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# INSTITUTE OF TERRESTRIAL ECOLOGY

ROOT AND MYCORRHIZAL DETERMINATIONS OF THE CERL LIPHOOK FIELD FUMIGATION EXPERIMENT

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INSTITUTE OF TERRESTRIAL ECOLOGY

(NATURAL ENVIRONMENT RESEARCH COUNCIL)

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Report to Central Electricity Generating Board

ROOT AND MYCORRHIZAL DETERMINATIONS OF THE CERL LIPHOOK FIELD FUMIGATION EXPERIMENT

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#### SUMMARY

This report gives baseline, pre-fumigation data on the root biomass and mycorrhizal populations of whole tree root systems excavated from the Liphook experiment in October 1985.

Root biomass showed considerable between plot variability, but this variability in Norway spruce and Scots pine fitted closely with that found in 1st year extension growth. The variability in Sitka spruce, however, deviated somewhat from the 1st year extension growth and even more from the initial height.

Mycorrhizal data for all 3 tree species show considerable within and between plot variability. It is suggested that the effects of the pollutant gases will probably only be able to be reliably assessed on the dominant mycorrhizal types. Full descriptions of the characteristics of the mycorrhizal types identified are provided, although detailed observations of embedded and sectioned material have yet to be carried out.

A further study is seen to be necessary to evaluate future sampling techniques. Due to the rates of growth of the trees it will no longer be possible to excavate whole root systems, but, as a result of site preparation, coring is severely hampered by the distribution of stones in the upper soil horizons. ١

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#### 1 INTRODUCTION

The aim of the field fumigation experiment at Liphook is to study the effects of  $SO_2$ ,  $O_3$  and a combination of these gases on the growth and physiology of a new stock of Sitka spruce, Norway spruce, and Scots pine. In addition, abiotic and biotic changes of soil will also be monitored in terms of chemistry, decomposition rates and arthropod populations under these treatments. Details of the main aims, site history, site peparation and preliminary background data are to be found in Shaw (1986).

The Merlewood involvement in this project is to examine the changes in mycorrhizal composition of the root system as a result of the pollutant gas treatments, and to supply some information on the rate of growth of the root system. This report provides the baseline, pre-fumigation data on root biomass and mycorrhizal associations of whole root systems excavated at the end of the 1st year after planting.

# 2 ROOT SAMPLING AND OBSERVATIONS

# 2.1 Root hervest

Whole tree root systems were extracted from ...the.2 halves of each of the treatment blocks on October 28-29 1985. Trees were taken from random co-ordinates on the planting grid pattern, with half the replicates for each treatment block taken from the southern half by one pair of workers, and the other 5 trees from the northern half of the block by 2 other workers. Root systems were removed by digging around the tree base with a fork and gently easing as much of the root system as possible from the soil. The areas disturbed were dependent upon the extent of lateral spread of the root systems and extended to approximated 0.5 m out from the tree base in the larger Sitka spruce trees.

Trees were separated on site into roots and shoots by severing the main axis at ground level. Roots and shoots were placed separately into pre-labelled self-seal polythene bags for transportation to the laboratory.

## 2.2 Root sample preparation

Root samples were stored in their polythene bags at 4°C whilst awaiting processing. The root washing and sampling process commenced immediately on return to the laboratory and was completed within 3 weeks.

Root systems were washed free of adhering soil particles using a 'washing machine' consisting of oscillating water jets washing the roots which are held over a series of mesh grids. Coarse particulate matter, stones and dead organic debris were removed from the samples by hand and loose root fragments recovered from the fine mesh screen.

Fresh root material was divided into coarse and fine root fractions, with the division occurring at approximately 2 mm root diameter. Coarse root material was placed in a pre-labelled paper bag and oven dried at  $80^{\circ}$ C to constant dry weight. The fine root fraction of each root was weighed fresh, and 5 random samples (totalling about 2 g of material) were removed and placed into a pre-labelled vial of 1% aqueous glutaraldehyde fixative; the remaining fine root fraction was reweighed, placed in a labelled paper bag and dried at  $80^{\circ}$ C to constant weight. Dry weights of coarse and fine root material were measured, and the latter corrected for the dry weight equivalent of the root samples taken for mycorrhizal observation.

# 2.3 Mycorrhizal observations

The samples of root fixed in glutaraldehyde were used to quantify the proportional contribution of different mycorrhizal types to the population on the individual tree root systems. A random sample of 5 root systems of each tree species was first observed, to characterize the main mycorrhizal types occurring on each tree species. These types were distinguished by morphological characteristics visible under a stereomicroscope at a magnification of 20-30 times. The mycorrhizas were photographed and a brief list of diagnostic features appended to the photograph to aid subsequent identification. These random samples were returned to their appropriate storage vials.

Each tree species was assessed for its mycorrhizal population one at a time, in order to facilitate characterization of the mycorrhizas. The root sample from each tree was spread out in a square petri dish which was marked, on its base, with a 1 cm grid (82 grid squares per dish). Fifteen arandom grid squares were assessed, by counting the number of mycorrhizas of each type in each square. Squares containing no fine root material were rejected and a new square selected. From these counts, the percentage contribution of each mycorrhizal type to the total population of mycorrhizas on the root system was calculated.

