A QUANTITATIVE APPROACH TO NITROGEN FIXATION AND THE DECAY OF TIMBER IN SOIL CONTACT

A thesis submitted in part fulfillment of the requirement for the degree of Doctor of Philosophy of the University of London and for the Diploma of Imperial College

by

Edmund Francis Baines

Department of Pure and Applied Biology
Imperial College of Science and Technology
London SW7 2BB

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Abstract

The occurrence of nitrogen fixing bacteria in decaying Scots pine, beech and Douglas fir exposed to soil was investigated using acetylene reduction and ^{15}Nitrogen incorporation to assay nitrogen fixation, and was found to require a high soil and wood moisture content. A system was developed to expose wood blocks, 50 x 25 x 15 mm, of beech, birch, spruce and Scots pine sapwood and heartwood to controlled soil moisture contents. Samples were taken at two week intervals up to 28 weeks from zones above, at and below the ground line and the acetylene reduction rate, weight loss due to decay and wood moisture content determined. Samples were also taken monthly from stakes of Scots pine and birch, untreated and treated with a copper-chrome-arsenic timber preservative, after field exposure. It was concluded that nitrogen fixing bacteria were not significant in providing nitrogen for the fungal decay of timber in ground contact. Wood moisture content was found to be important in the occurrence of nitrogen fixing bacteria and the uptake of water by wood in soil contact was found to have three phases. Computer analysis of the wood moisture content results using regression and curve fitting , and the exposure of wood to an artificial soil of known moisture retaining properties, revealed a relationship between soil water potential and wood moisture content. A dynamic equilibrium between wood moisture content and the moisture content of the environment surrounding the wood was discovered, together with 'wick action', where water moved through wood in soil contact from below to above ground, where the water evaporated. The significance of these results to the decay of timber in soil contact are discussed. Nine computer programmes used in the quantitative analysis of the results are included in an appendix.

Contents

ILLUSTRATIONS

ABBREVIATIONS

α	proportion of ^{15}N atoms
α_1	angle of contact
Å	angstrom = 10^{-1}nm
atm	atmosphere
bar	bar = 100 kN m^{-2}(100kPa)
B.S.	British Standard
^oC	centigrade = Celsius
CCA	copper-chrome-arsenic timber preservative
cm	centimetre
cm Hg	cm of mercury
cm water	cm of water
cm^3	cubic cm
C:N	carbon to nitrogen ratio
d	day
dyn	dyne = 10 µN
f.s.p.	fibre saturation point
G	guage
g	gram
g_1	gravitational constant
h	hour
h_1	capillary rise
i.d.	inside diameter
J	joule
^oK	degree Kelvin (absolute)
kg	kilogram
L (l)	litre
ln	natural logarithm
$\log_e$	logarithm to base e
$\log_{10}$	logarithm to base 10
M	mole
M_1 (m)	molecular weight of water
m.w.	molecular weight
µg	microgram (10^{-6}gm)
µm	micrometre (10^{-6}metre)
min	minute
mL (ml)	millilitre
mm	millimetre
mm Hg	mm of mercury = 133 Nm^{-2} = 133 Pa = 1 Torr
mM	milliMole
nM	nanoMole (10^{-9}Mole)
N	Normal
o.d.	outside diameter
p	vapour pressure of water
p_o	vapour pressure of a reference pool of water at T^oK
p/p_o	water activity = R.H./100
P.A.R.	planed all round
pF	$\log_{10}$ free energy of water pFx = 10^x cm water
pH	negative log of hydrogen ion concentration
ppm	parts per million
Q_{10}	change per 10^oC rise
ρ	density of water at T^oK
r	radius of a tube
R	gas constant
R.H. (r.h.)	relative humidity
S.D.	standard deviation
σ	surface tension of a liquid
T	temperature in ^oK
T_1	water tension in a capillary tube
vpm	parts per million for a gas
v/v	volume per volume
w/v	weight per volume
w/w	weight per weight
wt	weight

CONVENTIONS

Acetylene	=	Ethyne
AR	=	Acetylene reduction rate (Ethylene production rate)
Beech	=	*Fagus sylvatica*
Birch	=	*Betula pendula* Roth.
CCA	=	Copper chrome arsenic preservative
Douglas fir	=	*Pseudotsuga menziesii*
Ethylene	=	Ethene
EPR	=	Ethylene production rate = Acetylene reduction rate
MC	=	Moisture content (see page 87 for formula)
^{15}N	=	Isotopically labelled nitrogen atom
$^{15}N_2$	=	Isotopically labelled nitrogen gas
Scots pine	=	*Pinus sylvestris* L.
Spruce	=	*Picea abies* (L.) Karst.
WC	=	Water content (see page 88 for formula)
WL	=	Weight loss (see page 89 for formula)
Weight	=	Mass
PCP	=	Pentachlorophenol based preservative
TBTO	=	Tributyl tin oxide based preservative
3D	=	Three dimensional (x, y and z axes)

STATISTICAL CONVENTIONS

18.4 ± 6.3 (9)

= mean $\pm$ standard deviation (number of samples)

INTRODUCTION

When wood is exposed to ground contact, an inevitable series of events is begun whose ultimate conclusion is the decomposition of the wood. When the wood is a railway sleeper, a fence post or a transmission pole, it is an economic necessity to delay that decomposition for as long as possible. The preservation of timber for ground contact, exemplified by treated softwoods, has been one of the successes of wood preservation. Nevertheless ground contact remains as the major hazard to which timber can be exposed. An understanding of the decay process in wood in ground contact is of fundamental and major importance.

The attack of wood by fungi has been extensively investigated and considerable information has been collected on the mode of action and requirements of wood destroying fungi. The role of nitrogen in the timber decay process is of particular importance. Cowling and Merrill (1966) suggested that wood destroying fungi must be adapted to growth on the wood, which is low in nitrogen, and put forward 3 possible mechanisms.

Firstly, the preferential allocation of nitrogen to the enzymes and metabolic pathways involved in wood degradation. Secondly, the re-use of nitrogen in growing portions of the mycelium obtained by the autolysis of older portions. Thirdly, the fixation of atmospheric nitrogen by fungi. The ability of certain wood destroying fungi to grow on media containing nitrogen compounds occurring in wood or those which may be available as a result of autolysis of the mycelium indicates that wood destroying fungi could indeed conserve nitrogen by re-distribution. The ability of these fungi to grow on substrates with C:N ratios of 20:1 to 2000:1, and to adapt the level of N in the mycelium to the amount of nitrogen available is evidence for preferential allocation. The third hypothesis, concerning the possibility of nitrogen fixation by fungi can now be discounted as Millbank (1969) has shown, using the ^{15}N isotope technique, that putative nitrogen fixing fungi are more likely to be efficient scavengers of combined nitrogen than able to fix gaseous nitrogen. The rapid and sensitive acetylene reduction technique for the detection of nitrogen fixation (Postgate, 1972) has been used extensively in the investigation of such organisms and systems and as no nitrogen-fixing eucaryote has yet been found, it would appear that the ability to fix nitrogen is confined to the procaryotes.

The procaryotes, particularly bacteria, were once regarded as troublesome contaminants but are now considered as being of fundamental importance. Their ubiquitous occurrence, diverse chemistry, rapid

generation time and extended dormancy are properties of an ideal opportunist organism and wood colonist. Their presence and action in wood has been reviewed extensively (Rossell, Abbot and Levy, 1971; Holt, Gareth Jones and Furtado, 1979). Bacteria are a part of the wood decay ecosystem.

Nitrogen can be a limiting nutrient in wood decay, bacteria occur in decaying timber and some bacteria can fix nitrogen under certain conditions. In 1972 Seidler et al described the isolation and identification of bacteria from the decayed zones of living white fir trees. Some of the isolates were found to have characteristics similar to nitrogen-fixing Klebsiellas, and on subsequent testing using the acetylene reduction technique, of 145 isolates, 40 exhibited nitrogenase activity. Nitrogen fixing bacteria were present in decaying wood.

In 1973 Cornaby and Waide measured the rate of acetylene reduction in decaying chestnut logs and came to the conclusion that nitrogen fixation could serve as a source of nitrogen for decomposer organisms and have an important role in regulating the rate of log decomposition.

Sharp and Millbank (1973) measured the acetylene reduction rate of a number of woods exposed to ground contact. Activity was found in beech veneers and centimetre cubes, and in Scots pine and oak veneers, all exposed for 20 days. Activity was also found in a Scots pine stake after $3\frac{1}{2}$ years exposure in the field. Highest values were found in woods exposed to soil augmented with glucose.

In 1974 Aho, Seidler, Evans and Raju reported the isolation from living, but decayed, white fir trees of 130 gram negative bacterial isolates of which 68 reduced acetylene. Nitrogen-fixing bacteria were isolated from 31 different trees at 16 sites and were associated with decay in all cases. Five of these cultures were found to fix atmospheric nitrogen, using the ^{15}N technique. Bacterial populations in decaying wood were estimated by a most probable number estimate of serial dilutions of expressed sap using AR as a criterion. The number of colonies per ml of sap varied with the fungus present in the tree, from 7×10^6 associated with Hieracium abietis, to 39.3×10^6 with Phellinus and from 1.4×10^3 to 6.6×10^5 with Echinodontium tinctorium.

Identification of the isolates revealed only 20 which were biochemically typical of previously described species, which hampered identification. On the basis of 20 biological tests, the groups of isolates were assigned to Enterobacter agglomerans, E. aerogenes, Klebsiella pneumoniae, Enterobacter cloaca, other Enterobacter species and unknowns.

In 1975 Sharp extended these initial observations of the occurrence of nitrogen-fixing bacteria in decaying wood. He used a perfusion system and investigated the effect of antibiotics (both antifungal and antibacterial), different temperatures, different pH's and wood preservatives upon nitrogen-fixing activity. This initial survey, involving a small number of samples (veneers or centimetre cubes) exposed to a range of conditions revealed that the temperature optimum was 35°C although acetylene reduction activity was recorded at 15, 25, 45 and 55°C. Sharp postulated the existence of specialised or adaptable species for the more extreme conditions. The acetylene reduction rate was higher in veneers soaked in 0.4M phosphate-citrate buffer at pH's from 2 to 3.5 than from 4 to 6. The optimum pH was found to be 3.5, with little activity above pH6. Veneers perfused with antifungal actidione showed higher rates of AR than untreated veneers and both were higher than those perfused with antibacterial antibiotics, from which Sharp infers that the presence of fungi reduces nitrogen-fixing activity by bacteria.

The results from Scots pine and beech blocks treated with creosote in which greater activity is observed after 30 days than at 15 days is interpreted as being a lag effect in which bacteria gradually acquire a tolerance to the preservative. The activity in beech cubes treated with 2% PCP and 5% TBTO is suggested to be due to an uneven distribution of toxicant. The effect of sealing different faces of centimetre cubes of a range of wood species gave variable results and presented considerable difficulties in their interpretation. The only firm conclusions reached were that whichever face was exposed, the bacteria entered the cube, and that their occurrence in all the woods tested implied that they were unaffected by wood microstructure.

Sharp attempted to demonstrate the transfer of fixed ^{15}N from a bacterium to a fungus by measuring the ^{15}N incorporation into mycelium growing out of perfused veneers, but found no enrichment.

Isolation from the perfused veneers revealed no change in the pattern of deterioration.

The undoubted occurrence of nitrogen-fixing bacteria in decaying timber suggests that they have a role in the ecology of timber decay. It would be of considerable interest and no mean importance to discover the role and significance, if any, of nitrogen-fixing bacteria to timber decay in ground contact. Sharp's work suggests a number of questions.

Are they capable of penetrating timber of larger dimensions than centimetre cubes and veneers or are they merely confined to the surface? If they are able to penetrate wood, how does the wood microstructure affect their penetration? Are there differences in penetration between hardwoods and softwoods, between species, or between sapwood and heartwood of the same species, or do nitrogen-fixing bacteria occur in all timbers? Is their distribution localised within a stake, as suggested by Levy (1968), or do they occur at and above the ground line? Which factors affect the occurrence and activity of bacteria in timber in ground contact, in the laboratory and in the field? Is their presence a prerequisite to the decay of timber, and are they eliminated from timber by the presence of preservatives? If nitrogen-fixing bacteria are facultative anaerobes, but only fix nitrogen in anaerobic conditions, while fungi are obligate aerobes, are the organisms spatially or temporally isolated, and can nitrogen-fixing bacteria contribute to the nitrogen economy of the fungus?

The aim of the work was to investigate the role and significance of nitrogen-fixing bacteria to the decay of timber in ground contact.

1.1. Attempt to isolate nitrogen-fixing bacteria from wood exposed to different soil conditions.

1.1.1. Introduction

Sharp (1975) had suggested that different strains of bacteria were active in deteriorating wood exposed to soil at different temperatures. The isolation of these different strains from wood exposed to different soil conditions , i.e high and low temperatures and wet and dry soil, was attempted. Both a hardwood and a softwood were used to examine the effect of different wood substrates upon the occurrence of the bacteria. To ensure that the organisms had penetrated the wood, rather than being merely surface contaminants, the outer surface of the wood was sterilised on sampling and only the inner portion of the block used for isolations. Nitrogen fixing bacteria were detected using the acetylene reduction technique.

1.1.2. Materials and Methods

Blocks of birch (Betula pendula Roth.) and spruce (Picea abies (L.) Karst.) sapwood 20mm long, with the transverse face 10mm square were sterilised by autoclaving and then exposed to soil contact. Sixteen blocks of each species were buried in soil at 50% of saturation contained in plastic bags in the laboratory at approximately 22°C. Two blocks of each species were exposed to 4°C or 30°C in soil at 50% saturation and to soil at 100% and 25% saturation at 22°C.

After 14 days incubation, four blocks of each species were removed from the soil at 50% saturation and 22°C, and surface sterilised by washing with sterile distilled water followed by swift passage through a bunsen flame. The ends of the block were removed with a sterile saw, leaving a centimetre cube. The outer surfaces were sliced off with a sterile scalpel and then cut into slices. Some slices were plated out on 0.08% w/v Nutrient Broth agar (Oxoid) in petri dishes, and some were placed in sterile McCartney bottles which were then assayed for the presence of nitrogen-fixing organisms by the acetylene reduction technique.

1.1.3. Results

The results of the isolations and the acetylene reduction assay are given in Table I. Autoclaved soil, uninoculated agar and

sterile bottles did not produce any ethylene in the AR assay.

Table 1 Occurrence of bacteria and AR activity in Birch and Spruce blocks after exposure to soil under different conditions.

AR Activity : Acetylene reduction activity in chart recorder units.
Growth :
0 = No growth
1 = Few colonies
2 = Numerous
3 = Many
+ = Fungal growth

	Birch				Spruce			
	Growth	No. Isolates	AR Activity 0 hrs	AR Activity 3 hrs	AR Activity 24 hrs	Growth	No. Isolates	AR Activity 0 hrs	AR Activity 3 hrs	AR Activity 24 hrs
Moist 50 % saturation 22°C	3	2	80	70	70	3	1	70	75	70
	2	4	75	70	70	3	1+	80	80	75
Wet 100 % saturation 22°C	2	3	110	105	110	3	1	80	75	70
Hot 50 % saturation 30°C	2	2	75	65	60	3	2	75	70	70
Cold 50 % saturation 4°C	1	1	75	65	65	3	1	80	75	70
Dry 25 % saturation 22°C	2	3	70	70	65	3	1+	75	70	70

The 22 bacterial isolates were subcultured and tested for AR activity; none of the strains exhibited acetylene reduction. Fungal growth was observed on only two isolation plates. Cold incubation appeared to reduce the colonisation of birch wood by bacteria, but not of spruce.

1.1.4. Discussion

More bacterial colonies were observed growing from spruce wood samples than from birch, although fewer strains were isolated from spruce. No AR activity was recorded from any wood sample. Perhaps the sterilisation of the outer surfaces by flaming was too drastic, killing the nitrogen-fixing bacteria? However, when the slices which were used in the AR assay were plated out, bacterial colonies grew from them, implying that either bacteria or spores in the wood were capable of growth. Nevertheless, perhaps a milder sterilisation procedure was required.

Bacteria could be isolated from the inner regions of blocks exposed to soil, although the plating technique failed to reveal quantitative differences between the treatments. The lack of AR activity

by the isolates and the wood indicated that the conditions for the colonisation and development of nitrogen-fixing bacteria in wood were more specific than indicated by Sharp (1975) and that precise control of the soil environment was necessary to achieve reliable AR activity in wood in soil contact.

1.1.5 Conclusion

Further work was required to establish a reliable procedure of soil exposure and of the surface sterilisation of timber from soil contact.

1.2. Investigation of methods of surface sterilisation of wood exposed to soil contact prior to isolation and AR assay.

1.2.1. Introduction

To ensure that acetylene reducing organisms penetrated wood in soil contact rather than being merely surface contaminants required that the surface of the wood was sterilised before being cut through to expose the interior of the wood for isolation or assay. Five methods were investigated for efficacy : flaming, washing, sulphuric acid dip, contact with a hotplate and β-propiolactone immersion. The outside and inside of blocks removed from soil contact were plated out to determine the efficacy of the sterilisation procedures. The effect of sterilisation upon abiotic ethylene production by the wood was also investigated.

1.2.2. Materials and Methods

Blocks of birch (*Betula pendula* Roth.) and spruce (*Picea abies* (L.) Karst) were taken from the experiment described in 1.1 after 21 days exposure to moist soil at 22°C. Some blocks were rolled on a plate of nutrient broth agar, and all were then subjected to one of the sterilisation treatments shown below and in Table 2 :

(i) Flaming in a bunsen flame for 5 seconds.
(ii) Flaming in a bunsen flame for 2 seconds.
(iii) Surface washing with sterile distilled water.
(iv) Dip in concentrated sulphuric acid for 30 seconds.
(v) Each surface placed on a red-hot steel plate for 5 seconds.
(vi) Immersion in β-propiolactone for 4 hours (this compound sterilises on contact but becomes inert after 3 hours) (Sykes, 1965).

Control blocks were (i) a sterile block which was rolled on the surface of a petri dish inoculated with bacteria and then flamed for 5 seconds, (ii) sterile blocks of each species, and (iii) unsterilised blocks taken direct from soil.

The blocks were cut up as described in Section 1.1.2. To determine whether the sterilisation procedure had effectively sterilised the outer surface without affecting the interior of the block, the outer and inner slices were plated out on Nutrient broth agar. Slices from the interior were also transferred to McCartney bottles for AR analysis. The ethylene content of the bottles was determined immediately, and again 24

hours later. The bottles were evacuated, the gas atmosphere replaced with Argoshield '5' (BOC Ltd), and the ethylene content determined. Acetylene was then injected and the ethylene content determined after a further 24, 72 and 96 hours incubation.(See Section 1.3).

1.2.3. Results

The results of the plating out of the outer and inner slices and of the AR analysis are given in Table 2.

1.2.4. Discussion

The initial ethylene content values are high and variable because the bottles were autoclaved capped with metal caps and rubber washers, which can produce ethylene.

The isolation results indicate that flaming is ineffective. Surface washing with distilled water increases growth, presumably due to the spreading out of colonies over the surface of the wood prior to plating out. The sulphuric acid dip, not surprisingly, is very effective, although in the birch sample the surface print of the block on agar plates prior to the acid dip showed no bacterial growth, so that the lack of growth after dipping is not unexpected. The hot plate treatment was ineffective, due to the difficulty of sterilising by making contact with the convoluted wood surface. The β-propiolactone treatment appeared ineffective, and also gave rise to enhanced ethylene content in the AR assay. The claim that this compound is initially toxic and that its toxicity rapidly decreases is not strictly tested in this experiment. Convincing proof using wood would be necessary before its use as a surface sterilisation compound could be recommended.

The growth of bacteria from the interior slices of a sterile block which had been rolled in bacteria not only indicated the ineffectiveness of the flaming procedure but also the possibility that surface organisms could be transferred to the interior during the slicing procedure.

Slices from sterile blocks produced no growth on the agar and no AR activity. An unsterilised spruce block did exhibit AR activity, which indicated that nitrogen-fixing bacteria may well have colonised the block. All six sterilisation procedures adversely affect

subsequent AR assay presumably because the sterilant penetrates to the centre of these relatively small blocks.

1.2.5. Conclusion

A sulphuric acid dip is an effective surface sterilisation procedure, but any form of surface sterilisation may well affect the interior of small blocks. Thus the alternatives for the investigation of penetration are either a more mild sterilisation technique or the sealing of the block prior to burial to prevent colonisation other than in the desired direction. Care must then be exercised in the removal of the sealant and the subsequent slicing of the block to avoid contamination of the interior from the surface.

Table 2 Occurrence of Bacteria and AR activity in Birch and Spruce blocks after exposure to soil and different surface sterilisation treatments.

Key

AR Activity : Acetylene reduction activity in chart recorder units.

Growth :

0 = No growth

1 = Few colonies

2 = Numerous

3 = Many

F = Fungal Growth

Birch Sterilisation Procedure	Outer Growth (see Key)	Outer No. Isolates	Inner Growth (see Key)	Inner No. Isolates	No Acetylene injected	24 hrs later	Evacuated	+Acetylene 24 hrs	72 hrs	96 hrs
5 second flame	2	2	2	1	45	40	-			
	2	1			5	5	0			
2 second flame	3	1F	2	2F	15	15	0			
			2	1F	80	80	0			
			2	3	105	105	0			
Surface washed	3	4F			60	60	5	720	660	740
	3	2F	3	3	50	50	0	125	115	150
Untreated	0	0								
Sulphuric acid dip	0	0	0	0	240	230	10			
			1	1	10	30	10	80	95	100
Hot plate	1	2	1	3	50	50	5	0		
β-propiolactone	2	3F	2	3F	320	330	15	170	515	640
Sterile block + bact. Flamed	3	1	3	1	10	5	0			
Sterile block	0	0			2400	2150	50	10	70	60

Spruce

5 second flame	3	2	3	2	3650	3250	165	20	70	80
			2	2	135	70	15			
2 second flame	2	3F	2	2	620	650	0			
			2	2	7250	7100	140	20	80	65
			1	2	6500	6300	135	30	115	60
Surface washed	3	3F			2100	2050	100	15	80	80
Untreated	3	3F								
Surface wash	3	3								
Cut up	3	3F	2	1	80	80	0	5	70	65
Sulphuric acid dip	0	0	1	1F	2450	2500	60	10	90	70
Hot plate	2	2	2	2	310	300	60	5	50	50
β-propiolactone	2	1	2	3F	5	5	0			
Sterile block			0	0	660	670	20	5	60	60
Unsterile block					750	720	20	10	740	1125
Sterile bottle					15	20	0	10	50	40

1.3. The Penetration of Nitrogen-fixing Bacteria into Wood in Ground Contact.

1.3.1. Introduction

This experiment was designed to determine if nitrogen-fixing bacteria could penetrate wood in ground contact or were confined to the surface layers. This was achieved by sealing five faces of an orientated wood block leaving only the tangential face exposed. It was assumed that the sealant prevented the ingress of organisms, which would thus only enter from the tangential face. After exposure to ground contact the block was removed, the sealant removed and slices taken parallel to the tangential face. The slices were then assayed for the presence of nitrogen-fixing organisms by the acetylene reduction technique. Progressive colonisation and penetration were monitored by sampling after increasing periods of exposure.

1.3.2. Materials and Methods

Blocks of Scots pine (*Pinus sylvestis* L.), (*Fagus sylvatica*) beech, and Douglas fir (*Pseudotsuga menziesii*), all sapwood, 50x25x15mm with no visible defect and the growth rings parallel to the 50 x 15 face were selected. The blocks were coated on five faces with a silicone rubber sealant (Silastic 738 RTV, Dow Corning Ltd) leaving the tangential face unsealed. The blocks were sterilised by autoclaving and buried with their tangential face vertical in a vessel containing moist soil, a brown loam, taken from the Old Farm Site at Imperial College Field Station, Sunninghill, Berks. The vessel was sealed in a plastic bag and incubated at 20°C.

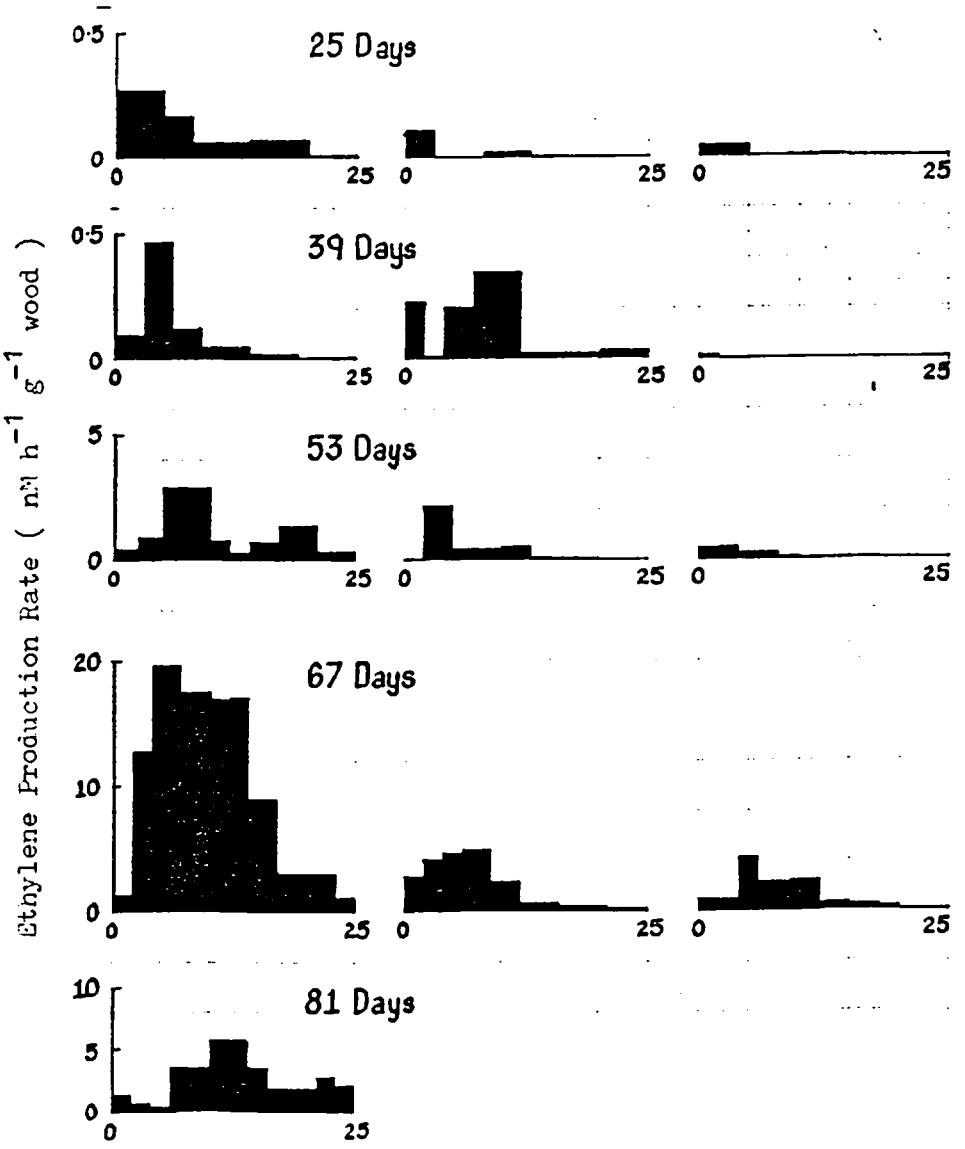
Three blocks of each species were removed at 25 days, and then at 14 day intervals. The sealed faces of the blocks were washed in distilled water and the uncoated face wiped to remove adhering soil. The surfaces were dried and then swabbed with ethanol. The sealant was removed with a sterile scalpel under aseptic conditions. Each block was then split longitudinally into a number of slices parallel to the exposed tangential face. Slices were first cut from the unexposed zone to minimise the transfer of organisms from the exposed face to slices deeper into the block during slicing. The thickness of each slice was measured to the nearest millimetre, and the slice chopped into a number of sticks which were transferred to sterile McCartney bottles and capped with a sterile suba-seal closure before acetylene reduction analysis.

Samples taken from some of the slices after analysis were weighed and oven-dried for the estimation of moisture content. Soil samples were taken at 53 days and their moisture content determined.

1.3.3 Results

No AR activity was found in any of the beech or Douglas fir slices at any sample time. Figure 1 shows the AR rate of Scots pine samples taken at a number of depths away from the exposed, tangential face after increasing periods of exposure. At each sample time except 81 days, three replicate blocks were removed and sliced. The thickness of each slice was measured and plotted on the horizontal axis, and its AR rate expressed in nM $h^{-1}g^{-1}$ wet wood plotted vertically.

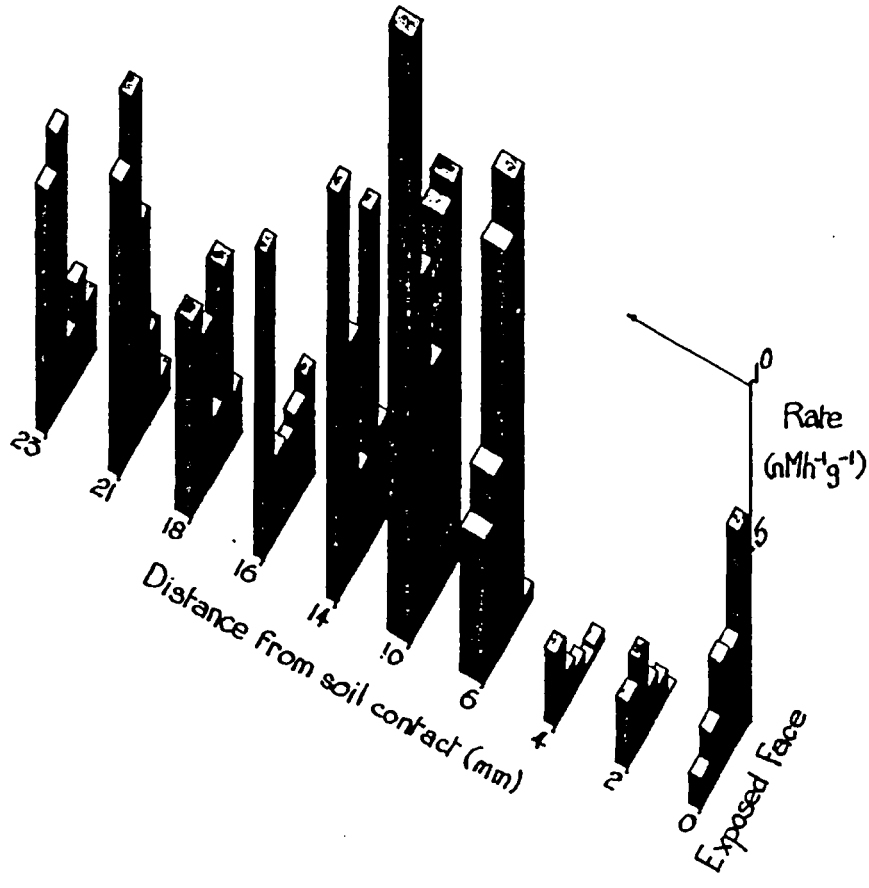
Figure 1 Ethylene production in Scots pine sapwood blocks with one tangential face exposed to soil. Samples taken from 0 - 25 mm in 3 replicates at 2 week intervals from 25 days.



The 25 and 39 day samples revealed higher activity in the slices near the exposed face than deeper into the block, implying that the activity and thus the organisms had penetrated into the wood from the exposed face. At 53 days, where the vertical scale has been increased by a factor of 10, the activity was not only found throughout the block, it was considerably higher than in the 25 and 39 day samples. By 67 days, where the scale has been further increased by a factor of 2, the rate of AR had further increased throughout the block. By 81 days, on the same scale, the activity had either declined or remained at the level found in the second or third replicate blocks of the 67 day sample.

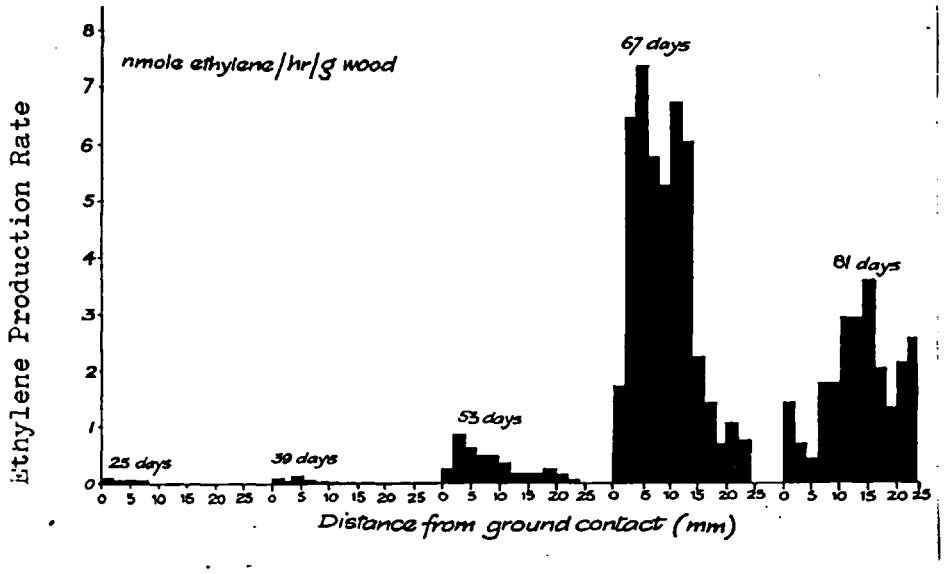
The values of AR recorded varied within a block and between replicates. The variability was presumably due to the disjunct distribution of the nitrogen fixing bacteria within the wood sample, shown in Figure 2, where the AR rate measured in the slices from the 81 day sample after further subdivision showed 10-fold differences in AR rate in samples only 1 mm apart.

Figure 2 Acetylene reduction rate in slices and subsamples of a Scots pine block after exposure of one tangential face to soil for 81 days.



If the results shown in Figure 1 are converted to a standard slice thickness of 2mm, pooled and plotted on the same scale (Figure 3), then the penetration of activity and the overall increase of activity with time is obvious.

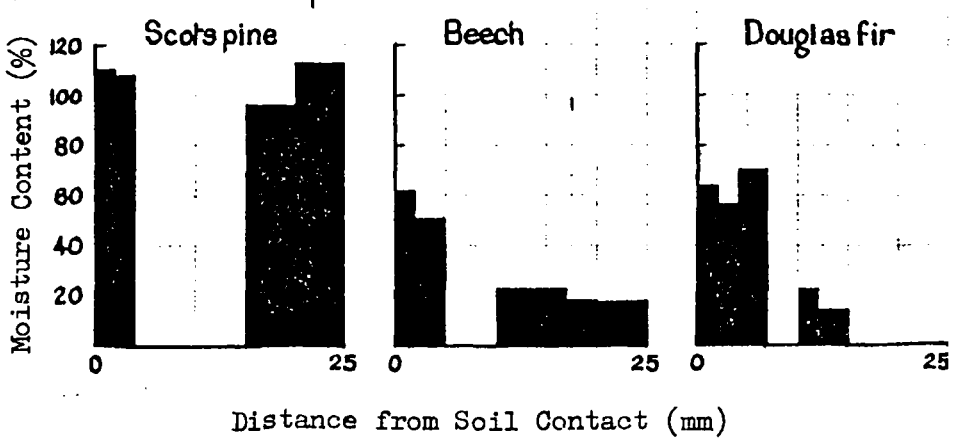
Figure 3 Average Ethylene production rate against time and distance from soil contact in Scots pine sapwood blocks with one tangential face exposed.



If the rate of activity measured in each slice at a sample time was assumed to have increased linearly since the previous sample, and the conversion factor for nM of acetylene reduced to nM nitrogen fixed is 3 (see Section 2.4.5 for discussion) then a crude estimate would be 20 µg nitrogen fixed per gm of wood, representing an overall 1.65 % increase in the nitrogen content of the wood, achieved in 81 days.

The soil moisture content after 53 days was 44.8 % ± 6·5 (8). The moisture content of the samples removed after this time period is shown in Figure 3.1.

Figure 3·1 Moisture content of slices taken from blocks with one tangential face exposed to soil contact for 53 days. No samples were taken where zero moisture content is plotted.



The hardwood beech and the Douglas fir, were, as Figure 3.1 shows, considerably drier than the softwood Scots pine and were wetter on the exposed tangential surface than towards the centre. This may have been due to the distilled water wash but it is unlikely that this had a large effect upon the block moisture content: the MC in the Scots pine blocks showed no difference between inner and outer regions. At 81 days the MC of the Scots pine had decreased to 65 % ± 10 (12) possibly due to the drying out of the soil and also the wood.

1.3.4. Discussion

The penetration of nitrogen-fixing bacteria into Scots pine sapwood in soil contact has been demonstrated. The lack of penetration into Beech and Douglas firwas not expected in view of Sharp's work (1975), but perhaps the activity described there was confined to the surface layers only and did not penetrate into the wood. If the major pathway in the tangential direction is via the rays, then the anomalous behaviour of beech,in which colonisation of the rays did not occur, (Greaves and Levy, 1965) may explain the absence of penetration in beech.

Presumably the bacteria are carried into the wood with the water as the block gets wet or a continuous water film from the soil is extended to allow the motile bacteria to penetrate. The low MC of Douglas fir samples may explain the absence of activity in this species. Water would seem likely to be a major factor affecting activity, especially as high MC may produce anaerobic conditions which favour nitrogen fixation by nitrogen-fixing bacteria. The decrease in activity at 81 days, if not a sampling artifact may be due to the decrease in MC accompanying the drying of the soil.

The observed "peak" of activity may be an artifact and the activity may reach a plateau and continue at the same rate; further work is required to establish how long nitrogen fixing activity may continue in the colonisation sequence. The variability of the activity in adjacent samples and in different blocks reflects the complexity of wood in soil contact. The volume occupied by the bacteria is only a tiny fraction of the wood volume sampled, and the colonies could easily be scattered throughout the timber. The assessment of their significance is a major problem.Is the activity due to relatively few,very active organisms, or to many relatively inactive organisms? The assay conditions (oxygen

tension, temperature, humidity, moisture content, nutrient concentration) may not be the same as those in vivo and thus the AR rate measured may bear no resemblance to the nitrogen fixation rate in vivo. Thus the assessment of nitrogen fixed in the time of exposure may be very crude, but it does indicate the order of magnitude of nitrogen fixing activity in Scots pine exposed to soil contact. The 1.65% increase in nitrogen content may be significant if it is localised in small areas within the wood, but this assumes that the fixed nitrogen is available to colonising fungi, implying that either the bacteria leak nitrogen, or autolyse, releasing usable nitrogen. Even those small amounts of fixed nitrogen may be important if the rate is continued and the time of exposure is months or even years.

Further work is necessary to determine the reason for the difference in penetration of Beech, Scots pine and Douglas fir, and to determine if penetration can occur in all three directions (tangential, radial and longitudinal) in Scots pine or if wood anatomy affects penetration. It is also necessary to assess the influence of water upon activity and penetration. Considerable work is required to assess the relevance, accuracy, and reliability of the AR technique when applied to wood in soil contact; the experiments on this aspect are collected together and discussed in Section 2.

1.3.5. Conclusion

Nitrogen fixing bacteria, assayed by the AR technique, were present in Scots pine in soil contact, but not in Beech or Douglas fir. They were found to penetrate wood when a tangential face was exposed and the AR activity increased with time of exposure, although the rates and distribution were disjunct and variable. A crude assessment of the amount of nitrogen fixed suggested that the nitrogen fixing bacteria may be of significance in the nitrogen budget of timber in soil contact.

1.4. The tangential, longitudinal and radial penetration of nitrogen fixing bacteria into Scots pine sapwood in soil contact.

1.4.1. Introduction

This experiment was designed to monitor the differences in the rate of tangential, longitudinal and radial penetration into Scots pine sapwood exposed to soil contact out of doors in fluctuating, uncontrolled conditions.

1.4.2. Materials and Methods

Blocks of Scots pine sapwood (Pinus sylvestris L.) 50 x 25 x 15mm were selected with the growth rings parallel to the 50 x 15mm face. The blocks were sealed with silicone rubber sealant, except for an area 15 x 15mm on one of the tangential, radial or transverse faces of each block. They were autoclaved and buried in a trough containing soil from the Old Farm Site at Imperial College Field Station, Sunninghill, Ascot, Berkshire, exposed on the roof of the Zoology Department in South Kensington, London.

Blocks were recovered at intervals, washed in distilled water and the surfaces wiped with ethanol. The seal was removed and the blocks cut transversely with a circular saw, under aseptic conditions. The sawn pieces were then sliced longitudinally and the segments assayed for acetylene reduction activity.

1.4.3. Results

Only one block of each sealing arrangement was sampled and analysed at each sample time, which, in view of the variability exhibited in the previous experiment, must limit the reliability of the results. Nevertheless qualitative differences can be seen in the rate of penetration of nitrogen fixing bacteria in the three major directions. Figures 4, 5 and 6 have four components and show:-

1. The position of the exposed face on the block and the shape and position of segments into which the blocks were cut, together with their code numbers.

2. The acetylene reduction rate measured in these segments (plotted vertically) against the time of exposure in months.

3. The moisture content of certain segments in the same format as 2.

4. A graph of the moisture content of a segment plotted against the AR rate of that segment.

The letter A represents a 4 month sample, letters B, C and D, 6, 7 and 8 month samples respectively.

TANGENTIAL FACE EXPOSED Figures 4.1 to Figure 4.4.

The blocks with only a portion of the tangential face exposed showed a pattern of radial penetration similar to that of the previous experiment. Activity was recorded on the exposed face after 1.5 and 2 months in Segment 1, while activity only began to occur within the blocks (in segments 3 and 4, 5 and 6) after 4 months. This implies that radial and longitudinal penetration occur at the same rate. A peak of activity occurred at 5 months in Segment 1.

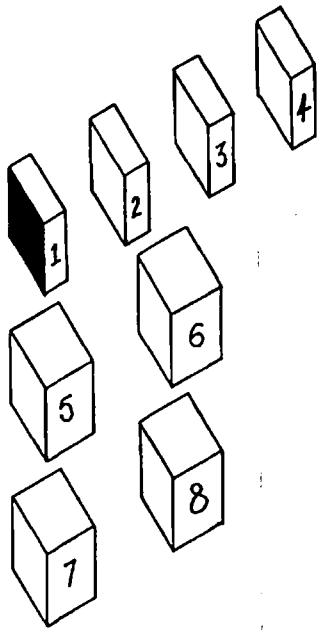
The moisture content of all the segments at all the sample times was over 100%, with the highest MC at 6 months and the lowest at 7 months. The graph of moisture content against AR showed no correlation between the two values. Most samples had relatively low rates of AR, with fewer samples having high AR rates between 2 and 8 nM $h^{-1}g^{-1}$. The moisture content of the samples covered a large range, centred on 150%. There were differences between sample times perhaps reflecting the fluctuating soil moisture content.

TRANSVERSE FACE EXPOSED Figure 5.1. to Figure 5.4.

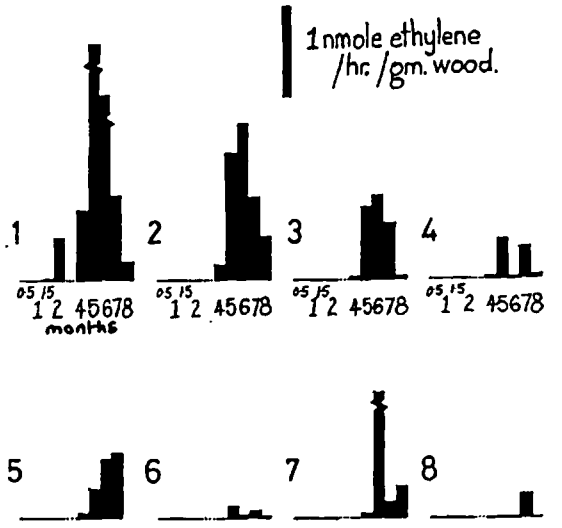
Penetration of nitrogen fixing bacteria in a longitudinal direction from an exposed transverse face resulted in the presence of AR activity in Segment 1(with the exposed face) after 2 months and in Segments 2, 3 and 4 at 4 months. This implies, as before, an equal rate of penetration in radial and longitudinal directions. Despite the variability of the activity in any one segment at different sample times, the overall pattern was that higher activity was recorded near the exposed face in segments 1 and 2 than deeper into the block, with segments 7 and 8 having the lowest activity.

