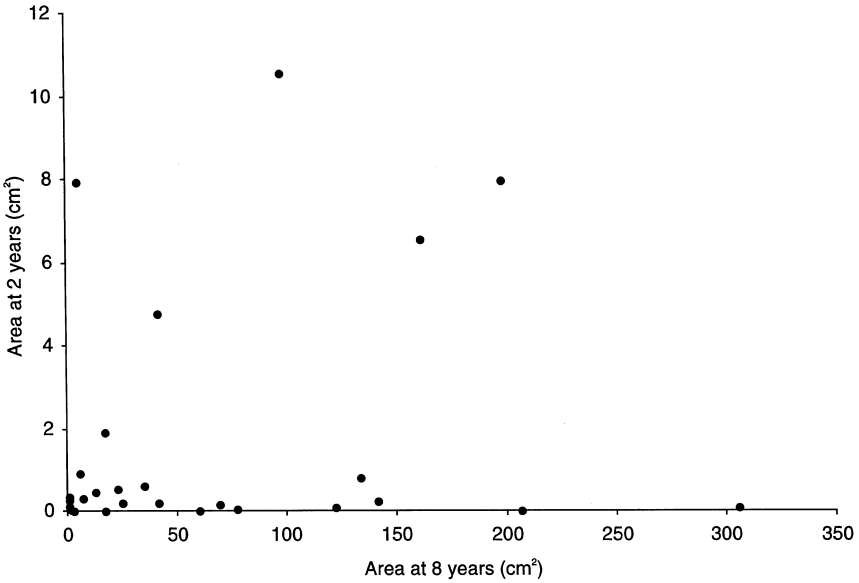

Figure 4. Relationship between the cross-sectional area occupied by *H. annosum* in naturally infected Sitka spruce stumps 2 years and 8 years after cutting. Data shown only for stumps with *H. annosum* at 8 years. Those stumps for which the area occupied by *H. annosum* is shown as zero at 2 years contained one or more colonies <1 mm^2 in area.

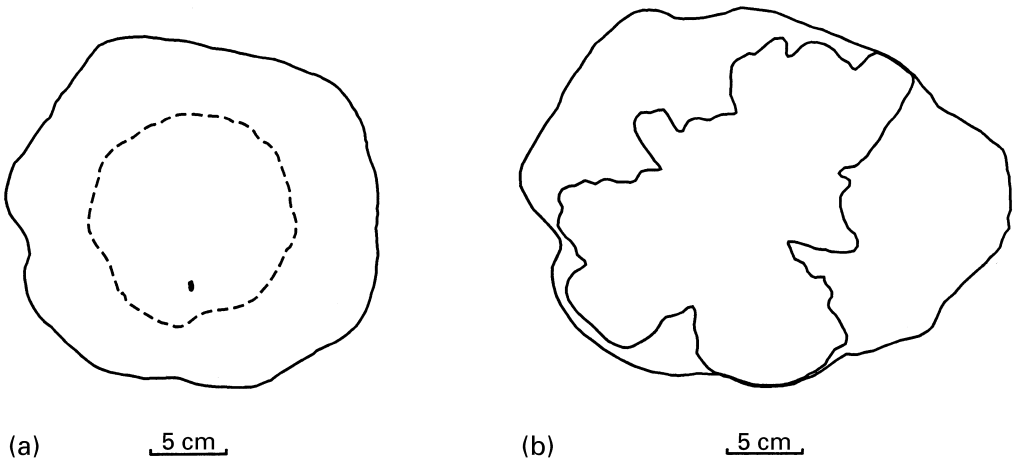

Figure 5. Extent of colonization in a Sitka spruce stump naturally infected by *H. annosum*, 2 years (a) and 8 years (b) after cutting. The 2-year-old colony was a heterokaryon.

Discussion

As in earlier work (Redfern, 1993), the amount of *H. annosum* in inoculated stumps after 2 years shows that in general there is potentially a much higher risk of stump infection than the natural levels presently recorded at most sites. Although ambient spore loads at the experiment sites were unknown, the numbers of spores used for inoculation probably far exceeded natural spore deposition at most sites unless basidiocarps were present in the immediate vicinity. Even so, the potential for infection revealed by inoculation seems likely to be realistic, since the three highest rates of natural infection recorded (52.0, 60.0 and 66.7 per cent) were well within the range obtained by inoculation. Even if stump protection is widely practised and effective, logs cut from healthy trees can become infected if left in the forest, allowing basidiocarps to form. The low spore loads previously recorded in western forests (Gladman and Low, 1963) can therefore be expected to rise as harvesting increases, and the risk of infection will increase in future beyond the overall average of 10.5 per cent recorded in this study.