> During the counting of mycorrhizas, examples of all previously determined and new types of mycorrhiza were transferred to storage vials of glutaraldehyde as type specimens. The characteristics of these type specimens were futher investigated by preparing temporary root squashes and free-hand transverse sections for observation by high power microscopy. Root squashes were mounted in lactophenol/glycerol and sections stained in 1% trypan blue in lactophenol and mounted in lactopehnol/glycerol. These samples were observed for the following characteristics:

- (i) degree of sheath development
- (ii) nature of sheath development (degree of aggregation of hyphae into pseudoparenchymatous tissue)
- (iii) characteristic orientations of surface hyphae

(iv) nature of extramatrical hyphae (hyphal diameter, surface characteristics, presence, absence and nature of clamp connections)

- (v) extent of Hartig Net
- (vi) presence of other distinguishing chracters (eg sclerotia)

Extensive observations of embedded and microtome sectioned material has not yet been undertaken.

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#### 3 RESULTS AND OBSERVATIONS

#### 3.1 Root biomass

Fine, coarse and total root weights for individual trees are given in Appendix 1. Mean dry weights of 5 trees from each half (N or S) of each the plot are given in Table 1 and a summary of an analysis of variance on the data\_is\_given in Table 2.

These data show that the major difference in root weight is a factor of the tree species, where Sitka spruce is significantly larger (P <0.001) than either Norway spruce or Scots pine. The fine root component of Norway spruce was significantly greater (P <0.001) than that for Scots pine, but this was not so for the coarse root component. This difference in fine root biomass, however, is enough to cause a significant difference in the total root weights between these 2 species.

Significant differences in root biomass between plots were evident for the fine root component (P <0.001) and the total root biomass (P <0.05). The ranking of total root weight across plots, for each species can be seen in relation to other parameters (Shaw, 1986) in Table 3.

### 3.2 Mycorrhizas

The characteristics on which the mycorrhizal types were separated are listed in Appendix 2. Seven types were recognized in Sitka spruce, 8 in Norway spruce and 9 in Scots pine. The mean percentage contribution of each mycorrhizal type to the total population on the root system of each plant is given in Appendix 3 and a table of means for each plot for each tree species is given in Tables 4, 5 and 6. It must be noted here that the classification of the mycorrhizal types was carried out for each tree species separately and, thus, for example, mycorrhizal type A for Sitka spruce cannot be equated to mycorrhizal type A for either Norway spruce or Scots pine.

From Table 4 it can be seen that type A mycorrhiza is dominant on Sitka spruce with type C being sub-dominant. Mycorrhizal types F and G occur only spasmodically and contribute least to the total population. Variations between plots are shown mainly by a change in the proportions of types A and C. Plots 2, 3, 4 and 5, however, show an elevated contribution of type E to the population, this type is very low in number in the other plots. Plot 7 has an anomalously high proportion of type D mycorrhizas. The proportion of type B mycorrhizas is fairly constant across plots, with an elevated value for plot 4 and lower values for plots 6 and 7.

Table 5 shows the proportional contribution of mycorrhizal types to Norway spruce root systems. These roots are dominated by type A mycorrhizas, with types C and B as sub-dominants. Apart from type H mycorrhizas, all other mycorrhizal types are almost always represented in each plot. Plots 5, 6 and 7 show a higher contribution of type . A to the mycorrhizal flora than plots 1 to 4 and are balanced by elevated proportions of type C in plot 1, type B in plots 2 and 4 and type F in plot 3.

Table 6 shows the equivalent data for Scots pine in which 9 mycorrhizal types were identified. Here type C mycorrhizas dominate, with types B, G and A being represented, on average, at greater than 10% of the

population. Type C mycorrhizas make a of remarkably constant contribution to the population, except for plot 4 where the value rises to nearly 48%. This rise is reflected in a lowering of the contribution of types A, D, E and F to the population of trees of plot 4. A higher proportion of type A mycorrhizas occurs in plot 2 than in other plots and high proportions of type ...E in plots 3 and 5. ...High levels of mycorrhizal types G are found in plots 5 and 7 and low levels in plots 6 and 3. Mycorrhizal type I has the lowest frequency of occurrence, being found in only 3 root samples.

The raw mycorrhizal data given in Appendix 3 show considerable variability in the proportional contribution of mycorrhizal types on the root systems of all tree species. In many instances where the mean proportion of a mycorrhizal type was below 10%, only a few replicate plants from each plot supported that type of mycorrhiza.

#### 4 DISCUSSION

This report has collated the baseline data for the root biomass and mycorrhizal observations of the 3 tree species planted at Liphook for the funigation experiment. In terms of root biomass, significant between plot differences were observed. These data need to be looked at in conjunction with Dr Mueller's data on above-ground biomass of the same plants in order to assess the full effect of plot on the growth of the trees. From the mechanics of excavating the root systems, differences in number of stoness between plots were evident, with plot 7 being very much less stony than other plots. Due to the drastic disruption of the original soil profile, data on the comparative contribution of stones to the bulk density of the soil in each plot would be of great value. The degree of stoniness will strongly influence the root growth pattern of the trees.

The ranking of plot mean data for 1st year shoot extension and total root biomass show remarkable similarity for Norway spruce, and reasonable similarity for Scots pine and Sitka spruce. Although some differences were detected between plots at planting (due to variation in planting stock) (Shaw, 1986), a considerable change in the rankings of, particularly, Sitka spruce indicate that variation in site characteristics between plots is an important determinant of tree performance.