Figure 4 Tangential Face Exposed to soil

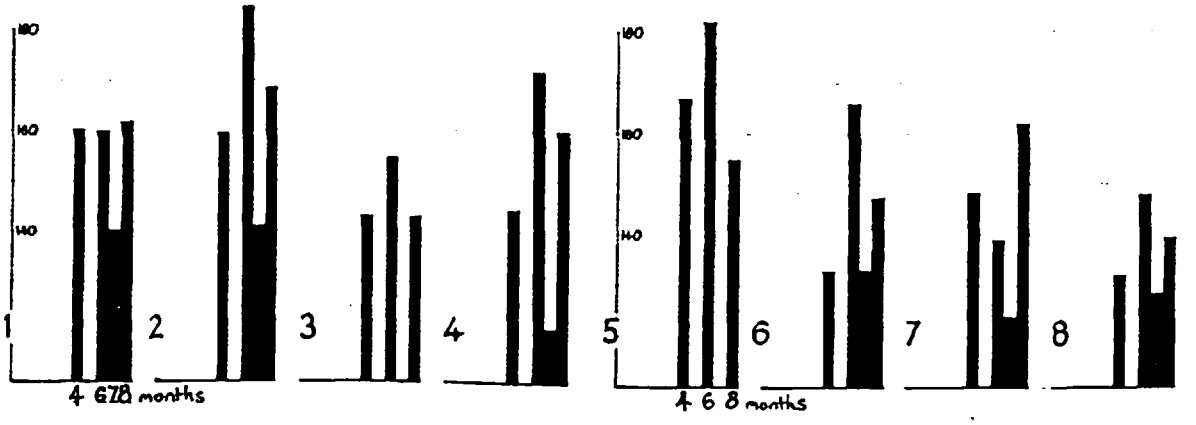
4·1 Segment arrangement



4·2 Ethylene production rate



4·3 Segment Moisture Content



4·4 Segment Moisture Content against Acetylene Reduction Rate

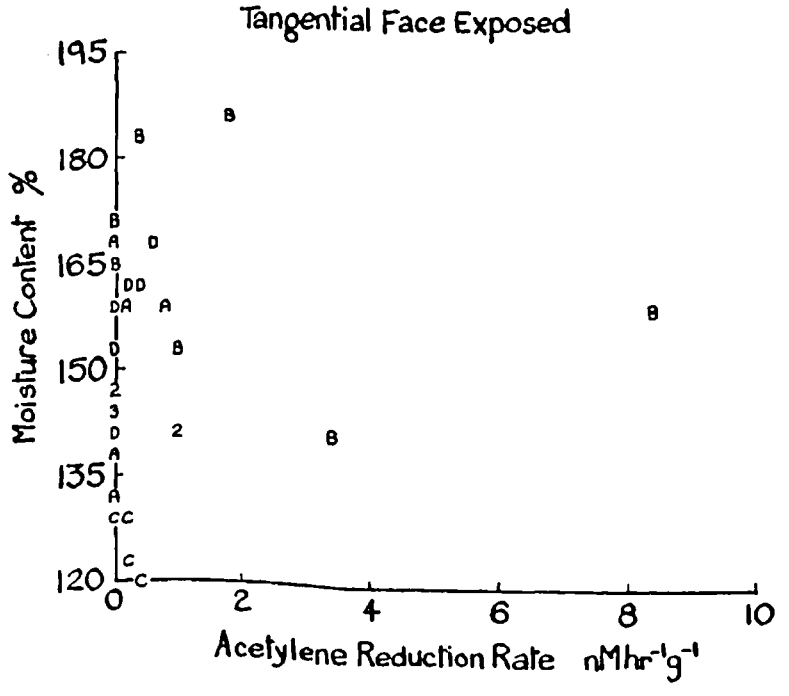
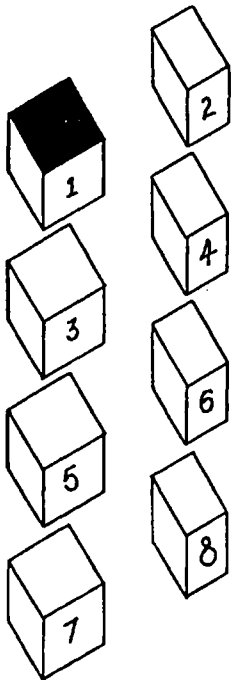
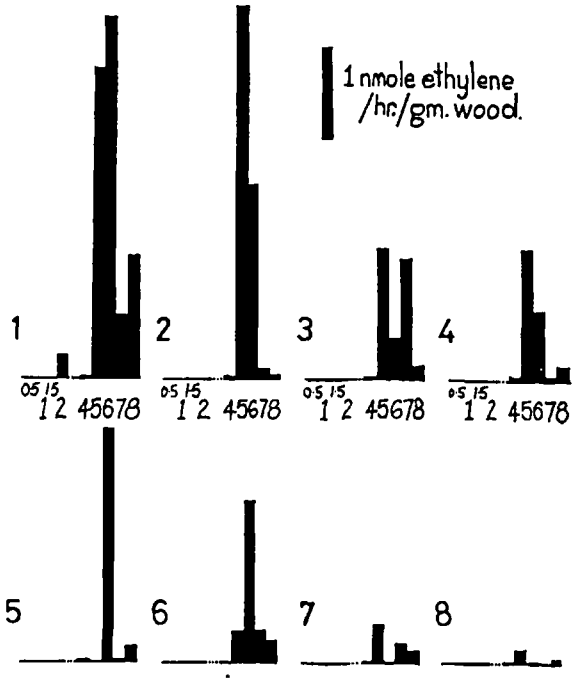


Figure 5 Transverse Face Exposed to soil

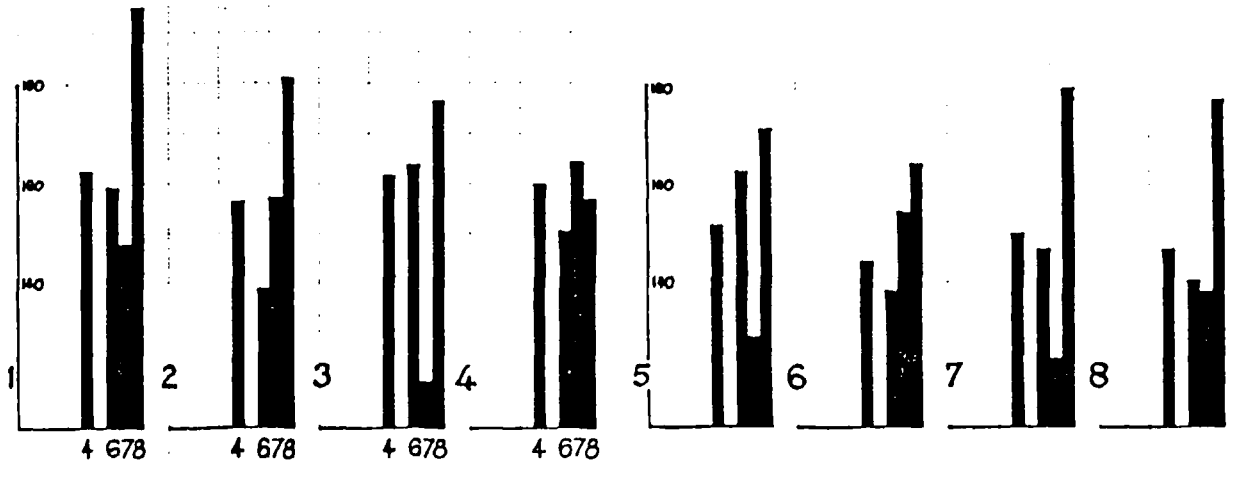
5·1 Segment Arrangement



5·2 Ethylene Production Rate



5·3 Segment Moisture Content



5·4 Segment Moisture Content against Acetylene Reduction Rate

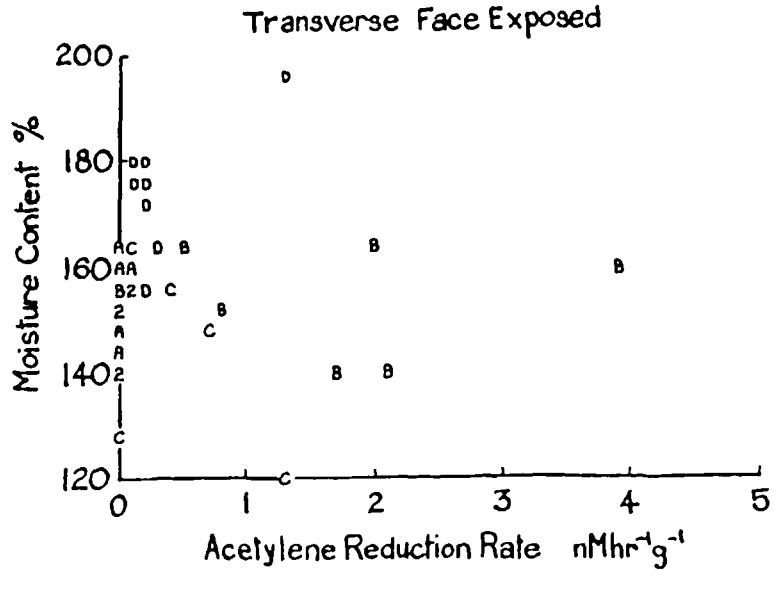
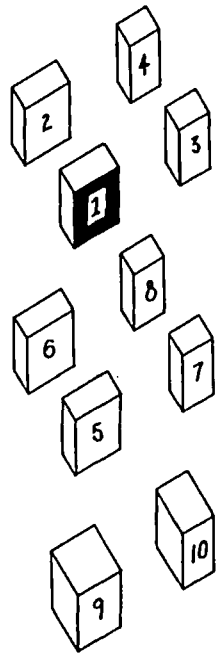
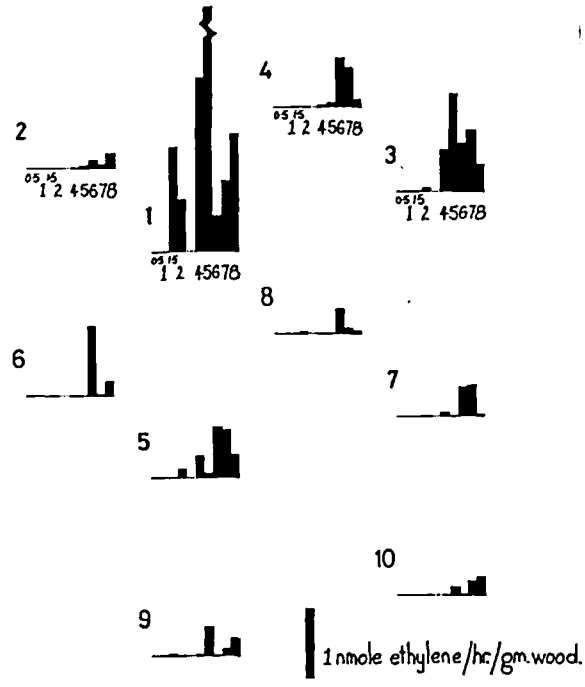


Figure 6 Radial Face exposed to soil

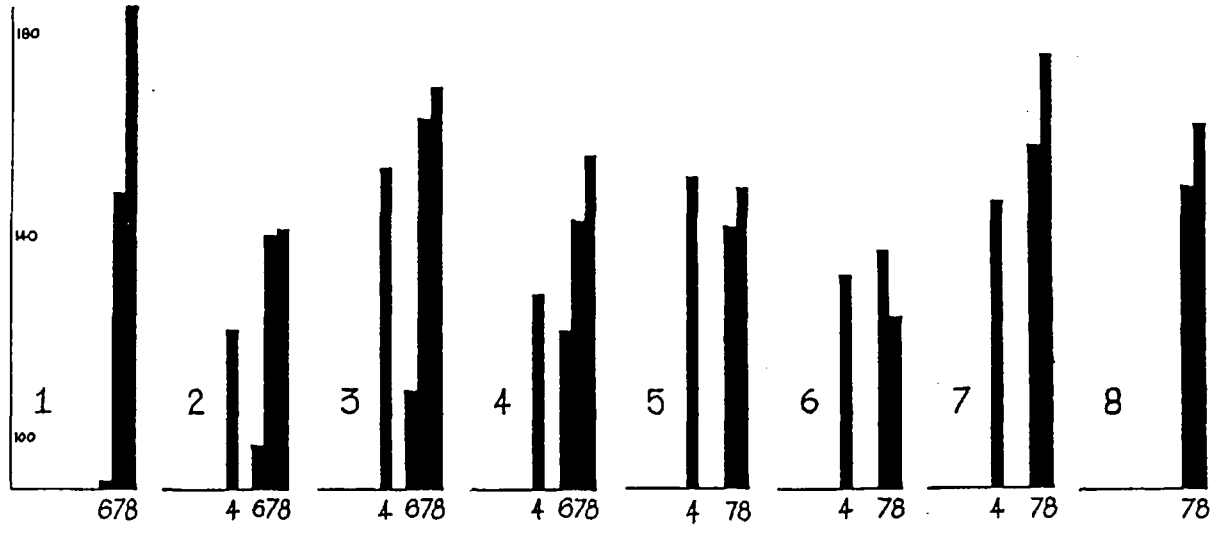
6·1 Segment Arrangement



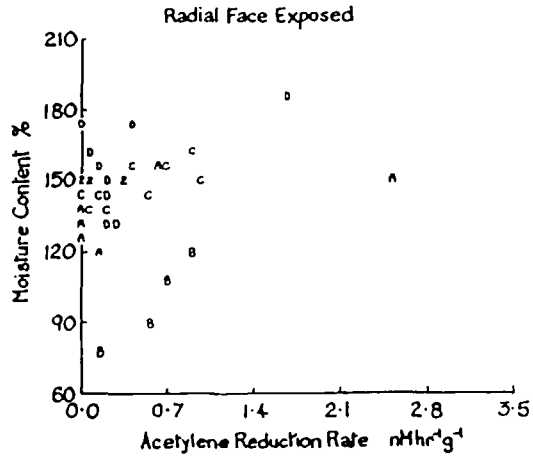
6·2 Ethylene Production Rate



6·3 Segment Moisture Content (%)



6·4 Segment Moisture Content against AR rate



The moisture contents were well above 100%, and highest in the 8 month sample, and the lowest at 7 months. There appeared to be little difference in the moisture content of the segments except that segment 1, exposed to the soil and washed on removal had a slightly higher moisture content than segment 8, the furthest segment from the exposed face. The graph of MC against AR again shows no correlation between the values, with many low AR rates and only a few high rates between 1 and 4 nM $h^{-1}g^{-1}$.

RADIAL FACE EXPOSED Figure 6.1 to 6.4

The results from these blocks having only a portion of the radial face exposed is shown in Figure 6. Considerable activity was first recorded in Segment 1 after only 1.5 months, before any activity was found in blocks with the other sealing arrangements. Activity was found in segments 3 and 5 at 2 months and not until 5 months in segment 2. This implies that radial and longitudinal penetration is faster than tangential. Segment 5 showed greater activity than 7, which was greater than 6, with segment 8 having least activity. The peak of activity on the exposed face occurred at 6 months.

The moisture content of segments 1 and 2 at 6 months were below 100% while all the others were over 100%. The MC appeared to increase at 6, 7 and 8 months in all segments except 6 and 9. The graph of segment MC against the AR rate shows no correlation. The range of MC was higher than in the other sealing arrangments, while the AR rate recorded was much lower, less than 2.8 nM $h^{-1}g^{-1}$.

1.4.4. Discussion

This experiment, by the combination of a careful selection of test blocks, an effective silicone rubber sealant, the removal of blocks after increasing periods of exposure and the slicing of these blocks into segments, enabled the penetration of nitrogen fixing bacteria into Scots pine sapwood in soil contact to be monitored. The results indicated that these bacteria penetrated more easily in longitudinal and radial directions than in a tangential direction. The inference is that the bacteria were able to move along the rays as easily as they moved along the tracheids, with restricted movement through the numerous bordered pits on the radial walls of the tracheids. If the bacteria, like the fungi,

preferentially colonised the rays as a source of easily obtainable nutrient then this would explain the AR activity found on the exposed radial face of blocks, where much ray tissue was exposed, before any activity was found on the exposed face of the other sealing arrangements.

The results from the previous experiment indicated that the moisture content of the wood was critical in the colonisation of the wood by nitrogen fixing bacteria, while in this experiment there was no correlation between wood moisture content and AR rate. This may be due to the fact that the MC of the wood was first measured after 4 months, when the blocks were presumably in equilibrium with the wet soil and virtually saturated. If the MC recorded represented the saturated MC of the segment, which is above the critical level required for colonisation, then there would be no correlation between MC and AR rate. The critical level would only be determined as the block became wet, requiring the measurement of MC and AR rate after shorter periods of exposure.

Soil, water, wood and organisms were inextricably linked. The movement of water into the wood could easily have carried bacteria into the wood. The "opening-up" of the pits by the bacteria (Greaves, 1969) and the attack of the wood by decay fungi could have allowed the influx of more water, carrying more organisms. However, if the wood was saturated and gaseous diffusion was restricted in wet soil, as suggested by Griffin (1968), then how did the decay fungi obtain their oxygen, and the nitrogen fixing bacteria obtain their nitrogen? Perhaps a sealed block was unrealistic, limiting oxygen diffusion and preferentially selecting facultative anaerobes, like certain nitrogen fixing bacteria.

1.4.5. Conclusion

The technique for the monitoring of the penetration of nitrogen fixing bacteria into wood in soil contact showed that radial and longitudinal penetration was faster than tangential penetration, presumably due to the influx of water from the soil. Further work is required, to examine penetration in hardwoods like beech, where the rays are not colonised, and to examine the influence of MC upon the penetration of the bacteria in more realistic situations than almost totally sealed blocks, totally buried in waterlogged soil.

1.5. The exposure of partially sealed Beech and Scots Pine sapwood blocks to soil contact.

1.5.1. Introduction

To investigate the lack of penetration of beech sapwood by nitrogen-fixing bacteria, blocks were sealed leaving two tangential, radial or transverse faces exposed, and buried in moist soil. They were sampled at intervals, sliced and assayed for AR rate. As controls, Scots pine blocks with the tangential face exposed were buried in the same soil and treated in the same way. In an attempt to limit the variability of the wood, B.S. 838 sized blocks were cut into 3 pieces which were sampled at different times. To determine if the disjunct distribution of activity was related to the distribution of early and late wood in Scots pine, these tissues were dissected out from one block and assayed separately.

1.5.2. Materials and Methods

Blocks of Beech sapwood 50 x 25 x 15mm with the tangential face on the 50 x 15 face or the 50 x 25 face and no visible defect were sawn transversely into 3 blocks each 15 x 25 x 15mm. Those with the tangential face 15 x 15mm were coated with 738RTV silicone rubber sealant to leave 2 tangential faces exposed, and those with the radial face 15 x 15mm were coated to leave 2 radial faces exposed. Blocks 50 x 25 x 15 mm were cross-cut into two blocks 24 x 25 x 15 mm with the transverse face 25 x 15mm. These blocks were coated to leave 2 transverse faces exposed.

As a control to ensure that nitrogen-fixing bacteria were active under the imposed experimental conditions, the tangential faces of Scots pine sapwood blocks 15 x 25 x 15mm, cut from blocks 50 x 25 x 15, were left exposed, and the other faces coated with silicone rubber.

The blocks were buried in shallow trays, containing soil from the Old Farm Site, Silwood Park, which had been sieved to remove large stones and plant roots, watered liberally and incubated at 25°C. The MC of the soil samples was determined at the start and again after 107 days.

At 18, 24, 31, 38, 48 and 59 days, blocks of Beech with tangential, radial and transverse faces exposed, and a Scots pine block with tangential faces exposed, were removed from the soil. They were washed to remove adhering soil, swabbed with methanol and, under aseptic conditions, the sealant was removed. Those blocks with the tangential and radial faces exposed were cut parallel with the exposed face into four segments. Those with the transverse faces exposed were cross cut, parallel to the exposed face, into two segments. The segments were sliced, transferred to McCartney bottles and assayed for AR rate. The moisture content of some wood samples was determined after assay.

After 73 and 87 days, only Scots pine blocks were sampled, and at 87 days the early and late wood were dissected and assayed separately.

1.5.3. Results

The MC of the soil at the start of the experiment was 31.24%, and after 107 days was 28.95%.

BEECH

The results from the sealing arrangements of the Beech blocks are shown in Table 3. The AR rate of the segments are given together with the MC of a small number of the segments after increasing periods of exposure. There was little AR activity recorded in those blocks with the tangential face exposed, and some activity in those with the radial face exposed. The MC of these samples after 18 days was from 24 to 37%. More activity was recorded in those segments cut from the blocks with the transverse face exposed and a MC of around 54%. The activity did not increase with time.

SCOTS PINE

Table 4 shows the results for the Scots pine blocks with 2 tangential faces exposed. AR activity was recorded in all the segments at all time intervals, increasing up to 73 days, but was lower at 116 days. The MC of the segments after 18 days in soil contact was over 110%, very much wetter than the Beech samples. The distribution of the activity was disjunct, with adjacent segments having widely different rates of AR activity.

Table 3 Ethylene Production Rate and Moisture Content in partially sealed Beech blocks with different faces exposed to soil.

Beech		Ethylene Production Rate (EPR) ($nMh^{-1}g^{-1}$) and Moisture Content (MC) (%)						
			Sample time (days)					
Exposed Faces	Block		18	24	31	38	48	59
Tangential	1	EPR	0	0·011	0	0	0	0 $nMh^{-1}g^{-1}$
		MC	37·7					%
	2	EPR	0	-	0	0	0	0
		MC	39·8		27·9			
	3	EPR	0	0	0	0	0	0
	4	EPR	0·060	0·036	0	0	0	0
Radial	1	EPR	0	0	0	0	0	0
		MC	31·7					
	2	EPR	0	0	0	0	0	0
		MC	24·6		24·6			
	3	EPR	0	0	0	0	0	0
	4	EPR	0	0	0	0	0	0
Transverse	1	EPR	0·020	0·003	0·071	0·004	-	0·004
		MC			47·9			
	2	EPR	0·016	0·008	0·007	0·003	-	0·008
		MC	44·8					

Table 4 Ethylene Production Rate and Moisture Content in partially sealed Scots pine blocks with two Tangential faces exposed to soil.

Scots pine		Ethylene Production Rate (EPR) ($nMh^{-1}g^{-1}$) and Moisture Content (MC) (%)									
			Sample Time (days)								
Exposed Faces	Block		18	24	31	38	48	59	73	87	116
Tangential	1	EPR	0·006	0	0	0·009	0·800	0·226	13·877	0·129	0 ($nMh^{-1}g^{-1}$)
		MC	140·3		102·1			173·2			(%)
	2	EPR	0·006	3·275	0·009	0·004	0·011	9·182	12·977	1·973	0·009
		MC						142·8			
	3	EPR	0·016	0·034	0·011	0·013	0·012	0·859	16·287	13·536	0·005
		MC	122·3					172·4			
	4	EPR	0·016	0·004	0·005	0·006	0·0 6	2·712	12·749	6·252	0
		MC						148·7			
	5	EPR	0·018	0	0·372	0·007	0·007	2·324	18·085	16·337	0·006
		MC	115·3					175·8			
	6	EPR	0·016	0	2·017	0·192	0·002	0·647	14·242	29·462	0
		MC				147·6		181·0			
	7	EPR	0·009	0·006	2·697	0·003	0·006	0·466	8·768	0·814	0·013
		MC	158·7		139·7			174·3			
	8	EPR	0·009	0	0·008	0·001	0·001	0·124	7·621	1·687	0
		MC						171·1			

There was a substantial increase in rate between 48 and 59 days and a further increase between 59 and 73 days. The values given for the 87 day sample are averages of late wood and early wood which were separated before assay. There is no significant difference in the rates exhibited by early and late wood. There was little activity recorded at 116 days.

1.5.4. Discussion

The results revealed the importance of water and wood anatomy upon the establishment of AR activity in wood in soil contact. AR activity was recorded in those Beech blocks with the transverse face exposed and a MC above 50%, and less recorded in the blocks with the tangential and radial faces exposed, and a lower MC. The profound difference between hardwood and softwood and their water uptake properties was indicated by the much higher MC of the softwood when buried in the same soil, at the same moisture content, as the hardwood. Differences in the AR activity exhibited by early and late wood of the Scots pine were not detected. The activity increased with time in the Scots pine, as observed and described previously in Section 1.3, with no further increase in wood MC. The lack of activity at 116 days can be attributed to the soil and the wood drying out.

There must be a complex and sensitive relationship between buried wood, water, soil, soil organisms and decay organisms which can only partially be investigated with this type of simple experiment. The relationship must be investigated under more controlled conditions in order to determine the quantitative significance of nitrogen-fixing bacteria to the decay organisms.

If the rate of AR is converted to a value of nitrogen fixed (See Section 2 for discussion) then the nitrogen increase of the block after 73 days will be 0.02% as a result of the activity of nitrogen fixing bacteria. The significance of this value remains to be assessed.

The sealant once more is shown to be effective in preventing the ingress of water and organisms through the sealed faces, although it may still adversely affect the natural, realistic behaviour of wood in soil contact.

1.5.5. Conclusion

Water and wood anatomy affect the colonisation of wood in soil contact by nitrogen fixing bacteria. AR activity was recorded in partially sealed, buried Beech blocks when their MC was greater than 50%, and in Scots pine was recorded after 18 days in the wood where the MC was greater than 100%. The activity in Scots pine increased up to 79 days, with no further increase in MC, but remained at a lower level in the Beech blocks. A loss of activity in the Scots pine was perhaps due to the drying out of the soil. The implication of these results is that there is a relationship between soil, wood, water and organisms which deserves further investigation.

1.6. Summary of Section 1

The experiments of Section 1 have shown that nitrogen-fixing bacteria can occur at depth in Scots pine in soil contact, that their penetration is affected by the anatomy of the wood and that their activity is affected by both soil moisture content and wood moisture content.

The next step in the investigation of the significance of nitrogen-fixing bacteria to timber decay in soil contact was to determine their occurrence in different timber species under different soil moisture conditions and to obtain further information on their distribution within wood, the effect of MC upon their activity, and the effect of that activity upon the decay of wood.

2. MEASUREMENT OF NITROGEN FIXATION IN DECAYING WOOD

2.1. INTRODUCTION

This section is concerned with the measurement of the rate of acetylene reduction in decaying wood, and how this value relates to the amount of nitrogen fixed by micro organisms in the wood. The background to the acetylene reduction (AR) technique and some of its general characteristics are briefly described. The procedure for the measurement of AR rate in decaying wood is both described and evaluated. The measurement of nitrogen fixation using the ^{15}N isotope technique and the problems associated with it are described. The technique applied to decaying wood is evaluated. The experiments involving calibration of AR against nitrogen fixation are described and discussed.

2.1.1. Background to the measurement of nitrogen fixation

The fixation of nitrogen by organisms is not quantified easily. The measurement of total nitrogen by Kjeldahl digestion is laborious and insensitive: it cannot distinguish a nitrogen increase caused by the fixation of atmospheric nitrogen gas from the uptake of combined nitrogen from the surroundings. The use of isotopically enriched nitrogen gas allows these two sources of nitrogen to be distinguished and is 1000 times more sensitive than Kjeldahl analysis. However, enriched compounds are expensive, considerable expertise and equipment is required to generate uncontaminated ^{15}N gas, to expose organisms to it, and the subsequent procedure to measure ^{15}N enrichment and thus nitrogen fixation is laborious and requires access to an expensive mass spectrometer which is working at the limits of detection. It is thus fortunate that the nitrogenase enzyme which catalyses the reduction of N_2 to NH_3 in the presence of a suitable hydrogen donor system, also catalyses the reduction of acetylene to ethylene. The reaction is irreversible, quantitative and is the basis of the AR assay for nitrogen fixation which has been widely used since its suggestion by Schollhorn and Burris (1966) and Dilworth (1966).

The technique was described by Postgate (1972) and reviewed by Hardy, Burns and Holsten in 1973. The advantages of using AR as a measure of enzyme activity is that it is 1000 times more sensitive than the ^{15}N technique, the product is a gas and is stable, allowing non-destructive sampling, and can be analysed rapidly and quantitatively using a simple and inexpensive gas chromatograph. Its disadvantages are that acetylene is explosive, the handling of the gases must be quantitative and ethylene can be produced from sources other than the reduction of acetylene.

2.1.2. Characteristics of Acetylene Reduction

Only nitrogen fixing organisms reduce acetylene to ethylene and nitrogenase is the only known enzyme to reduce acetylene to ethylene. The product of the reduction is ethylene, which does not inhibit further acetylene reduction and is not further reduced. The reaction is inhibited by carbon monoxide.

Theoretically, the rate of AR can be divided by 3 to determine the rate of nitrogen fixation as 2 electrons are involved in AR, and 6 electrons in nitrogen fixation.

$$N_2 \xrightarrow{6e} NH_3$$

$$C_2H_2 \xrightarrow{2e} C_2H_4$$

However, experimentally determined conversion factors vary considerably, from 3.6 for nitrogenase in vitro to 4.3 for bacteria and up to 25 in anaerobic soils (Hardy, Burns and Holsten, 1973). Each particular AR system must thus be calibrated using ^{15}N incorporation (Rennie, Rennie and Fried, 1977).

A number of factors affect the rate of AR and also the conversion factor (Bergersen, 1970). Because no nitrogenous products are formed during AR, their shortage may restrict growth of the organism during prolonged incubation. Similarly, there can be no feedback inhibition of the nitrogenase system, leading to overestimation of the nitrogen fixation rate calculated from the AR rate. The presence of combined nitrogen can cause the diminution of nitrogenase synthesis, nitrogen fixation and AR. Temperature has its usual effect upon enzyme catalysis;the optimum temperature is within a range from 4°C to 37°C. Light is obviously critical when photosynthetic organisms are involved. Oxygen tension is most important, as nitrogenase is inactivated by oxygen. Nitrogen fixing organisms are adapted to prevent permanent inactivation in their normal environment and ideally the assay conditions in vitro reproduce the normal conditions in vivo. Acetylene concentration is not particularly critical, as acetylene inhibits nitrogen fixation and 2-10% v/v is sufficient to saturate the enzyme. Water affects AR rate because dehydration affects the organism, while saturation can reduce gas diffusion rates

A major problem is the production of ethylene from sources other than acetylene. It is commonly produced by plants, fungi and bacteria in amounts which could be confused with low rates of AR. Care must be taken to ensure that the ethylene is being produced by AR alone.

Thus to use the AR technique for the estimation of nitrogen fixation, the tissue suspected of possessing nitrogenase is incubated with acetylene in an appropriate atmosphere and ethylene is assayed after a suitable time. The rate at which the tissue would fix nitrogen under similar conditions can be calculated. Although simple in concept and principle, it rapidly became apparent that considerable care must be exercised to achieve accurate, quantitative results, and that it was necessary to evaluate how the AR technique could be applied to wood from soil contact.

2.2. ACETYLENE REDUCTION IN WOOD FROM SOIL CONTACT

The aim was to determine whether the rate measured by the technique was a true measure of the rate occurring in wood and whether the rate of AR varied in the same piece of wood at different times. Also, what factors might affect that variability, such as temperature and gas atmosphere, with the intention of modifying the procedure to reduce variability. The final aim was to calibrate the derived AR procedure against ^{15}N uptake, so that it would be possible to convert AR rates to rates of true nitrogen fixation.

2.2.1. The Variability of Acetylene Reduction

Introduction

The aim was to examine the variability of AR rate within one wood sample.

Materials and Methods

The individual AR rate of 50 "sticks", taken from a block of Scots pine sapwood exposed to soil contact for 81 days, was determined.

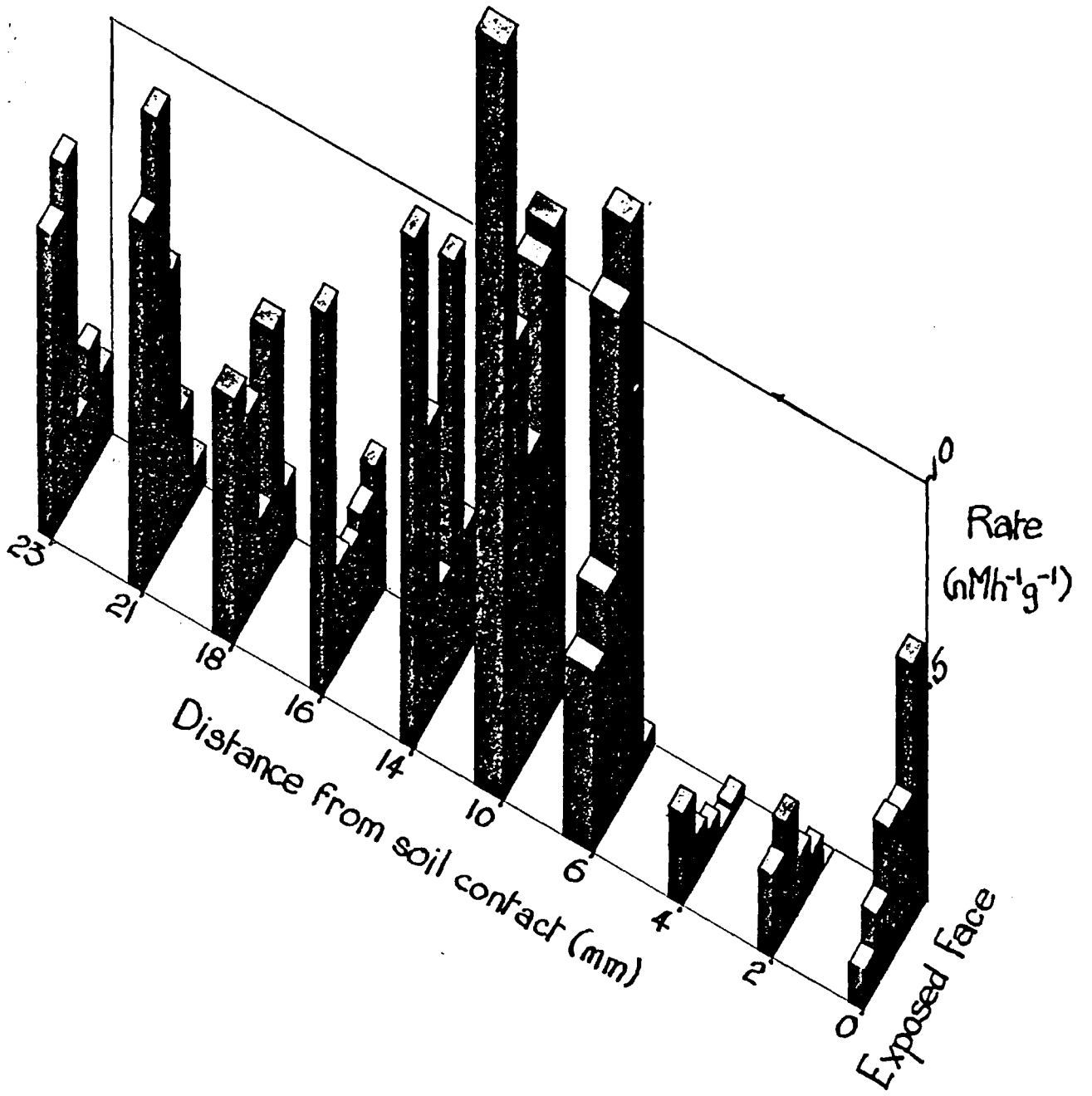
Results

The results, given in Section 1.3.3. are presented again in Figure 7. The AR rate was very variable , even between adjacent segments, ranging from 0.0 to 1.81 $nMh^{-1}g^{-1}$.

Discussion and Conclusion

The variability in AR rate within one block of wood and between adjacent segments is perhaps a reflection of the disjunct distribution of nitrogen-fixing bacteria within the wood. A sizeable colony of a million individuals may only occupy $1.57 \times 10^{-6} cm^3$, a tiny fraction of the wood sample volume. The activity of that colony may vary, being more active in optimal conditions and less active under limiting conditions. Those conditions may vary throughout the wood and also vary with time. It is obviously of importance to investigate those conditions which cause variation in AR activity, both in space and time.

Figure 7 Acetylene reduction rate in slices and subsamples of a Scots pine block after exposure of one tangential face to soil for 81 days.



2.2.2. The Variation of Acetylene Reduction rate with Time

Introduction

The aim of this experiment was to determine how the AR rate measured in wood samples from soil contact varied during the assay period of 24 hours.

Materials and Methods

The amount of ethylene produced from Scots pine wood samples taken from soil contact after 73 days exposure (section 1.5) was measured after $2\frac{1}{2}$, $4\frac{1}{4}$, 6, $7\frac{1}{2}$ and 24 hours incubation in the presence of acetylene.

In addition three samples from Scots pine blocks exposed to soil contact were analysed for AR rate at 30 minute intervals from 21 to 30.5 hours. One sample was from the segment with the radial face exposed, after 120 days exposure (section 1.4). The other samples were from the exposed and unexposed segments of blocks with two tangential faces exposed to soil for 59 days (section 1.5).

Results

The results are shown in Figure 8. The amounts of ethylene produced were converted to nM $h^{-1}g^{-1}$ for the 0-$2\frac{1}{2}$ (Histogram A), $2\frac{1}{2}$ to $4\frac{1}{4}$ (B), $4\frac{1}{4}$ to 6 (C), 6 to $7\frac{1}{2}$ (D), and $7\frac{1}{2}$ to 24 (E) hour periods. The hourly rate was calculated from the $7\frac{1}{2}$ hour ethylene concentration alone (Histogram F), and from the 24 hour reading alone (Histogram G). The average of the five measured rates is shown in Histogram H.

Figure 8

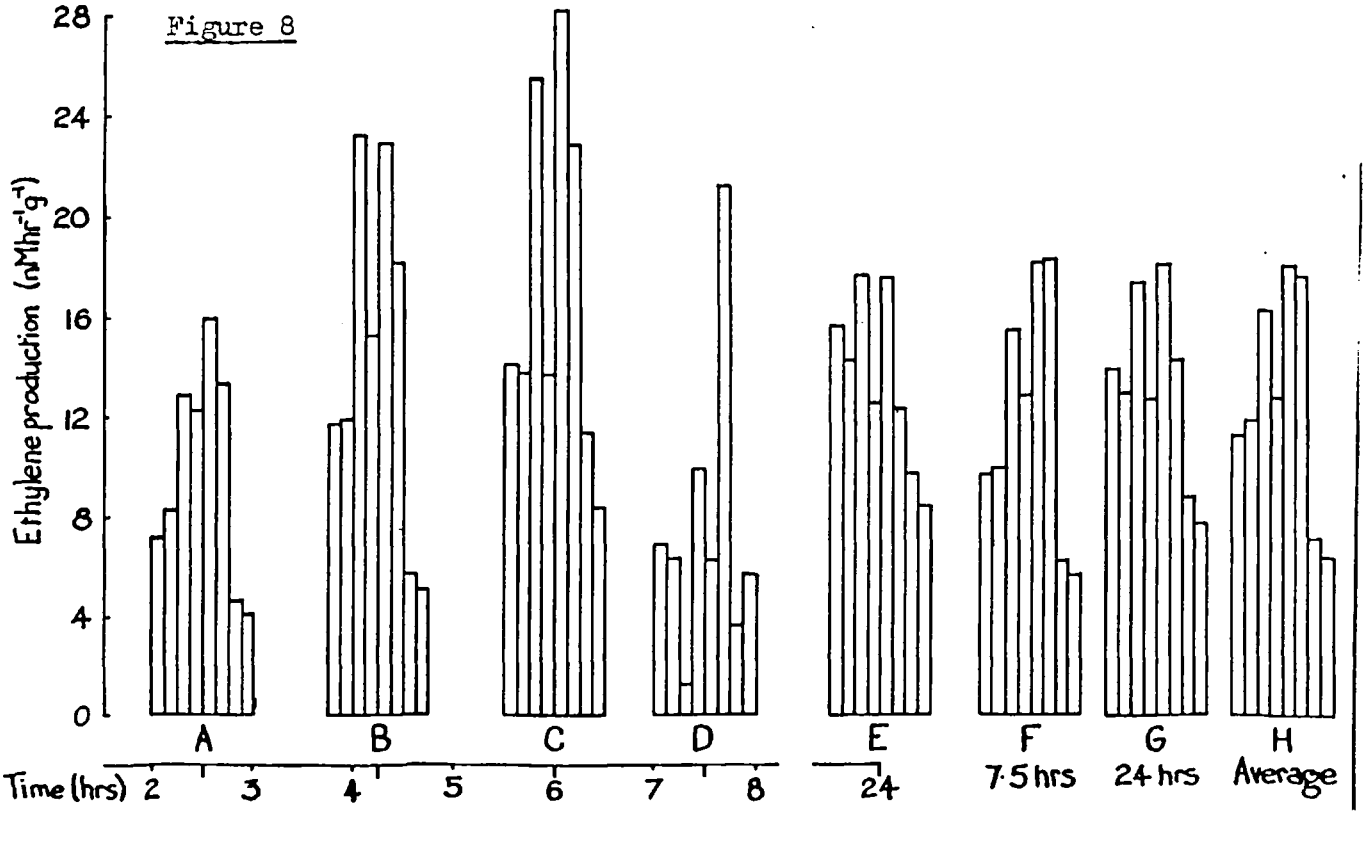
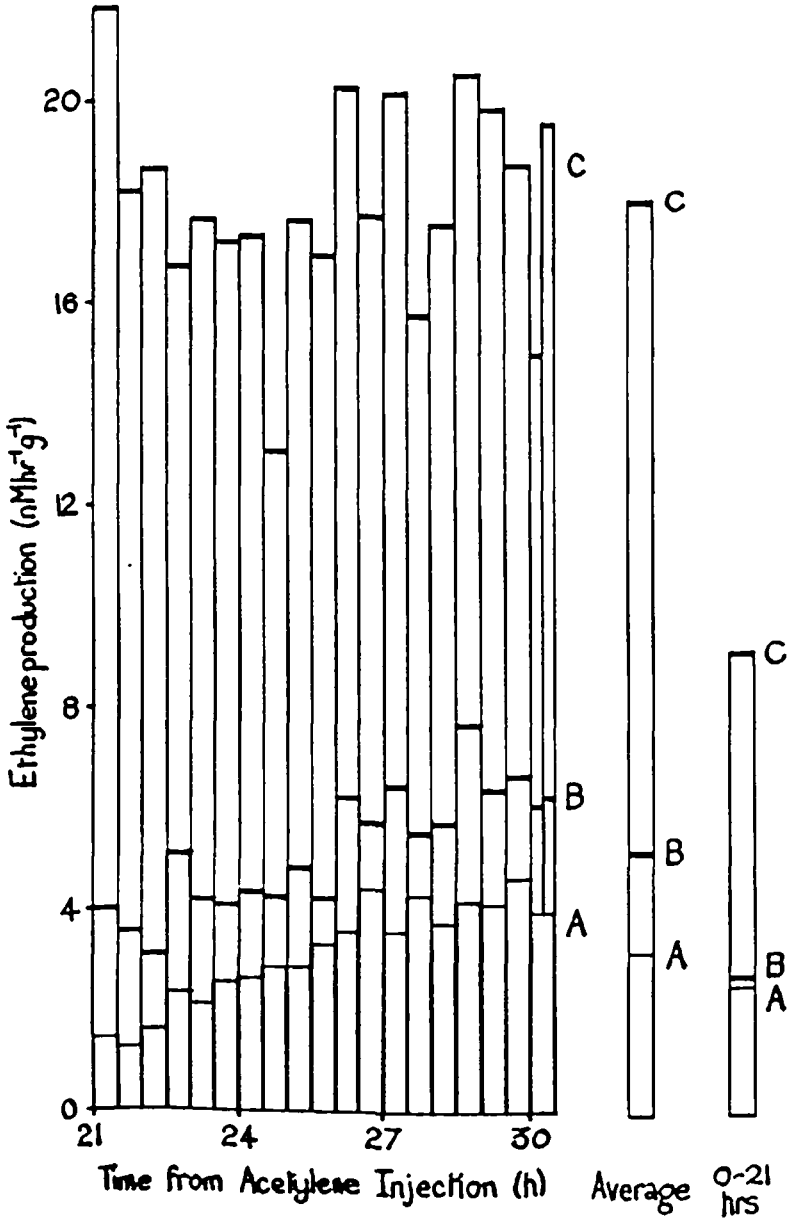


Figure 9 Ethylene production in samples of Scots pine sapwood after soil exposure. Samples A,B and C analysed at 30 minute intervals from 21 to 30·5 hours after acetylene injection.



These results showed that the AR rate in wood from soil contact varied with time, e.g. Sample 3 increased in rate from 13 to 23 to 25 nM $h^{-1}g^{-1}$ in the first 6 hours, dropped to 1 in the next $1\frac{1}{2}$ hours and increased to 17 nM $h^{-1}g^{-1}$ in the next $16\frac{1}{2}$ hours. The other samples showed a similar, if less dramatic, trend.

Discussion

The calculation of an hourly rate from a single 24 hour reading would appear adequate as a measure of the average rate of AR over the 24 hour incubation period. The variability of the rate within individual samples was not greater than the difference between samples, so that if the samples were ranked in order of activity at each time, the order was broadly similar although the absolute rate of activity differs. The 24 hour reading represented an adequate summary of the rankings over the 5 time periods. It would appear that the order of activity obtained from the 24 hour reading alone could be used to determine a difference in activity between different samples.