Natural infection was apparently lower under conditions of high rainfall than on drier sites. However, this association may be confounded by the relationship between rainfall and geography, since rainfall is higher in the west than the east. It seems likely therefore that the effect is related more to low ambient spore loads in western forests than to high rainfall *per se*. The importance of geographical location can be appreciated more readily by noting that the five sites (15, 35, 36, 43 and 44) with the highest incidence of natural infection were all located in north-east Scotland (out of 12 sites in that region), whereas of the 17 sites in the experiment where no infection was recorded only one was located in the north-east. For stumps receiving high spore loads by artificial inoculation, there was no relationship between infection and rainfall.

These results confirm earlier reports, based on inoculation experiments, that infection of Sitka spruce stumps is extremely variable, both among stumps on the same site and among sites (Redfern, 1982, 1993). For stumps exposed to natural infection, comparison with the results obtained by inoculation on the same site shows that much of this variation is related to the availability of spore inoculum. No information was gathered about spore deposition rates at the experiment sites but basidiocarps were observed near some of them and it was notable that two of the three sites with the highest levels of natural infection had basidiocarps in the immediate vicinity. Although inoculation increased infection on all sites compared with natural levels, between-site variation was still high, suggesting that other factors were involved. Among inoculated stumps, infection was less frequent on peat soils than on mineral soils, and among naturally infected stumps the extent of colonization was also lower on peat soils. The effect of rainfall was not clear-cut, though a combination of poor drainage and high rainfall appeared to restrict infection and colonization. Otherwise little of the variation can be explained. There was in fact evidence of a more uniform response to inoculation among stumps on nearby sites that were paired to compare soils (Figure 1) than among replicate sites on the same soil. Thus, in Table 8, whereas infection on all six sites was initially similar, subsequent survival on each site was related much more closely to that of the other site in the pair (15 and 16, 35 and 36, 43 and 44) than to soil type. Observations in natural and semi-natural forests elsewhere have shown that other fungi, particularly *Armillaria* spp. and *Resinicium bicolor*, are effective competitors for *H. annosum* in spruce (Holdenrieder and Greig, 1998). In this experiment *R. bicolor* was most common on high rainfall sites but there was no obvious relationship between the incidence and extent of colonization by this fungus and the success of infection by *H. annosum*.

Wood moisture content is an important factor determining the success of stump infection in Sitka spruce. The corollary of earlier findings, that on wet sites heartwood provides a more favourable environment for infection than sapwood, is that under drier conditions sapwood infection should increase (Redfern, 1993). In a later, unrelated, experiment on a mineral soil in an exceptionally low rainfall area, infection occurred equally in both heartwood and sapwood (Redfern *et al*., 1997). Rishbeth (1970) also found infection in sapwood on this site type. Sapwood colonization has also been reported for Sitka spruce from southern France (Delatour *et*

al., 1998; Soutrenon *et al*., 1998). However, in the work reported here from 48 sites, despite a tendency for greater sapwood colonization on dry sites and for greater heartwood colonization on wet sites, the overwhelming majority of infection, whether of natural origin or resulting from inoculation, took place in the heartwood.

Other work has suggested that colonies established in the heartwood may not be capable of long-term survival: Morrison and Redfern (1994), using similar methods of sequential sampling, found that only stumps having infection initially in the sapwood contained the fungus after 8 years. However, in the work reported here *H. annosum* was originally located in the sapwood in only four of the 30 naturally infected stumps in which it survived for 8 years, and in none of the 43 corresponding inoculated stumps. Sapwood colonization appears not to be a prerequisite for long-term survival. The only major difference between the two pieces of work was that the earlier study was designed to compare the effect upon infection of covering stumps against rain. The results were not analysed separately for the two treatments because survival was much poorer in the uncovered stumps (9 per cent) than in those protected against rain (55 per cent). Our study of natural infection suggests that the earlier finding should be modified. Although the conditions associated with substantial initial colonization in sapwood may also be those that most favour long-term survival, survival is not precluded where colonization is initially restricted to heartwood, even when it comprises only a single, small colony.

Overall, *H. annosum* survived for 8 years in 26.3 per cent of naturally infected stumps and in 30.9 per cent of inoculated stumps in which it had been present 6 years earlier. This compares with a figure of 9 per cent given for the same time period, for inoculated stumps, by Morrison and Redfern (1994). However, the higher figures reported here were probably influenced by greater survival on dry sites, particularly on mineral soils; Morrison and Redfern's figure applied to soils in a high rainfall area. On comparable sites in this study survival was only 10.7 per cent (Table 7). It seems unlikely, in either case, that poor survival could be accounted for by removal of *H. annosum* in the first sample since other work has shown that growth in Sitka spruce stumps following spore inoculation can exceed 10 cm in 5 months (D.B. Redfern, unpublished). Similar declines have also been observed in several other North American conifers (Morrison and Johnson, 1978). However, it should be acknowledged that exposure of a fresh surface might itself prejudice survival.