The mycorrhizal data show that numerous different mycorrhizal types can be identified by morphological characteristics on each of the tree species. The variability between trees within a plot and between plots is large, particularly for the less frequently occurring types. At this stage it is difficult to find clear patterns in the mycorrhizal populations and the data will need to be interrogated further in order to plan sampling strategies for future years.

The planning of sampling strategies for future phases in the programme is not only to be based on statistical considerations of variability within plots, but also on the ability to collect suitable samples. The sampling in 1985 was from whole root systems which were excavated by careful digging. Even after one season's growth, however, the degree of spread of the root mass was considerable. Lateral spread of roots was often 0.5 m or more from the stem base. On this basis, with trees initially planted on a 1 m grid spacing pattern, by the end of the second season's growth it will be much less practical to sample whole root systems, without incurring considerable site damage and influencing the growth of trees adjacent to the one being harvested. This problem had been partially foreseen and it had been the intention, in October 1985, to run a parallel series of harvesting techniques. This harvesting was to involve removing a series of soil cores from pre-determined distances from the stem base of a tree and to correlate the root biomass from the cores with the total root biomass of the tree. Due to the destruction of the iron pan and redistribution of Carr stones in the upper soil layers during site preparation, it was found impossible to extract 2.5 cm diameter cores from even the least stony plot. It is, therefore, planned to re-visit the site in May 1986 to assess the possibilities of alternative methods of sampling by using either larger diameter corers or a hand excavation system over a limited defined area. Initial trials would be conducted on the unplanted edge of the plot and actual determinations made on a number of edge trees which do not form part of the main experimental area.

In summary, at this stage of the programme it appears that there are considerable differences between plots in the growth and mycorrhizal

status of root systems of all 3 tree species. It is thought that the effect of the pollutant gas treatments on mycorrhizal communities of the roots will only be detectable for the dominant mycorrhizal types. Between and within plot variability will probably be too large to assess the seffects on infrequently occurring mycorrhizal types.

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# 5 ACKNOWLEDGEMENTS

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# REFERENCES

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SHAW, P.J.A. (1986). The Liphock forest fumigation experiment description, and project plan. CEGS Technology and Planning Research Division Report TPRD/L/2985/R86...

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Table 1. Mean root weight of fine, coarse and total root fractions of whole root systems harvested from Liphcok in October 1985. (Weights are dry weights expressed in g after drying at 30°C, means of 5 individual root systems).

	Sitka spruce		Norway spruce			Scots pine			
Plot	Fine	Coarse	Total	Fine	Coarse	Total	Fine	Coarse	Total
1 N	11.84	8.55	20.46	5.81	3.11	8.92	3,16	2.86	6 02
S	16.34	16.10	32.44	5.58	3.53	9.12	5.32	2.36	7.68
2 N	8.57	5.64	14.21	10.92	2.70	13.62	1.21	3 77	8 01
S	13.88	20.43	34.32	5.62	4.86	10.48	4.13	4.57	8.70
3 N	14.60	9.35	23.95	10.08	3.35	13.43	6.12	3.70	9,90
S	12.82	10.03	22.85	7.04	7.17	14.21	3.50	1.97	5.47
4 N	8.69	14.53	23.22	8,60	6.39	14.99	2.11	2.47	4.59
S	9.45	16.47	25.92	4.09	2.72	6.81	1.12	1.33	2.45
5 N	14.07	14.13	28.20	4.44	2.95	7.39	2.79	2.84	5.64
S	9.75	12.93	22.68	4.12	3.51	7.62	4.80	2.89	7.69
6 N	10.62	13.44	24.05	5.85	3.94	9.79	4.61	6.17	10,78
S	13.67	19.25	32.92	4.99	4.17	9.16	2.98	3.79	6.77
7 N	13.30	12.47	25.77	14.68	6.18	20.85	4.78	5.43	10,20
S	20,51	17.09	37.61	15.77	7.51	23.27	5.18	4.81	10.39

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Source of	, d.f	ទា	₽the		arse	Total	
variation		vr	 2	vr	Р	VT	P
Species	2	36.6	<0.001	57.4	<0.001	73.8	<0.001
Plots	6	3.8	<0.001	1.0		2.6	<0.05
Sub-plots	1	0.0		3.5		1.6	
Species.plots	12	0.9		0.6		0.7	
Species.sub-plots	2	1.9		4.0	<0.05	4.1	<0.05
Plot.sub-plots	6	0.8		1.0		1.0	
Species.plots.sub-plot	12	0.5		0.7		0.8	

Table 2. Summary table of analysis of variance of root biomass of whole root systems harvested from Liphook in October 1985.

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Table 3. Ranking of mean total root biomass at the end of the first growing season with initial tree height and first year shoot extension for each plot at Liphook.

Species	Parameter	Rank	order.	of plot order o	nuaber of plot	'in de: mean	scendi;	ng
Sitka spruce	Initial height	3	1	4	2	7	5	6
	Extension (yr1)	2	6	1	5	3	7	4
	Root biomass	7	6	1	5	4	2	3
Norway spruce	Initial height	3	7	4	6	1	2	5
	Extension (yr1)	7	3	4	2	1	6	5
	Root biomass	7	3	2	4	6	1	5
Scots pine	Initial height	7	6	5	2	1	4	3
	Extension (yr1)	7	6	4	3	1	2	5
	Root biomass	7	6	2	3	1	5	4

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4. Mean percentage contribution of each mycorrhizal type to the total population on roots of Sitka spruce harvested from Liphook in October 1985 (mean of 10 root samples).