The results from the analysis of 3 samples at 30 minute intervals from 21 to 30.5 hours after acetylene injection are given in Figure 9. Once again the rate changed with time. In samples A and B the rate was increasing, while in C it fluctuated considerably about a mean level. Sample C had increased in rate from 0-21 hours as seen from the adjacent histogram giving the rate over 0-21 hours. Despite the fluctuation and variability, the difference between the three samples was maintained.

Conclusion

The 24 hour reading, converted to an hourly rate, was an adequate average, or rather an integral, of the rate during the period of incubation. Despite differences in the rate at different times, an overall difference in rate between samples was determined. From these results, it can safely be assumed that a difference in AR rate between two samples, calculated from a 24 hour reading of ethylene concentration is a reliable indicator of a real difference in activity between the two samples.

2.2.3. The Effect of Temperature on Acetylene Reduction Activity

Introduction

This experiment was designed to determine the effect of temperature upon the AR rate measured in wood from soil contact.

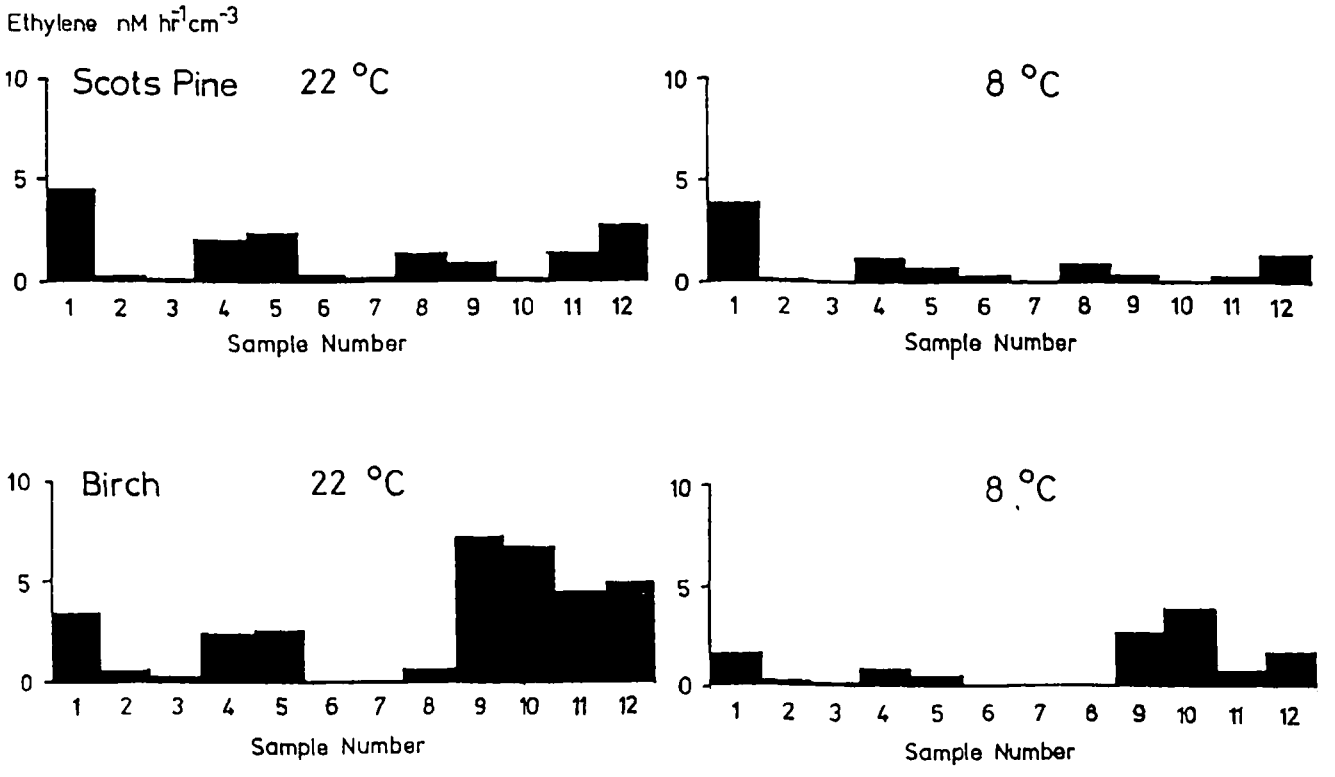
Materials and Method

Blocks of Scots pine and birch exposed to soil contact at 45 % soil moisture content (very wet) for 16 weeks were cut into 12 segments and then assayed for AR rate after incubation at 22°C. The 12 samples were then placed in a refrigerator at 8°C for 24 hours and their AR rate determined once again.

Results

The results are shown in Figure 10. There was in all samples a reduction in rate following incubation at the lower temperature, although the degree of reduction was variable.

Figure 10 Acetylene reduction rate in Scots pine and birch samples incubated at 22°C and then at 8°C.



Discussion

The reduction in AR rate as a result of incubation at a low temperature was expected, as most processes involving enzymes are reduced in rate when the ambient temperature is lowered. However, the reduction in rate was not the same in each sample, again reflecting the variable and heterogeneous nature of the AR activity of the bacteria in the wood. Perhaps a localised, highly active colony of bacteria would be less affected by a fall in temperature than a diffuse colony of less active bacteria. The activity may be less affected by low temperatures if the bacteria are well supplied with nutrients or have energy reserves, or perhaps the differences in the effect of lowering the temperature is due to a reduction in the rate of gas diffusion to the organisms. No more information is available from this simple experiment to explain the difference in response in different samples.

Conclusion

The conclusion from these results is that AR rate is reduced at low temperatures, that temperature affects AR rate, and that any assay of AR rates should be carried out at a constant, defined temperature.

2.2.4. The effect of oxygen concentration on Acetylene Reduction Activity

Introduction

This experiment was designed to examine the effect of oxygen concentration upon AR rate. The nitrogen-fixing bacteria are presumed to be anaerobic (see section 2.1) so that the presence of oxygen should inhibit AR activity.

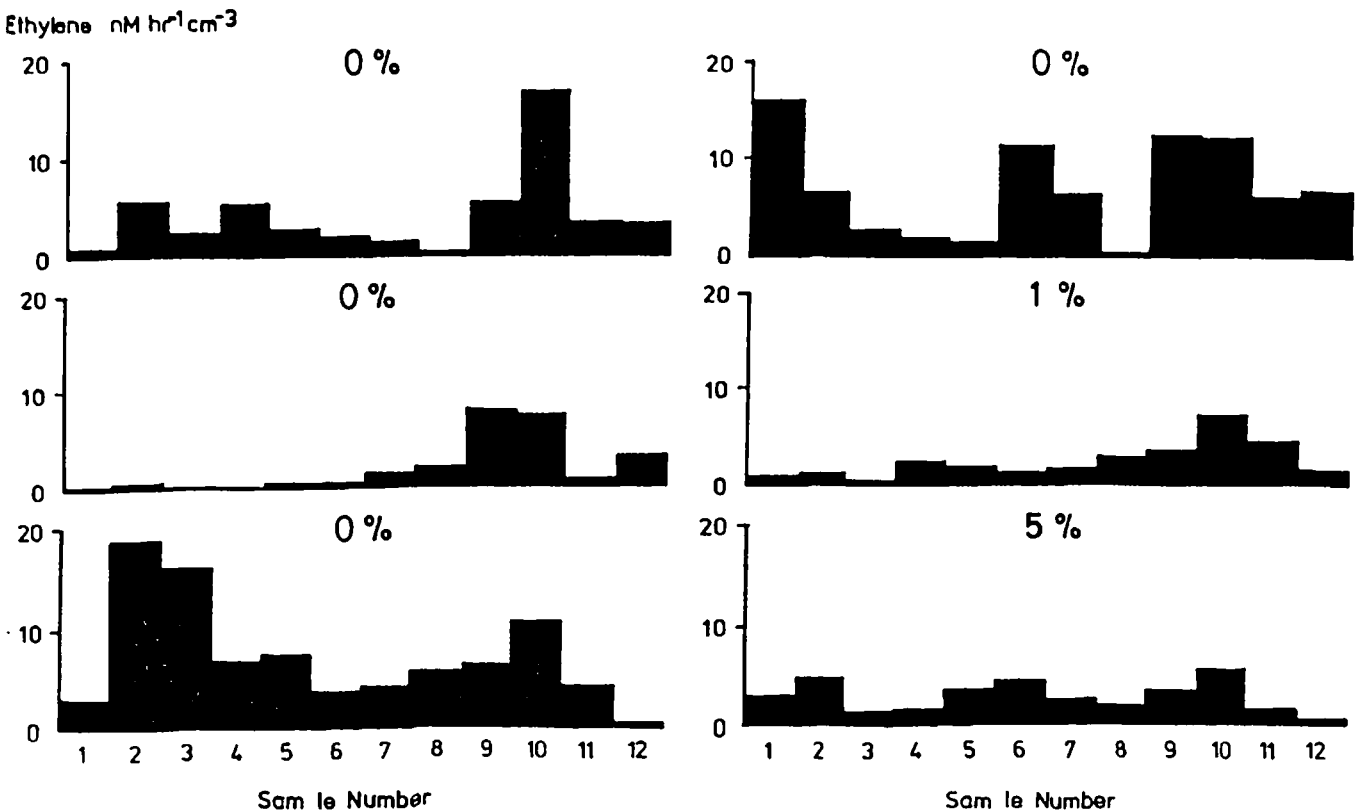
Materials and Methods

Samples of Scots Pine sapwood, exposed to soil contact at a soil moisture content of 45% (very wet) for 112 days, were assayed for AR rate in an Argon (90%)/Acetylene (10% v/v) atmosphere. One set of 12 sample vessels was then evacuated and refilled with argon once again, one set with 1% oxygen in nitrogen, and one set with 5% oxygen in nitrogen. The AR rate was then measured.

Results

The results are shown in Figure 11, where the rate of AR in those samples which were refilled with argon increased, but not uniformly. In the samples refilled with 1% oxygen, some samples increased their rate, and some decreased. In those samples refilled with 5% oxygen, the rates of AR decreased.

Figure 11 The effect of oxygen concentration on acetylene reduction activity in Scots pine sapwood.



Discussion

The effect of 5% oxygen was as expected, a decrease in AR rate. However, the rate was not zero, implying that some organisms remained active or were still active in the presence of 5% oxygen. This may have been due to the low rate, or perhaps even the absence, of oxygen diffusion into parts of the wood.

The results for 0% and 1% are not expected. One interpretation is that to achieve 0% oxygen is difficult and in the presence of wet wood, 0% oxygen must be a nominal value, and some oxygen may remain in the vessel after evacuation and refilling with argon. Conceivably its concentration may be as high as 1%, so that histograms A and D, and B and E represent changes of AR with time, rather than changes in rate due to oxygen concentration; the gas atmosphere in the four sets of samples may have been similar. Alternatively, as with temperature in the previous experiment, the response of the organisms may have differed. Highly active localised colonies may have been inhibited sooner and more effectively than less active or diffuse colonies, which may explain why these segments with high rates decreased their AR rate, and those with low rates increased activity.

Conclusion

Oxygen concentration affected the AR rate of wood samples from soil contact, although the response was variable. The evacuation of the vessels should be uniform to achieve a reliable, uniform gas atmosphere of known constitution. The measured AR rate will then be a reliable indicator of the presence of anaerobic organisms able to reduce acetylene to ethylene.

2.3. THE DETERMINATION OF ACETYLENE REDUCTION RATE

2.3.1. Equipment

The Pye Unicam 104 gas chromatograph was fitted with a gas flow controller for two column operation and two flame ionisation detectors. The two glass columns were 1m x 6mm i.d., packed with "Poropak R", 100-120 mesh. The carrier gas was oxygen free nitrogen flowing at 60mlmin^{-1}, and the ionisation detector was supplied with hydrogen flowing at 60 mlmin^{-1} and air at 600 mlmin^{-1}. The oven was maintained at 50°C. Under these conditions the retention time for ethylene was approx. 1¼ mins and for acetylene 1½ mins. A peak after 15 seconds in aerobic incubation was identified as methane, and a negative peak after 1⅓ minutes was identified as carbon dioxide.

The usual arrangement is for the output of columns A and B to be connected together but in opposition, so that any background signal from the column packing is automatically subtracted from the signal coming from the column into which the test sample is injected. This instrument was modified by the insertion of a switch to enable either column A or column B to be connected to the amplifier. This allowed the next analysis in the second column while the acetylene, with a relatively long retention time and high concentration (producing a long 'tail' on the chart record) came off the first column. This arrangement allowed the analysis of up to 50 samples per hour.

The output from the amplifier was recorded on a Leeds and Northrup Speedomax "W" pen recorder with a chart speed of 25 mm min^{-1}.

2.3.2. Calibration

The instrument was calibrated using ethylene standards: 0.65 ml of 99% ethylene (B.O.C. Ltd) in a glass bottle of known volume (298 ml), or by the direct injection of 1 ml of a specially prepared gas mixture (B.O.C. Ltd) of 117 vpm ethylene in nitrogen. A 1 ml sample from the former gave a peak height of 57 tenths on an attenuation of 1 x 10^4, and was equivalent to 1 nM of ethylene. A careful and exhaustive investigation of the factors affecting sensitivity revealed that hydrogen gas flow rate was critical and could profoundly affect the sensitivity of the instrument. A change in flow rate from 60 ml min^{-1} to 50 ml min^{-1} reduced the peak height for an ethylene standard from 8800 units to 7400 units. Throughout this work the instrument was calibrated before and after any analyses. The instrument had a linear response to ethylene concentration but was susceptible to contamination, resulting in instability and zero drift.

2.3.3. Analysis

Quantitative gas handling was achieved by the use of Gillette Scimitar 1 ml disposable plastic syringes and 23G needles. With a smear of silicone grease around the piston and the luer fitting, and with a rubber band looped around the needle and over the syringe body, these syringes proved adequately accurate and gas-tight. Gas samples could be stored, prior to analysis, by the insertion of the needle into a rubber bung, although the plastic syringe body was able to adsorb gases over an extended period of time. A 0.5 ml sample was removed from the incubation vessel after mixing the atmosphere by "pumping" the piston a number of times. The sample time was recorded on the chart roll together with the sample number. The recorder chart feed was switched on.

The sample was then injected into the column through the rubber septum in the injection port. This septum was prone to leakage and care was taken to insert the needle through the same hole in the septum if possible. Leaks were detected with a rubber-tube connected to a bubbler held over the injection port. Septa were punched from the rubber wads inside McCartney bottles with a No. 6 cork borer.

The amplifier attenuation was set on range 10, giving a stable baseline. The first peak was ignored. The ethylene peak came off after $1\frac{1}{4}$ minutes and as the chart recorder pen neared fullscale deflection the attenuation was switched to the next range and repeated until the pen reached its maximum excursion. The attenuation range was recorded on the chart record and the attenuation turned to 10×10^4 for the acetylene peak which at a concentration approaching 10% of the gas volume, was much higher than the product concentration of ethylene. After the acetylene peak, which served as an internal standard for the detection of major leaks from the vessel and column, the second column was connected to the amplifier and recorder, and the next sample injected into this column. Care was taken to ensure that the injection was into the correct column, that the column was connected to the amplifier and that the column into which each sample was injected was recorded. In these investigations, odd numbered vessels were injected into column A, and even numbered vessels into column B. The two columns were not entirely independent, and a part of the signal of the disconnected column was detected by the connected column, causing a falling baseline as the "tail" of the acetylene peak came off the disconnected column. This was no serious problem and was allowed for in the calculation of the ethylene concentration.

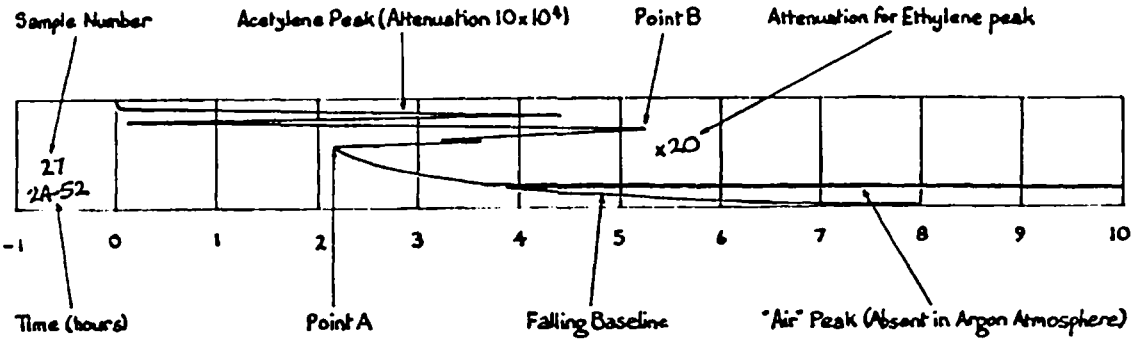
The chart record was stored and the ethylene concentration and acetylene reduction rate calculated later.

2.3.4. The calculation of ethylene concentration

Ideally, the pen was zeroed on the baseline and the attenuation was set before injection of the sample. The peak height could then be measured directly and multiplied by the attenuation to give the peak height in units. However, because samples were injected in rapid succession, the baseline could not be zeroed before injection and the attenuation could not be preset. The peak height could not be measured directly as the baseline was not zero, and thus the calculation of peak height in units was not direct.

A typical chart record for one analysis is shown in Figure 12.

Figure 12 Chart Record from Acetylene Reduction Analysis



The chart was 11 inches wide, subdivided into inches and tenths. One tenth was one "unit". The number of tenths from the baseline to the point at which the pen began the ethylene peak (Point A) was measured (22 tenths). This was the position of the baseline on x 10 attenuation. The ethylene peak height was 53 tenths to point B on x 20 attenuation. However on x 20, the baseline would have been (10/20) x 22 tenths. The baseline on x 20 would have been 11 tenths and the actual ethylene peak height 53-11= 42 tenths. This was multiplied by the attenuation to give 840 units. The baseline correction obviously became less important as the attenuation increased, or if the point A was arranged to be close to zero.

These calculations were made easier by the use of tables generated from the specially written computer programme ARDATA (see Appendix 1). The baseline position and attenuation were read off the tables and subtracted from the ethylene peak height times the attenuation to give the number of ethylene units produced.

The ethylene concentration in the vessel was given by

$$\text{Ethylene concentration in vessel (in nM)} = \frac{\text{Units} \times \text{Vessel volume}}{\text{number of units equivalent to 1nM} \times \text{Gas sample volume}}$$

The number of units equivalent to 1nM was usually approximately 6000, and the sample volume usually 0.5ml. Because vessel volume entered the equation and inaccuracies in estimating the vessel volume would cause inaccuracies in the final estimate of ethylene concentration, the volume of each vessel used was determined. The McCartney bottles were weighed clean dry and empty with a Suba-seal closure. The vessel was completely filled with water and reweighed, from which the vessel volume was calculated. The range of variation in vessels of nominal 28ml capacity was from 26.09 to 28.47ml with a mean of 27.13 and standard deviation of 0.72 (100). No allowance was made for the volume of the wood in the vessels as it was relatively small and varied relatively little in comparison with the vessel volume. The sample volume was usually 0.5ml, a compromise between a volume large enough to contain a measurable quantity of ethylene and small enough to avoid the withdrawl of too large a proportion of the gas atmosphere in the experimental vessel, reducing the gas concentration.

2.3.5. Summary

The analysis of AR by gas chromatography was rapid and sensitive. Typically 100 samples were analysed in 2 hours by this routine procedure every week, and some 11000 samples analysed in all. Each phase of the analytical procedure was investigated experimentally to improve the efficiency, accuracy and precision of the measurement of acetylene reduction rate.

The next section is concerned with the calibration of AR rate against nitrogen fixation rate determined by the use of isotopically labelled nitrogen.

2.4. NITROGEN FIXATION: THE USE OF ISOTOPICALLY LABELLED NITROGEN

2.4.1. Introduction

The amount of nitrogen which has been fixed by an organism can be measured by using isotopically labelled nitrogen, specifically ^{15}N (Burris and Wilson, 1972).

Nitrogen atoms may have an atomic mass of 13, 14, or 15 depending upon the number of neutrons in the nucleus. The isotope ^{13}N is very unstable having a very short half-life, and its natural occurrence is insignificant. When nitrogen gas is generated from combined nitrogen, the nitrogen molecules are formed by the random collision of atoms of ^{14}N and ^{15}N. Thus the gas is composed of molecules of masses 28, 29 and 30:

$^{14}N - ^{14}N$: Mass 28 $^{14}N - ^{15}N$: Mass 29 $^{15}N - ^{15}N$: Mass 30

If the combination is random, then the proportion of each type of molecule in a sample of nitrogen gas depends upon the proportion of ^{15}N to ^{14}N atoms in the sample. If α is that proportion of ^{15}N atoms then the proportion of each type of molecule in the sample can be calculated:

Type of molecule	Proportion
$^{14}N - ^{14}N$	$(1-\alpha)^2$
$^{14}N - ^{15}N$	$2\alpha(1-\alpha)$
$^{15}N - ^{15}N$	α^2

The natural occurrence of the ^{15}N isotope is between 0.36 and 0.37%. This is the "atom % ^{15}N", a commonly measured and used value being 0.3663 for naturally occurring nitrogen. If α is 0.3663, then the proportion of molecules with mass 28 is 99.22687%, of mass 29 is 0.7299% and of mass 30, 0.0013%.

The principles and practicalities of the technique have been described by Hitch (1975). It involves the generation of nitrogen gas from a commercially available "enriched" combined nitrogen compound with a higher proportion of ^{15}N atoms than found naturally. The nitrogen gas is then supplied to the organism or tissue suspected of being able to fix nitrogen, where, if fixation occurs, the nitrogen gas is incorporated into the cells of the organism by conversion to combined nitrogen (ammonia, amino acids, protein etc). At the end of the period of incubation, the

organism is harvested and digested, using the Kjeldahl procedure, to convert all the nitrogen containing compounds in the organism to ammonium sulphate, and to remove the carbon as carbon dioxide. The nitrogen content of the digest is usually determined by the conversion of the ammonium sulphate to ammonia (by the addition of alkali), its collection in boric acid buffer and back-titration with hydrochloric acid. The enrichment of the resulting solution can then be measured using the mass spectrometer (for a full description, see Hitch 1975) .

The solution is acidified and evaporated to dryness. The addition of lithium hypobromite solution generates nitrogen gas which is admitted to the mass spectrometer at a constant rate. The molecules of gas are ionised and the positively charged ions accelerated towards a uniform electromagnetic field. In this field the ions move in a circular path, with heavier ions following a line of larger radius than lighter ions. The separated beams of ions fall on collector plates, generating a current. This current is amplified and displayed on a galvanometer whose deflection is proportional to the number of ions hitting the collector.

In the mass spectrometer there are two collectors. The ionisation current and electromagnetic field are adjusted to focus ions of mass 29 on one collector and mass 28 on the other. With a galvanometer connected to each collector, the ratio of the galvanometer deflections is a measure of the ratios of ions of mass 28 to mass 29 in the gas sample.

Now , the proportion of ^{15}N (atom % ^{15}N) can be calculated from this ratio of mass 28 molecules to mass 29 molecules.

$$28/29 \text{ Ratio} = \frac{\text{Proportion of } {}^{14}N - {}^{14}N}{\text{Proportion of } {}^{14}N - {}^{15}N} = \frac{(1-\alpha)^2}{2\alpha(1-\alpha)} = R_{28/29}$$

Now, by dividing by $(1-\alpha)$

$$\frac{(1-\alpha)}{2\alpha} = R \quad \therefore \quad 1-\alpha = R.\ 2\alpha \quad \therefore \quad 1 = R.\ 2\alpha + \alpha$$

By taking out α

$$1 = \alpha(2R + 1) \quad \therefore \quad \alpha = \frac{1}{2R_{28/29} + 1}$$

Similarly, for the 29/30 ratio $\alpha = \dfrac{2}{R_{29/30} + 2}$

and for the 28/30 ratio $\alpha = \frac{1}{1 + \sqrt{R_{28/30}}}$

In unenriched nitrogen gas, if α is 0.003663, the 28:29 ratio is 136 and the 29:30 ratio is 544. Thus from the ratio of the galvanometer current due to mass 28 to that due to mass 29, the atom % ^{15}N can be deduced. If the background "natural" atom % ^{15}N is subtracted, the result is the "atom % ^{15}N excess". It is generally accepted that an atom % excess of 0.015 is evidence for nitrogen fixation by the organism or tissue. The atom % excess can then be converted to a value of µg nitrogen fixed, from the atom % excess of the gas to which the organism was exposed, and the nitrogen content of the digest.

$$\text{Amount of nitrogen fixed} = \frac{\text{atom \% excess } ^{15}N \text{ in the digested sample}}{\text{atom \% excess } ^{15}n \text{ in the gas phase}} \times \text{Total nitrogen in the digested sample}$$

From the length of exposure to the gas, the rate of nitrogen fixation can be calculated.

The accuracy of the technique depends upon the generation and supply of a known gas mixture of known ^{15}N enrichment, the accurate recovery and measurement of fixed nitrogen from the organism or tissue under investigation, and the accurate measurement of the 28:29 ratio. There are numerous problems in achieving the required level of accuracy and precision.

2.4.2. Problems of the ^{15}N isotope technique

2.4.2.1. Expense

A major problem associated with the ^{15}N technique is expense. Enriched nitrogen compounds are expensive at approximately £ 35 per g for 95%, equivalent to £ 140 per litre of gas. Thus although the ideal is a continuous supply of enriched gas to the organism or tissue under investigation, the cost of the gas is prohibitive. The rate of fixation may be low, so to produce the minimum quantity of fixed ^{15}N which can be analysed, a gas of high enrichment or a long incubation period, and often both, must be used. During long incubations of days or even weeks the environment in the exposure vessel may change and the organism may deteriorate, which is to be avoided if an accurate nitrogen fixation rate is to be obtained. As it is not possible to determine whether the tissue is fixing nitrogen before exposure to $^{15}N_2$ gas, a large amount of material is usually exposed to ensure that some of it

is active, and to allow for deterioration during exposure. The volume of the vessel is kept to a minimum to reduce the amount of gas required, and only a portion of the gas phase is occupied by $^{15}N_2$ gas. The balance is usually made up with an inert, inexpensive gas such as helium or argon.

The exposure vessel, quantity of tissue and gas phase is thus a compromise to achieve a measurable fixation rate under realistic conditions with a minimum incubation time and at a reasonable cost.

2.4.2.2. The generation of $^{15}N_2$ gas

The generation and supply of a gas phase of defined composition and known ^{15}N enrichment requires an all glass apparatus under a high vacuum. The chemical reaction which produces nitrogen gas must be carefully controlled to ensure that no ammonia is produced - because organisms exposed to such a contaminated gas could absorb the enriched ammonia directly, giving an enrichment not derived from nitrogen fixation. Oxides of nitrogen and water vapour must be removed by freezing to leave pure, uncontaminated, ^{15}N enriched, nitrogen gas. This can then be admitted into the evacuated experimental vessel containing the organism or tissue and the balance made up to atmospheric pressure with helium, argon, carbon dioxide and oxygen to the desired composition and partial pressure using a manometer.

2.4.2.3. Air Contamination

The contamination of the gas phase by air, which contains 80% nitrogen, by the leakage of even a small quantity of air (obviously containing mainly ^{14}N - ^{14}N molecules) "dilutes" the ^{15}N enriched gas phase. The "atom % excess" of the gas phase is reduced and leads to an underestimation of the fixation rate. It is obviously of importance to prevent air contamination during the generation of the enriched gas. If the experimental vessel is large, and whole, usually moist, organisms or tissues are used, it may not be possible to remove all the air by evacuation. The remaining air contaminates the gas phase and needs to be allowed for in the calculation of the atom % excess of the gas phase. If a sample of the gas phase is removed at the start and end of the incubation, and its 28:29 and 29:30 ratio measured, the air contamination can be allowed for.

Let the 28:29 ratio be R_1, and the 29:30 ratio be R_2
Let the proportion of 28 = A, 29 = B, 30 = C

$$\therefore \quad A + B + C = 1$$

$$\frac{A}{B} = R_1 \qquad \frac{B}{C} = R_2$$

Now R_1 and R_2 can be determined by mass spectrometric analysis of the gas samples so that A, B and C can be calculated. Thus the proportions of masses 28, 29 and 30 in the gas phase can be determined and the proportion of ^{15}N calculated.

$$\frac{B}{2} + C = \alpha$$

2.4.2.4. The Recovery of Nitrogen

The accurate recovery of nitrogen from the experimental tissue after exposure can be difficult, particularly if the C:N ratio is high, as in wood (Uju, 1979). The usual procedure is Kjeldahl digestion involving the oxidation of the tissue with hot concentrated sulphuric acid in the presence of a copper and/or a selenium catalyst, and potassium sulphate to raise the boiling point of the acid. The basic technique is well-established, although there are numerous modifications and adaptations (Bradstreet, 1965), so that a particular method requires verification that good recovery of nitrogen is obtained with minimal contamination by nitrogen in the reagents, and that the long digestion period (up to 50 hours) necessary to remove the carbon from the wood samples does not cause loss of nitrogen. The Markham distillation procedure for the estimation of ammonia is well established but susceptible to inaccuracy (Humphries, 1956). Variables such as steam supply rate, alkali strength, quantity and speed of addition, washing procedure, condensation rate, and collection procedure all need to be optimised for the particular operator and apparatus to achieve reliable, accurate results. Similarly the indicator, titration procedure and end-point are a matter of personal preference. The accurate assessment of nitrogen content is very important and considerable care was taken to ensure that these measurements were as reliable as possible, evaluating and eliminating losses of nitrogen, inaccuracy, and carry over from one sample to the next.

2.4.2.5. The Mass Spectrometer

The measurement of the 28:29 ratio depends upon the accuracy and performance of the mass spectrometer. It is essential that a high vacuum is maintained in the instrument necessitating the use of cold traps, diffusion pumps and rotary vacuum pumps. Considerable damage may be caused by failure of the vacuum system, and elaborate precautions are required to ensure that the instrument shuts down in the event of failure. Electronic

stability is also essential, so that a constant ionising current, accelerating voltage and field current are maintained, together with the accurate and linear amplification and display of the collector current. Leakage of the ambient atmosphere into the inlet system must be eliminated. Under ideal conditions, the AEI MS2 used was both sensitive and accurate. However it was prone to spurious failure of the safety circuitry causing unnecessary shutdown, to vacuum failure and shutdown, to electronic instability and failure, and to leakage of the inlet system.

2.4.2.6. Summary

These problems were overcome thanks to the combined skill, experience and expertise of Dr. J.W. Millbank and Dr. C.J.B. Hitch whose work with nitrogen fixation in lichens using the ^{15}N technique was of enormous value in the identification and solution of technical problems. The procedure adopted in this investigation was that developed and described by Hitch (1975), a modification of a technique described by Ross and Martin (1970). Because the assessment of nitrogen fixation by the ^{15}N isotope technique has not previously been applied to decaying wood, it was necessary to ensure that the mass spectrometer was sufficiently accurate and reliable to measure low enrichments on a large number of samples, that the digestion and distillation procedure would provide a reliable measure of the nitrogen content of wood samples and that the titrated solution would then be accurately analysed for ^{15}N enrichment.

The next 5 subsections describe this evaluation of the ^{15}N isotope technique.

2.4.3. The Accuracy and Precision of the Mass Spectrometer

Introduction

The accuracy of sample analysis and the determination of the isotope ratio was tested by analysing a number of standards containing a known ratio of ^{15}N, and the precision tested by analysing identical standards on different days.

Materials and Methods

Solutions of enriched ammonium sulphate and unenriched ammonium sulphate were made up. These solutions were mixed in different proportions to produce a range of standard ammonium sulphate solutions of known nitrogen content and whose enrichment would be calculated. 0.1ml of each standard was transferred to a 2 dram glass vial (Johnsen and Jorgensen Ltd., London) acidified by the addition of 2 drops sulphuric acid and evaporated to dryness. One set of standards were then assayed for ^{15}N enrichment using the mass spectrometer. For each sample ten readings of the galvanometer deflection due to mass 28 and mass 29 were measured and the average ratio calculated.

The readings were repeated with another set of dried samples on the following day.

Results

Figures 13 and 14 show the mean and standard deviation of the measured 28:29 ratios for each standard plotted against the calculated ratio. There is a reasonable correlation between the measured and calculated ratio, perhaps better on the first day than the second, although the s.d. is less on the second day.

Discussion

The results show that the mass spectrometer can provide a measure of the ^{15}N enrichment. However it is susceptible to long term variability (shown by a difference in the ratio measured for identical samples on different days), and short term variability (shown by variation in the ratio measured in similar samples on the same day). There may be many causes of such variation, including vacuum leakage, electronic instability and inexperience in the operation of the instrument. To eliminate the errors caused by the variability in the instrument, standards must be analysed at frequent intervals. In addition, standard samples can be interspersed with

experimental samples to monitor the performance of both instrument and operator.

Conclusion

The mass spectrometer can measure nitrogen isotope ratios accurately. Precautions must be taken to ensure that accuracy and precision are maintained during the analysis of experimental samples.

Figure 13 Mean and S.D. of the measured 28:29 Ratio of ^{15}N standards against the calculated Ratio. Day 1.

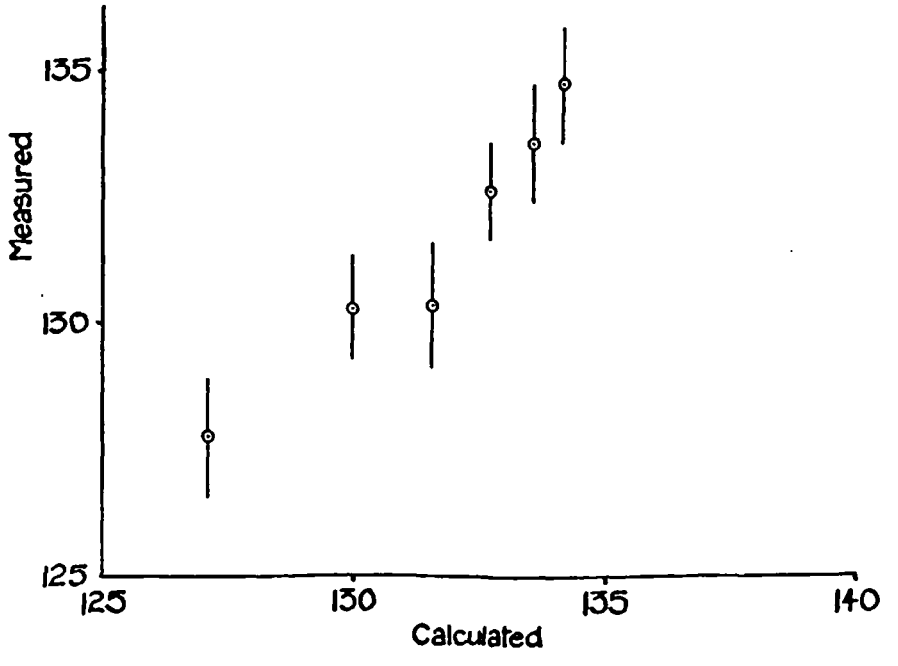
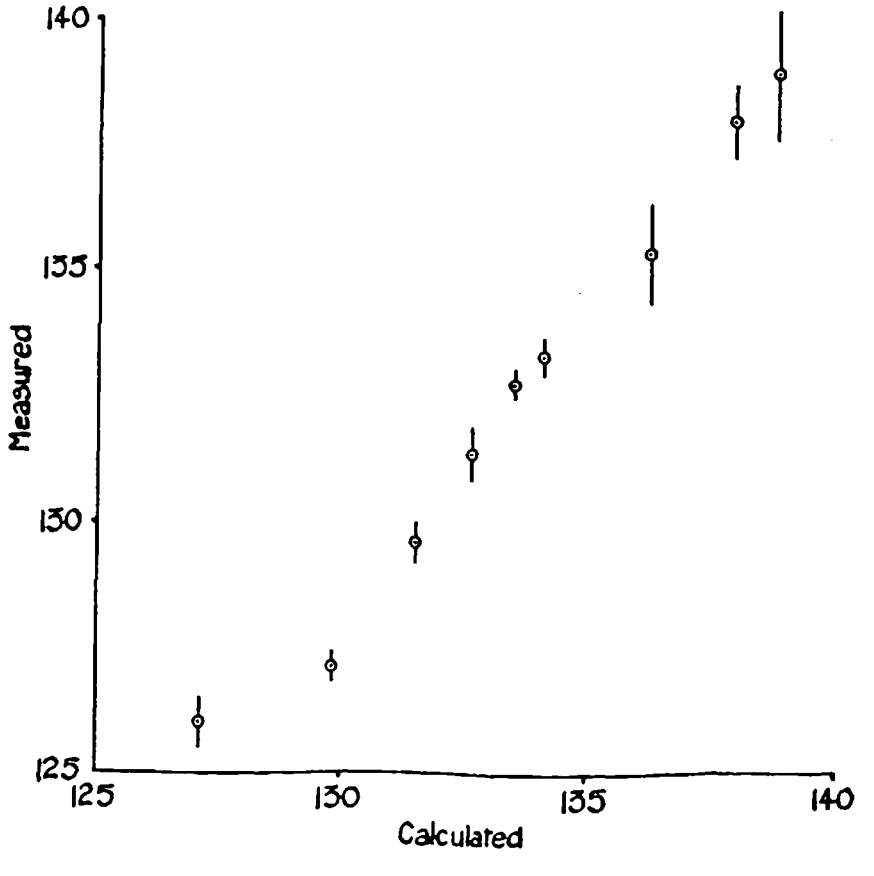


Figure 14 As above. Day 2.



2.4.4. The Accuracy of Nitrogen Content Determination

Introduction

It is essential to have an accurate measure of the nitrogen content of the tissue or organism which has been exposed to ^{15}N gas in order to calculate the rate of nitrogen fixation accurately. The accuracy and reproducibility of the technique can be tested by the analysis of standards containing a known amount of nitrogen in the range to be expected in the analysis of experimental samples.

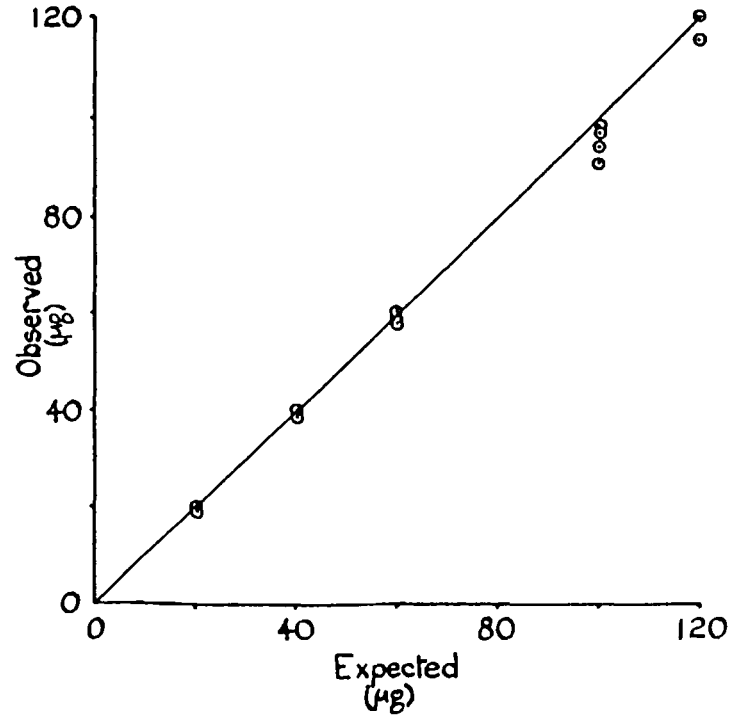
Materials and Methods

4.714g of dry "Analar" ammonium sulphate were made up to 1 litre and then diluted to produce solutions containing 20, 40, 60, 80, 100 and 120 $\mu g\ ml^{-1}$ nitrogen. One ml of each solution was transferred to a test tube, the digestion acid ($10NH_2SO_4$) and catalyst ($CuSO_4.5H_2O + Na_2SO_4$) (Umbreit and Burris, 1957) added, and mixed. The solution was then steam distilled, using the Markham apparatus, into boric acid buffer[*] (see Footnote page 71) The solution changed colour from red to green. After cooling, the solution was titrated against N/28 hydrochloric acid to the first pink tinge. One ml of acid is equivalent to 500 mg of nitrogen (Humphries, 1956).

Results

Figure 15 shows the observed nitrogen content plotted against the expected, known nitrogen content of the standard solution.

Figure 15



They correspond very closely. The deviations are perhaps due to slight differences in the assessment of the end point in different samples. Experience and familiarity were necessary to achieve accuracy with this technique; 300 trial samples were tested before any experimental samples were analysed.

Conclusion

The technique is capable of the accurate determination of the nitrogen content of wood, with care and experience.

Footnote : Indicator for Titration (Humphries, 1956)
6 ml methyl red (0.16% in 95% alcohol)
12 ml bromo-cresol green (0.04% in water)
6 ml 95% alcohol
Turns faint pink at the end point, pH 4.9

2.4.5. The Accuracy of Nitrogen enrichment determination

Introduction

Although the Markham distillation technique can be made accurate for unenriched nitrogen samples, there is the possibility, when analysing enriched experimental samples consecutively, that carry over from one sample to the next may occur, seriously affecting the accurate measurement of the sample's enrichment. This possibility was investigated.

It is generally assumed that ^{15}N and ^{14}N have identical chemical properties, so that there is no preferential loss of either ^{15}N or ^{14}N, as obviously any such preferential loss would affect the measurement of enrichment. The effect of prolonged digestion, necesary in wood samples, was investigated to determine whether preferential loss of ^{15}N occurred, resulting in a reduction in the 28:29 ratio.

After titration, the solution must be dried to a crystalline deposit before analysis in the mass spectrometer, and as heating can liberate ammonia unless the solution is acidic, the effect of acidification on nitrogen loss, and the 28:29 ratio, was investigated.

Materials and Methods

Samples from the solution of enriched ammonium sulphate with a calculated 28:29 ratio of 131.50 were used. Samples of this and an unenriched standard with a ratio of 138.75 were distilled and titrated alternately and the 28:29 ratio of the solution measured after evaporation. In addition the apparatus was flushed with steam for 2, 4 or 8 minutes between enriched and unenriched samples to examine the effect of prolonged washing on carryover.

Results

The results are shown in Table 5. There was no evidence of carryover affecting the measurement of 28:29 ratio in the unenriched samples.

The difference between the calculated ratio and the ratio measured on the mass spectrometer may have been due to error, particularly as the mass spectrometer was particularly unstable during the measurements.

There was no difference in the ratio of acidified and non-acidified samples, although the absolute quantity of nitrogen decreased in non-acidified samples. Although nitrogen had been lost, both ^{14}N and ^{15}N had

Table 5 The accuracy of nitrogen content determination.

Test and Sample Type	Unenriched Ratio Measured (Calc = 138·80)	Enriched Ratio Measured (Calc = 131·53)
Test of Carryover		
1 Enriched		131·08 ± 0·39
2 Unenriched	138·49 ± 0·48	
3 Enriched		130·06 ± 0·43
4 Unenriched	137·82 ± 0·36	
5 Enriched		130·68 ± 0·39
6 Unenriched	137·56 ± 0·68	
Test of Washing		
1 Enriched - 2 min wash		130·71 ± 0·41
2 Unenriched	138·36 ± 0·67	
3 Enriched - 4 min wash		130·69 ± 0·45
4 Unenriched	138·22 ± 0·52	
5 Enriched - 8 min wash		130·85 ± 0·41
6 Unenriched	138·40 ± 0·21	
Test of Acidification		
1 Enriched - No acid		131·40 ± 0·62
2 " "		130·08 ± 0·33
3 " "		130·44 ± 0·20
4 Enriched - With acid		130·03 ± 0·43
5 " "		130·44 ± 0·27
6 " "		129·89 ± 0·34
Test of Digestion Time		
1 Enriched - 2 hr digestion		132·43 ± 0·69
2 Enriched - 4 hr digestion		131·95 ± 1·62
3 Enriched - 14 hr digestion		132·38 ± 0·52
4 Enriched - 26 hr digestion		132·22 ± 0·60

been lost in the same proportions, and did not affect the overall ratio.

The effect of prolonged digestion on the 28:29 ratio was minimal, with no difference in the 28:29 ratio measured.

Conclusion

There was no evidence of carryover between samples during distillation. Acidification is to be recommended, but is not essential, and prolonged digestion does not affect the measurement of the 28:29 ratio in enriched nitrogen samples.