The decline cannot be attributed simply to colony size or genetic composition. While it was largely accounted for by the extinction of small colonies, almost one third survived, some expanding to occupy almost the entire stump; conversely some large colonies died. Similarly, while homokaryons might seem less well equipped to survive than heterokaryons (Platt *et al*., 1965; Korhonen, 1981) the latter were equally represented among colonies that died.

Stenlid (1994) found that in 4-month-old Norway spruce stumps the proportion of homokaryons was inversely related to the number of colonies. In this work with Sitka spruce, the higher proportion of homokaryons in naturally infected stumps compared to those that were inoculated is consistent with a much lower spore load and fewer opportunities for heterokaryotization. However, 56 per cent of 340 colonies tested were homokaryons. This is even higher than the 'unexpectedly high proportion' of 38 per cent recorded by Stenlid, despite the apparently greater opportunity for heterokaryotization between nearby colonies in these older stumps. There was also no relationship with colony density, even though the range, which varied from 20 to 249 colonies m^{-2} among the 26 sites, was similar to that in the Norway spruce (J. Stenlid, personal communication, 1999). No information is available about the earliest stages of colonization in either species, but after about 12 months, and in contrast to Sitka spruce in northern Britain, *H. annosum* is present mainly in the sapwood of Norway spruce (Johansson and Brandtberg, 1994) and this may have an influence. Similarly the decline and fragmentation of colonies in Sitka spruce may also restrict heterokaryotization. It is also difficult to identify truly separate colonies if those that initially constituted a single physical and genetic entity become fragmented. Nevertheless general support for the relationship reported by Stenlid (1994) is provided by the rarity of homokaryons on inoculated stumps compared to those that were infected naturally.

Although the largest homokaryon was much smaller than the largest heterokaryon, this work shows that homokaryons can persist for at least 2 years. The data in Table 6 suggest that a few could have survived much longer, but since genetic information is lacking for the later sampling time they could have been heterokaryotized by individuals that were either not detected earlier or were not tested. Garbelotto *et al*. (1997) found that homokaryons could be isolated from dead and diseased trees in infection centres and showed that there was no difference in virulence on *Abies grandis* Lindl. between homokaryotic and heterokaryotic isolates. Thus, although the former may become heterokaryotized readily where ambient spore loads and stump infection rates are high, failure to do so may not of itself reduce disease development – other than in the long term by limiting basidiocarp formation.

Whilst the method of sequential sampling seems unlikely to have prejudiced long-term survival of *H. annosum*, it limits the conclusions that can be reached about colony development in stumps since the extent of colonization recorded at 8 years was determined for a level 5 cm deeper than that recorded at 2 years. Thus a failure to record the fungus at 8 years in stumps in which it was present 6 years previously may only mean that it failed to penetrate to the 8-year sampling depth. Similarly, stumps in which the fungus was recorded on both occasions also present difficulties of interpretation. An apparently large increase in the area occupied by *H. annosum* was recorded for some stumps between the two sampling times, in one case from a 'dot' colony to one of 306 cm^2. This could represent radial growth of colonies that were as small at the lower level at year 2 as the area actually recorded 5 cm above. On the other hand, it could equally well represent a decline at the upper sampling level that had taken place by year 2 in colonies that were originally cylindrical in shape and with the same diameter as that recorded at the lower level at year 8. Alternatively, there may have been no change, at either level, between the two sampling times. There is some evidence for decline in the upper portions of stumps (Redfern, 1982, 1993). In this study the additional samples taken during the 2-year assessment (disc 3) showed that whereas there was no difference overall in the incidence of *H. annosum* at the two levels, on some sites it was recorded less frequently at the upper level. The apparent fragmentation of some genets at the upper level (Figure 3c, d) also implies decline. On the other hand, there is also evidence that colonies can expand radially (Redfern *et al*., 1997). It therefore seems likely that the size, shape and number of colonies in a stump after 8 years is determined through a process involving both decline and expansion.