			M	ycorrhizal :	type	· · · ·	
Plot	A	З	С	D	Ξ	F	G
1	71.7	7.9	13.5	6.9	0	0	0
2	47.0	7.3	27.3	3.3	15.2	0	0
3	27.2	8.4	39.4	11.6	13.5	0	0
4	59.7	13.9	10.5	0	15.9	0	0
5	46.7	6.6	19.3	1.4	15.9	10.2	0
6	77.2	4.4	9.6	0	0.9	2.3	5.6
7	54.8	3.6	10.5	20.9	3.0	5.7	0
Overall mean	54.9	7.4	18.6	6.3	9.2	2.6	0.8

Table 5	5.	Mean percentage contribution of each Eycorrhizal type to the total
		population on roots of Norway spruce from Liphook in October 1985 (mean of
		10 1000 Sampres/.

				Mycorrhi	zal type			
Flot	A	в	С	D	Ξ	F	G	Ξ
1	49.0	9.0	30.5	6.0	3.4	0	2.1	0
2	46.7	27.2	9•5	5.2	0	0	8.1	3.3
3	43.1	8.5	18.6	3.8	1.0	23.7	1.3	0
4	40.9	23.5	12.5	9.8	2.6	6.7	4.1	0
5	58.6	5.6	18.8	6.6	1.7	0.6	8.1	0
6	59.8	2.1	16.7	4.9	0	6.8	1.4	8.3
7	74.9	1.5	2.0	0.7	0.2	14.3	4.3	2.2
Overall mean	53.3	11.1	15.5	5.3	1.3	7.4	4.2	2.0

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Table	6.	Mean popul: 10 roc	percent ation on ot sampl	age cont roots di les).	tributio E Scots	n of e pine fro	ach myc m Liphoo	orrhizal k in Oct	type to ober 198	the tota 5 (mean o	1 f
		Mycorrhizal type									
Plot		A	В	С	D	Ε	Ŀ	G	Ħ	I	
1		5.5	24.1	26.1	8.8	9.4	4.0	19.8	3.6	0	
2		33.1	7.0	23.2	0	6.8	13.3	6.8	7.9	0	
3		12.1	30.8	29.2	5.6	4.2	9.2	3.7	5.5	0	
4		6.6	14.6	47.6	. 0	2.3	2.9	14.6	10.6	0.8	
5		8.3	27.7	21.5	2.0	3.1	0	34.9	2.4	0	
6		11.3	17.6	26.8	8.0	15.6	7.6	0.4	7.5	5.5	
7		13.8	5.9	23.0	5.1	10.4	9.9	25.5	4.0	2.6	
Overal] mean	1	13.0	18.2	28.2	4.2	7.4	6.7	15.1	5.9	1.3	

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APPENDIX 1. Root biomass data for individual trees harvested from Liphook in October 1985.

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LIPHOOK 1985 ROOT BIOMASS DATA (SITKA SPRUCE)

BLOCK	S.BLOCK	CO-ORDINATE	LARGE ROOT DRY WT (g)	FINE ROOT DRY WT (g)	TOTAL ROOT DRY WT (g)
1 1 1 1 1 1 1 1 1 1 1	l l l 2 2 2 2 2 2	11 6 12 7 10 9 13 3 15 2 18 18 21 20 21 26 19 28 20 28	7.92 5.48 13.50 7.53 8.32 13.78 37.44 18.28 1.97 9.01	5.46 5.16 6.79 14.00 27.78 11.68 27.15 17.54 10.18 15.16	12.38 11.00 20.29 21.53 36.10 25.46 64.59 35.82 12.15 24.17
2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	1 1 1 2 2 2 2 2 2 2 2	13 4 13 10 12 11 10 4 15 2 • 25 23 20 21 19 24 19 28 20 28	8.80 3.50 7.32 5.39 3.17 34.59 45.13 7.23 6.79 8.43	7.57 4.43 8.09 12.56 10.20 23.69 16.38 7.28 8.91 13.15	16.37 7.93 15.41 17.95 13.37 58.28 61.51 14.51 15.70 21.58
3 3 3 3 3 3 3 3 3 3 3 3 3 3 3	1 1 1 1 2 2 2 2 2 2 2 2	13 6 14 5 14 11 14 3 11 3 21 26 17 26 20 25 19 28 20 28	13.56 6.17 18.78 5.43 2.80 12.48 12.29 8.80 8.59 8.00	19.14 11.92 20.21 14.35 7.39 14.28 8.37 16.40 10.83 14.23	32.70 18.09 38.99 19.78 10.19 26.76 20.66 25.20 19.42 22.23
4 4 4 4 4 4 4 4 4 4 4 4	1 1 1 2 2 2 2 2 2 2	9 8 8 9 7 10 15 3 10 4 17 26 19 22 19 26 18 27 20 27	13.23 25.24 13.65 4.74 15.80 6.29 15.45 42.44 8.45 9.71	3.95 8.14 6.18 9.44 15.75 10.44 15.02 7.32 6.10 8.37	17.18 33.38 19.83 14.18 31.55 16.73 30.47 49.76 14.55 18.08
5 5 5 5 5 5 5 5 5 5 5 5 5 5	1 1 1 1 2 2 2 2 2 2 2	7 8 12 7 14 8 10 4 7 5 24 23 22 25 18 21 23 26 20 26	13.94 36.02 8.22 9.59 2.88 5.92 11.19 21.25 6.31 20.00	16.87 16.98 15.34 13.18 7.96 23.75 3.27 8.42 5.27 8.02	30.81 53.00 23.56 22.77 10.84 29.67 14.46 29.67 11.58 28.02