2.4.6. The calibration of Acetylene Reduction Rate against ^{15}Nitrogen Fixation Part 1

Introduction

In order to calculate the rate of nitrogen fixation from the rate of AR it is necessary to determine the rate of AR and of isotopic nitrogen fixation in the same tissue under the same conditions. Only when the ratio of acetylene reduced to nitrogen fixed has been experimentally determined is it possible to convert AR to amounts of nitrogen fixed. The theoretical ratio is three (section 2.1.2.).

Materials and Methods

Twenty-one slices of wood taken from Scots pine blocks exposed to soil contact for 6 months were assayed for AR rate. The slices were sliced again, separated into 50 McCartney bottles and the AR rate of each determined after 14, 19, 24 and 48 hours. Each slice was then transferred to the ^{15}N exposure vessel described by Hitch (1975). An atmosphere of ^{15}N (20% nominal 95% atom % excess $^{15}N_2$) Oxygen (20%) and argon (60%) was introduced into the vessel and the vessel incubated at 25°C for three weeks.

The slices were then removed from the vessel and their AR rate determined after 24, 48 and 72 hours. They were oven dried, weighed, sliced into 10 sticks and transferred to 1" diam. boiling tubes. The wood was digested. The solution was then steam distilled in a Markham apparatus and the ammonia collected in boric acid buffer. This was titrated against N/28 hydrochloric acid to the first pink tinge and the nitrogen content of the digest calculated (Humphries, 1956). The solution was acidified, partially evaporated on a hotplate, and the remaining solution transferred to a 2 dram glass vial (Johnson and Jorgensen Ltd) before being evaporated to dryness at 80°C. The contents of the vial were then assayed using the procedure described by Hitch (1975), to measure the 28:29 ratio.

Results

In none of the 50 samples was any enrichment detected. The AR rate of the samples was greatly reduced after exposure, implying that the organisms had been damaged during exposure to the ^{15}N, argon, oxygen atmosphere.

Discussion

The lack of any enrichment in the wood slices which exhibited AR may have been due to inhibition by the oxygen in the gas atmosphere (which

can inhibit nitrogen fixation, (section 2.1.2.)) or due to the presence of low concentrations of acetylene in the atmosphere which can also prevent nitrogen fixation by competitive inhibition of the nitrogenase enzyme.

Conclusion

It was necessary to repeat the experiment under anaerobic conditions and using wood which has not been exposed to acetylene before incubation in the ^{15}N-containing atmosphere (See Section 2.4.7).

2.4.7. The calibration of Acetylene Reduction Rate against ^{15}N Nitrogen Fixation Part 2

Introduction

The aim of this experiment was to measure both the acetylene reduction rate and nitrogen fixation rate of the same wood samples. The measurement of the ratio of acetylene reduced to N_2 fixed would allow measurements of AR to be converted to amounts of nitrogen fixed, which would obviously be of importance in the assessment of the significance of nitrogen fixing bacteria to the nitrogen economy and the decay of wood in ground contact.

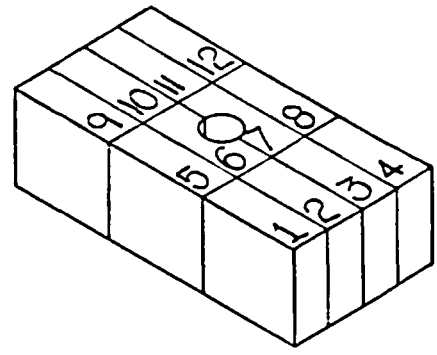
The wood samples were to be incubated in a $^{15}N_2$ atmosphere containing no oxygen (see Section 2.4.6). The AR rate of the sample could not be determined before incubation because nitrogen fixation can be inhibited by acetylene. Due to the variability and disjunct distribution of AR observed previously, it was necessary to expose as much experimental material as possible to $^{15}N_2$ gas in order to ensure that some wood did contain nitrogen fixing bacteria. Some of the material was obtained from the experiment described in Section 1.4. The rest was a number of Scots Pine blocks which had been exposed in a cooling tower for six months. Some blocks were sliced before exposure to $^{15}N_2$, some were left whole and sliced after incubation. The AR rate of the samples was determined after incubation and those exhibiting pronounced activity were subdivided, assayed again for AR rate and then the ^{15}N enrichment determined using the mass spectrometer.

Materials and Methods

Wood samples of Scots pine sapwood, from the experiment described in Section 1.4, were removed after 13 months exposure to soil contact. One block, with part of the transverse face exposed, was sliced, as described in Section 1.4. Each segment was sliced, but the slices were not separated. Each set of slices was transferred to a numbered compartment of a Repli dish (Sterilin Ltd). One additional block of each sealing arrangement was removed and cleaned, but not sliced.

Scots pine sapwood blocks which had been exposed in a cooling tower were also used in this experiment. Six blocks 50x25x15mm, with a 6mm hole drilled through the centre of their 50x25mm faces, were threaded onto nylon cord and suspended in the mist eliminator area of a cooling tower. After six months exposure, the blocks were removed, and one block was sawn into 12 segments as shown in Figure 16. The segments exhibited AR activity when tested.

Figure 16 Segment arrangement in cooling tower blocks.



Three of the blocks were cut into 12 segments. Two blocks were cut into segments, and segments 1,2,3,4,9 and 10 from each were sliced and transferred to the Repli dish. Segments 5,6,7,8,11 and 12 from each were sliced and transferred to McCartney bottles, and assayed for AR. One block was not cut into segments.

Exposure to $^{15}N_2$ gas

The apparatus used for exposure of the wood to $^{15}N_2$ was that described by Hitch (1975), a glass dessicator lid with a neoprene gasket tightly clamped to a 30 mm thick perspex base. A 6 mm hole was drilled in the base, a suba-seal inserted, and a piece of filter paper cut to cover the base. The two repli dishes, containing 50 sliced segments, and the four unsliced blocks were arranged in the base, and, in order to reduce the amount of $^{15}N_2$ required, any excess space filled with aluminium oxide grit (Aloxide TPX30, Carborundum Ltd). The lid was clamped in position and the vessel evacuated before being filled with the gas atmosphere (20% nominal 95% atom % excess $^{15}N_2$) with the balance helium. A sample of the gas in the vessel was taken for later analysis. The filter paper was moistened by the injection of 2 ml of distilled water (degassed by boiling). The vessel was incubated for exactly 11 days at 25°C.

Sampling

A second sample of the gas phase in the vessel was taken at the end of the exposure period and the 50 samples transferred to McCartney bottles for AR assay. The four unsliced blocks were cut into segments and assayed for AR. Those segments exhibiting pronounced AR activity were sub-divided into slices, and each slice transferred to a bijou bottle (nominal 7 ml

capacity) for the AR analysis. The slices were removed after assay, oven dried and weighed again. They were then digested, the digests steam distilled and the nitrogen content determined by titration. The solution was acidified, partially evaporated, transferred to vials and evaporated to dryness before measurement of the ^{15}N enrichment using the technique described by Hitch (1975).

For each sample and a number of standard solutions of known enrichment, six readings of the deflection due to masses 28 and 29 were measured and the average ratio calculated.

Results

The 28:29 and 29:30 ratios of the gas samples taken from the exposure vessel at the start and end of the incubation period were determined and are given in Table 6. Using the equations derived in Section 2.4.1., and assuming that the natural occurrence of ^{15}N is 0.3663, then the atom % excess at the start of the experiment was 93.86 %, and at the end, 72.12 %.

Table 6 ^{15}N Ratios at the start and end of the incubation period.

	28 : 29 Ratio	29 : 30 Ratio	% ^{15}N Excess
Start	0•38	0•07	93•68
End	4•68	0•08	72•12

The % ^{15}N excess of the gas phase declined during the experiment presumably due to the dilution of the supplied atmosphere with the nitrogen of the air contained in the wood blocks. It was assumed that equilibration of the gas inside, and outside, the wood would occur rapidly, and the % ^{15}N excess of the gas phase was assumed to be 72.12 % during the exposure.

Table 7 shows the origin, AR rate, moisture content, nitrogen content, 28:29 ratio, final nitrogen content, nitrogen fixation rate and acetylene reduced:nitrogen fixed ratio of each sample analysed. The AR rate is expressed in nM h^{-1}, nM d^{-1} and nM $h^{-1}g^{-1}$ (obtained by dividing the hourly

rate of AR by the oven dry weight of the wood samples). The nitrogen content of the sample is expressed in µg nitrogen and as a percentage of the oven dry weight of the sample. The 28:29 ratio given is the mean of six estimates of the galvanometer deflections on the mass spectrometer when tuned to masses 28 and 29. If the ratio of the deflections is R_1 the amount of nitrogen fixed in µg during the exposure period of 11 days is given by:

$$\frac{\frac{100}{2R_1 + 1} - \text{Natural abundance of } {}^{15}N \ (0.3663)}{\text{Atom \% excess } {}^{15}N \text{ in gas phase } (72.121)} \times \text{Total Nitrogen content of sample in µg} = \text{Amount of Nitrogen fixed in µg}$$

The amount of nitrogen fixed is divided by the atomic mass of the N_2 molecule (28) to obtain µM nitrogen fixed, then by 11 to obtain µM nitrogen fixed d^{-1}, and then multiplied by 1000 to obtain nM nitrogen fixed d^{-1}. This value is divided into the AR rate in nM d^{-1} to obtain the ratio of acetylene reduced:nitrogen fixed.

Discussion

The profound effect of air contamination in reducing the % ^{15}N excess of the gas phase is apparent from Table 6. Obviously it is essential that the enrichment of the gas phase is measured, rather than assumed, so that its actual ^{15}N excess can be calculated.

In Table 7, in only one sample (4150) was there any AR in the absence of nitrogen fixation. In the remaining samples, measurable nitrogen fixation was accompanied by measurable AR. In one sample (4206) there was no nitrogen fixation and no AR was recorded in this sample. The implication is that AR exhibited by a wood sample is evidence of nitrogen fixation in that sample. The ratio of acetylene reduced to nitrogen fixed was very variable, ranging from 1.01 to 19, 668 with a mean of 1273.97 ± 3638.08 (42), vastly different from the theoretical ratio of 3. In samples with rates of AR less than 0.01 nM h^{-1}, e.g. 4204, 4205, 4207, the average ratio was 1.30 ± 0.50 (3). Samples 4160 and 4212 have ratios of 19,668 and 13,077 respectively, because the amount of nitrogen fixed in these samples is very low (less than 0.0004 µg). Perhaps in these samples, the nitrogen could not penetrate the wet wood during the $^{15}N_2$ exposure, but after removal and slicing, acetylene could reach the organisms, allowing substantial AR. Alternatively,

these two results are anomalous and should be disregarded. For samples with medium rates of AR, between 0.01 and 1 nMd^{-1}, the average ratio was 392.64 ± 509.36 (21). In those samples whose AR rate exceeded $1nMh^{-1}$, the average ratio was 695.48 ± 1060.07 (17) The ratio appears to be close to the theoretical value of 3 at low rates of AR, and much higher than 3 at high rates of AR.

This deviation of the measured from the theoretical ratio has been observed (Rennie et al., 1977). Bergerson (1970), in a paper describing some experiments on the quantitative relationship between nitrogen fixation and the AR assay in soybean nodules and in bacterial cultures, wrote:

"The measurements of the input of nitrogen into ecosystems has been a major difficulty in the measurement of nitrogen balance. It has been hoped that the acetylene-reduction method would provide an adequate measurement for this purpose and it has been used for nitrogen-fixation studies in field situations. Before the potential of the method can be realised it must be established that there is a constant quantitative relationship between these two reactions of nitrogenase and the numerical value of the relationship must be accurately known. If substrate, energy supply, and reductant supply are not limiting, the ratio of the products for equal numbers of electrons transferred should be 1.5:1 according to the reactions:

$$3C_2H_2 + 6H^+ + 6e \longrightarrow 3C_2H_4,$$

or

$$N_2 + 6H^+ + 6e \longrightarrow 2NH_3.$$

The alternative of expressing the relationship as the ratio of nitrogen to acetylene reduced (3:1 above) has been commonly used (e.g. Hardy et al 1973; Klucas et al 1968). Values close to this theoretical value have been obtained by a number of workers with cell-free extracts of nitrogen-fixing organisms but there is little information available from intact living systems. Preliminary experiments in our laboratory indicated that considerable variations in the ratio could be encountered. These may have been due to some fundamental differences between the two reactions. For example, although acetylene is iso-electronic with nitrogen and has similar molecular dimensions, it is about 65 times more soluble in water than nitrogen (at 1 atm and 25°C, acetylene in solution is 41.9mM and nitrogen in solution is 0.64mM). In whole cell systems, fixed nitrogen enters the nitrogen pools of the cells and contributes to protein synthesis,

while acetylene reduction measures the activity of the nitrogenase system only and the product makes no contribution to the metabolism of the cells. The two substrates may also have differing abilities to enter living cells through lipoprotein membranes. In addition, nitrogenase is a fairly unstable enzyme system and failure to match adequately environmental conditions in the acetylene-reduction assay with those of the nitrogen-fixing system in nature may have a differential effect".

Thus although the theoretical ratio may be approached in cell-free systems, in an intact living system such as decaying wood, the ratio may not be 3 and can be variable for any or all of the reasons suggested by Bergersen. In this experiment it would appear that nitrogen fixation is limited, perhaps by feedback inhibition of the nitrogenase by the fixed nitrogen, whereas AR is not limited in the absence of feedback. Presumably the nitrogen fixed by the nitrogenase is metabolised in those organisms capable of nitrogen fixation, being converted to ammonia, amino acids, protein etc. and utilised by the organism during development. The amount of nitrogenase in the organism and its activity is determined by its requirement for nitrogen - if the supply of combined nitrogen is adequate, no nitrogenase is synthesised and no nitrogen fixation occurs. If the supply is inadequate, nitrogenase can be synthesised or "switched on" and fixation occurs if the environmental conditions are suitable. In the presence of acetylene, the nitrogenase enzyme preferentially reduces this compound to ethylene, but it is not metabolised by the organism. Thus no fixed nitrogen is made available to the organism, which presumably still requires combined nitrogen for growth. It may synthesise more nitrogenase or switch on more of the existing enzyme, promoting even more acetylene reduction but no nitrogen fixation. Presumably AR can continue while nitrogenase and energy is available in the organism, even though the organism ceases growth and may even deteriorate in the absence of a nitrogen supply. Despite this simplistic view of the nitrogen physiology of the nitrogen-fixing organism, it can be seen that the rate of nitrogen fixation is adjusted to the demands of the organism, and integrated into the entire process of development and growth. The rate of AR is not related to the biology of the organism, merely to the amount of nitrogenase enzyme present.

Thus in a cell-free system with isolated nitrogenase and no control of the enzyme's activity by the organism, the theoretical ratio of AR to nitrogen fixed is obtained. However in the living organism, where nitrogen fixation is controlled and constrained, while AR is not, the ratios of AR to

Table 7 The ratio of acetylene reduction to nitrogen fixation in Scots pine sapwood after exposure to soil or in a cooling tower.

Sample Number	Origin	Acetylene Reduction Rate			Moist.Cont.	Nitrogen Content		28:29 Ratio	µg N fixed		$C_2H_2 : N_2$ Ratio
		$nM\ h^{-1}$	$nM\ d^{-1}$	$nM\ h^{-1}\ g^{-1}$		µg	%			$nM\ d^{-1}$	
4150	Radial 7	0.67	16.18	5.78	123	81.9	.070	136.0	0.000	0.000	-
4152		1.77	18.58	9.66	122	85.5	.107	134.0	0.006	0.021	884.7
4154	Radial 10	0.17	4.13	1.08	157	101.2	.064	135.4	0.002	0.008	516.0
4155		0.56	13.48	3.23	34	109.7	.063	135.2	0.003	0.010	1348.1
4156		0.23	5.60	1.85	147	77.3	.061	132.8	0.009	0.031	180.7
4157		0.28	6.82	1.63	126	77.6	.068	135.5	0.001	0.004	1705.2
4159	Radial 11	0.01	0.06	0.02	136	94.2	.078	133.7	0.008	0.027	2.4
4160		0.82	19.67	6.43	162	77.0	.060	135.8	0.001	0.001	19668.0
4161		0.58	13.82	11.12	149	48.4	.094	134.1	0.003	0.011	52.4
4165	C.Tower 1 : 6	1.73	41.53	24.90	85	77.0	.111	134.3	0.003	0.010	173.0
4166		2.29	54.96	62.18	70	86.5	.108	121.3	0.053	0.172	319.6
4169	C.Tower 1 : 10	1.27	30.43	10.67	75	136.3	.115	96.3	0.284	0.922	33.0
4170		0.73	17.59	8.40	82	100.7	.115	126.9	0.037	0.119	147.8
4171		0.23	5.46	3.69	72	78.1	.127	81.2	0.266	0.864	6.3
4172		0.19	4.65	2.39	70	91.7	.113	86.9	0.262	0.849	5.5
4174	C Tower 3 : 3	2.49	59.80	25.98	108	92.5	.096	32.9	1.451	4.710	12.7
4176		2.79	67.02	35.98	92	73.2	.094	45.5	0.731	2.374	28.2
4177		2.21	53.00	28.35	101	89.5	.115	131.7	0.015	0.048	1104.2
4181	C Tower 3 : 8	2.91	69.81	30.24	93	98.5	.103	133.0	0.011	0.036	1939.1
4182		1.63	39.02	13.55	92	104.5	.087	53.4	0.814	2.642	14.7
4184	C Tower 3 : 10	1.10	26.52	15.08	92	72.4	.099	134.4	0.004	0.014	1894.6
4185		0.03	0.76	0.35	89	89.5	.098	134.0	0.007	0.022	34.5
4186		0.19	4.56	1.71	89	107.5	.097	131.9	0.017	0.055	82.8
4187		1.66	39.95	14.53	87	105.3	.092	133.7	0.009	0.029	1377.7
4189	C Tower 4 : 1	0.80	19.27	11.15	119	83.8	.116	26.0	1.766	5.732	3.4
4190		0.74	17.87	8.68	101	77.2	.090	129.5	0.019	0.063	283.7
4191		0.74	17.69	9.49	89	97.4	.125	31.1	1.642	5.330	3.3
4192		0.32	7.73	2.37	112	117.1	.086	70.2	0.553	1.797	4.3
4194	Tngtl 1	3.51	84.25	32.66	127	93.4	.087	66.3	0.495	1.608	52.4
4195		6.14	147.43	67.06	121	86.7	.095	96.4	0.180	0.585	252.0
4196		2.25	53.92	22.69	115	63.3	.064	96.5	0.131	0.425	126.9
4197		1.07	25.75	9.31	123	97.7	.085	91.3	0.241	0.783	32.9
4200	Trnsvrse 1	0.77	18.42	9.30	160	80.9	.098	128.8	0.023	0.075	245.5
4201		2.54	60.85	27.17	132	88.4	.095	122.6	0.049	0.159	382.7
4202		0.52	12.52	5.43	131	86.1	.090	114.0	0.084	0.273	45.9
4204	Trnsvrse 3	0.01	0.09	0.04	142	68.8	.077	130.6	0.014	0.046	1.9
4205		0.01	0.02	0.01	138	72.8	.076	133.9	0.006	0.019	1.0
4206		0.00	0.00	0.00	116	80.6	.068	137.4	0.000	0.000	---
4207		0.01	0.02	0.01	123	72.0	.102	133.8	0.006	0.019	1.0
4209	C Tower 5 : 2	1.01	24.31	8.96	149	86.1	.076	134.6	0.005	0.015	1621.0
4210		0.41	9.73	3.09	114	103.8	.079	134.3	0.005	0.015	648.8
4211		2.29	54.84	18.32	140	123.4	.099	135.1	0.004	0.014	3917.3
4212		0.54	13.08	4.75	144	52.3	.046	135.8	0.003	0.001	13077.6

nitrogen fixed can easily deviate from 3. In this experiment the ratio is not 3 and is also very variable. Perhaps in dealing with not only entire organisms, but also presumably colonies and perhaps ecosystems within the wood samples, it is not surprising that the theoretical cell-free ratio is not obtained and that variability is large.

In addition, the different solubilities of nitrogen and acetylene in water may be important in wet wood, where the gases must diffuse through water to reach the organisms within the wood. The higher rate of AR compared to N fixation in some samples maybe partially due to the higher rate of acetylene diffusion to the enzyme.

Finally, the environmental conditions in which the $^{15}N_2$ exposure and AR assay were carried out may not have been identical. Despite the considerable care with which the temperature and gas phase were controlled, there is the possibility that the micro-environment surrounding the organisms may have been very different during the $^{15}N_2$ exposure and AR assay and totally different to the conditions in vivo. As the environmental conditions profoundly affect the rates of fixation and AR, the comparison of two rates measured under different conditions could lead to massive variability.

The implication of this experiment is that AR can be used as a measure of the amount of nitrogenase in the sample, i.e. the assay of an individual enzyme over a short time period, while the measurement of ^{15}N enrichment measures the amount of nitrogen incorporated into the organisms over a period of time i.e. a complete process. Although the enzyme is the same in both reactions and the reactions can be compared theoretically, in whole organisms and in living systems, the enzyme assay cannot be compared directly with a process of development. Thus it appears from this experiment that it is not possible to convert the AR rate to a rate of nitrogen fixation by dividing by 3, or any other factor. The occurrence of AR merely shows that nitrogen fixing organisms are present.

However if the amount of nitrogen fixed, given in Table 7, in samples taken from similar sources is averaged and compared with the average AR rate in the same samples (Table 8), there is some relationship apparent between AR rate and the amount of nitrogen fixed; a high average rate of AR is found in those samples which have the highest average amount of nitrogen fixed, particularly in those samples taken from soil exposure, although the variability associated with the means is large. The large variability caused by adjacent samples having very different amounts of fixed nitrogen

Table 8 Average amounts of nitrogen fixed and average acetylene reduction rate in sliced and unsliced wood samples.

	No. of Samples	µg Nitrogen fixed in 11 days.(Mean)	Acetylene Red. Rate($nMh^{-1}g^{-1}$)
Transverse face exposed (uncut)	3	0·006 ± 0·006	0·014 ± 0·018
Transverse face exposed (uncut)	3	0·052 ± 0·031	13·96 ± 11·60
Radial face exposed (sliced)	8	0·004 ± 0·003	4·53 ± 3·94
Tangential face exposed (uncut)	4	0·262 ± 0·162	32·93 ± 24·68
Cooling tower (uncut)	4	0·003 ± 0·002	3·78 ± 6·82
Cooling tower (sliced)	19	0·418 ± 0·593	16·30 ± 15·37

presumably reflects the disjunct distribution of nitrogen-fixing organisms within the block, which parallels the variability of the AR measurements in Section 2.2.1., and in this experiment. The nitrogen results confirm the pattern and distribution of organisms and activity observed in those experiments using AR alone, e.g. there is a difference in the amount of fixed nitrogen in the two uncut blocks with part of one transverse face unsealed. The sample with the highest amount of fixed nitrogen was from the segment with the exposed face, while the other sample, with the lowest amount of fixed nitrogen, is taken from a segment with no exposed face.

As with AR, the differences in amount of fixed nitrogen cannot be explained by moisture content, which is very similar in the samples (See Table 7), except for those samples from cooling tower blocks; which were once presumably saturated but dried out slightly in transit.

The effect of cutting the blocks before $^{15}N_2$ incubation was particularly noticeable in the cooling tower blocks. The amount of nitrogen fixed in the sliced samples was considerably higher than in the uncut samples, presumably due to the increased ability of the $^{15}N_2$ to diffuse to the nitrogen

fixing organisms in sliced blocks. The AR rate of samples from blocks sliced after $^{15}N_2$ incubation was high and similar to the rate found in samples from blocks sliced before $^{15}N_2$ incubation.

It would appear that the measurement of fixed nitrogen confirms the conclusions obtained using AR alone and that AR rate can be a measure of the amount of nitrogen fixed, despite the variability. The implication is that if, of two wood samples, sliced up similarly and incubated under the same conditions, one exhibits a high rate of AR activity and the other a low rate, then there are two alternatives; either the former has more nitrogen fixing organisms present than the latter, or that the same number of organisms are present in both samples, but the organisms in the former are more active than the latter. To decide which was the case would be difficult and perhaps of no consequence, as the net effect is the same, the former is likely to have fixed more nitrogen than the latter. So, although there is no direct quantitative relationship between AR rate and nitrogen fixation rate, some subjective comparison is possible. At low rates of AR, less than 1 $nMh^{-1}g^{-1}$, the amount of nitrogen fixed per day is around 0.0004 µg, at medium rates from 1 to 10 $nMh^{-1}g^{-1}$, around 0.02 µg d^{-1}, and at high rates greater than 10 $nMh^{-1}g^{-1}$, around 0.05 µg of nitrogen are fixed per day. Although these values are subjective and based on meagre and variable data, they are the only values available of the amounts of nitrogen fixed in wood from soil contact and must be used to assess the significance of nitrogen fixing bacteria to timber decay in soil contact.

If it is assumed that the rate of fixation is maintained despite changes in the environment, in stage of decay, in the growth of the organism and in the development of the ecosystem in which it lives, and that the rates measured in vitro under anaerobic conditions at 25°C are similar to those occurring in vivo, and that the activity is uniform throughout the wood, then the amount of nitrogen fixed in 1g of wood in one day is from 0.0004 to 0.04 µg. However these are large assumptions and this range could easily be optimal rather than actual. Perhaps the actual rate is closer to that observed in the uncut blocks, where the activity is limited by gas diffusion through wet wood. The increase in nitrogen at these low rates is equivalent to an overall increase of nitrogen content of the wood of 0.00004% per day or 0.0146% per year. Although the overall nitrogen content is increased, it is not certain that the fixed nitrogen is available to wood colonising and wood-degrading fungi. Sharp (1975) was unable to show any fungal uptake of nitrogen fixed by bacteria.

The significance of nitrogen fixing bacteria to wood decay in soil contact cannot be assessed from this experiment alone. Their significance depends upon whether they occur naturally in all timbers, how active they are and for how long that activity is maintained. Further work is required to establish the occurrence of these organisms in decaying wood under different natural environmental conditions.

Conclusion

The occurrence of AR in wood samples is evidence of the presence of nitrogen fixing organisms in the wood. The AR rate can be a measure of the amount of nitrogen which is fixed in the wood, although the relationship is subjective and has not been precisely defined. The amount of nitrogen fixed in wood in soil contact by nitrogen fixing bacteria may be of significance to timber decay fungi, and further work is required to establish the occurrence of the organisms in decaying wood in soil contact.

3. A LABORATORY SYSTEM FOR THE INVESTIGATION OF NITROGEN FIXING BACTERIA IN WOOD IN SOIL CONTACT

3.1. Introduction

From the preliminary results of Section 1 it can be seen that nitrogen fixing bacteria are part of a complex process of timber decay, and that to assess their role and significance in the entire process is difficult without further information on their distribution, occurrence and activity in decaying wood. The need for a soil exposure system in which the variables can be controlled or measured is of paramount importance for this type of investigation and could be of value in the testing of preservatives for use in ground contact. There are a number of problems which must be taken into consideration in the design of such a soil exposure system.

3.1.1. Complexity

One method of unravelling a complex web of factors is to control as many variables as possible and to measure the others. One variable can be changed, and the effect upon those being measured can be assessed. In this investigation, the soil type, soil moisture content and wood species were varied independently, and the effect upon wood MC, weight loss and AR rate determined, in an attempt to discover the effect of those variables upon nitrogen fixing bacteria and the effect of those bacteria upon the decay of the wood.

3.1.2. Variables : Water

Water is essential for wood decay. Griffin's review (1977) points out the relationship between wood MC and water potential (see also Section 11), and that the water potential profoundly affects the activity of wood decay fungi and the mechanism of the decay process. Obviously, in view of the results obtained from the experiments in Section 1 and of the general importance of water in timber decay, some measurement of the amount of water in wood is vital.

The usual measure of the amount of water in wood is moisture content:

$$\text{Moisture Content (MC) (\%)} = \frac{\text{Wet weight - Oven dry weight}}{\text{Oven dry weight}} \times 100$$

Although it can be measured accurately and relatively easily, if destructively, there are disadvantages in using this value. Decayed and wet timber may have a MC over 100%, reflecting the large volume of the wood occupied by water relative to the small volume occupied by cell wall material, and percentage values greater than 100% are difficult to visualise. The MC depends

upon wood density, in that two different blocks of different density may contain the same weight, and thus the same volume of water, while their calculated MC would be different, giving a misleading impression of a difference in the amount of water in the two blocks, e.g. a wood block 50 x 25 x 15 mm may weigh 9g at room temperature having a density of $0.48 gcm^{-3}$. A similar block of density $0.5 gcm^{-3}$ would weigh 9.38g. If both contained 4.5g water, then the former would have an MC of 50% and the latter, 48%. The MC is also dependent on weight loss due to decay, e.g. if the block has a weight loss of 10%, although the amount of water may be the same, then the calculated MC would be 55.56%. Expressing the amount of water as a concentration in gg^{-1} wood gives the same result numerically and suffers the same disadvantages.

A measure of the amount of water in wood which overcomes these disadvantages is "water content", the weight of water in a block of wood expressed as a percentage of the block volume.

$$\text{Water Content (WC) (\%)} = \frac{\text{Wet wt of Wood - Oven dry wt of Wood}}{\text{Wet wood volume}} \times 100$$

e.g. the water content of the blocks in the previous example would be 24.00%, irrespective of their density and weight loss. However, water content is dependent on block volume, and the volume increases with moisture content due to swelling, e.g. if the dimension of the dry block in the previous examples increases by 5% in both tangential and radial directions between dry and fibre saturation point (f.s.p.), then the volume increases by 10.25%, and if the amount of water in the block is 4.5g, then the calculated water content would decrease from 24.00 to 21.77%. Nevertheless, despite the slight complications of volume changes during swelling below f.s.p. (around 30% MC) water content would seem a more meaningful and understandable measure of the amount of water in wood than is MC. It allows the comparison of the relative volume occupied by water, cell wall material and air during soil exposure, while water is taken up and decay proceeds, e.g. before exposure, at room temperature and room humidity, the theoretical block may be at around 9% MC and weigh 9.38g. Its volume would be $18.75 cm^3$, having been cut to its final dimensions under these conditions, and at 9% MC it would be about one-third swollen. The water content would be 4.16%, and if the dry cell wall material is assumed to have a density of $1.5 gcm^{-3}$, then it would occupy 30.57% of the block volume. However the density of water adsorbed onto the cell wall is not 1 (Kollmann and Cote, 1968), and varies from 1.28 at 2% MC to 1.11 at 30% MC.At 9% MC its density would be 1.207, so that the volume of the water would only

be 0.65cm^3 and the block water content would be 3.47 rather than 4.16%. If the block is then exposed to soil contact, it takes up water and becomes fully swollen. If the total swelling from the dry state is 5%, the radial and tangential dimensions would have increased by 3.25% during soil exposure and the block volume would now be 19.99cm^3. If the block contained 4.5g water then its water content would be calculated as 22.51%. If there was no decay and no loss of wall substance, then the volume occupied by cell wall material would be the same as before exposure, but now represent 28.68% of the block's volume! If there had been 10% weight loss, then only 25.81% of the block volume would be cell wall material. The remaining volume would presumably be air and water vapour. As the block continued to take up water, its water content would approach a maximum, when all of the air was displaced by water. If no decay occurred, then the maximum water content in this example would be 58.34%. However, any decay and thus loss of cell wall material would allow water to occupy a larger proportion of the block. In this way, water content presents a more meaningful and more easily visualised picture of the relationship between water uptake and decay of the wood.

An additional source of water, other than the environment, is so-called metabolic water. This is water produced as a result of cellulose breakdown by fungi. Griffin (1977) has suggested that the theoretical production of 0.555g water for each 1g of cellulose decomposed may be important as a source of water in decaying wood, e.g. if the theoretical block has a 10% weight loss due to decay, and the decay is assumed to be due to cellulose breakdown alone, then 0.86g cellulose would have yielded 0.48g water. If the block contained 4.5g water after exposure, then 10.66% of the water in the wood is metabolic water. However this is the maximum theoretical yield which may be attained and in the investigation of the effect of moisture content upon decay, metabolic water is usually ignored.

Decay

The usual measure of timber decay is weight loss, given by:

$$\text{Weight Loss (WL) (\%)} = \frac{\text{Oven dry weight before exposure} - \text{Oven dry weight after exposure}}{\text{Oven dry weight before exposure}} \times 100$$

Weight loss is dependent upon wood density, e.g. if theoretical blocks having densities of 0.48gcm^{-3} and 0.50gcm^{-3} decayed, such that 1g of cell wall material was lost from each block, the weight loss of the former would be 11.11%

and of the latter, 10.66%.

The calculation of weight loss does not allow for the weight of micro-organisms within the wood. Those wall components which are metabolised to carbon dioxide and released as gas are measured, but not those which are merely solubilised or incorporated into hyphae and bacteria. Butcher (1975) has advocated the use of leaching by Soxhlet extraction to remove "loosened" material from decayed wood before drying.

An additional factor is a gain in weight due to the uptake of soluble substances in soil water, e.g. if the soil can be assumed to contain a total inorganic ionic concentration of between 1000 and 5000 ppm (Russell 1973), then one cm^3 of soil water would contain between 1 and 5mg of soluble material. If the block takes up 4.5ml of water, its final oven dry weight would be increased between 4.5 and 22.5mg, the weight of the soluble substances in that volume of water. Despite the complications, weight loss is a reliable and accurate measure of the amount of decay which has occurred in a block of wood.

Nitrogen Fixation

The measurement of nitrogen fixation rate in decaying wood by the AR technique has been discussed in Section 2. The technique is considered to measure the rate of AR in decaying wood and to give a measure of the presence of nitrogen fixing organisms, although the amount of nitrogen fixed cannot be estimated using the AR technique in deteriorating wood under the conditions used. The units in which the AR rate is usually expressed depends upon the type of system under investigation: in a cell free enzyme system the rate is expressed in nM $min^{-1}mg^{-1}$ protein, in single cells as nM $min^{-1}g^{-1}$ fresh or dry weight, in whole tissues as $nMh^{-1}g^{-1}$ fresh or dry weight. In this investigation, because the fresh wet weight depended upon moisture content, and dry weight depended on the degree of decay, and were unsuitable, the AR rate was expressed relative to wood volume as $nMh^{-1}cm^{-3}$ wood. This allowed the rate obtained from different sizes of wood sample to be compared directly.

3.1.3. Realism

A laboratory exposure system should be realistic and be completely representative of service exposure. Thus wood blocks, rather than veneers or sawdust, were used, and they were not sealed or autoclaved prior to exposure to natural soil maintained under conditions which could be expected in the field. The blocks were only partially buried, rather than totally buried, to produce a ground line zone, where any failure occurs in timber exposed to soil contact in the field. The blocks were subsampled to allow the comparison of above, at, and below, ground line zones. The timber species used were representative hard and softwoods, selected because of their frequent use in laboratory and field investigations of timber decay. Finally, a field experiment was set up to allow the comparison of the laboratory results with realistic field exposure. Preservative treated stakes were included in this experiment to determine the effect of preservatives upon the nitrogen fixing bacteria under natural conditions.

3.1.4. Variability

Both wood and soil are notoriously variable, in that apparently similar samples treated in a similar way can give different results. In this series of experiments, the soil was taken from a site with known history; the Old Farm Site at Silwood Park, Sunninghill, Ascot, Berkshire, where ground contact exposure stakes were first installed in 1958. Soil from this site has been used extensively at Imperial College, and the range of organisms and performance of timber in this soil well documented. (Butcher 1970; Banerjee and Levy, 1971). The second soil was of a different type, selected for the presence of nitrogen fixing bacteria. Both soils were carefully handled, prepared with a minimum of disturbance, and maintained at a controlled temperature and moisture content. The control of soil moisture content allowed the effect of changes of MC upon the wood and decay organisms to be investigated.

The wood was also carefully selected and prepared to ensure that all the blocks used in an experiment were as similar as possible. The nitrogen content of wood varies from the bark to the centre of a plank (Merrill and Cowling, 1966) and the soluble nitrogen in wood can be redistributed during drying (King, Oxley, Long 1974) affecting its decay (King, Oxley, Long 1976). Thus the edges of the plank were removed and discarded to eliminate redistributed soluble nitrogen from the experimental blocks. Blocks were cut

from the same set of annual rings within a plank, where possible, and the position of each block within the plank recorded. Those blocks with visible defects, such as knots, inaccurate orientation, wavy grain, resin pockets, splits or shakes were discarded. The remaining blocks were randomly assigned between treatments and additional blocks retained as representatives of the different regions of the plank, for later analysis if required.

A further attempt to overcome the problem of variability of wood and soil necessitated the use of replicate samples, the accurate quantitative measurement of moisture content, AR rate and weight loss, and the application of suitable statistical analysis.

3.1.5. Time

Because the sequence of events leading to failure often takes years, to investigate the process in a matter of weeks or months necessitates the acceleration of the sequence. The laboratory experiments were carried out at the unrealistically high temperature of 25°C to accelerate decay. Sampling was frequent to monitor the occurrence and activity of nitrogen fixing bacteria after increasing periods of exposure.

A major constraint in an investigation of this type with a number of wood species, two soils, a range of soil moisture contents, many replicates, each cut into subsamples with a number of measurements being made on each sample at frequent sampling times, is the time involved in collecting and analysing the data. Thus care was taken to streamline the sampling and data recording, and the computer was used extensively in the analysis of the results.

3.1.6. Computing

During the calculation of the initial results using a hand calculator it became apparent that a major problem in this investigation was to be the amount of time necessary to calculate the results from the data, and that the data would have to be analysed using the computer. During the programming of the computer it was realised that the results of four measurements from each of 300 blocks cut into more than 4000 segments would be difficult to compare and contrast, and that the computer could be used to display results in a way which would facilitate their examination and comparison. Having produced the results as graphs, histograms, density maps and three dimensional pictures (See Appendix 1) differences and similarities between segments, zones, blocks

and different wood species at different soil moisture contents could be observed more easily than from numerical data. Any subsequent statistical comparison or data manipulation could be performed fairly easily on the numerical data stored in the computer. Access to the computer has been essential during this investigation, not only for its speed and accuracy in calculation, but also in the display of the results to facilitate examination.

3.2. Materials and Methods

3.2.1. Wood Preparation

Figure 17 shows the procedure adopted for the conversion of the tree to experimental blocks and their exposure to soil contact.

Trees of the required species were felled from the same area at the same time of the year(A), sawn into planks approximately 50mm thick, and kiln dried by a schedule appropriate to the species (B). Planks having no major visible defect and with the bark still attached were selected and the end 20mm sawn off and retained(C). Sticks, 15 x 25mm were carefully sawn from the plank, avoiding the outer surfaces, so that the 15mm dimension was tangential (D). Each stick was then given a code number and the position of the stick in the plank marked on the end section which had been removed. The sticks were then cross-cut into blocks 50mm long, each block being labelled sequentially, as it left the saw (E). Every block then had a unique reference number which identified its original position in the plank and relative to all the other blocks cut from the same plank. Those blocks with a wavy grain, knots, resin pockets and inaccurate orientation were rejected.

3.2.2. Soil

Soil was collected from the upper 150mm of the Old Farm Site at Imperial College Field Station, Sunninghill, Berkshire and passed through a 15mm sieve to remove large roots and stones. The water holding capacity of the brown loam soil was determined using the vacuum method of Savory (1972), and the MC of a sample determined by oven drying. Weighed plastic bins 270 x 197x 100mm (Stewart Plastics Ltd., Purley Way, Croydon), with the lid vents taped to limit evaporation were half-filled with soil, weighed and adjusted to the required MC by the addition of distilled water according to the equations:

$$\text{Water in soil at start} = \frac{(\text{Weight of soil used} \times \text{soil moisture content at start})}{(\text{Soil moisture content at start} + 100)}$$

$$\text{Water required} = \frac{(\text{Required soil moisture content} \times (\text{Wt soil used} - \text{water in soil at start}}{100}$$

$$\text{Water to be added} = \text{Water required} - \text{Water in soil at start}$$

Figure 17 Conversion of tree to experimental blocks for soil exposure.

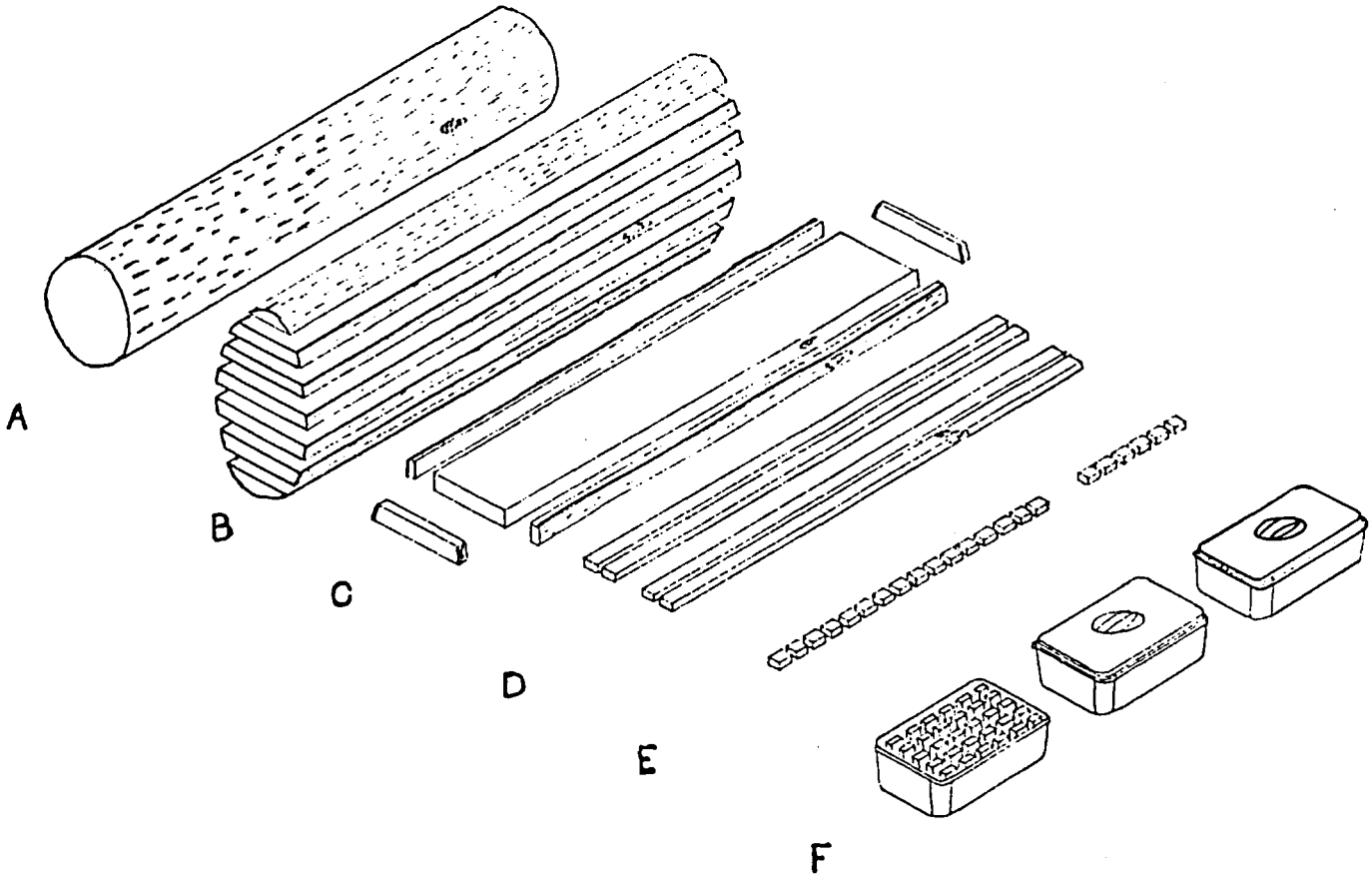


Figure 18 Soil contact exposure recording form.

Wood Species		Bin Number		Block Number		Block Position		Sample Time		Date of Sample		Oven Dry Weight		Removal Weight		Standard A		Standard B	
												.		.		.		.	
Segment	Bottle No.	Bottle + Wet Wood		Bottle + Dry Wood		Time 1 Units		Time 0 Units		Time 1		Time 0		Segment Length	
01		.		.		.		.		.		.		.	
02															
03															
04															
05															
06															
07															
08															
09															
10															
11															
12															

The bins were incubated at 22°C for two weeks to allow the soil to recover after disturbance and to reach a stable equilibrium before the wood was exposed to it. The bins were periodically weighed and water lost by evaporation made up by the addition of distilled water.