Despite the uncertainty about colony development, it is clear that whereas *H. annosum* failed to survive in more than two-thirds of naturally infected stumps, a few became colonized extensively (Figures 4, 5). There was a similar pattern among inoculated stumps, though the general level of colonization was greater. This supports an earlier suggestion that a small proportion of stumps may be particularly susceptible to colonization (Redfern *et al*., 1997). Alternatively only rare genets may be successful in Sitka spruce. Tests on isolates obtained from basidiocarps throughout Britain (J. Stenlid, personal communication, 1992, 1994; Korhonen *et al*., 1998) suggest that only the 'P' intersterility group (ISG) (*sensu* Korhonen 1978; Mitchelson and Korhonen, 1998) is present. There is some evidence that Sitka spruce stumps show selectivity to infection by particular ISGs (Delatour *et al*., 1998). The relative resistance of Sitka spruce stumps to infection compared to *Pinus* spp. (Phillips and Greig, 1970; Redfern, 1982), and the poor long-term survival of the fungus, might therefore reflect poor adaptation of the 'P' ISG to Sitka spruce, at least as a saprotroph. The evidence so far available from studies of the clonal composition of colonies in both basidiospore-inoculated and naturally infected stumps provides evidence for both possibilities. In well-colonized stumps large areas can be occupied by a single genet but on the other hand 10 genets or more may be present even in naturally infected stumps, with much larger numbers following inoculation (Morrison *et al*., 1994; Redfern *et al*., 1997; Table 5).

On those sites where naturally infected stumps were resampled to determine long-term survival, the proportion of infected stumps fell from 30.6 to 8.0 per cent. If applied to the experiment as a whole this implies a decline in the overall level of natural infection from a mean of 10.5 per cent to 2.8 per cent. In Sitka spruce plantations, trees adjacent to inoculated stumps can become

infected within 8 years of inoculation with spores (Morrison and Redfern, 1994) and within 5 years of inoculation with wooden dowels (Redfern, 1998). In this experiment, stumps in which *H. annosum* survived for 8 years would therefore have had the potential to cause infection. The risk depends on a number of factors: the extent to which the fungus invades and retains possession of the stump roots, the frequency of root contacts with surrounding trees and finally on the successful transfer of infection. *H. annosum* colonized only one-third of the available roots on those stumps in which it survived. However, this figure was based on samples obtained from the proximal portions of roots; other work has shown that recovery is poorer from the periphery of root systems (Redfern, 1984). Even where the fungus is present at a root contact, successful transfer is not assured: in one study only about 20 per cent of such contacts resulted in infection (Morrison and Redfern, 1994). In the work reported here, *H. annosum* was frequently confined to the central tissue and was surrounded by uncolonized wood, probably reflecting its preponderance in the heartwood higher in the stump. Under these circumstances spread could only occur following invasion of the outer uncolonized tissue or exposure of the inoculum contained in the central core of wood.

Since about 1960, the build-up of *H. annosum* in conifer stands has been prevented by treating freshly cut stumps with a chemical or biological control agent. Treatment was formerly recommended on all sites, but recently it has been shown that the risk of serious disease in pure Sitka spruce is much lower on peat soils than on mineral soils (Redfern *et al.* 1994; Redfern, 1998). In the disease model used to estimate losses, probabilities were assumed for the risk that stumps would become infected by spores and for the risk that *H. annosum* would become established and survive long enough to cause infection in adjacent trees, i.e. that stumps would be infective (Pratt *et al.*, 1989). Losses were calculated for spore infection risks of 5 and 50 per cent, and a single probability (0.40) was used for the likelihood that stumps would be infective. It was concluded that on strictly commercial grounds, the cost of protection could be justified only on mineral soils subject to a high risk of stump infection by spores (Redfern *et al.*, 1994). The work described in this paper shows that for much of upland Britain the lower probability for stump infection would be the most appropriate one to use in a model at the present time, but this is likely to increase in future. Also, in view of the substantial failure of *H. annosum* to exploit those stumps that became infected or even to survive in them, the probability assumed for stump infectivity may have been too high, particularly for crops in high rainfall areas. Loss predictions based on lower probabilities for these initial stages in the life cycle of *H. annosum* would therefore be lower than earlier estimates, and in turn this would decrease the economic benefit of stump protection.

These results refer only to thinning stumps. No information is available for Sitka spruce about the susceptibility of stumps created by clear-felling. Both the microclimate and possibly the number of spores available for infection may differ greatly between stumps in the open and those within a stand. In Sweden, Bendz-Hellgren (1998) found that Norway spruce stumps on a clear-fell were less susceptible than thinning stumps. Nevertheless, the results presented here reinforce the conclusions reached earlier that the risk of serious disease on peat soils in the uplands is low (Redfern *et al.*, 1994). Considering only the first rotation, they also suggest that the risk of successful colonization of Sitka spruce stumps is so low on wet sites, particularly on peaty soils, that the fungus may not become permanently established in stands on these site types, even if ambient spore loads increase. By contrast, on mineral soils in low rainfall areas, the risk of a build-up of inoculum in unprotected thinning stumps and the transfer of infection to residual trees in the stand is much higher.

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