I	1	78	10.87	7.16	18.03
)	1	13 9	15.34	11.53	27.17
)	1	96	24.47	13.50	39,93
)	i	11 3	5.65	9.28	15.93
1	1	14 3	9.85	9.32	19.17
1	2	21 22	24,45	10.92	35 37
I	2	19 18	21.48	22,49	43.07
1	2	18/21	27.99	Q_Q1	37 90
1	2	15 28	3.95	8 42	12 37
•	2	21 27	18.27	16.63	35.00
			10.27	10.05	55.00
	1	14 7	22.01	7,52	29 53
	1	14 13	18.04	13.74	31.78
	1	88	4.97	5,94	10 91
	1	15 2	12.47	12.20	24 67
	1	11 3	4.88	27.10	31 98
	2	23 24	6,90	7,99	14 89
	2	18 26	15.97	9 32	25 32
	2	21 24	34.57	7 38	61 95
	2	17 28	11,95	11 01	22.96
	2	20 27	16.06	66.87	82.93

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ELOCK	S.BLOCK	CO-ORDINATE	LARGE ROOT DRY WT (g)	FINE ROOT DRY WT (g)	TOTAL ROOT DRY WT (g)
1 1 1 1 1 1 1 1 1	1 1 1 2 2 2 2 2 2	7 24 6 24 11 26 9 27 13 28 23 10 18 7 24 7 26 7 27 8	1.09 2.73 7.00 2.50 2.21 2.73 0.73 6.27 2.72 5.21	5.03 3.93 6.83 4.75 8.52 1.56 3.06 4.25 5.88 13.17	$\begin{array}{c} 6.12 \\ 6.66 \\ 13.82 \\ 7.23 \\ 10.73 \\ 4.29 \\ 3.79 \\ 10.52 \\ 8.60 \\ 18.38 \end{array}$
2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	1 1 1 2 2 2 2 2 2 2	$ \begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	3.13 1.30 2.64 3.91 2.53 1.08 4.04 5.98 5.67 7.54	4.39 6.75 8.91 27.22 7.33 1.18 10.74 5.37 4.95 5.86	7.52 8.05 11.55 31.13 9.86 2.26 14.78 11.35 10.62 13.40
3 3 3 3 3 3 3 3 3 3 3 3 3 3	1 1 1 2 2 2 2 2 2	10       25         9       24         14       27         11       28         15       29         21       6         20       5         24       7         27       8         26       7	2.44 5.56 2.36 2.07 4.32 15.26 6.08 2.81 5.99 5.72	9.09 7.53 6.92 10.31 16.53 8.45 7.78 5.05 10.48 3.43	11.53 13.09 9.28 12.38 20.85 23.71 13.86 7.86 16.47 9.15
4 4 4 4 4 4 4 4 4	1 1 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	14 27 8 23 12 23 11 28 14 28 18 9 19 6 24 9 24 3 24 5	15.12 2.21 3.20 3.03 8.38 3.73 1.50 3.46 2.04 2.88	5.64 4.87 5.93 7.66 18.92 3.24 3.68 6.17 3.36 3.98	20.76 7.08 9.13 10.69 27.30 6.97 5.18 9.63 5.40 6.86
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LIPHOOK 1985 ROOT BIOMASS DATA (SCOTS PINE)

BLOCK	S.BLOCK	CO-ORDINATE	LARGE ROOT DRY WT (g)	FINE RCOT DRY WT (g)	TOTAL ROOT DRY WT (g)
1 1 1 1 1 1 1 1	1 1 1 2 2 2 2 2 2	5 16 6 13 12 15 3 19 3 21 19 16 23 14 23 16 29 15 28 14	4.45 2.32 2.74 2.52 2.25 1.21 1.95 1.62 2.90 4.12	2.41 1.92 4.55 2.33 4.59 2.52 3.30 3.02 6.21 11.54	6.56 4.24 7.29 4.85 6.84 3.73 5.25 4.64 9.11 15.66
2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	1 1 1 2 2 2 2 2 2 2	7 14 7 18 11 16 3 11 2 15 28 12 25 16 28 18 28 19 29 16	3.26 0.87 6.21 5.14 3.35 2.44 6.68 10.39 2.53 0.83	4.57 0.79 5.71 3.43 6.72 3.74 4.69 7.82 2.51 1.89	7.83 1.66 11.92 8.57 10.07 6.18 11.37 18.21 5.04 2.72
3 3 3 3 3 3 3 3 3 3 3 3 3 3	1 1 1 1 2 2 2 2 2 2 2 2	6 15 4 19 9 18 3 10 2 15 21 16 27 20 19 16 29 15 28 14	9.41 0.70 3.65 3.65 1.07 1.91 1.14 1.64 2.90 2.26	17.39 0.81 5.82 4.44 2.57 3.60 2.61 2.36 6.05 2.89	26.80 1.51 9.47 8.09 3.64 5.51 3.75 4.00 8.95 5.15
4 4 4 4 4 4 4 4 4 4 4 4	1 1 1 2 2 2 2 2 2 2 2	7 12 4 21 10 15 3 12 2 16 26 15 28 13 25 20 28 18 28 17	5.04 2.15 0.77 1.87 2.54 0.78 1.55 1.17 1.34 1.83	3.21 0.44 2.48 1.69 2.74 0.80 0.46 1.11 0.78 2.44	8.25 2.59 3.25 3.56 5.28 1.58 2.01 2.28 2.12 4.27
5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	1 1 1 1 2 2 2 2 2 2	11 14 5 16 5 12 3 12 3 19 21 16 25 14 25 12 28 12 28 14	5.71 1.55 0.64 4.01 2.31 1.99 0.52 3.31 0.73 7.92	2.99 3.04 1.08 3.09 3.76 3.59 2.50 3.81 1.89 12.19	8.70 4.59 1.72 7.10 6.07 5.58 3.02 7.12 2.62 20.11