3.2.3. Exposure

A number of the experimental blocks were oven dried and their moisture content determined. The oven dry weight of each experimental block was calculated and recorded as the initial weight before burial:

$$\text{Oven dry weight} = \text{Room temperature weight} - \frac{\text{Moisture content} \times \text{Room temp wt}}{\text{Moisture content} + 100}$$

The experimental blocks were allocated randomly, 30 blocks to each bin. Each block was then partially buried, with the grain perpendicular to the soil surface and with 15mm exposed above the soil surface. The bin was weighed immediately after all 30 blocks were buried and the bin incubated at 22°C with the lid in position and weighed again at intervals. The weight of the bin system was made up to the original weight by the addition of distilled water. At 2 week intervals the blocks in the bin were sampled.

3.2.4. Sampling

Blocks were selected at random, removed from the bin, and adhering soil scraped off and returned to the bin. The blocks were weighed and their weights recorded on the recording form (Figure 18). They were then cross cut twice (Figure 20), once at the ground line, and once halfway between the ground line and the buried end of the block. The length of the three zones was recorded and then each zone chopped into segments with the aid of the apparatus depicted in Figure 19, a modified drill stand, which had equally spaced knife blades (Stanley Trimknife No. 5901) which split the zone into four equal segments. Each segment was then chopped into 11 slices using the single blade, and transferred to a McCartney bottle positioned under the funnel. The numbered bottles were stoppered with a suba-seal and the bottle number containing each segment of the block recorded. The position of the segments within a block are shown in Figure 20.

Figure 19 Apparatus for chopping wood zones into segments and then into slices.

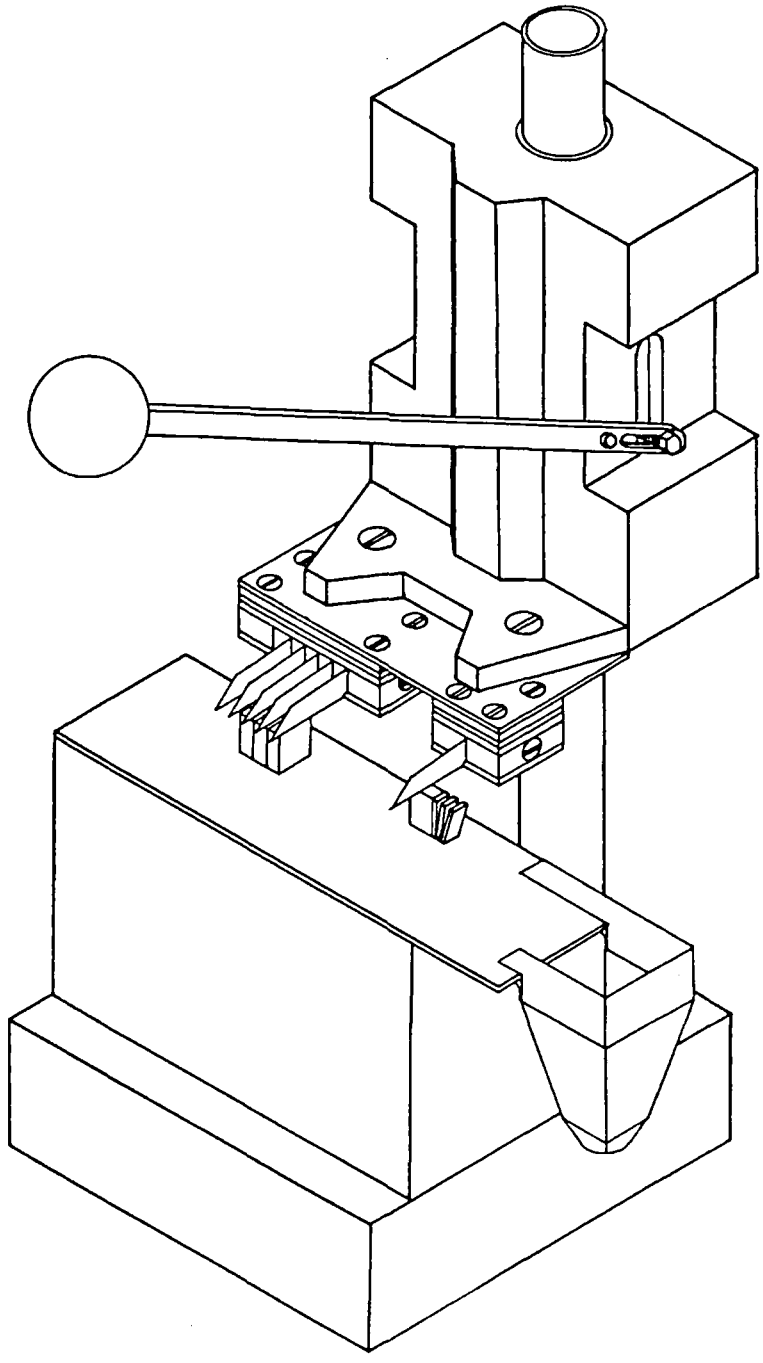
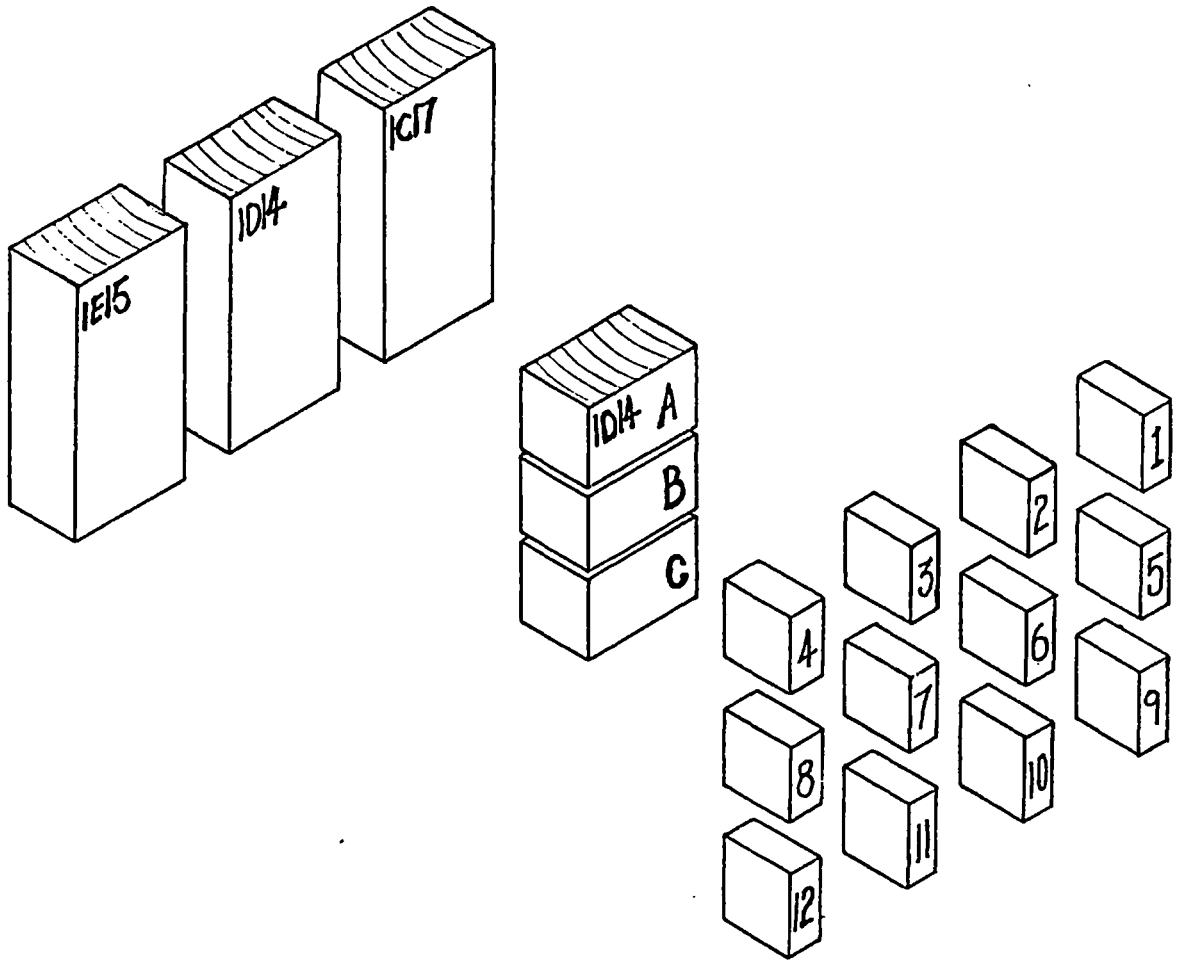


Figure 20 The position of zones and segments within a sampled wood block.



The zone above the ground line was designated Zone A, immediately below the ground line Zone B, and the deepest zone, Zone C. The segments were numbered from 1 to 12; 1 to 4 in Zone A, with 4 as the segment having the outer tangential face and 1 with the inner tangential face; 5 to 8 in Zone B, with 8 having the outer tangential face, and similarly with 9 to 12 in Zone C. This sampling procedure was repeated for each of the replicate blocks from a bin, and for each of the bins to be sampled. The sliced wood segments were then assayed for acetylene reduction activity.

3.2.5. Acetylene Reduction Assay

The atmosphere in the bottles was replaced with an anaerobic atmosphere of argon from a cylinder (BOC Ltd.). The bottles were attached to a manifold via a hypodermic needle and shortened plastic syringe. (Gilette Scimitar, 1ml). The manifold was connected to a vacuum pump and argon cylinder as shown in Figure 21. The vessels were evacuated for 2 minutes and the vacuum pump disconnected. The cylinder was connected and

the vessels filled with argon. This procedure was repeated twice to remove most of the air in the bottles and replace it with an anaerobic atmosphere of argon. The vessels were removed from the manifold and incubated overnight at 22°C.

1ml of acetylene was injected into each vessel. Immediately after injection into those bottles whose code number was a multiple of 5, a 0.5ml gas sample was withdrawn and the ethylene background determined in the gas chromatograph. Samples from odd numbered bottles were injected into column B, and even numbered into column A. The time of sampling was recorded in hours and decimal fractions of an hour. This was aided by the re-marking of a clockface in hundredths of an hour rather than sixtieths. The peak height and attenuation of the ethylene peak produced by each sample was recorded. An ethylene standard was injected before and after each batch of analyses and the peak height corresponding to one nMole of ethylene recorded. Each bottle was sampled 24 hours later and the time of sampling and ethylene peak height recorded.

3.2.6. Weight Loss and Moisture Content Determination

After the assay of acetylene reduction rate, the suba-seal was removed from each bottle and the bottle weighed. They were then oven dried at 105°C overnight and weighed again. The weights were recorded. The total wet weights of the segments from each block plus their bottles, and the total dry weights of the segments plus bottles, were calculated.

The weight loss, moisture content and weight of water in each block at the time of sampling were then calculated. The values provide an immediate impression of the progress of the experiment and are necessary for the calculation of the amount of water to be added to the bin to maintain the soil moisture content at the desired level.

Sum of Wet Wood Segments + Bottles : (1)

Sum of Dry Wood Segments + Bottles : (2)

Sum of Empty Bottles : (3)

Initial Calculated Oven Dry Wt of Block before burial : (4)

Block wt on Removal from Bin and before Sawing : (5)

Ratio of Block Length before Sawing to Length after Sawing (50mm ÷ 45mm) : (6)

2 saw cuts of 2.5mm kerf = 50mm ÷ 45mm = (6)

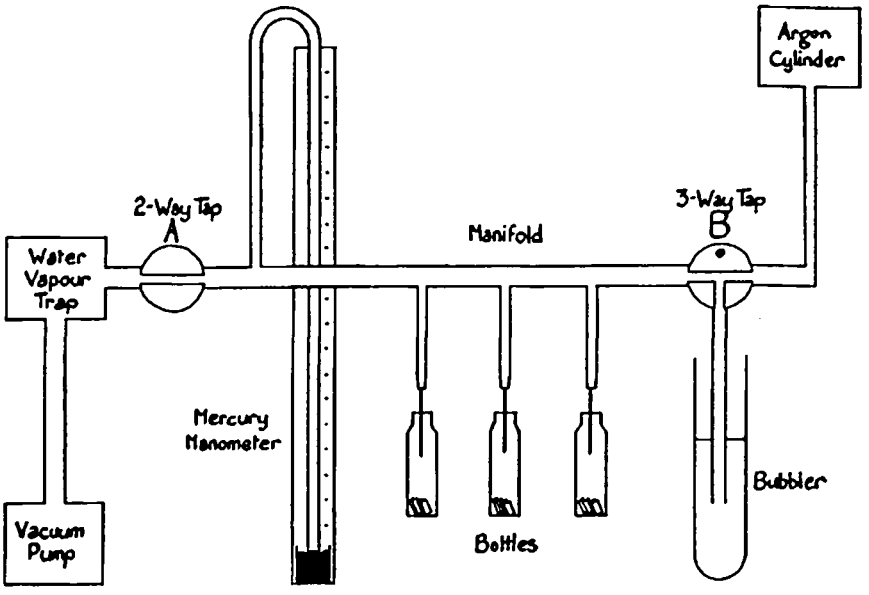
Oven Dry Weight of block after exposure (7) = ((2) - (3)) x (6)

$$\text{Weight Loss of Block (\%) (8)} = \frac{(4) - (7) \times 100}{(4)}$$

Wt of Water in Block on Removal from Bin (9) = (5) - (7)

The values (7) - (9) were determined for each sampled block, and the average weight loss and water content of the replicate blocks calculated.

Figure 21 Apparatus for filling vessels with Argon prior to acetylene reduction assay.



Operation :

1. Close A. Switch on Vacuum Pump.
2. Turn dot on B to 9 o'clock - Anticlockwise.
3. Regulate Argon supply to achieve a regular stream of bubbles.
4. Connect bottles.
5. Open Tap A - Manometer level rises to 760 mm.
6. Leave for 2 minutes.
7. Close Tap A. Meniscus should remain at 760 mm if there are no leaks.
8. Turn dot on Tap B to 6 o'clock - Anticlockwise
9. Manometer meniscus level falls to 0 mm as Argon enters.
10. Turn dot on B to 9 o'clock - Clockwise.
11. Leave for 30 secs.
12. Repeat 5 to 11 two more times.
13. Remove bottles.

3.3. Bin Moisture Adjustment

The amount of water needed to be added to the bin to maintain the desired soil moisture content was calculated. It was assumed that there were five components which made up the overall weight of the experimental system;

(i) the bin and lid, which did not change weight
(ii) the soil particles, which did not change weight
(iii) the soil water, which decreased due to evaporation and by uptake into the wood
(iv) block water, which came from the soil
(v) wood, which lost weight as decay proceeded

The humus content of the soil was ignored, as were any changes of humus weight. The increase of water in the block caused by the production of water from cellulose degradation was ignored, together with the weight of the micro-organisms in the wood and soil. It was also assumed that all the blocks in the bin behaved similarly in their weight loss and water uptake characteristics. With these assumptions, the weight of water to be added to maintain the required soil moisture content was calculated.

Average Block Weight Loss : (10)
Average Initial Oven Dry Weight of a Block : (11)
Number of Blocks Remaining in the Bin after Sampling : (12)
Average Weight of Water in Block : (13)
Overall Bin System Weight : (14)
Weight of Soil Particles : (15)
Weight of Empty Bin + Soil Particles : (16)

((15 and (16) were determined at the start of the experiment).
(14) was the weight to which the bin system was made up before sampling.

$$\text{Wt. of wood remaining in one block (17)} = (11) - \frac{(11) \times (10)}{100}$$

Total Wt of Wood in bin after weight loss (18) = (12) x (17)
Total Wt of Water in blocks in bin (19) = (12)x (13)
Soil Water = Overall Bin Wt - (Total wood wt. + Total water wt. in wood + bin + soil particles)

$$\therefore (20) = (14) - ((18) + (19) + (16))$$

$$\text{Soil Moisture Content (21)} = \frac{(20) \times 100}{(15)}$$

Hence water to be added :

$$\text{Water to be added} = \frac{\text{Required soil MC} \times \text{Soil particle weight (15)} - (20)}{100}$$

This amount of water was added to the soil by spraying and the new system weight recorded. This weight was maintained by the addition of distilled water until the next sample time.

This procedure was repeated at each sampling time.

3.4 Presentation of Results

The results for the seventeen combinations of soil, soil moisture content and wood species are presented in the next seven sections, and the last six are presented in the same format, described below.

3.4.1 Wood Block and Soil Data Tables

There is a Table (e.g. Table 9, and Example 1) to compare the weight, density, void volume, initial and maximum moisture content of the wood blocks used in the experiments.

Example 1 Wood Block and Soil Data Table

Wood Species	: Birch	Soil Type	: Loam
Initial Block Wt.	: 9·52 ± 0·65 g	Initial Moist. Cont.	: 7·75 %
Calc.Oven Dry Wt.	: 8·83 g	Void Volume	: 62·00 %
Initial Density	: 0·507 g cm^{-3}	Max. Moist. Cont.	: 125·11 %

Soil Moisture Conditions
Nominal : 28 % (Dry)

Code Number of Blocks Sampled at each Sample Time

2	4	6	8	10	12	14	16	18	20	22	24	26	28 weeks
OS06	OT15	OS07		OT07	OT06	OT10	OT08	OT04		OS15			
OT15	OT12	OS13		OT09	OS08	OT13	OT11	OS13		OT10			
					OU09	OT05	OS09	OS05		OT03			
								OS04		OS14			

The initial block weight was the mean of 30 blocks. The initial moisture content was determined from replicate blocks which were oven dried. The other values were calculated from the equations below.

$$\text{Calculated oven dry weight} = W_1 - \left(\frac{M_1 \times W_1}{M_1 + 100} \right)$$

where W_1 is the weight of the block at room temperature and humidity, and M_1 is its moisture content, (Kollmann and Cote, 1968).

$$\text{Void volume (\%)} = 100 \left(1 - S_1 \left(\frac{1}{1.46} + \frac{\frac{M_1}{100}}{S_2} \right) \right)$$

where S_1 is the specific gravity of the wood at room temperature and humidity, and M_1 is its moisture content. S_2 is the density of water in the cell wall at a MC of M_1, taken to be 1.2 g cm^{-3} (Kollmann and Cote, 1968).

$$\text{Maximum moisture content} = 100 \left(\frac{1}{S_3} - 0.65 \right)$$

where S_3 is the specific gravity of the wood when oven dry (Brown, Panshin and Forsaith, 1952).

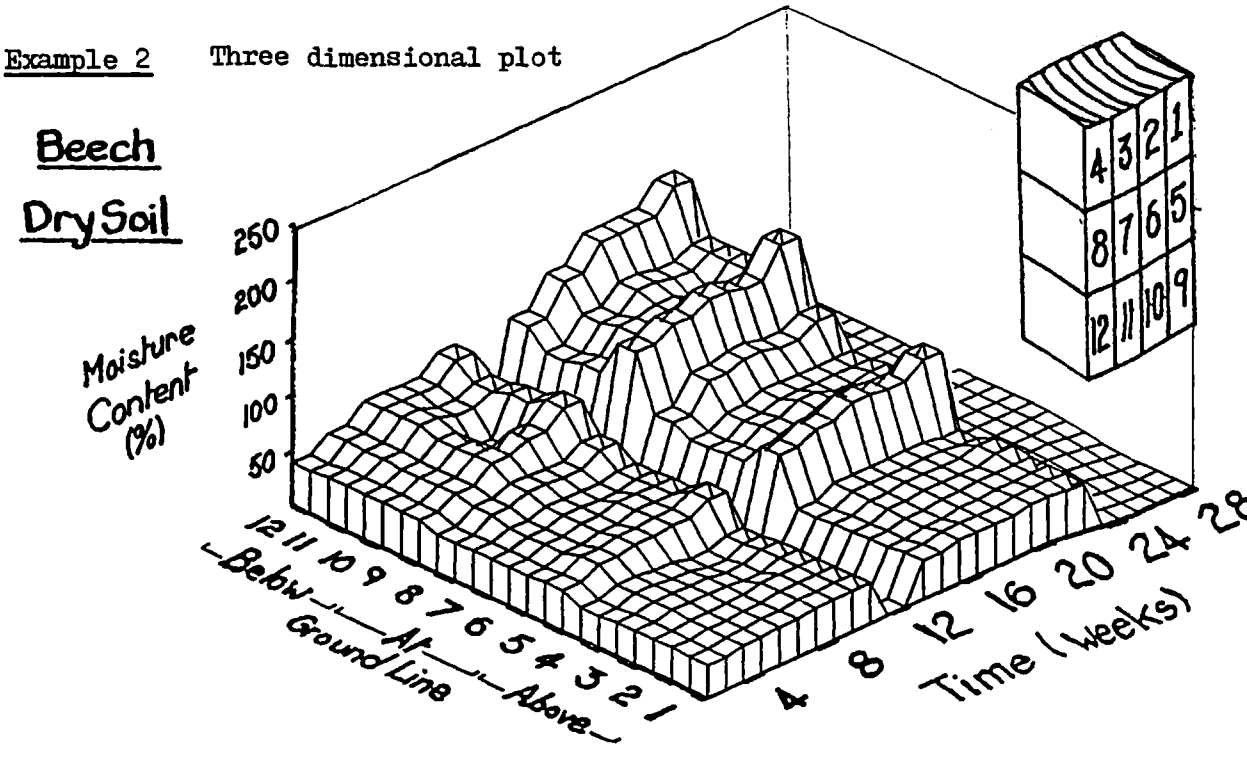
The soil moisture conditions are given in the table, both as percentages and as a subjective assessment (i.e. dry, wet, very wet, waterlogged). The code numbers of the wood blocks sampled at each sample time are given. The letters of the block code refer to a single stick cut from a plank, and the numbers refer to the position of a block in that stick. Thus sticks OS, OT and OU were adjacent sticks from the same plank; blocks OS12, OS13 and OS14 were consecutive blocks from the same stick, and OS12, OT12 and OU12 were adjacent blocks in the plank. The table shows the number of replicate blocks removed at each sample time ; e.g. two blocks were removed after 2 weeks, 3 at 12 weeks, and 4 at 18 weeks.

3.4.2 Three-dimensional Plots

A typical three dimensional plot of the results for moisture content in the twelve segments of wood blocks taken from soil exposure after increasing times of soil exposure is Figure 31, from

which Example 2 is taken.

Example 2 Three dimensional plot



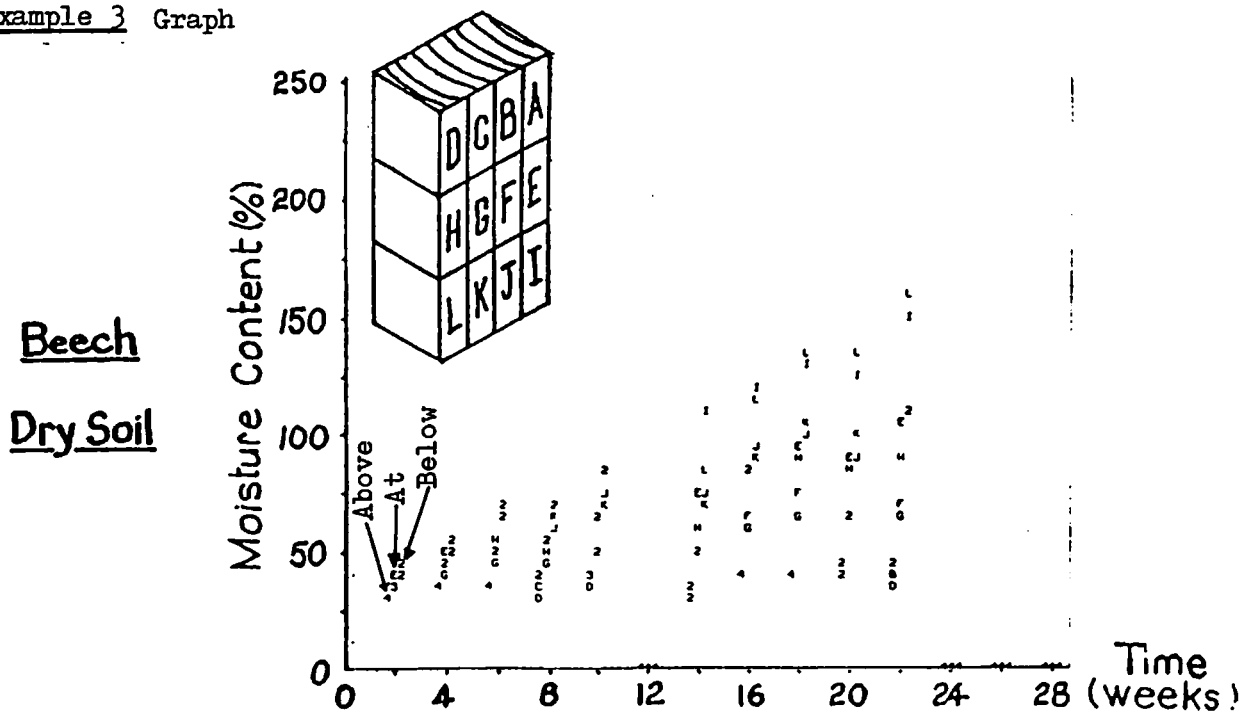
The right hand(x) axis shows sample time in weeks. The left hand axis (y) shows the segment number from 1 to 12. 1 to 4 were above ground, 5 to 8 at the ground line, and 9 to 12 below ground. The vertical (z) axis shows the average moisture content, water content, weight loss or acetylene reduction rate of the segments from the replicate blocks. Thus the average moisture content of blocks OD17 and OD19 was nearly 50 % in all 12 segments after 2 weeks exposure to soil. The moisture content in segments 1 to 4 was lower than in segments 5 to 8, and their moisture content was lower than segments 9 to 12. After 4 weeks, the moisture content in all 12 segments was slightly higher than at 2 weeks. The value for each segment and sample time was plotted as a flat surface and the contours filled in by the computer program to give a surface rather than isolated points. This representation gives a good overall impression of the variation in MC, WL, WC and AR with time in the 12 segments (MC is shown in Example 2), and allows easy comparison between the trends in different wood species exposed to different soils and soil conditions. However it lacks accuracy in that numerical values cannot be read off and compared very easily. Thus the same data was also plotted as conventional graphs.

The inset diagram shows the position of the 12 segments in the wood block.

3.4.3 Graphs

A typical graph, showing moisture content against time for beech blocks exposed to dry soil is given in Figure 32, from which Example 3 is taken.

Example 3 Graph



The x axis is the exposure time in weeks, and the y axis is MC, WL, WC or AR, in this case, MC. For each sample time the results were printed on the graph in 3 columns. The left hand column showed the zone above ground, the centre column showed the zone at the ground line, and the right hand column showed the zone below ground. Within each column, the value for segment 1 was plotted as an A, segment 2 as a B, segment 3 as a C, and segment 4 as a D. This was necessary because the two digit values 10, 11 and 12 could not be plotted as one symbol on the graph. In the centre column segment 5 was plotted as an E, 6 as an F, 7 as G and 8 as the symbol H. Segment 9 in the right hand column wsa represented by I, 10 by J, 11 by K and 12 by L. The inset shows the position of the segments in the block, with their code letters. Where more than one symbol occurred at the same coordinates, the number of symbols coinciding was plotted. Where no blocks were removed at a sample time, a 4 in each column on the baseline at that sample time was plotted. Thus the MC in all four segments in the zone above ground after 2 weeks exposure was around 30 %. The MC in segment 5, represented by the symbol E was slightly higher than the other 3 segments of the zone at the ground line.

The graphs for MC, WL, WC and AR allowed numerical values for each segment to be read off and compared with some accuracy, but generally the graphs presented a more confusing display of the data than the three dimensional plots.

3.5 Conclusion

The method described in this section enabled the values of the four variables, moisture content, weight loss, water content and acetylene reduction rate, to be measured at 14 day intervals over a period of 28 weeks in the 12 segments and three zones of two, three or four replicate blocks. Scots pine sapwood and heartwood, and birch, beech and spruce sapwood were exposed to 25, 35, 40, 45 and 50 % soil moisture contents at 22°C. Seventeen combinations of soil type, soil moisture content and wood species were examined, involving over 300 blocks cut into more than 4000 segments.

The next seven sections describe and assess the results.

4. THE EFFECT OF SOIL MOISTURE CONTENT ON ACETYLENE REDUCTION IN SCOTS PINE BLOCKS

4.1. Introduction

The aim was to examine the effect of different soil moisture contents upon the colonisation and development of nitrogen fixing bacteria in Scots pine sapwood. To monitor radial penetration, blocks were coated with epoxy resin before burial. Epoxy resin was used, rather than silicone rubber, because it was easier to apply in a uniform layer. The weight of the blocks before and after coating were recorded so that the weight of the resin could be subtracted in the final estimate of weight loss in the blocks. Acetylene reduction rate and moisture content were also determined in order to attempt a correlation of decay, nitrogen fixation and wood and soil moisture content.

A full description of the experimental design and procedure is given in Section 3. Only the modifications to this basic method are described here.

4.2 Materials and Methods

Wood

The block size was 15 x 25 x 15mm with the 15 x 15mm face tangential. Five faces of each block were sealed with 2 coats of Araldite epoxy resin applied with a paint brush and taking the usual safety precautions when using epoxy resins.

Epoxy resin formula (information from Ciba-Geigy Technical Bulletin TB 4.4).

Araldite GY 250	41%	Resin
HY 830	8%	For low temperature and high humidity cure
HY 850	24%	For rapid cure
GY 298	27%	Plasticizer

Soil

Three soil bins were set up as described in Section 3.2.2, at 27.25% (dry), 39.20% (wet) and 49.13% (waterlogged) soil moisture content. Forty blocks were buried in each bin, with one transverse face horizontal and approximately 10mm below the soil surface.

Maintenance and Sampling

These operations are described in Section 3.2.4. Three blocks from each bin were removed at 14 day intervals up to 98 days. No samples were removed at 42 or 84 days.

Weight Loss and Moisture Content

The computer program allowed for the weight of the resin on each block. It was assumed that the resin was of uniform thickness and that each block was cut into four equal segments, and that the resin did not change in weight during exposure or drying. As the weight of the resin coat and the surface area of each block was known, the weight of the resin on each segment could be calculated. This weight was subtracted from the wet and dry weights of each segment before the weight loss and moisture content were calculated.

4.3 Results

Figures 22 to 25 show the moisture content, weight loss, AR rate and water content of each segment of each of the three replicate blocks removed from the three bins at 14 day intervals. The pictures are generated by the computer linked to a graphics terminal (Appendix 1).

Figure 22 shows the moisture content of the segments. In those blocks exposed to dry soil the moisture content of the blocks increased with time. In those blocks sampled at 14 days the exposed face had a higher MC than the other segments, but at 28 days, the MC of all four segments was similar. At 56 days, one block had a much higher MC than the other two, and the exposed outermost and the innermost segments had the highest MC, a difference which was maintained at 70 and 98 days. In those blocks exposed to wet soil, a similar pattern of moisture uptake and distribution was apparent, with a slightly lower MC in those blocks removed at 56 days. In those blocks exposed to waterlogged soil, the MC after 14 days was very similar to that of the blocks removed from the dry soil after the same time. At 28 days the exposed segments had a higher MC than the other segments, unlike the blocks removed from the other soil conditions. By 56 and 70 days the blocks had a higher MC than those exposed to the drier soils, and the difference was more marked at 98 days.

Figure 22 Moisture Content of the three blocks of Scots pine after exposure to soil.

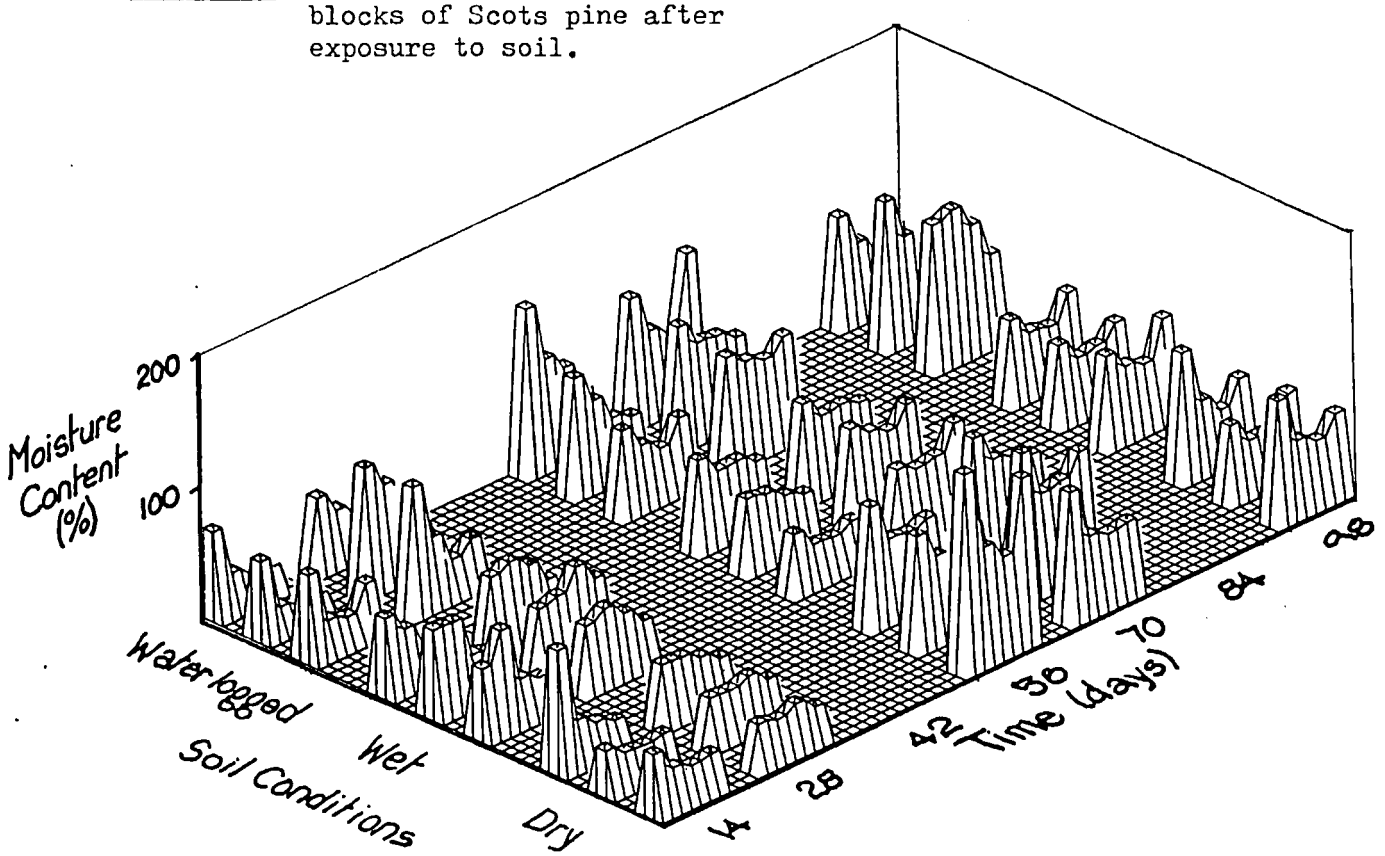


Figure 23 Weight Loss of the three blocks of Scots pine after exposure to soil.

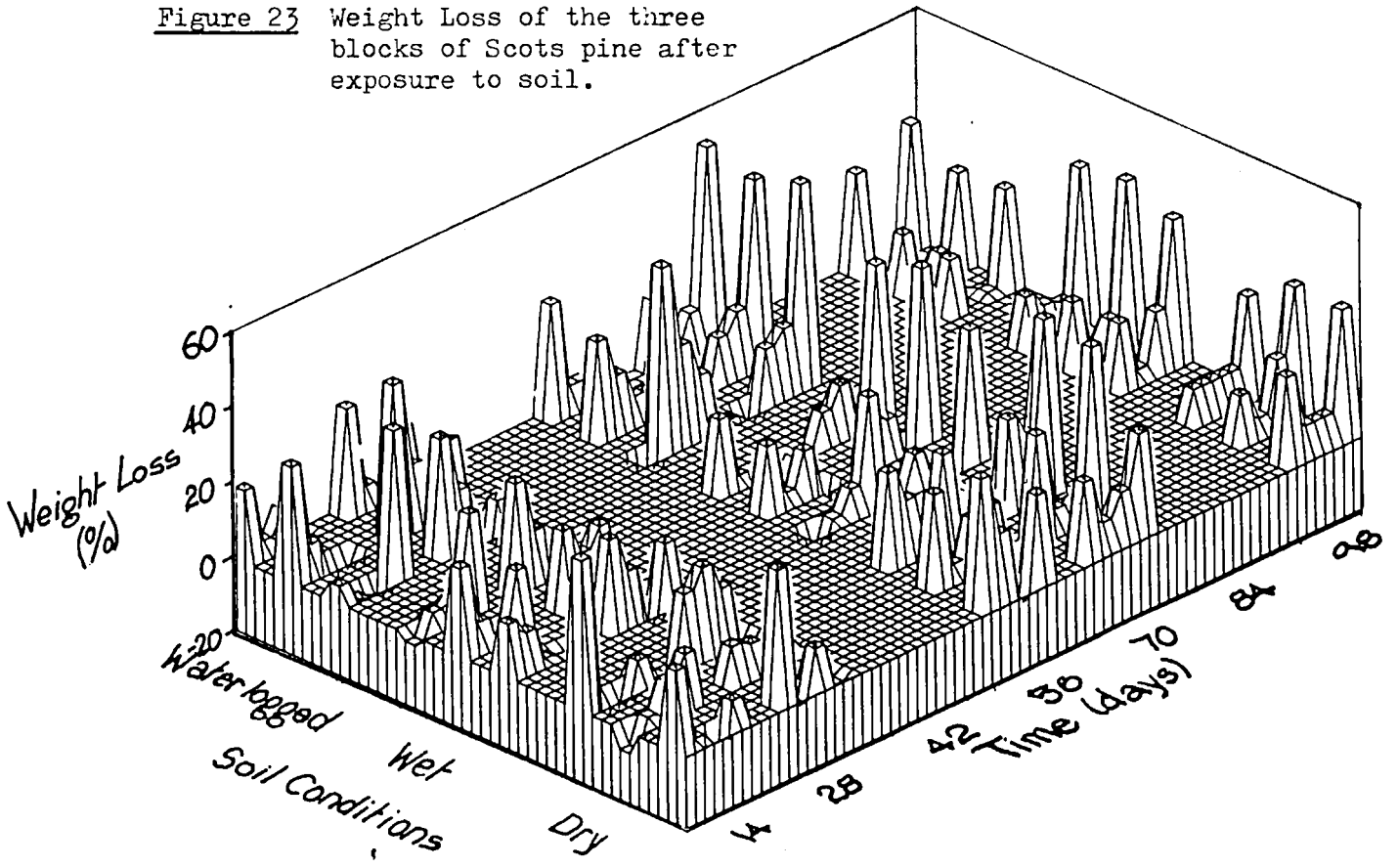


Figure 23 shows the weight loss of the segments. The overall impression is of extreme variability with weight losses ranging from 35% to weight gains of 55%. An overall trend is from weight gains and low weight loss at 14 days in all three treatments, to higher weight loss and no weight gain at 98 days. There was no major difference in weight loss between the three treatments. At 70 and 98 days, the innermost, unexposed segments had a higher weight loss than the outer, exposed segments in all 18 sampled blocks.

Figure 24 shows the AR rate of the segments. In those blocks exposed to the driest soil there was some activity at 14 days but then no activity recorded until 70 days, when there was slightly more activity in the segment adjacent to the one with an exposed face. Slight activity was recorded at 98 days, again in unexposed segments. In these blocks exposed to wet soil, some activity was recorded at 56 days. At 98 days there was activity in all three sampled blocks, although of different magnitude. In those blocks exposed to the wettest soil, activity was recorded on the exposed face of all three sampled blocks at 56 days although very much greater in one block than the others. At 70 days, two blocks exhibited activity, one did not. At 98 days the AR activity of the exposed face was high, with some activity recorded in the unexposed segments. The greatest activity in the blocks exposed to the wettest soil was evident at 98 days.

Figure 25 shows the water content expessed as a percentage of the segment volume for each segment. The overall pattern of water distribution was similar to that of MC. The outer exposed face often had the highest water content of the block, although the water content of the innermost unexposed segments was usually lower, or similar to the water content of the central two segments. The greatest water content of those blocks exposed to the wettest soil was most apparent in the 98 day sample. One segment of one block taken from dry soil at 70 days was unusual in that it had a higher water content than its replicate segments and blocks.

Despite the visual appeal of the three-dimensional pictures, if the results are pooled and calculated for the entire block rather than as four individual segments, the variability due to inaccurate cutting of the segments is removed and the similarities and differences between treatments can be examined more easily.

Figure 24 Ethylene production rate in the three blocks of Scots pine after exposure to soil.

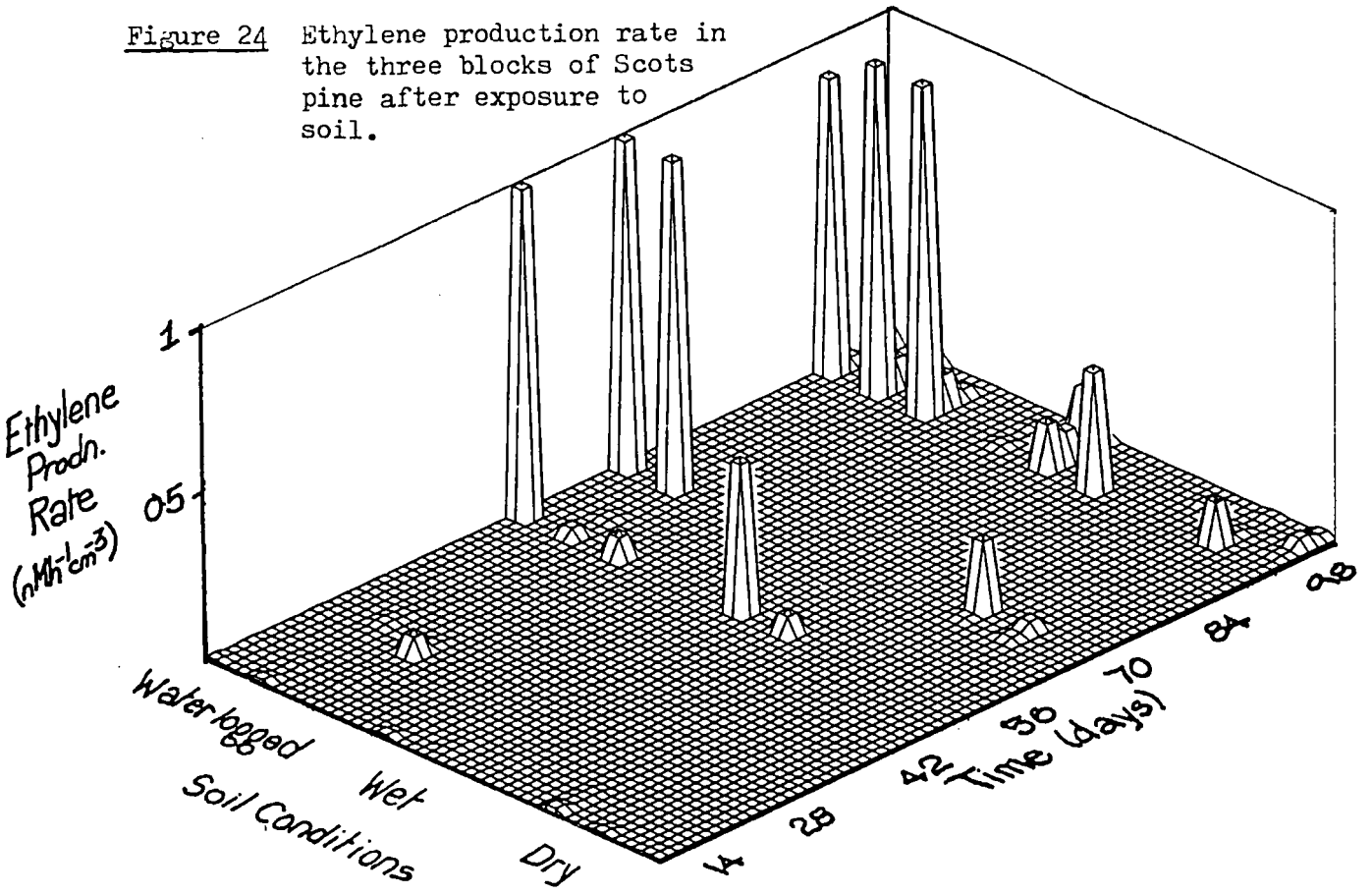


Figure 25 Water Content of the three blocks of Scots pine after exposure to soil.