6 6 6 6 6 6 6	i 1 1 1 2 2	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	5.12 1.21 12.83 8.22 3.47 1.51 4.86	4.35 2.70 3.37 7.34 5.30 1.08 4.96	9.47 3.91 16.20 13.56 5.77 2.59 9.82
5	2	25 14	9.17	4.51	12.68
5	2	28 15	1.85	2.53	4.38
6	2	29 16	2.56	1.81	4.37
7	1	6 15	7.06	7.71	14.77
7	1	6 13	3.94	5.82	9.76
7	1	9-16	1.68	3.49	5.17
7	1	3 10	2.31	2.92	5.23
7	1	3 21	12.15	3.94	16.09
7	2	20 17	2.59	5.65	8.24
7	2	24 15	9.06	8.19	17.25
7	2	19-16	3.44	4.09	7.53
7	2	27 21	1.04	2.99	4.03
7	2	27 22	7.93	4.97	12.90

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APPENDIX 2. Characterization of the mycorrhizal types found on Sitka spruce, Norway spruce and Scots pine from whole root systems harvested from Liphook in October 1985.

#### 1 SITKA SPRUCE

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- TYPE A Slightly swollen short lateral roots of reddish brown colouration and a grained surface texture with some emergent hyphae. In squash and sectional material there was little evidence of organized sheath structure.
- TYPE B <u>Cenococcum</u> slightly thickened black mycorrhiza with distinct black emergent hyphae. Sheath 150um thick, of organized hyphae tending towards and pseudoparenchymatous structure.
  - TYPE C Elongate swollen laterals which were somewhat flattened, having a buff brown to grey colour and non-reflective, felt-like surface appearance. A loose weft of surface hyphae was present giving a silver appearance in patches and aggregating into strands. The emergent hyphae have clamps. The sheath was thick (300-400um) and consisted of highly structured pseudoparenchyma of irregularly shaped hyphal cells. The Hartig Net was extensive.
  - TYPE D Elongate laterals with distal swelling, never extending back to subtending axis. Surface was very smooth with no visible emergent hyphae under the stereomicroscope. Colour was pale creamy-yellow with a dull surface texture. The sheath (200-300um thick) was a highly organized pseudoparenchymatous arrangement of cuboidal and slightly tessellated hyphal cells. Fine reticulate emergent hyphae were present and these had clamp connections. The Hartig Net was extensive but difficult to see.
  - TYPE E Slightly swollen elongate laterals covered in a mass of hyaline surface hyphae giving a silvery appearance over orange-brown sheath surface. This extensive extramatrical hyphal outgrowth extended proximally over the subtending root axis and readily aggregated to produce stands in which numerous sclerotia were observed. The emergent hyphae were reticulate and had clamp connections. The sheath was of loose structure (350-400um thick), of fairly undifferentiated hyphal cells.
  - TYPE F Very swollen short-intermediate length short roots with a rufous brown colour and smooth (slightly reflective) surface. Sheath was very thick (300-400um) of very small rounded pseudoparenchymatous cells tending to run around the root. Numerous fine emergent hyphae, having clamp connections, were evident in root squash preparations.
  - TYPE G Elongate, slightly thickened grey-brown coloured mycorrhizas (some appeared dead). Sheath thick (300-350um), pseudoparenchymatous with a looser outer layer consisting of tessellated, cuboidal cells and less differentiated elongate

hyphal cells. Some emergent hyphae were present which have clamp connections.

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#### 2 NORWAY SPRUCE

TYPE A Unthickened terminal short roots. Root branching was pinnate and there was little colour differentiation between main root axis and lateral short roots.

Little to no indication of hyphae on root surface, and no formation of sheath structure when prepared as a squash or section.

TYPE B Short roots elongate and distinctly swollen to 1.5-2 times the diameter of non-mycorrhizal roots. Swelling was principally distal. Colour was pale orange to buff, becoming pinkish under intense illumination. Surface was smooth with fine wefts of emergent hyphae evident. Some of the surface hyphae had a hyaline appearance.

In a squash and section the sheath was shown to be loosely pseudoparenchymatous 150-200um thick with emergent hyphae having obvious clamp connections. Sheath cells only partly differentiated - Hartig Net extensive and obvious.

- TYPE C Very similar in appearance to TYPE B but colour tending towards buff-brown and having a very smooth and more reflective sheath surface than B. Surface hyphae were somewhat hyaline and produced a few emergent hyphae which bear clamp connections; these were short and terminal, with no extensive ramification from root. Sheath was of similar thickness to B, being 250-200um thick and appeared to be constructed of hyphae running around the root rather than a true pseudoparenchymatous structure. A more organized sheath than B with an extensive Hartig Net.
- TYPE D <u>Cenococcum</u> spp. Intense black sheath with thick emergent black hyphae.
- TYPE E Short roots distally swollen with a buff-orange brown coloured sheath. Emergent hyphae fairly prominent, tending occasionally to produce strands. Often secondarily colonized by <u>Cenococcum</u>. Sheath ill structured, consisting of large cells and reticulate hyphae with sparse clamp connections.
- TYPE F Dense pinnate branching with elongate and thickened short roots. Extensive cover of buff brown surface hyphae which extended proximally along main axis of root. Here, hyphae were hyaline and loosely packed and readily develop into strands. Clamp connections present. Sheath, in section, was a very loose surface arrangement of hyphae, becoming more organized beneath, fairly thick (200-300um) of somewhat undifferentiated cells (not pseudoparenchymatous). Extensive Hartig Net.
- TYPE G Similar in appearance to TYPE F but appeared to be less well developed. Surface hyphae buff-brown with a covering of very white hyphae which age to buff. Some development of strands was evident. The emergent hyphae were reticulate and had clamp connections.

TYPE H

Pinnately branched, very greatly thickened and more elongate than B or C but with similar characteristics. Sheath colour buff-orange with a distinct losse weft of outer hyphae being smooth with clamp connections. Surface had an almost gelatinous appearance. Sheath was uniform and thin (ca 125-150um thick) becoming pseudoparenchymatous, but somewhat undifferentiated and fairly disorganized, with extensive extramatrical hyphal extension. Extensive Hartig Net.

# 3 SCOTS PINE

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TYPE A Dishotomous to slightly more than dichotomous branching of short root tips. Distally, slightly enlarged with a crean to rufcus brown colour. Surface smooth with few emergent hyphae. As a squash preparation most of these roots showed presence of we root hairs and no fungal association, whereas others showed a thin sheath of disorganized surface hyphae with obvious clamp connections. Hyphal extensions from surface appeared to be terminal rather than extensive (see Norway Spruce type C).

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- TYPE B Loosely to tightly coralloid mycorrhiza with short terminal root segments. A distinctly swollen, pale cream-coloured sheath was present, having a smooth surface with fine emergent hyphae, giving it a slightly woolly appearance at higher magnification. The sheath was pseudoparenchymatous and 200-300um thick, with an outer layer of surface hyphae tending to form loose aggregations around the root. The emergent hyphae were reticulate in appearance and had clamp connections.
- TYPE C Dichotomous to loosely coralloid rufous brown or paler form. Thickening of the short root was slight with a somewhat 'beaded' appearance. There was little evidence of a fungal sheath. As a root squash many roots showed little to no fungal material and a number of root hairs. Where fungus was present a thin sheath (100-150um) had formed, with regularly arranged, though poorly differentiated hyphal cells wrapping around the root. Emergent hyphae were present and were reticulate with clamps.
- TYPE D Elongately coralloid orange-orange/brown coloured mycorrhizas with a smooth surface which sometimes appears scaly. Emergent hyphae scarce. The thin sheath (200-250um) was a well developed pseudoparenchymatous structure with an outer layer of more diffuse cells. Very fine emergent hyphae were present which bore clamps. The Hartig Net was distinctly visible.
- TYPE E A dichotomous (rarely more) branching mycorrhiza with pale orange-buff colour. The sheath gave a distinct thickening of the root in the distal portion only. The sheath surface appeared smooth but with short emergent hyphae visible. The sheath was thin (200um) and loose textured.
- TYPE F Elongately corolloid and swollen, more or less evenly, along length. Dense covering of loose sheath hyphae of orange-brown colour with a pink-grey surface mat of hyaline hyphae. These emergent hyphae coalesced to form strands and were present not only on short roots, but extend back to proximal root axes. These emergent hyphae bore clamps and were reticulate. The sheath was very thick (300-350um) with a very diffuse and loose structure of relatively undifferentiated hyphal cells.
- TYPE G A closely dichotomous to coralloid mycorrhizal form with short, terminal root swellings. Sheath surface was pale buff - hyaline with a woolly appearance given to it by a mass of extramatrical hyphae which aggregate into strands (secondary invasion by <u>Cenococcum</u> was evident on a number of samples).

The sheath was extremely thick (450-650um) of well organized pseudoparenchymatous tissue.

- TYPE H <u>Cenococcum</u> spp. Distinctly unbranched to dichotomous black mycorrhizas with a sheath and emergent thick-walled black hyphae.
- TYPE I A type very-similar-to D, of loosely coralloid or dichotomous mycorrhizas which were distinctly swollen, brown, and with a rufous tint. The surface was smooth and shiny. The thin sheath (75-200um) was loose in construction and of relatively undifferentiated hyphae.

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APPENDIX 3. Percentage contribution of each mycorrhizal type to the population on roots of individual trees harvested from Liphcok in October 1985.