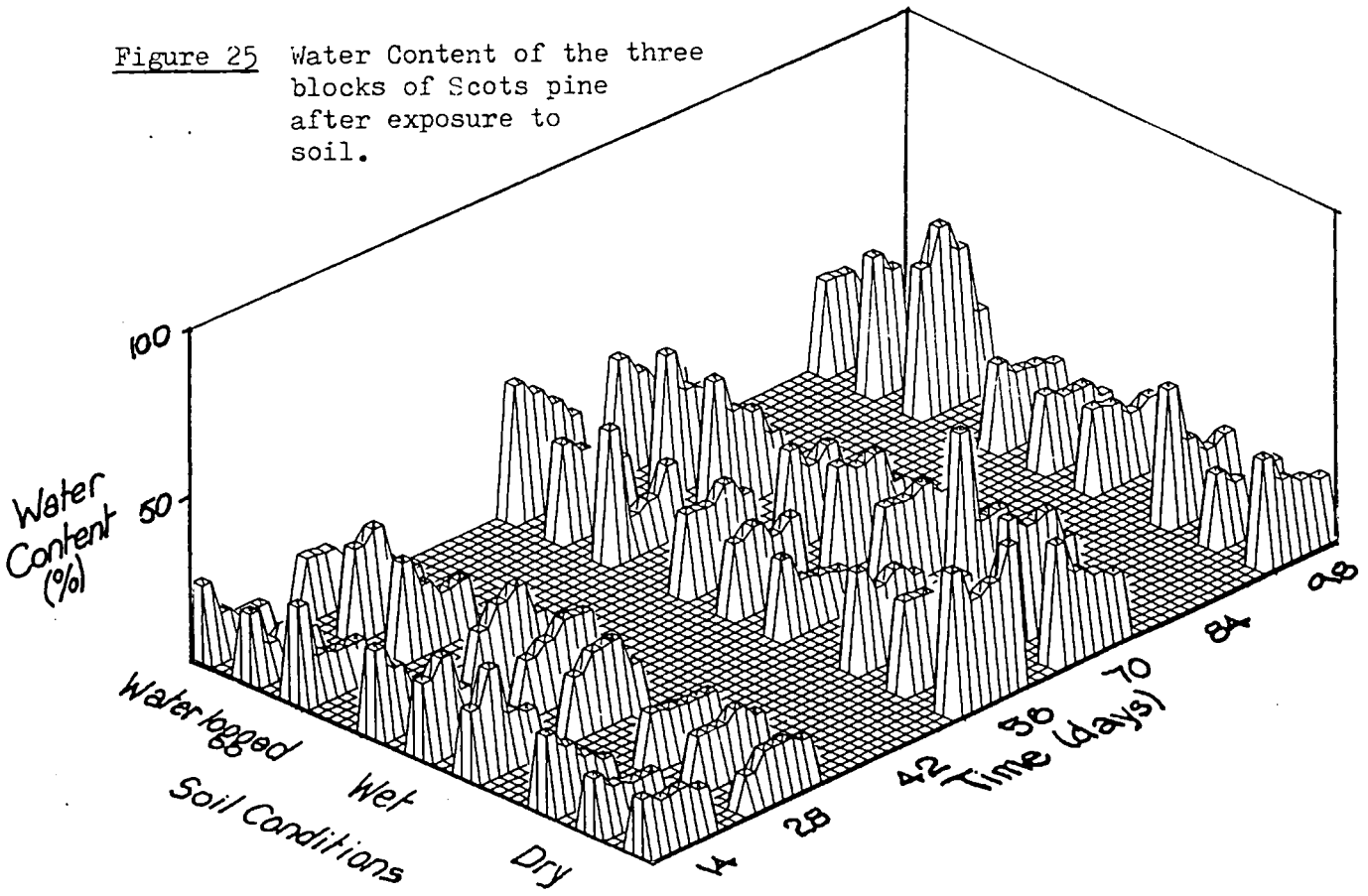


Figure 26 shows the moisture content of the blocks exposed to dry, wet and waterlogged soil at successive sample times. The major difference in block MC, which was expected after exposure to soils at different moisture contents, was not apparent. The wood in the wet soil reached 80% faster than in the other soils, while the highest MC recorded was after 56 days in the driest soil. The 56 day sample taken from the wet soil was drier than the blocks removed at the other sample times. The water content results showed a similar pattern to the MC results and are not presented.

Figure 27 shows the weight loss of the blocks against time. Weight loss occurred in all three soils, reaching a maximum of around 22%. There was low weight loss recorded in the blocks taken from wet soil and waterlogged soil at 56 days and in blocks taken from dry and waterlogged soil at 98 days. There was little difference between the rate and extent of weight loss in the blocks exposed to the dry and wet soils. In the blocks exposed to wet soil, the weight loss increased rapidly at first, but then less rapidly, approaching the maximum observed in the other soils at 98 days.

Figure 28 shows the AR rate in the blocks. Only in the wettest soil was any significant activity observed which was first present at 56 days and increased with time.

4.4 Discussion

The MC of the blocks take from the three soil conditions was very similar, which may have been due to having five faces sealed and only one tangential face exposed to the soil. If it was the rate of water penetration into the block which had determined the wood MC rather than the amount of water available in the soil, particularly in the initial stages of wetting, then this could explain the similarity in wood MC despite the differences in soil MC. Only at 98 days was any difference apparent, presumably because by that time, the water had penetrated the whole block and the wood MC was then determined by the amount of water available in the soil.

The large variability in the weight loss of the segments may be due to the difficulty of slicing the blocks accurately into equally sized regular segments. Slight variations in the volume of the segments or in the thickness of the resin coating would affect the calculated value of weight loss. The high weight loss regularly recorded in the segment furthest from

Figure 26 Average Moisture Content of partially sealed Scots pine sapwood blocks after exposure to soil.

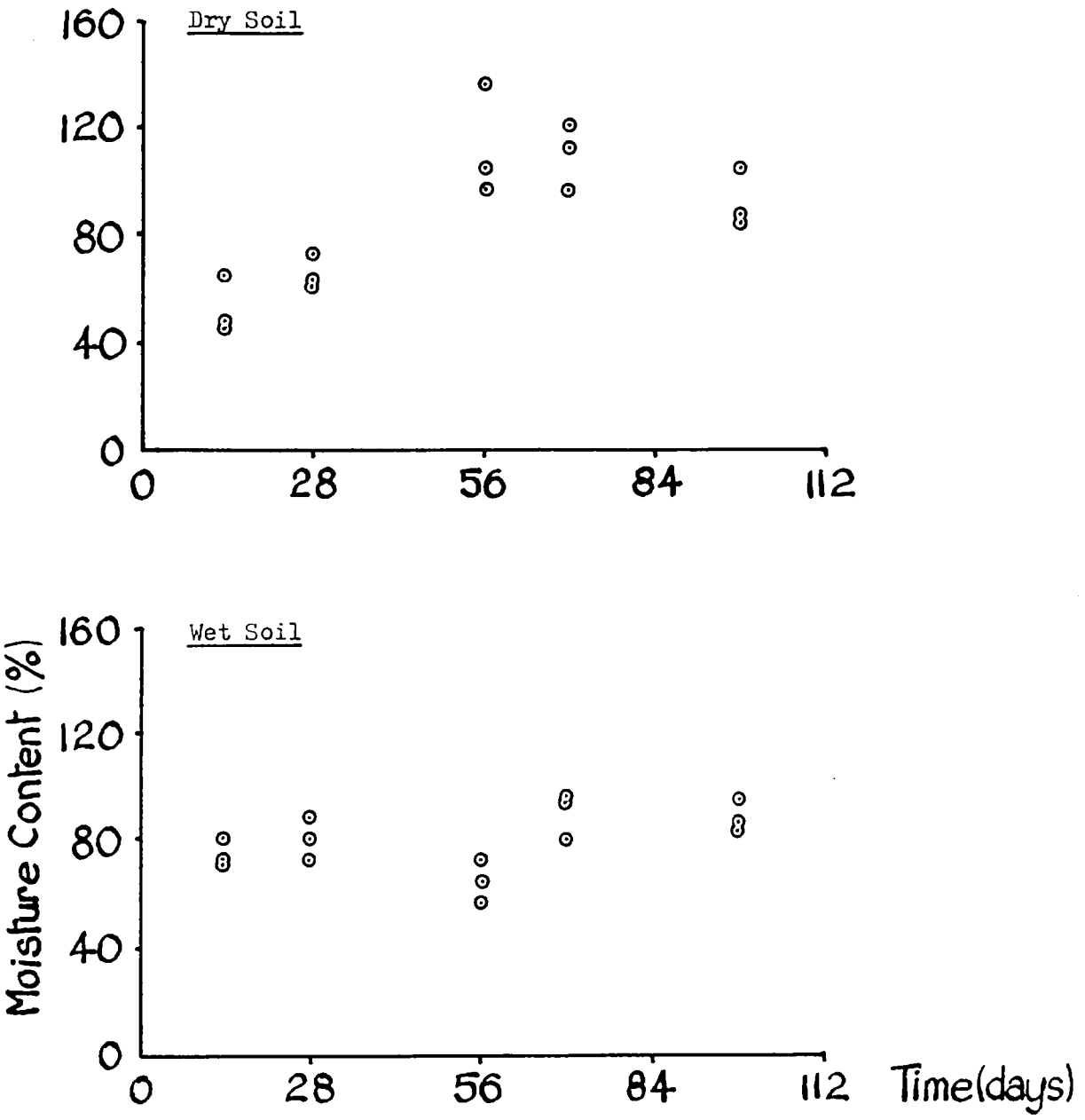


Figure 27 Average Weight Loss of partially sealed Scots pine sapwood blocks after exposure to soil.

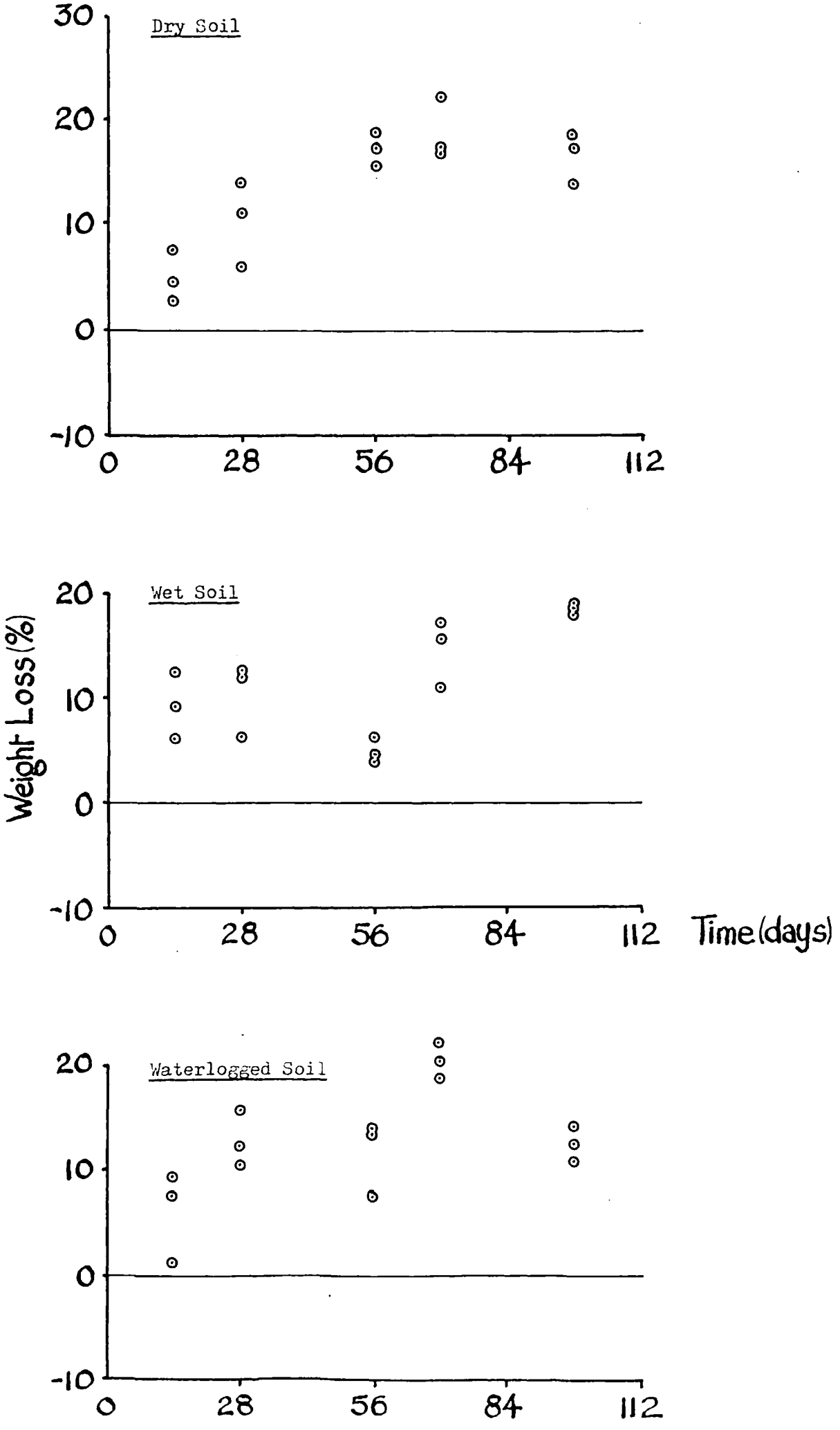
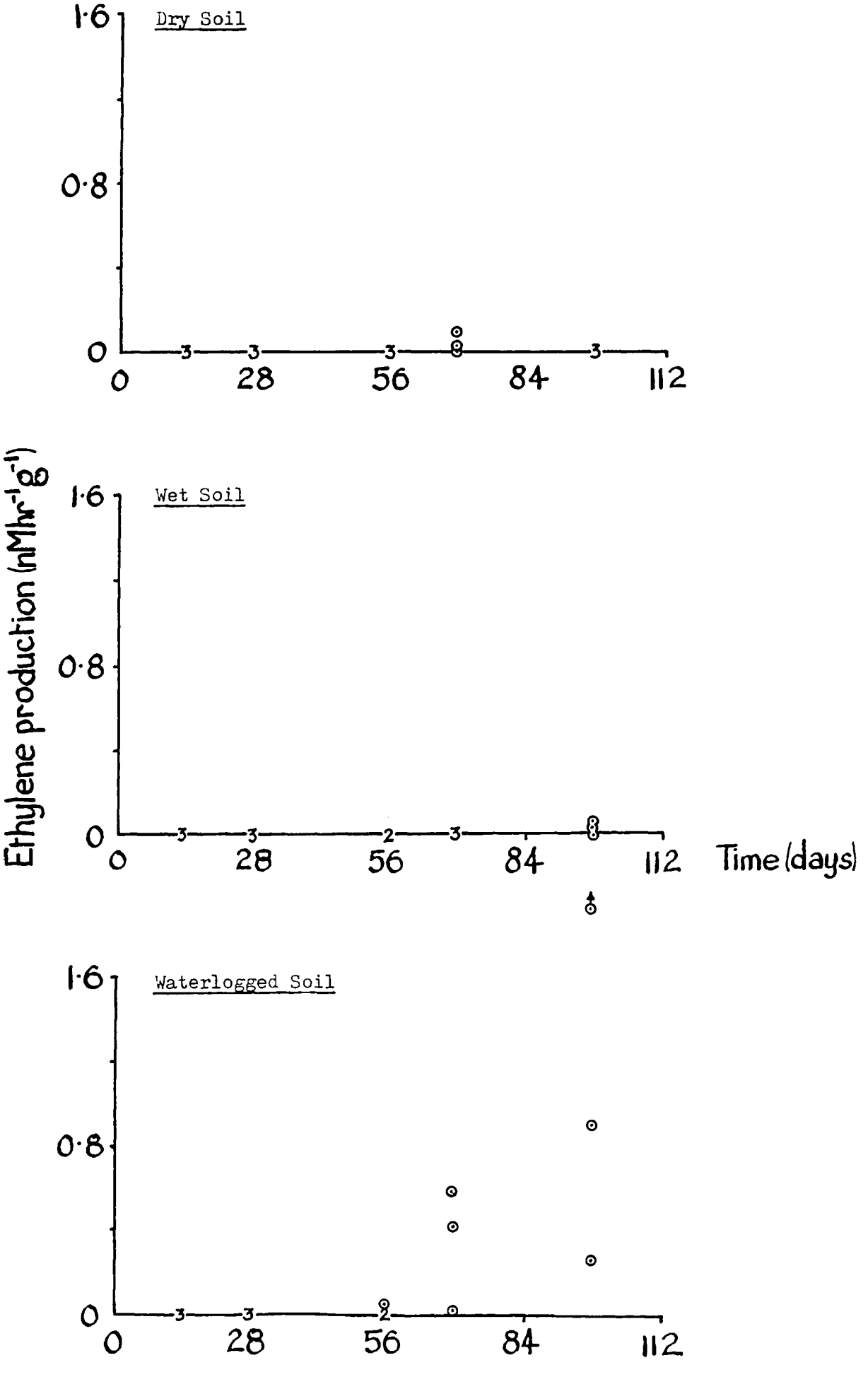


Figure 28 Average Ethylene production rate in partially sealed Scots pine sapwood blocks after exposure to soil.



ground contact was probably due to the effect of inaccurate slicing which caused this segment to be smaller than the other three, and as this segment had five faces covered in resin, any variation in thickness would affect this segment more than the other three.

The weight loss calculated for the block as a whole was more reliable as it was less variable than the weight loss calculated for each segment individually. It showed little difference in the amount of decay in the wet and dry soil, a result which was unexpected. The weight loss of the blocks exposed to an intermediate soil moisture content appeared to have a faster rate of weight loss initially, which decreased with time, and after 98 days was similar to the weight loss in the other two soils. There is more than one interpretation of this similarity of weight losses after exposure to different soil conditions.

There may be similar conditions in the wood, regardless of the conditions in the soil, an interpretation which is supported by the similar MC of the blocks from the three situations. The implication is that the conditions in the wood determine the colonisation and degrade by microorganisms, rather than the soil conditions. Because the conditions in the wood are the same in all three situations, the same group or groups of organisms are selected from the soil and colonise the wood and the rate and extent of decay is similar in all three situations.

Alternatively, different groups of organisms may be active in the three soil conditions. Their net effect upon the wood is identical, although the rate at which it is achieved may differ. The graph of weight loss against time supports this view as the rate of weight loss differs in the three conditions In addition, the occurrence of significant AR activity in only those blocks in the wettest soil supports the idea of different conditions in wood exposed to the three different soil moisture contents.

A third possibility is that a single group of organisms, able to tolerate a range of soil moisture contents, may be involved in the decay, so that regardless of the environmental conditions, their capacity to colonise and degrade the wood remains the same.

Which of these possibilities, if any, actually occurred cannot be determined from this experiment. However, the results do indicate that the decay of wood in ground contact may be determined by a combination of the prevailing soil conditions, the relationship between these soil conditions and the conditions inside the wood, and the physiology of the organisms

themselves.

The occurrence of significant AR activity in only those blocks from the wettest soil has been observed previously (Section 1.4). If the AR activity of each segment is plotted against MC, (Figure 29) there is a range of MC over which AR activity occurs, from 90-120%, although the blocks exhibiting significant activity were all from the wettest soil. High MC, over 100%, does not necessarily mean that AR activity, and nitrogen fixing organisms, will be present, merely that if the soil is wet, and presumably anaerobic, then these organisms can proliferate in the soil and may be able to penetrate the wood. The high AR rate of the exposed face, the penetration of activity with time and the variability and distribution of the activity have been observed previously (Section 2.2.1). The implication is that the soil conditions influence the population of organisms which enters the wood in addition to being affected by the wood itself.

Perhaps the wettest soil was anaerobic, favouring the growth of obligate and facultative anaerobes such as nitrogen fixing bacteria, to the exclusion of aerobes, such as wood decay fungi. However, with a 22% weight loss in the blocks in the wettest soil, the implication is that both aerobes and anaerobes are active simultaneously in the wood. Although they could conceivably be active in localised, separate areas within the wood, there is the possibility that they could exist in close association. Line and Loutit (1973) found AR in a mixture of Clostridium butyricum, which only shows AR under anaerobic conditions, and Pseudomonas azotogenesis, because the aerobe used the oxygen, creating anaerobic conditions around the other organism. Perhaps the fungi utilise all the available oxygen in the sealed wood, allowing a weight loss of 22% before the conditions become anaerobic and the fungi die. The inference is that the total burial of partially sealed wood is unrealistic and misleading. Decay in timber in soil contact is usually at the ground line (Levy, 1968) which is presumably aerobic, while a deeper zone of the timber may be below the water table and presumably anaerobic. This separation of the zones would allow both groups of organisms to be active in the same timber.

The decay of the blocks exhibiting significant AR activity is no greater than those in which AR is minimal, suggesting that AR activity and the presence of nitrogen fixing organisms is not essential for decay, and that timber decay can occur in the absence of nitrogen fixing organisms.

Figure 29 Acetylene reduction activity plotted against the moisture content of partially sealed Scots pine blocks exposed to soil. A represents blocks from dry soil, B from waterlogged, and C from wet soil.

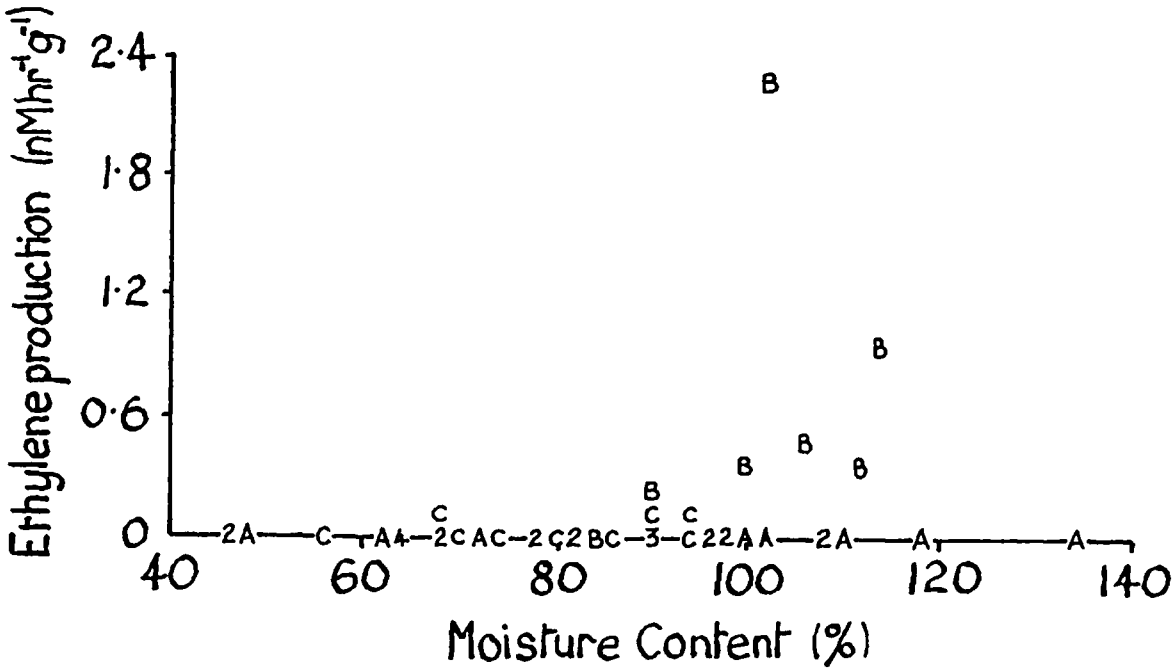
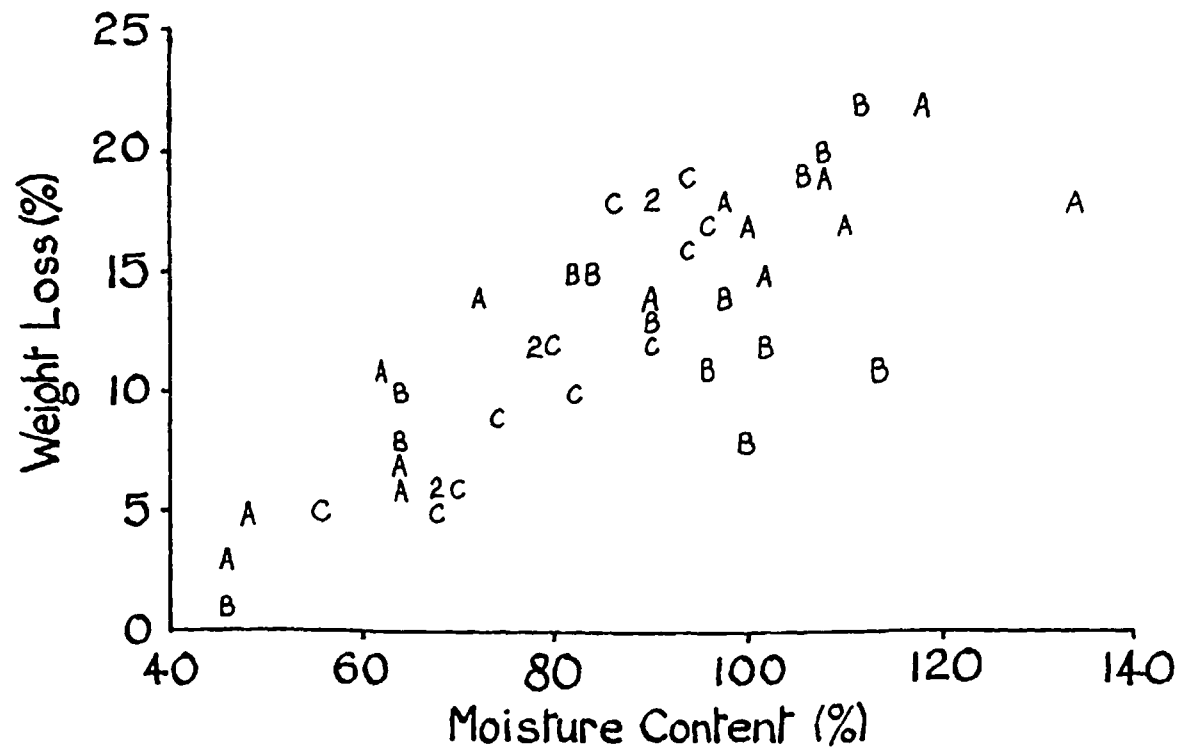


Figure 30 Weight loss plotted against the moisture content of partially sealed blocks of Scots pine sapwood after soil exposure. A represents blocks from dry soil, B from waterlogged soil, and C from wet soil.



If the weight loss is plotted against MC, there is an apparent correlation (Figure 30). However, as explained in Section 3.1.2, the correlation is spurious. These samples with the lowest weight loss have the highest density, suggesting a correlation between weight loss and block density. However, if the weight of wood lost, rather than weight loss, is plotted against initial block weight, the correlation coefficient is only 0.404 (r^2 = 14.4% (43)), showing that there is no correlation, in this experiment, between the two values.

4.5 Conclusion

The sealant was very effective in preventing the penetration of micro-organisms and water through the sealed faces, but increased the variability of the weight loss results. The differences in wood MC which would be expected from the exposure to different soil MC did not occur until the end of the experiment, suggesting that water penetration of the blocks was a limiting factor in the onset of colonisation and decay, rather than soil MC. However, the influence of soil conditions upon wood MC, decay and AR rate was observed.

Further work is obviously required using unsealed blocks, partially buried, and of different wood species exposed to different soil conditions. The careful monitoring of soil MC, wood MC, decay rate and AR rate should allow the examination of the relationship between these factors in the decay of wood in soil contact.

5. A COMPARISON OF BEECH, BIRCH AND SCOTS PINE EXPOSED TO DRY SOIL

5.1. Introduction

The aim of this experiment was to assess the occurrence and activity of nitrogen-fixing bacteria in beech, birch and Scots pine sapwood exposed to moist soil, and the effect of these organisms upon decay. Beech was selected as a frequently used test hardwood, despite the anomalous absence of ray attack (Greaves and Levy, 1965), and birch, selected as a non-durable hardwood which is being used increasingly in preservative testing. Scots pine was selected as it is the usual softwood test species, and because nitrogen fixing organisms had been shown to colonise this timber in ground contact. No sealant was used on the blocks and the soil was maintained at just below the field holding capacity of the soil (28%) and appeared 'dry.'

5.2. Materials and Methods

The preparation of the wood blocks and soil, and the setting-up, maintenance and sampling of the experiment were as described in Section 3. Table 9 gives the average values of the block weight and density, and the MC, theoretical void volume and maximum MC of the blocks. The nominal and actual soil MC are given together with the code numbers of the blocks removed at each sample time.

5.3. Results

The technique allowed the comparison of moisture content, weight loss, acetylene reduction rate and water content in samples from zones below, at and above the ground line of beech, birch and Scots pine blocks after exposure to soil for periods up to 22 weeks. (See Fig. 20 for position of zones and segments in a sampled block.)

Moisture content - Figure 31

In the beech blocks, the moisture content above ground had reached 30% by 2 weeks and was maintained during the period of exposure. In the zone at the ground line, the moisture content reached 35% after 2 weeks and then increased with time. Overall, the segments with both tangential and radial faces in soil contact (Segs. 5,8,9,12) had a higher MC than those with the radial faces in soil contact. The below ground zone showed a similar pattern of MC, although this zone was much wetter than the zone at the ground line.

In the birch blocks, the overall MC was much higher than in beech. At

Table 9 Wood Block and Soil Data

Wood Species	: Beech	Soil Type	: Loam
Initial Block Wt.	: 10•94 ± 0•29 g	Initial Moist. Cont.	: 7•48 %
CalcOven Dry Wt.	: 10•16 g	Void Volume	: 56•49 %
Initial Density	: 0•583 g cm^{-3}	Max. Moist Cont.	: 99•74 %

Soil Moisture Conditions
Nominal : 28 % (Dry)

Code Number of Blocks Sampled at each Sample Time

2	4	6	8	10	12	14	16	18	20	22	24	26	28 weeks
OD17	OD07	OD20	OD13	OD10		OD30	OD14	OD16	OD03	OD26			
OD19	OD12	OD23	OD27	OD31		OD32	OD21	OD08	OD18	OD04			
							OD15	OD28	OD05	OD22			
							OD11	OD09	OD06	OD33			

Wood Species	: Birch	Soil Type	: Loam
Initial Block Wt.	: 9•52 ± 0•65 g	Initial Moist. Cont.	: 7•75 %
Calc.Oven Dry Wt.	: 8•83 g	Void Volume	: 62•00 %
Initial Density	: 0•507 g cm^{-3}	Max. Moist. Cont.	: 125•11 %

Soil Moisture Conditions
Nominal : 28 % (Dry)

Code Number of Blocks Sampled at each Sample Time

2	4	6	8	10	12	14	16	18	20	22	24	26	28 weeks
OS06	OT15	OS07		OT07	OT06	OT10	OT08	OT04		OS15			
OU15	OS12	OS14		OT09	OS08	OT13	OU11	OS13		OU10			
					OU09	OT05	OS09	OS05		OS03			
								OS04		OS16			

Wood Species	: Scots pine	Soil Type	: Loam

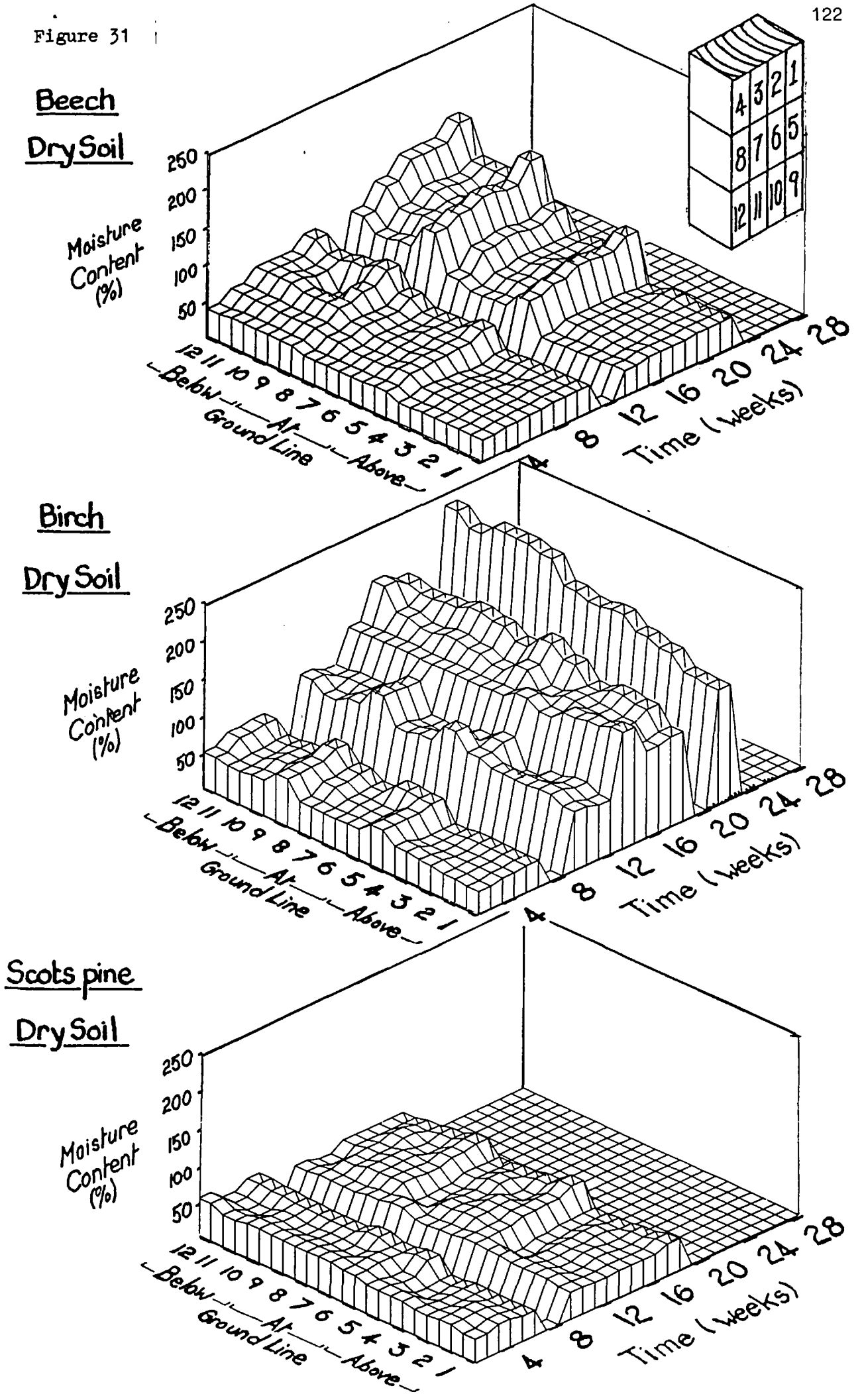
Soil Moisture Conditions
Nominal : 28 % (Dry)

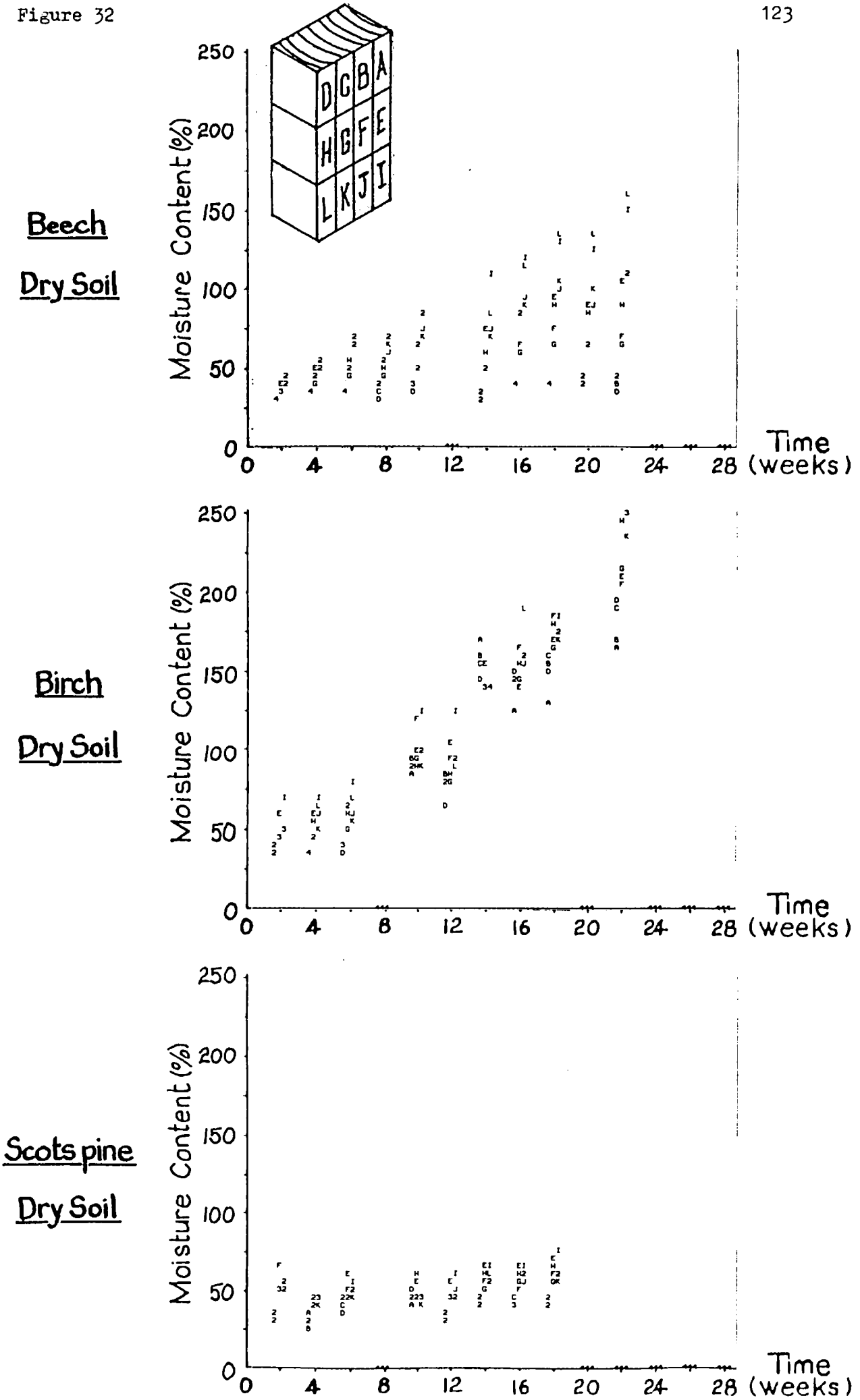
Code Number of Blocks Sampled at each Sample Time

2	4	6	8	10	12	14	16	18	20	22	24	26	28 weeks
OG11	OF06	OG01		OG12	OF12	OG10	OG14	OF01					
OG06	OG09	OF02		OG13	OG16	OG03	OF16	OG08					

(N.B. No initial block weights etc. were recorded for Scots pine)

Figure 31


Beech
Dry Soil
Moisture Content (%)
250
200
150
100
50
12 11 10 9 8 7 6 5 4 3 2 1
Below Ground Line
At
Above
4 8 12 16 20 24 28
Time (weeks)
4 3 2 1
8 7 6 5
12 11 10 9
Birch
Dry Soil
Moisture Content (%)
250
200
150
100
50
12 11 10 9 8 7 6 5 4 3 2 1
Below Ground Line
At
Above
4 8 12 16 20 24 28
Time (weeks)
Scots pine
Dry Soil
Moisture Content (%)
250
200
150
100
50
12 11 10 9 8 7 6 5 4 3 2 1
Below Ground Line
At
Above
4 8 12 16 20 24 28
Time (weeks)


Beech
Dry Soil
Birch
Dry Soil
Scots pine
Dry Soil
D C B A
H G F E
L K J I
Moisture Content (%)
250
200
150
100
50
0
0 4 8 12 16 20 24 28
Time (weeks)

2 weeks there was a distinct difference in MC between the three zones, with those segments having exposed tangential faces being wettest in the below ground zones. This difference was less marked after 14 weeks, where the MC of all 12 segments was similar and high, compared to beech.

In the Scots pine blocks, the overall MC was very much lower than in the hardwoods, with the differences between zones and between the four segments of each zone being less distinct.

Figure 32 shows the average moisture content of each segment plotted against time of exposure for the three species. The beech blocks showed a remarkably linear and regular increase in MC. The differences between the zones and between segments of the same zone were maintained throughout the period of exposure. In the birch blocks the differences between zones and between segments in the same zone were less marked, and the MC of all segments increased with time to well above the MC recorded in beech. In the Scots pine blocks, the difference between zones was apparent, even though there was little increase in wood MC during exposure.

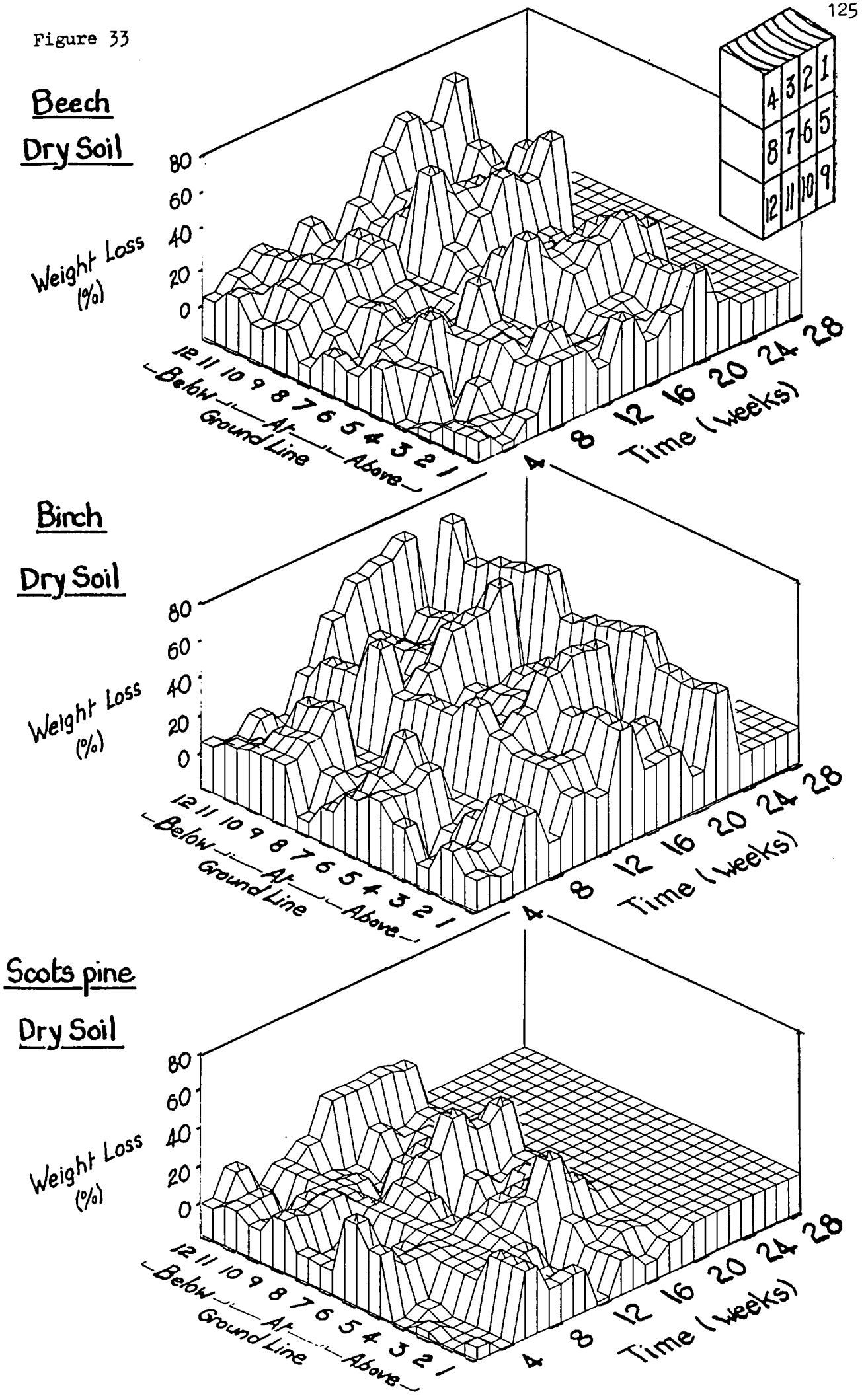
Weight loss - Figure 33

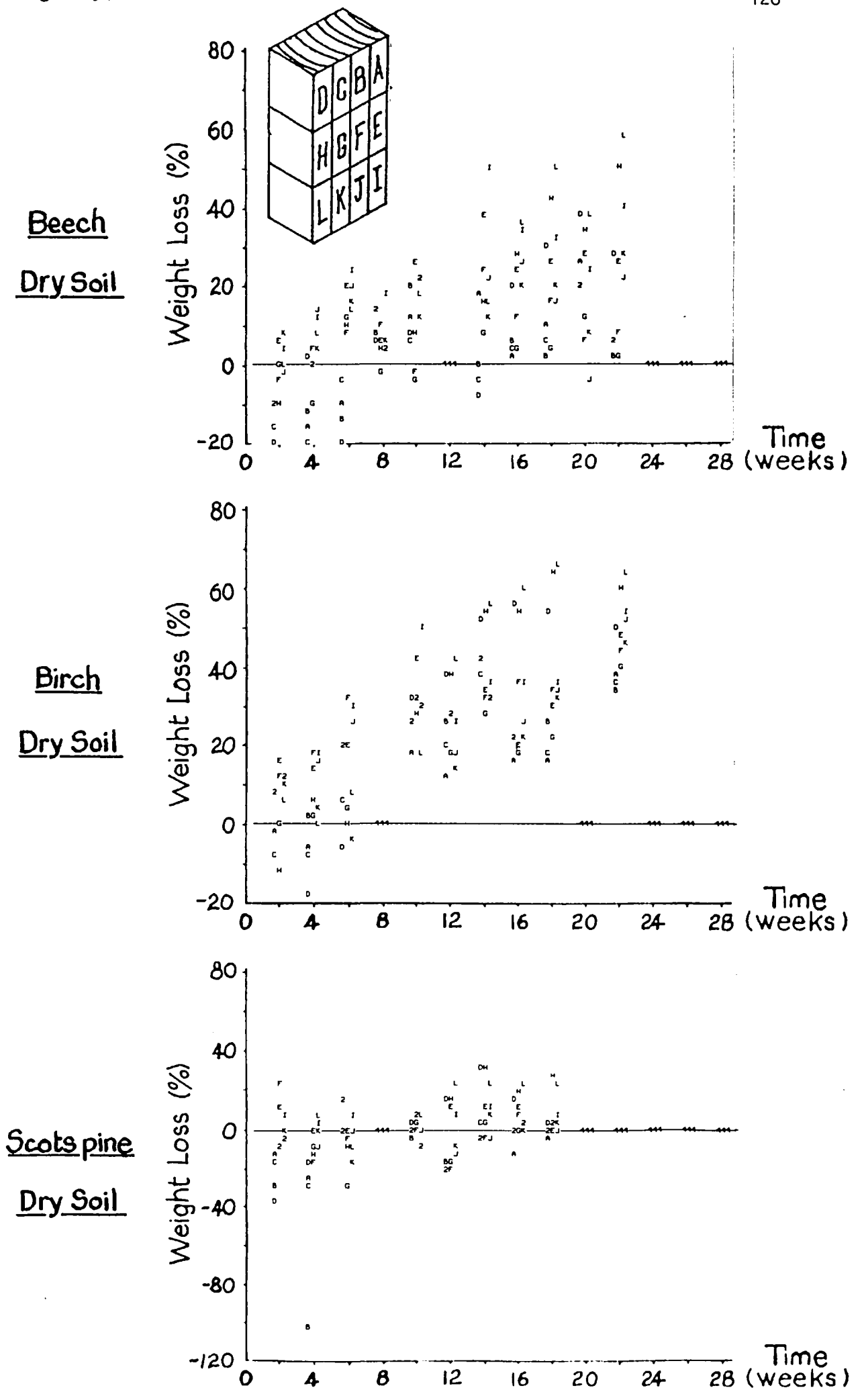
The overall impression is of variability with adjacent samples having very different weight losses, while values for the same segment were high, low or negative at successive sample times. Nevertheless, despite the variability some information can be extracted.