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L.I.PHOOK 1985

STTKA SPRUCE

% MYCORRHIZAL TYPES

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31.OCK	BI.OCK	CO-08	SHLVNICE	Υ,	13	C	Q	ப	Ċ	C
	-	Ξ	9	76.8	8.1	0.0	15.2	0.0	0.0	0.0
		12	7	26.9	0.0	59.6	13.5	0.0	0.0	0.0
	_	01	6	83.3	16.7	0.0	0.0	0.0	0.0	0.0
		<u>[]</u>	ň	63.8	6.4	29.8	0.0	0.0	0.0	0.0
	-	15	2	61.9	8.3	28.6	. 1.2	0.0	0.0	0.0
_1	2	18	18	87.1	12.9	0.0	0.0	0.0	0.0	0.0
	2	21	20	80.0	8.9	11.1	0.0	0.0	0.0	0.0
	~:	17	26	85.3	8.8	5.9	0.0	0.0	0.0	0.0
	.7	61	28	97.6	2.4	0.0	0.0	0.0	0.0	0.0
	.2	20	28	54.4	6.7	0.0	38.9	0.0	0.0	0.0
<u> </u>	-	E	4	9.40	14.6	20.8	0.0	.0.0	0.0	0.0
<u> </u>	,	13	10	32.5	7.5	60.0	0.0	0.0	0.0	0.0
~.		12	1	7.2	9.3	83.5	0.0	0.0	0.0	0.0
0.1		Ξ	4	39.2	15.2	45.6	0.0	0.0	0.0	0.0
<u> </u>	_	5	.~_	29.4	3.8	6.9	0.0	60.09	0.0	0.0
<b>~</b> .	2	5	23	6.6	1.8	0.0	0.0	91.6	0.0	0.0
<u> </u>	7	20	21	76.0	9.4	14.6	0.0	0.0	0.0	0.0
<b>~</b> ·	2	61	24	67.0	10.7	22.3	0.0	0.0	0.0	0.0
<b>-</b> ·	?	6	28	59.6	0.1	19.2	20.2	0.0	.0*0	0.0
•	4	07	28	87.5	0.0	0.0	12.5	0.0	0.0	0.0
-	-	13	6	0.0	2.9	44.6	0.0	52.6	0.0	0.0
~	-	14	ſ	21.2	10.1	42.4	0.0	26.3	0.0	0.0
~	-	14	=	0.0	7.6	36.6	0.0	55.7	0.0	0.0
~	-	14	e	45.0	0.0	55.0	0.0	0.0	0.0	0.0
~	<u> </u>	_	n -	34.2	9.2	56.6	0.0	0.0	0.0	0.0
~	2	<u>-</u>	26	34.8	14.6	6.7	43.8	0.0	0.0	0.0
~	~	17	26	30.3	15.8	53.9	0.0	0.0	0.0	0.0
	.∼1	50		60.6	23.9	15.5	0.0	0.0	0.0	0.0
_	C1 (	<u>6</u>	20	21.1	0.0	31.6	47.4	· 0•0	0.0	0.0
~	2	02	8.1	24.6	0.0	50.7	24.6	0.0	0.0	0.0

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IPHOOK 1985

NORWAY SPRUCE

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0.0 36.3	0.0		87.5	19.7	18.4	72.8	0.0	0.0	0.0	18.2	5.2	0.0	0.0	0.0	0.0	0.0	32.7	ר נ	- c - c	7.2	0.0	0.0	0.0	12.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.1	0.0	0.0	0.0	0.0	8.5
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J. PHOOK 1985

SCOTS PINE

TYPES

MYCORRHIZAL

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10.1	0.0	0.0	0.0	0.0	9.5	0.0	3.6	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0	23.0	8.3	0.0		24.4	8.1	71.0	0.0	32.3	0.0	1.4	18.6	0.0	0.0	4.7	0.0	0.0	23.1	7.2	23.9	0.0	11.0	0.0	33.8
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	•	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	19.5	0.0		0.0	0.0	0.0	0.0	0.0	0.0	13.8	44.2	21.7	0.0	7.0	0.0	19.4	0.0	24.3	0.0	0.0	0.0	0.0	0.0
61.0	1.7.1	42.4	11.3	95.6	13.7	(5.8	45.5	17.5	46.3		13.9	15.8	4.0	35.9	25.0	73.3	0.0	6.0	39.8	1.4	•	46.8	0.0	17.0	64.8	12.3	14.0	26.2	0.0	51.7	35.2	0.0	16.0	55.0	47.4	2.7	23.9	26.9	0.0	28.6	29.9
0.0	0.0	0.0	0.0	0.0	63.2	0.0	0.0	73.0	10.2		67.4	0.0	0.0	0.0	43.2	0.0	82.3	25,0	27.8	31.7	•	22.4	0.0	0.0	26.8	0.0	18.6	58.6	0.0	0.0	49.5	6.3	0.0	0.0	26.9	0.0	25.4	0.0	0.0	0.0	0.0
0.0	22.9	1.7	0.0	0.0	13.7	0.0	27.3	0.0	0.0		18.6	0.0	0.0	12.8	0.0	26.7	7.4	4.9	1.9	10.6		0.0	18.9	0.0	7.0	29.2	0.0	0.0	37.2	16.7	4.4	18.9	0.0	0.0	0.0	0.0	13.4	2.5	16.0	51.2	36.4
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