In beech blocks, at 2 and 4 weeks, there were weight gains in the zone above ground. At successive sample times, weight loss of all the segments increased, particularly in segments 4, 8 and 12, whose tangential face would have been in the outer sapwood closest to the cambium in the living tree. Segments 1, 5 and 9 would have been in the inner sapwood with their exposed tangential face closest to the heartwood.

In the birch blocks the overall WL was higher than that of beech, and less variable. The zone below ground exhibited the greatest WL, followed by the ground line zone and then the above ground line zone. The segments with the outer tangential face had the greatest WL in any one zone, even above ground. The weight gain in this zone at 2 and 4 weeks, was less than that observed in beech. At 22 weeks the difference in WL between the three zones was most distinct.

Figure 33


Beech
Dry Soil
Weight Loss (%)
80
60
40
20
0
12 11 10 9 8 7 6 5 4 3 2 1
Below Ground Line
At
Above
4 8 12 16 20 24 28
Time (weeks)
4 3 2 1
8 7 6 5
12 11 10 9
Birch
Dry Soil
Weight Loss (%)
Scots pine
Dry Soil
Weight Loss (%)


Beech
Dry Soil
Birch
Dry Soil
Scots pine
Dry Soil
Weight Loss (%)
Time (weeks)
D C B A
H G F E
L K J I

In the Scots pine blocks there was much less WL than in the hardwoods. The weight gains in the above ground zone were maintained throughout the exposure period, while the only consistent weight losses were in segments 8 and 12 with an outer tangential face exposed to soil contact.

Figure 34 shows the graphs of average weight loss in each segment against sample time, and shows the differences between the species very clearly. Birch decayed more rapidly than beech, notably in the above and ground line zones, where the WL of this zone in beech after 22 weeks was between 3 and 30%, while in birch it was 30-60%. It would appear that the rate of decay in the below ground zone of birch was declining at the later time periods, presumably as maximal decay had been reached. In Scots pine the WL was considerably less than in the hardwoods, with more weight gains than weight losses, although the outer tangential segments reached 30% WL after 18 weeks exposure. The vertical scale on this graph is compressed because there was one erroneous result. The occurrence of errors due to mispunched data cards was eliminated in the results in remaining sections by careful checking.

Acetylene Reduction - Figure 35

The occurrence of AR activity, implying the occurrence of nitrogen fixing bacteria, was sporadic, with very little activity recorded in any of the three species at any of the sample times.

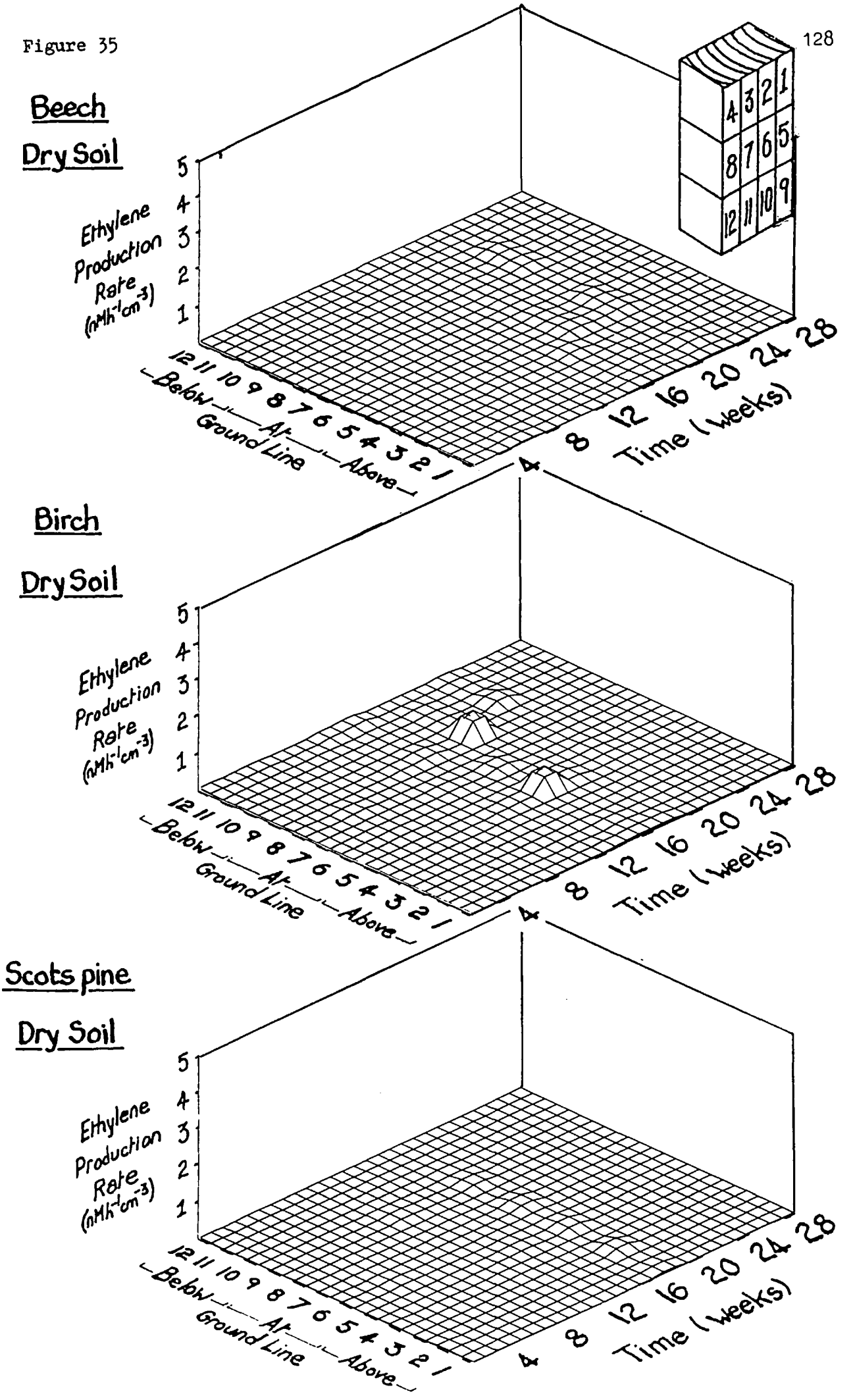
Water content - Figures 36 and 37

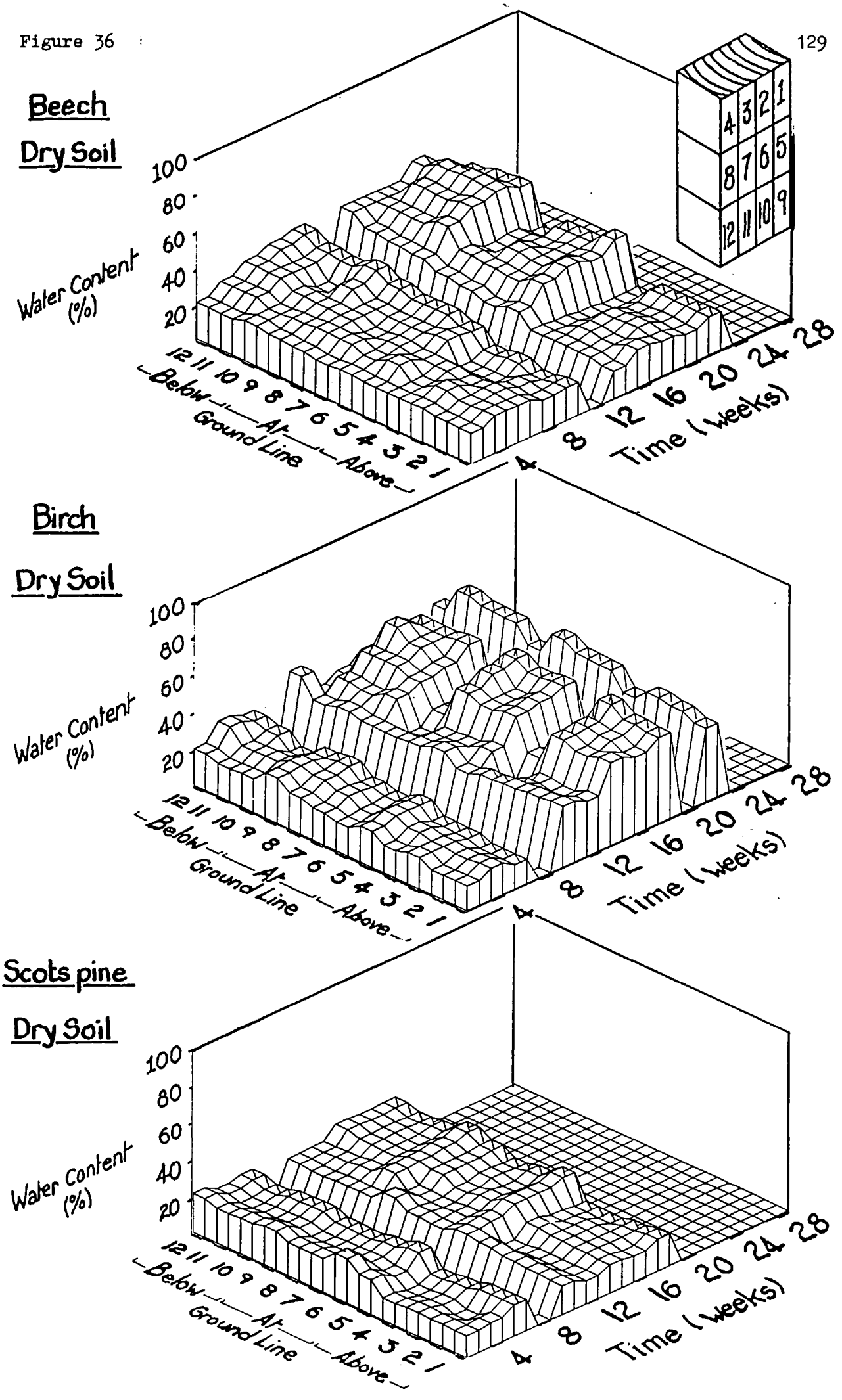
In the beech blocks, the zones had different water contents with the 4 segments in each zone being very similar, except for the outer tangential segments where the WC was lower than in the rest of the zone, unlike the MC values.

In birch, there was a higher WC than in beech, and although there was some difference between zones at the early sample times, there was little difference between zones at the later sample times. As with the beech blocks, and unlike the MC values, the outer tangential segment had a lower WC than the other segments in the same zone.

In Scots pine, the overall WC was lower than in the hardwoods, with, once again, differences between zones and between the outer tangential segment and the others in the same zone.

Figure 35


Beech
Dry Soil
Ethylene Production Rate (nMh⁻¹cm⁻³)
5
4
3
2
1
12 11 10 9 8 7 6 5 4 3 2 1
Below Ground Line
At
Above
4 8 12 16 20 24 28
Time (weeks)
4 3 2 1
8 7 6 5
12 11 10 9
Birch
Dry Soil
Ethylene Production Rate (nMh⁻¹cm⁻³)
5
4
3
2
1
12 11 10 9 8 7 6 5 4 3 2 1
Below Ground Line
At
Above
4 8 12 16 20 24 28
Time (weeks)
Scots pine
Dry Soil
Ethylene Production Rate (nMh⁻¹cm⁻³)
5
4
3
2
1
12 11 10 9 8 7 6 5 4 3 2 1
Below Ground Line
At
Above
4 8 12 16 20 24 28
Time (weeks)


Beech
Dry Soil
Water Content (%)
100
80
60
40
20
12 11 10 9 8 7 6 5 4 3 2 1
Below Ground Line
At
Above
4 8 12 16 20 24 28
Time (weeks)
4 3 2 1
8 7 6 5
12 11 10 9
Birch
Dry Soil
Water Content (%)
Scots pine
Dry Soil
Water Content (%)

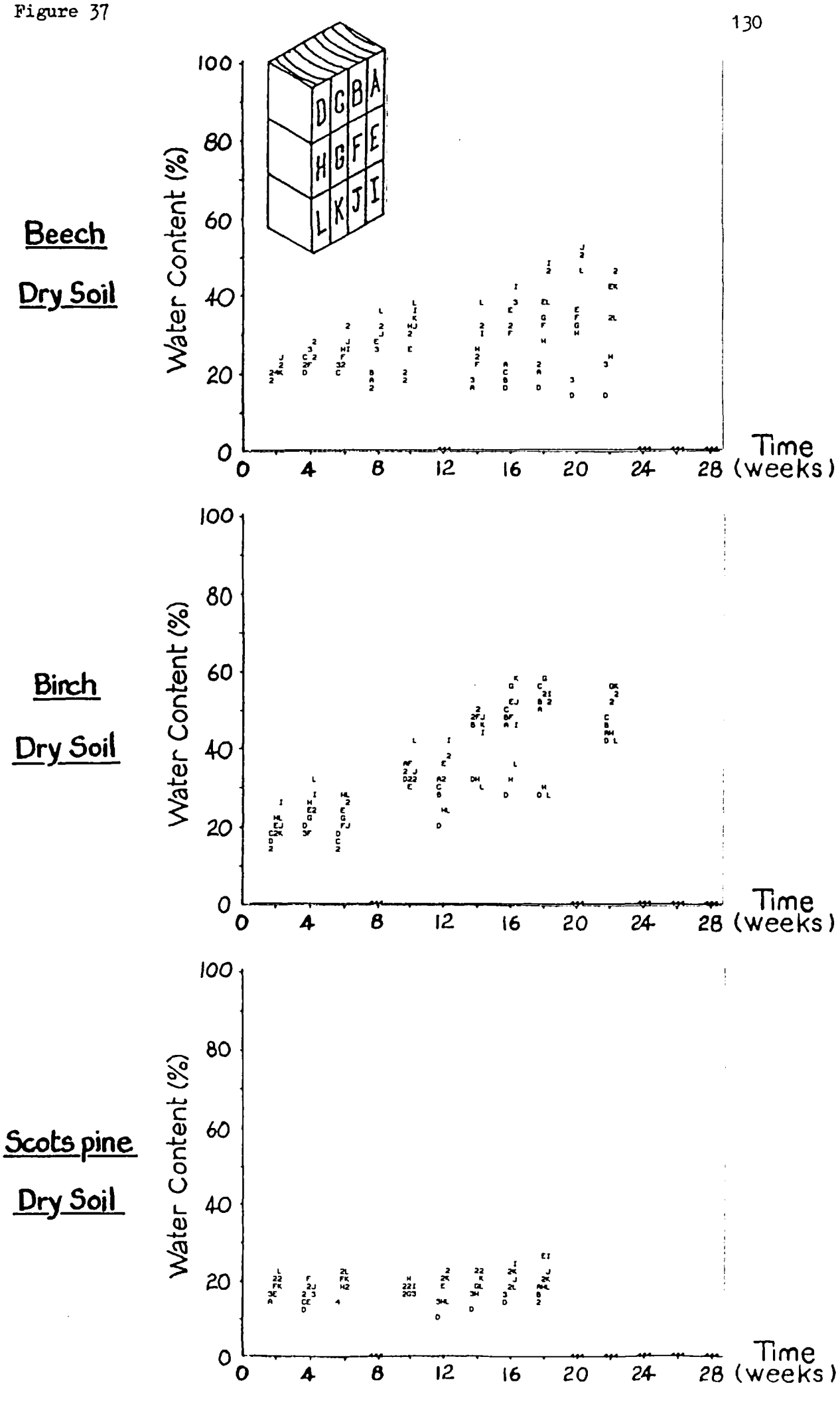

Beech
Dry Soil
Birch
Dry Soil
Scots pine
Dry Soil
Water Content (%)
Time (weeks)
0
20
40
60
80
100
0
4
8
12
16
20
24
28
D C B A
H G F E
L K J I

Figure 37 shows graphs of average segment water content, plotted against sample time for the three species. In the beech blocks the WC increased in the below ground zone only, and less rapidly than in birch, where all the zones increased in WC. In birch the rate of increase began to level off after 16 weeks at 40-50%, except in the above ground zone. The low WC of the outer tangential segment was very clear in both beech and birch. In the Scots pine blocks, the rate of water content increase was minimal, and only reached 15-25% after 18 weeks.

5.4. Discussion

The considerable weight loss in the beech and birch blocks in the presence of only sporadic and minimal acetylene reduction activity indicates that the presence of nitrogen fixing organisms is not essential for the decay of these hardwoods by fungi.

Decay in birch occurred faster and to a greater extent than in beech, and in both species there was greater decay in the outer tangential face, which would have been closest to the cambium in the living tree. Perhaps there was a larger proportion of living, active or nutrient-containing cells in the outer sapwood than in the inner sapwood. Perhaps it reflected a greater permeability of those outer regions which allowed the faster penetration of water longitudinally and radially, carrying nutrients, fungal spores and bacteria which would accelerate colonisation and decay in the outer sapwood. The importance of water movement is illustrated by the weight gains observed, particularly in the above ground zones of all the wood species, presumably due to an influx of soil water containing soluble salts which were deposited in the wood above ground when the water evaporated. As decay proceeded in the hardwoods, the blocks lost weight, but in Scots pine, there were was less decay, the weight gains persisted in the above ground zone. This deposition of soil salts may well influence decay, although little decay was recorded in Scots pine in this experiment.

In addition, little acetylene reduction activity was recorded in the Scots pine blocks, presumably because the soil or wood moisture content was too low for the colonisation of nitrogen fixing bacteria that was observed in previous experiments using this species. The implication is that nitrogen fixation may still be significant in the decay of softwoods, so that the weight loss observed in Scots pine would have been even higher in the presence of nitrogen fixing bacteria. The significance of this group of organisms to softwood decay still requires further investigation.

The results illustrated the value of the exposure system and sampling procedure for the investigation of timber decay in ground contact. The ability to specify and control the soil moisture content ensured that different species of wood could be exposed to the same conditions and that any difference in their response could be detected. Sampling at regular intervals showed the difference in decay rate between birch and beech, and that the rate was linear initially, but reached a plateau eventually. The ability to monitor the amount of water, decay and AR activity in different zones and segments of the blocks showed how wood, soil, water and decay affected each other, e.g. water uptake may be important in transporting nutrients and propagules from the soil into the wood. Once the wood is wet, differences in moisture content could develop in zones below, at and above the ground line which could influence the onset and development of decay in these zones. Fungal decay could increase wood permeability allowing the wood to absorb more water, perhaps carrying more nutrients and propagules.

Obviously wood in ground contact is in a very complex environment and when and how it decays must depend upon a number of interacting factors. The effect of some of these factors, such as position within the tree, natural wood susceptibility, water uptake and supply, nutrient content and position relative to the ground line can be seen in this experiment. The technique would appear to be capable of providing considerable information on the relationship between these factors, particularly on the relationship between wood species, soil, soil water and decay.

5.5. Conclusions

The presence of nitrogen fixing bacteria is not essential for the decay of hardwoods in the soil used, but may be of significance in the decay of softwoods. Further investigations of softwood decay in the presence and absence of nitrogen fixing organisms would be of value in determining the significance of nitrogen fixing bacteria to timber decay in soil contact.

6. THE EFFECT OF SOIL MOISTURE CONTENT UPON SCOTS PINE SAPWOOD DECAY

6.1. Introduction

The aim of this experiment was to investigate how different soil moisture contents, (dry, moist and wet), affected the MC of Scots pine sapwood blocks exposed to soil and how the wood MC affected decay, measured by weight loss, and the occurrence of nitrogen fixing organisms, measured by the AR technique.

6.2. Materials and Methods

The preparation, setting-up, maintenance and sampling of the experiment were as described in Section 3. Blocks cut from the same plank were randomly assigned to one of three bin systems, containing soil maintained at 25% (dry), 35% (moist) and 40% (wet). Table 10 gives the average values of the block weight and density, and the MC, theoretical void volume and maximum moisture content of the blocks. The nominal and actual soil MC are given together with the code number of blocks removed at each sample time. In the vessel containing soil at 25%, after 77 days the soil MC was increased to 50% (waterlogged) by the addition of distilled water.

6.3. Results: Moisture Content - Figures 38 and 39

In the dry soil bin, the effect of increasing the moisture content of the soil after 11 weeks was apparent and obvious, an increase in the MC of all the wood segments in the 12 weeks sample. When exposed to dry soil, the MC of all the segments was very similar at around 25-30% in all three zones, below, at and above the ground line. This increased only marginally to 27-32% after 10 weeks. After the soil had been wet to 50%, the MC of the wood increased to 120 to 200%. Although the overall MC of each zone was similar, there were differences in the MC of the segments within each zone. The outer segments (with a tangential face in soil contact) had a higher MC than the inner segments (with no exposed tangential faces). This difference in the MC of the segments was remarkably similar at 12 and at 16 weeks, although the absolute values were higher at 16 weeks.

In the moist soil the MC of the blocks at 14 days was similar to that of blocks in dry soil, but then the MC increased, particularly in the zones in soil contact. The outer tangential segment, which would have been closest to the cambium in the living tree was consistently wetter than the other segments in the same zone, while the segment which would have been closest to the heartwood was wetter than the two segments with no tangential face exposed, as was observed in

Table 10 Wood Block and Soil Data

Wood Species	: Scots pine	Soil Type	: Loam
Initial Block Wt.	: 9•42 ± 0•35 g	Initial Moist. Cont.	: 9•36 %
Calc. Oven Dry Wt.	: 8•61 g	Void Volume	: 61•73 %
Initial Density	: 0•502 g cm^{-3}	Max. Moist. Cont.	: 124•04 %

Soil Moisture Conditions
Nominal : 25 % (Dry) 50 % (Waterlogged)
Calculated :

2	4	6	8	10	12	14	16	18	20	22	24	26	28 weeks
22	25	25	25	26	44		49	51					

Code Number of Blocks Sampled at each Sample Time

2	4	6	8	10	12	14	16	18	20	22	24	26	28 weeks
ID29	ID18	IC33	ID04	ID25	IC13		IC06	ID11					
IB20	ID20	ID13	IB14	ID19	IC05		ID32	IB23					
IB33	IB15	IB34	IC14	IC29	IC07		IB28	IC19					
IB02	IB35	IC24											

Wood Species	: Scots pine	Soil Type	: Loam
Initial Block Wt.	: 9•42 ± 0•35 g	Initial Moist. Cont.	: 9•36 %
Calc. Oven Dry Wt.	: 8•61 g	Void Volume	: 61•73 %
Initial Density	: 0•502 g cm^{-3}	Max. Moist. Cont.	: 124•04 %

Soil Moisture Conditions
Nominal : 35 % (Moist)
Calculated :

2	4	6	8	10	12	14	16	18	20	22	24	26	28 weeks
32	34	34	35	35	35		35	35					

Code Number of Blocks Sampled at each Sample Time

2	4	6	8	10	12	14	16	18	20	22	24	26	28 weeks
IB24	IE35	IE15	IC23	IC18	IC26		IE30	IB16					
ID07	ID28	ID14	IE28	IC25	IB13		IB19	IC01					
ID24	ID17	IC17	ID27	IC20	ID21		IB08	IB17					
ID05	IE10	IB09											

Wood Species	: Scots pine	Soil Type	: Loam
Initial Block Wt.	: 9•42 ± 0•35 g	Initial Moist Cont.	: 9•36 %
Calc.Oven Dry Wt.	: 8•61 g	Void Volume	: 61•73 %
Initial Density	: 0•502 g cm^{-3}	Max. Moist. Cont.	: 124•04 %

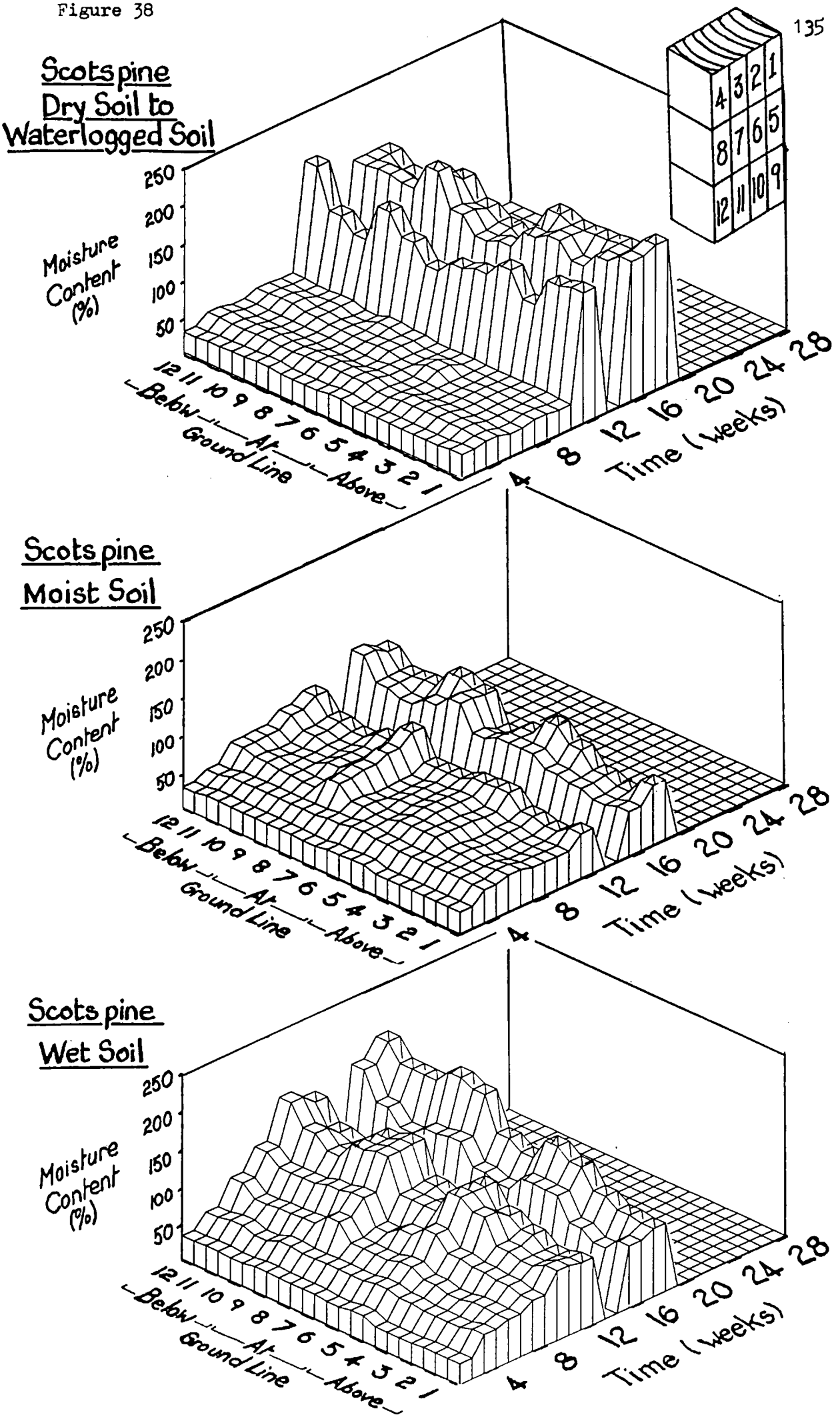
Soil Moisture Conditions
Nominal : 40 % (Wet)
Calculated :

2	4	6	8	10	12	14	16	18	20	22	24	26	28 weeks
37	38	38	39	36	38		38	39					

Code Number of Blocks Sampled at each Sample Time

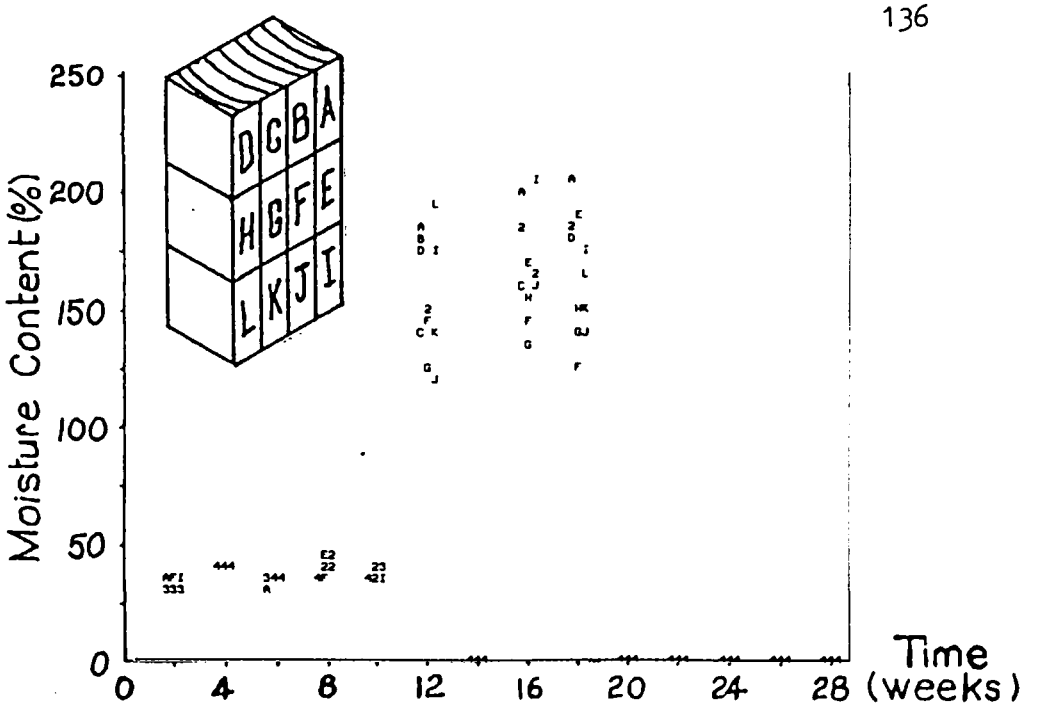
2	4	6	8	10	12	14	16	18	20	22	24	26	28 weeks
IE16	ID09	IE14	IEC3	IE20	ID10		ID16	IB36					
IB07	IE32	IB21	IE13	IC03	IE21		ID31	IE27					
IB05	ID22	IC10	IE33	IE29	IB11			IC08					
IC04	IC16	IE31											

Figure 38


Scots pine
Dry Soil to
Waterlogged Soil
Moisture Content (%)
250
200
150
100
50
12 11 10 9 8 7 6 5 4 3 2 1
Below Ground Line
At
Above
4 8 12 16 20 24 28
Time (weeks)
4 3 2 1
8 7 6 5
12 11 10 9
Scots pine
Moist Soil
Moisture Content (%)
250
200
150
100
50
12 11 10 9 8 7 6 5 4 3 2 1
Below Ground Line
At
Above
4 8 12 16 20 24 28
Time (weeks)
Scots pine
Wet Soil
Moisture Content (%)
250
200
150
100
50
12 11 10 9 8 7 6 5 4 3 2 1
Below Ground Line
At
Above
4 8 12 16 20 24 28
Time (weeks)

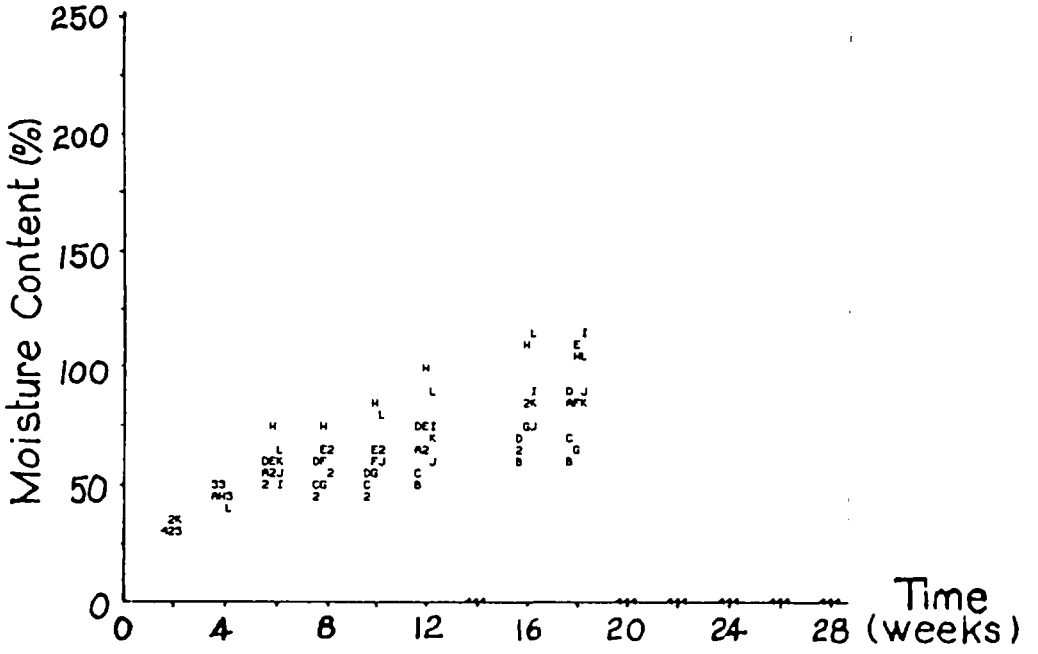
Scots pine

Dry Soil to Waterlogged Soil



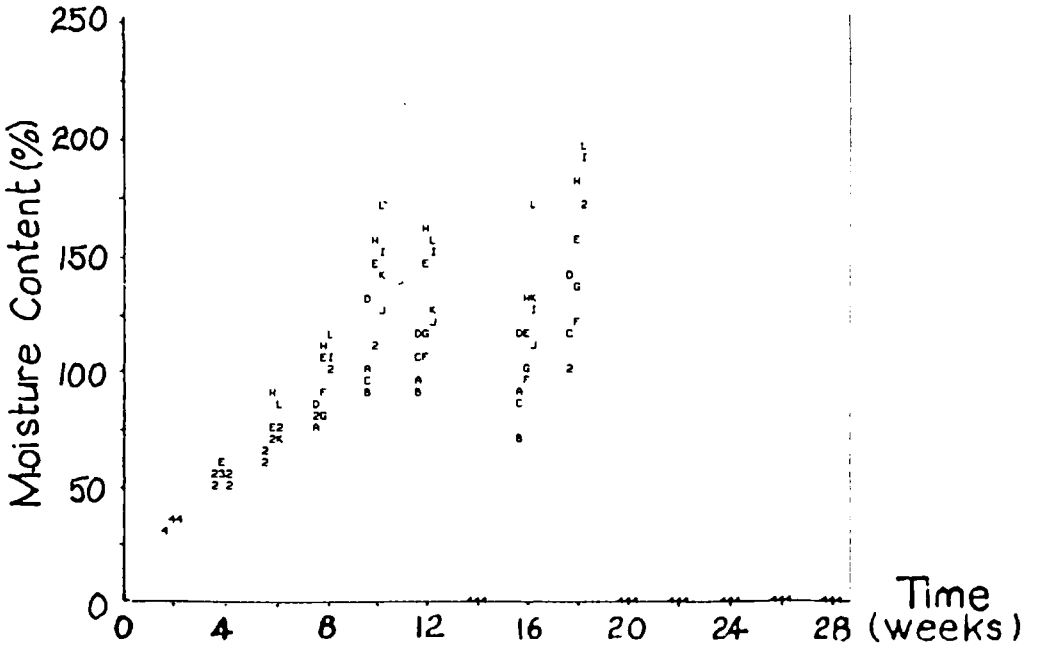
Scots pine

Moist Soil



Scots pine

Wet Soil



the beech blocks in Section 5. The MC of the three zones was different, particularly at later sample times.

In the wet soil, the increase of MC with time was evident, although the increase levelled off after 10 weeks.

Figure 39 shows the graphs of moisture content in each of the 12 segments against sample time for the dry, moist and wet soils.

In the dry soil the MC after 2 weeks was 30-35% in all the segments. This level was maintained up to 10 weeks with only slight variations, presumably reflecting the different equilibrium moisture contents of the different segments and blocks. However, 7 days after the soil had been wetted to 50%, the MC of all the segments had risen to around 150% and differences between the zones were apparent. Below ground there was no increase in MC with time although the MC in the segments became less variable. In the ground line zone the segment MC values became more variable with time, with some segments increasing and some decreasing in MC. Above ground, all the segments increased in MC.

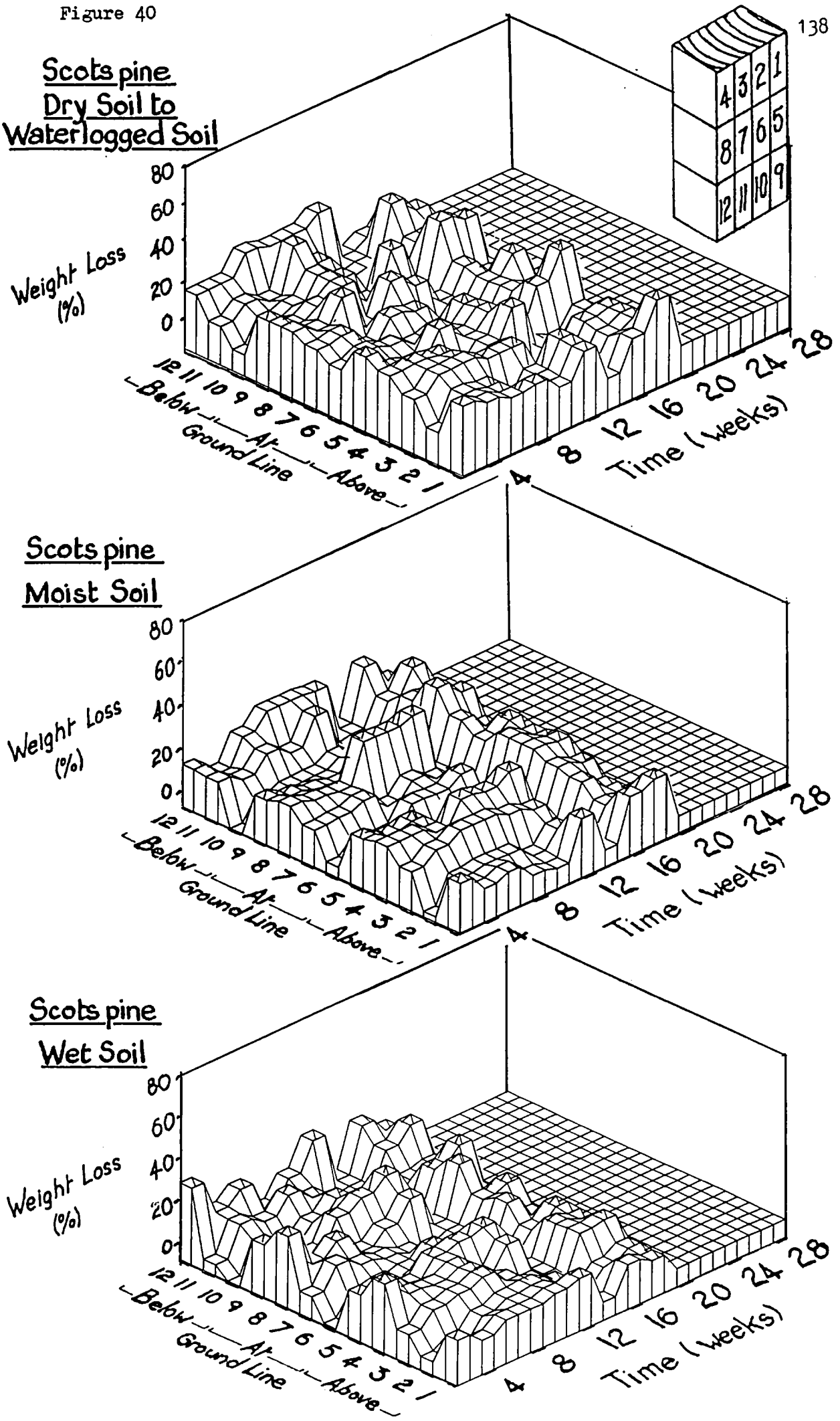
In the moist soil the MC results were remarkably regular and consistent, with a uniform increase in MC in all the segments with time, and the difference in MC between the segments of the same zone being maintained as the blocks got wetter. The two buried zones had a similar MC, although the deeper zone was marginally wetter, while the zone above ground was distinctly drier. The MC after 2 weeks was, once again, 30-35% in all the segments, rising to 50-100% after 12 weeks.

In the wet soil, the results were again remarkably regular. The rate of MC increase was higher in the wet soil than in the moist soil, from a MC of 33-40% at 14 days to 100-150% after 10 weeks. The low values at 16 weeks may have been due to the lack of soil moisture content adjustment at 14 weeks which allowed the soil to dry slightly, and was reflected in a lower wood MC. As in the moist soil, the difference between zones became more marked as the blocks got wetter, with the outer tangential segment consistently the wettest in each zone.

Weight loss - Figures 40 and 41

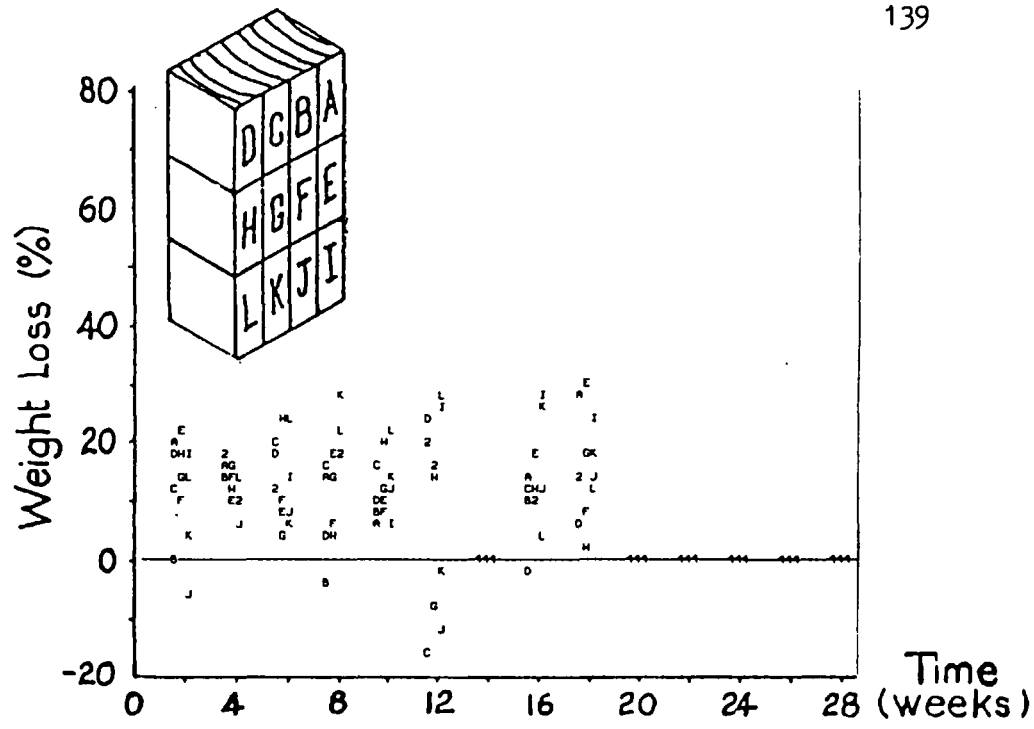
At 14 days in all 3 treatments, the pattern of weight loss in each zone

Figure 40


Scots pine
Dry Soil to
Waterlogged Soil
4 3 2 1
8 7 6 5
12 11 10 9
Weight Loss (%)
80
60
40
20
0
12 11 10 9 8 7 6 5 4 3 2 1
Below
At
Above
Ground Line
4 8 12 16 20 24 28
Time (weeks)
Scots pine
Moist Soil
Weight Loss (%)
Scots pine
Wet Soil
Weight Loss (%)

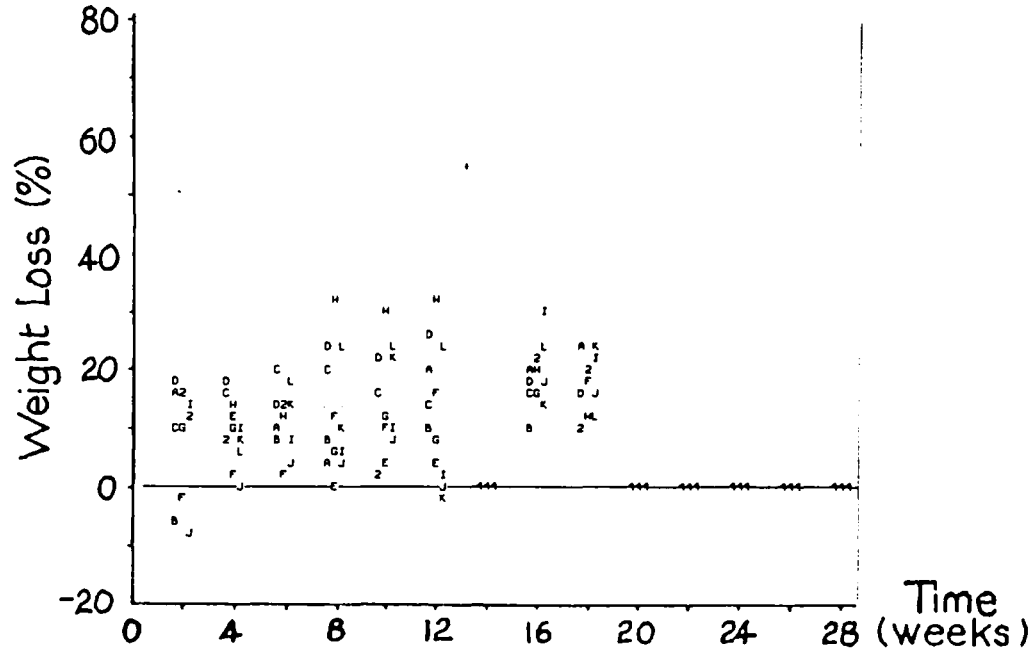
Scots pine

Dry Soil to Waterlogged Soil



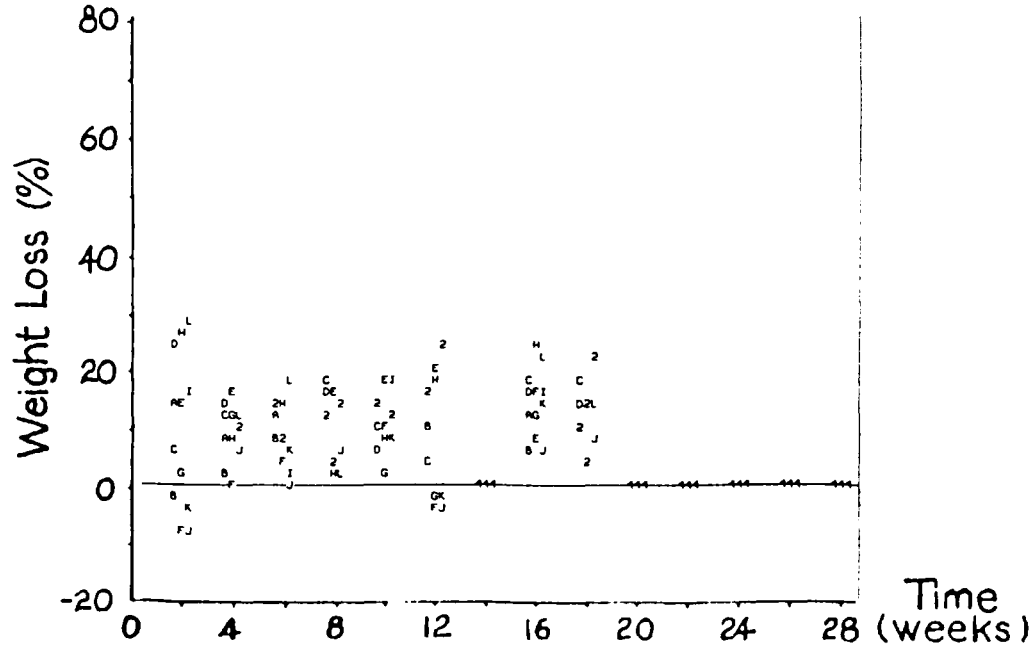
Scots pine

Moist Soil



Scots pine

Wet Soil



appeared very similar with the outer tangential segment having the highest WL, followed by the inner tangential, the outer central and lastly the inner central segment. This pattern was probably an artifact produced by variation in the density of each segment in a zone and the method of calculation of WL which assumed that each segment was of the same density as the entire block. Thus some of the variability in the WL results must have been due to differences in density within the block, rather than to weight loss due to decay.

Nevertheless, despite the variability, there appeared to be greater WL in the blocks exposed to moist soil than in those exposed to dry or wet soil, although even in the moist soil the WL was less than that found in Scots pine sapwood in Section 5.

Figure 41 shows the graphs of weight loss in the segments taken from blocks exposed to dry, moist and wet soil. In dry soil, there was no more WL after 10 wks than after 2. After the soil had been wet, some segments showed an increase in weight, but at 16 and 18 weeks, weight loss had occurred. The weight change at 2 weeks ranged from a weight gain of 3% to a weight loss of 22%, and at 18 weeks ranged from 2 to 26% weight loss.

In moist soil, the weight losses were very variable with weight gain in some segments. The range of weight change was from a gain of 10% to a weight loss of 30% at 2 weeks, to a weight loss in the range 10-24% after 18 weeks, a definite, if slight, loss in weight.

In the wet soil the weight losses became less variable with time, changing from a range of weight gain of 8% and a weight loss of 28% at 2 weeks, to a weight loss of 4-22% after 18 weeks, a definite weight loss, although less than that found in moist soil.

Table 11 shows the average WL of the entire blocks at each sample time in the three soil systems. If the 2 week value is taken as datum, then a weight gain occurred in the moist soil initially, but became a weight loss greater than that found in dry and wet soil. The WL in the blocks exposed to very wet soil appeared to be increasing.

Table 11 Weight Loss (%) in Scots pine exposed to soil.

Weeks of Exposure	Soil Moisture Level			
	Dry to Waterlogged 25%	50%	Moist 35%	Wet 40%
2	10•65		8•61	10•75
4	9•96		9•15	7•64
6	13•01		9•54	12•16
8	11•28		10•12	8•12
10	11•99		13•79	11•14
12		10•07	13•51	10•31
14				
16		13•03	19•14	16•00
18		15•92	17•07	13•00

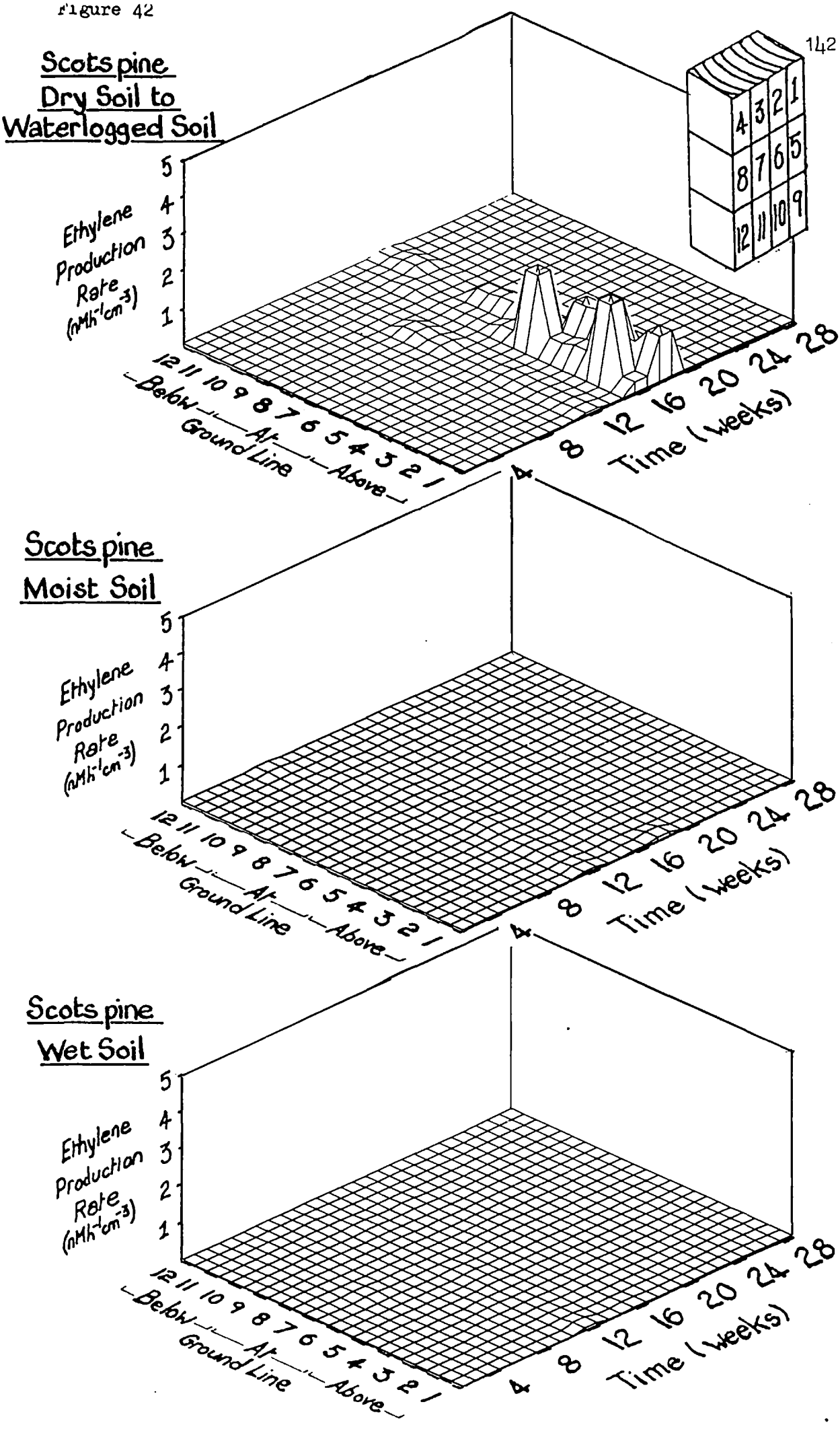
Acetylene Reduction - Figures 42 and 43

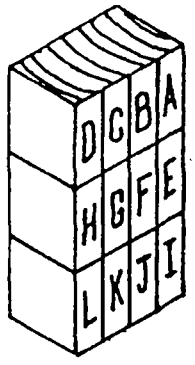
Figure 42 shows the acetylene reduction activity recorded in the segments taken from blocks exposed to dry, moist and wet soil. Significant activity was not recorded in the dry, moist or wet soil blocks, but was found after the dry soil moisture content had been increased to 50%. Some activity was apparent at 12 weeks and increased considerably, particularly above the ground line, at 16 and 18 weeks.

Figure 43 shows the graphs of acetylene reduction rate in the segments. The graphs have the same vertical scales. In the dry soil no activity was recorded in the wood until after the soil was wet at 11 weeks, when a rate of around 0.1 nMh^{-1} cm^{-3} was recorded in the upper 2 zones. Most activity was recorded at 16 weeks, particularly in the above ground zone where the rate ranged from 0.3 to 2.2. At 18 weeks, the difference between the zones was obvious with the activity in the above ground zone ranging from 0.8 to 1.3, at the ground line from 0.3 to 0.4 and below ground only up to 0.2 nMh^{-1} cm^{-3}.

In the moist soil the highest activity recorded was only 0.105nMh^{-1} cm^{-3}. Activity was detectable at 2 weeks and its amount and occurrence increased with time up to 12 weeks, particularly in the above ground zone. The activity decreased at 16 or 18 weeks to only 0.01 nMh^{-1} cm^{-3}.

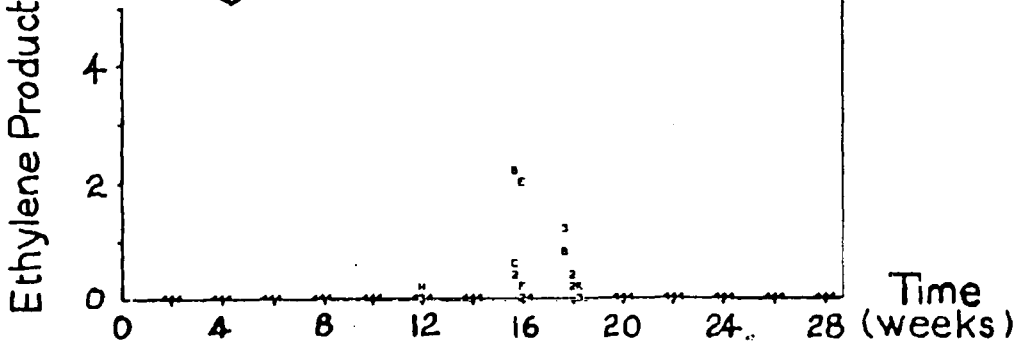
Figure 42


Scots pine
Dry Soil to
Waterlogged Soil
Ethylene
Production
Rate
(nMh⁻¹cm⁻³)
5
4
3
2
1
12 11 10 9 8 7 6 5 4 3 2 1
Below
At
Above
Ground Line
4 8 12 16 20 24 28
Time (weeks)
4 3 2 1
8 7 6 5
12 11 10 9
Scots pine
Moist Soil
Ethylene
Production
Rate
(nMh⁻¹cm⁻³)
5
4
3
2
1
12 11 10 9 8 7 6 5 4 3 2 1
Below
At
Above
Ground Line
4 8 12 16 20 24 28
Time (weeks)
Scots pine
Wet Soil
Ethylene
Production
Rate
(nMh⁻¹cm⁻³)
5
4
3
2
1
12 11 10 9 8 7 6 5 4 3 2 1
Below
At
Above
Ground Line
4 8 12 16 20 24 28
Time (weeks)



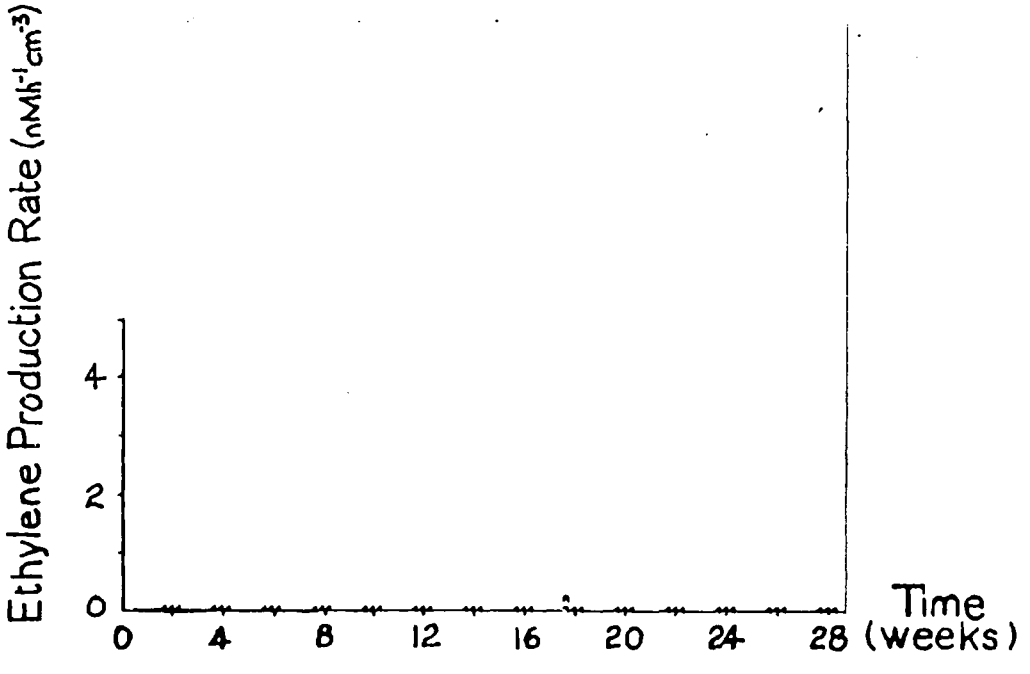
Scots pine

Dry Soil to Waterlogged Soil



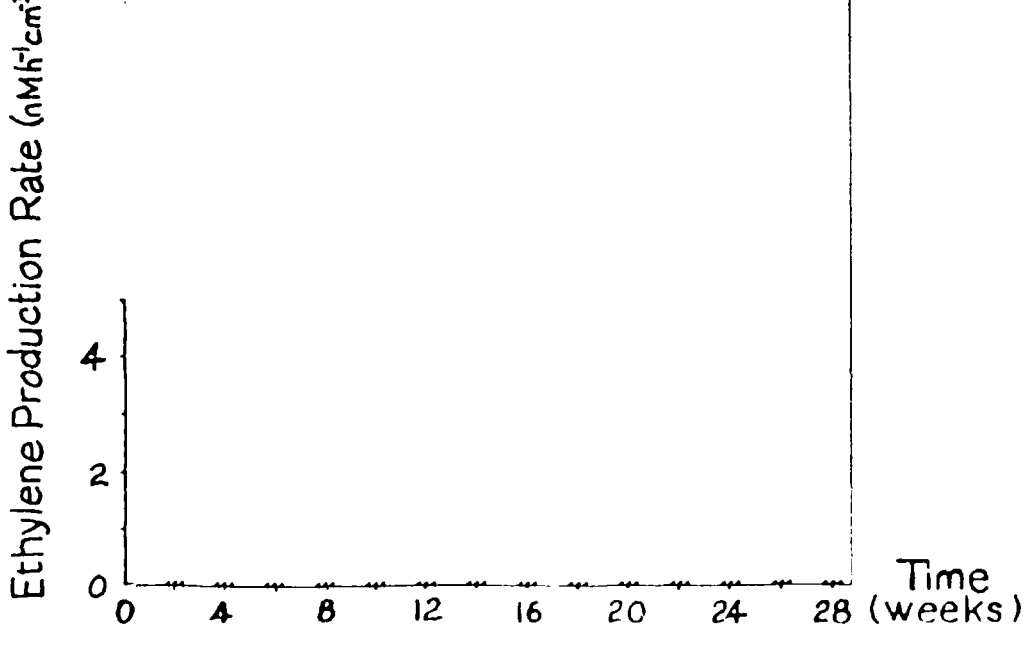
Scots pine

Moist Soil



Scots pine

Wet Soil



In the wet soil the highest activity recorded was 0.07. Activity was first detected at 6 weeks in the buried zones, which increased up to 12 weeks but was slightly lower at 16 and 18 weeks. The above ground zone exhibited a higher rate of activity than the two buried zones. The overall rate was approximately 0.0075 nMh^{-1} cm^{-3} ,too low to be plotted on Figure 43.

Water content - Figure 44

Figure 44 shows the water content (the weight of water expressed as a percentage of the wood volume) of the segments taken from blocks exposed to dry moist and wet soil.

In the dry soil, which was wet at 11 weeks to 50% soil moisture content, the increase in wood water content was obvious. At 2 weeks, segments 2, 6 and 10 had a higher WC than the other segments in the same zone. This was an effect of low density in these segments relative to the others, which artificially increased their value of WC. At 12 weeks the WC was similar in all 12 segments, unlike the MC values at the same time (see Figure 38).

The effect of a high soil moisture content was apparent from the blocks exposed to moist soil, where the WC increased with time. At 12 weeks, the outer tangential segment was wettest and the overall water distribution was the same as observed using moisture content as a criterion. Water content was of particular interest where massive decay had occurred, but in the absence of marked decay and weight loss, moisture content was adequate to describe and evaluate the water distribution and content. In the very wet soil the wood must have been approaching saturation, as the wood WC exceeded the average theoretical maximum of 56.96%.

6.4. Discussion

This experiment showed that there is a definite, if complex and indirect, relationship between wood moisture content and soil moisture content. If the rate of change of wood moisture content is plotted against soil moisture content, (Figure 45), the rate for the first 2 weeks of exposure is very similar in dry, moist and wet soils at around 2.5% d^{-1}. This implies that the initial uptake is independent of soil moisture content and determined by the wood. The MC after the first 2 weeks is around 30% in all three soils. However if the rate for 2 to 10 weeks is plotted for dry, moist and wet soils, together with the rate over the first 7 days exposure to waterlogged soil, the rates for the four soil moisture levels are different. There appears to be an exponential relationship

Figure 44

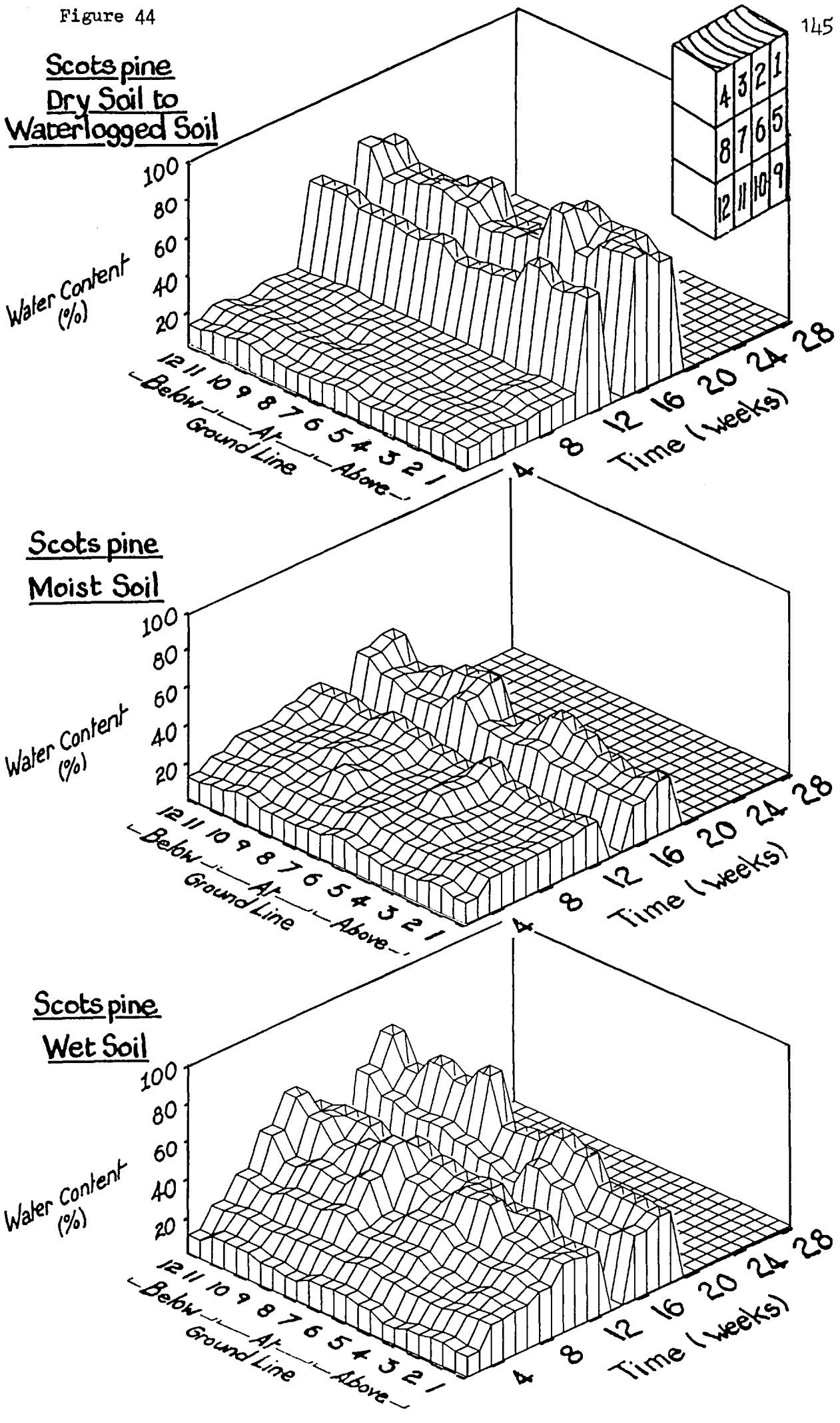

Scots pine
Dry Soil to
Waterlogged Soil
Scots pine
Moist Soil
Scots pine
Wet Soil
Water Content (%)
100
80
60
40
20
12 11 10 9 8 7 6 5 4 3 2 1
Below Ground Line
At
Above
4 8 12 16 20 24 28
Time (weeks)
4 3 2 1
8 7 6 5
12 11 10 9

Figure 45 Rate of change of wood moisture content against soil MC.

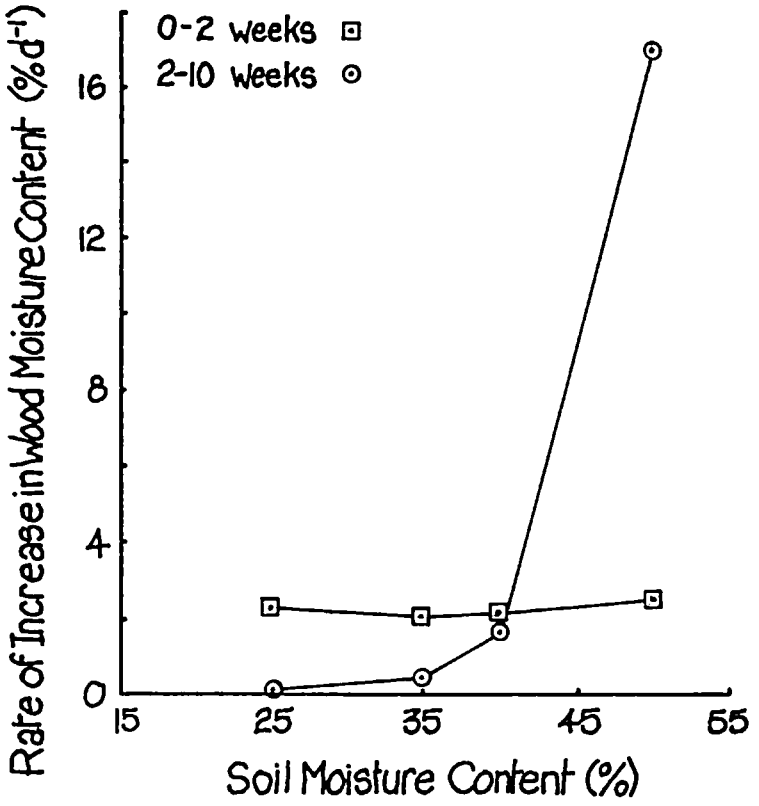
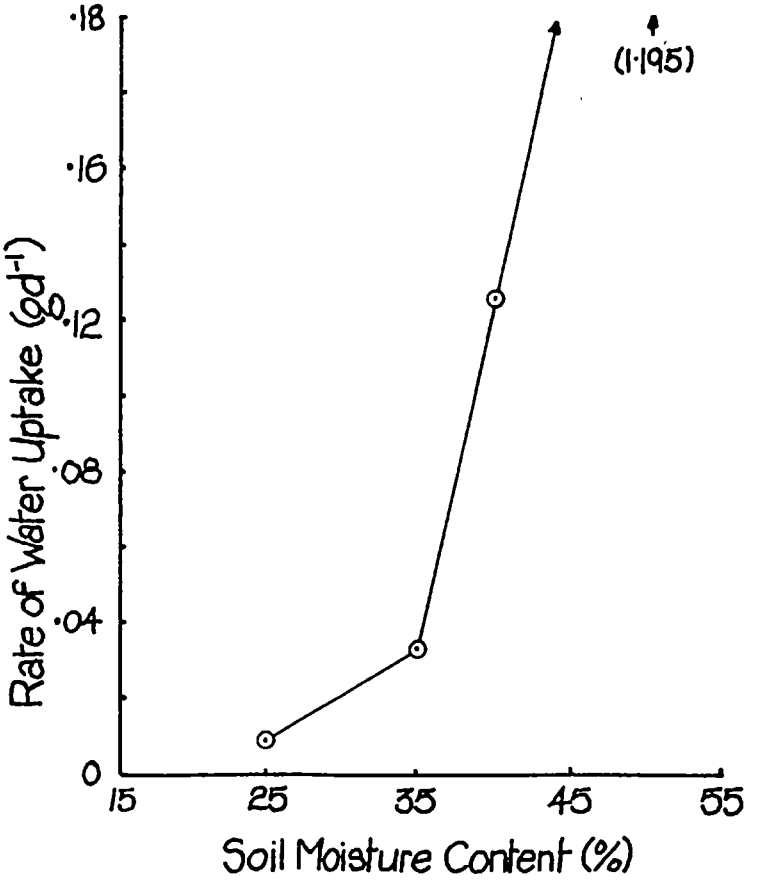


Figure 46 Rate of water uptake by wood against soil moisture content.



between the rate of wood MC increase and the soil moisture content. If the rate of water uptake in g d^{-1} is plotted against soil moisture content (Figure 46), the relationship between the rate of water uptake and soil MC is also exponential. Now if the final MC of the wood is plotted against soil MC, (Figure 47), the relationship is sigmoid. The inference is that wood has a maximum moisture content, presumably at saturation, which cannot be exceeded, and is only approached in wet soil but reached in waterlogged soil.

The implication of these results is that there is a precise relationship between wood MC and soil MC even above the water holding capacity of the soil, and that wood and soil interact to define the rate of uptake of water by wood in soil contact, and its final moisture content.

The uptake of water by wood in soil content can be divided into three phases on the basis of the results presented above (Figure 48). The initial wetting-up phase to around 30% is controlled by the wood. The closeness of this value to fibre saturation point (28%) may not be coincidental if this phase represents the wetting of the cell wall at a rate which is independent of the amount of water in the soil. In the second phase the rate of wetting-up is mainly controlled by the soil, presumably by the interaction of the water-filled capillaries and pores of the soil with the capillaries and pores in the wood. The rate of uptake of water by wood in this phase is dependent on the way the soil pores in contact with the wood,transport, retain and lose water. The implication is that the amount of <u>available</u> water and the way in which that water is held in the soil at any particular soil MC is of more importance in determining water uptake than is the total amount of moisture in the soil, i.e. its moisture content. Unfortunately, soil MC is considerably easier to measure and visualise than "available water", which has matric, gravitational, hydraulic and osmotic components. In Figure 48 the rate of MC increase would appear to be proportional to the amount of available water in the soil, rather than the soil MC.

The time taken to reach the equilibrium moisture content (EMC) of the wood in the soil is proportional to the amount of available water in the soil, so that the more water there is available in soil, the faster equilibrium is reached. The third phase is characterised by the attainment of EMC, where presumably the water-filled pores of the wood are in equilibrium with the water filled pores of the soil. The amount of water in the wood at this equilibrium point depends upon the amount of available water in the soil, so that the final MC of the wood between FSP (around 30%), and saturation (around 120% for Scots pine sapwood) is proportional to the water availability of the soil.

Figure 47 Final wood moisture content against soil moisture content.

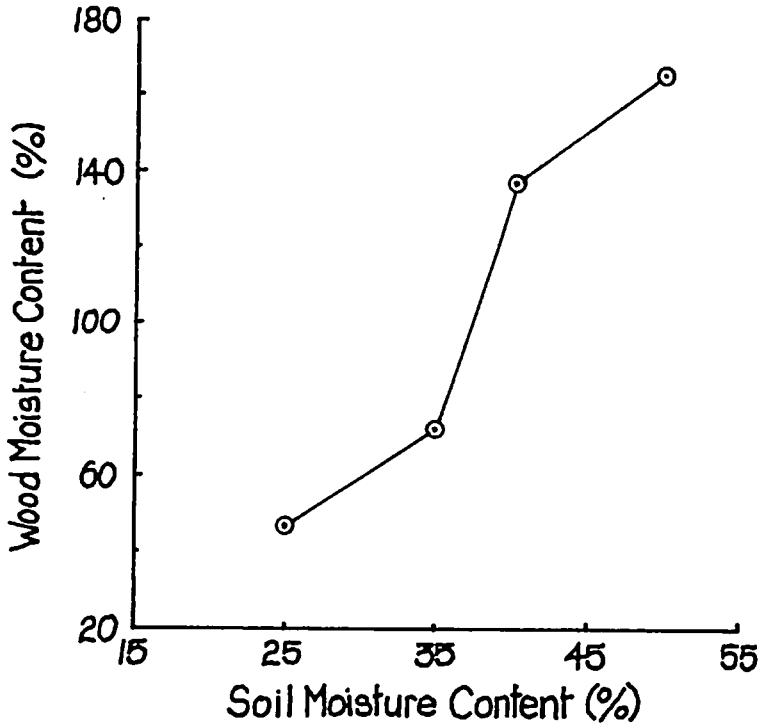
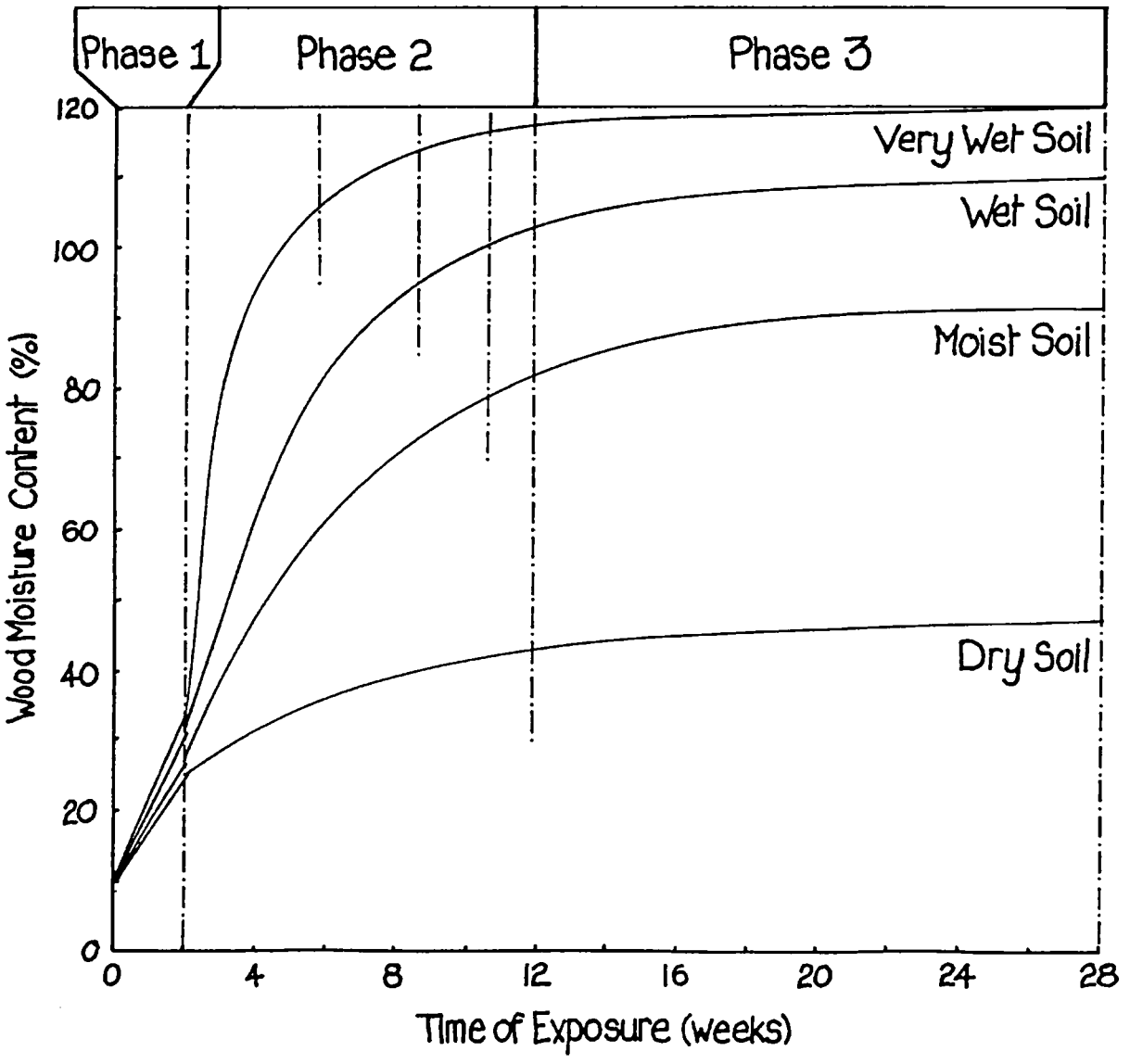
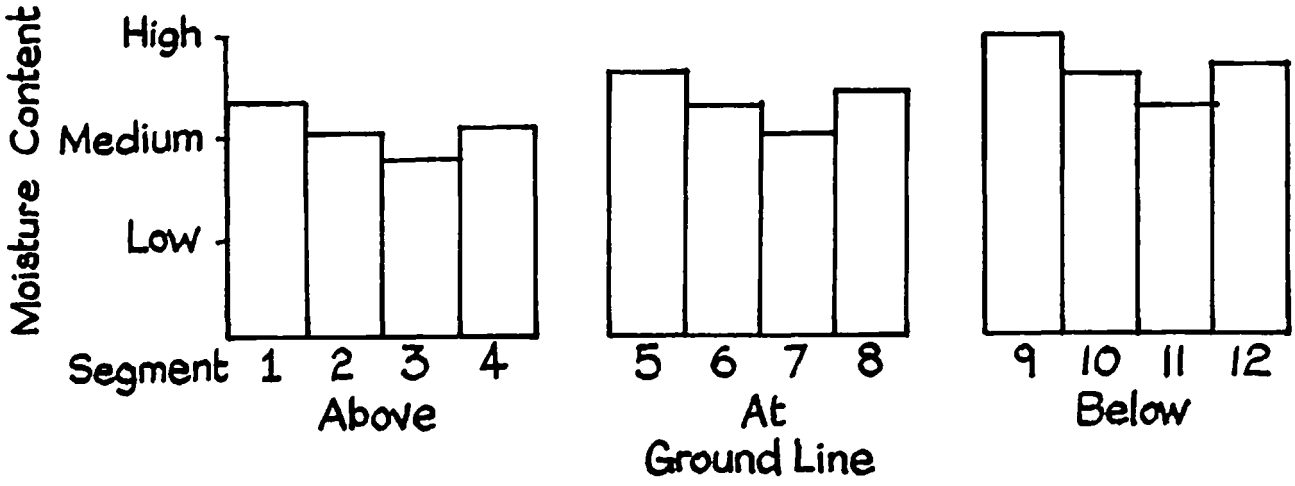


Figure 48 The uptake of water by wood in soil contact.



However, although the relationship between the average overall MC of a block of Scots pine sapwood and the surrounding soil moisture can be described, within the block the MC in zones above, at and below the ground line are different, and the MC differs in different segments of the same zone. There is a recurrent pattern of MC distribution within the blocks, represented in Figure 49.

Figure 49 Relative moisture content distribution in segments of Scots pine blocks after partial exposure to soil contact.



The distribution of MC has radial and longitudinal components. Within each zone the MC of the segment which would have been closest to the cambium in the living tree has the highest moisture content, with the MC of the adjacent segment rather less, and the next adjacent segment still less. The segment which was closest to the heartwood in the living tree has a MC similar to the wetter of the two inner segments. This distribution may be due to differences in segment density (so that different segments absorb different amounts of water)or to the way in which water penetrates in the radial direction. If water penetrated more easily from the cambium to the heartwood, than it did in the opposite direction, this would explain the observed distribution. The longitudinal component is shown by the difference in MC between zones. As there is less difference between longitudinally adjacent segments than between radially adjacent segments, the inference is that longitudinal water movement is greater than radial movement. The high MC in the above ground zone indicates that the buried zones influence the MC of the zone above ground, which must be achieved by longitudinal water flow.

Thus the MC of an area of wood, part of which is exposed to soil contact, is not random, but is precisely determined by an interaction of the wood and the soil. The implication is that the MC in wood exposed to soil can be predicted and controlled from a knowledge of some physical properties of the wood and the soil.

However, the relationship is complicated by the effect of micro-organisms on the wood, as bacteria can increase wood permeability and decay, and fungi can increase the ability of wood to hold water. The importance of wood MC to decay organisms is shown by the greater decay observed in the moist soil than in dry or wet soils. Presumably better conditions for decay occurred in this system than in the other soil systems. The effect of increasing soil MC upon the occurrence and activity of nitrogen-fixing bacteria was dramatic, with some evidence of enhanced decay in the presence of these bacteria. The greatest occurrence of these anaerobic bacteria above ground, which is presumably the least anaerobic zone, may be explained by the mechanism of their transport through the wood. If they are carried into and through the wood by water flow, and if the water evaporates above ground to be replaced by water from the buried zones and ultimately the soil, then the bacteria would be concentrated in the evaporation zone above ground. The loss of water from the bin system by evaporation and the drying of the surface layers of the soil is evidence of this evaporative flow through the soil, and presumably also the wood.

Changes in soil MC and wood MC have a profound effect on the activity of nitrogen fixing bacteria, and perhaps on the activity of other flora and fauna in the wood. Obviously soil MC and its effect on timber decay and nitrogen fixing bacteria requires further investigation. The effect of wetting the soil, exposure to already wet soil, and the effect of soil drying upon wood MC, nitrogen fixing activity and decay could be investigated using the bin exposure system, and combined with the exposure of Scots pine heartwood, (which is less permeable and more durable than sapwood) would supply considerable information on the relationship between wood moisture content, soil moisture content, water availability, nitrogen fixing activity and decay.

6.5. Conclusion

There is a definite, if complex and indirect, relationship between wood moisture content and soil moisture content. The uptake of water by wood in soil can be divided into three phases, the first up to 30% MC controlled by the wood, the second controlled by the interaction of wood and soil capillaries and pores, and the third by the wood's equilibrium moisture content in the soil. The amount of available water in the soil at any particular soil moisture content is of more importance in determining the rate of water uptake and wood MC than soil moisture content. The distribution of MC within the wood is dependent upon both radial and longitudinal components of water penetration. Although very variable, greatest decay was recorded in moist, rather than dry or wet soil. The occurrence and activity of nitrogen fixing bacteria was confined to very wet soil, and may increase decay under these conditions. The observed distribution of these bacteria in wood may be due to the longitudinal flow of water through the wood.

7. THE EFFECTS OF CHANGING SOIL MOISTURE CONTENT ON SCOTS PINE SAPWOOD AND HEARTWOOD

7.1 Introduction

The aim of this experiment was to examine both the effect of increasing the soil MC upon the MC, decay and AR rate in Scots pine sapwood (i.e. to repeat part of the previous experiment) and the effect of drying the soil upon these measurements. As a comparison, blocks of Scots pine heartwood, which is more resistant to decay and difficult to treat with preservatives (being less permeable), was also to be exposed to moist soil and to the effect of increasing soil MC.

7.2 Materials and Methods

Blocks of Scots pine sapwood were cut from planks from the same source and treated in the same way as described in Section 3. The Scots pine heartwood blocks were cut from a 4"x 3" P.A.R. plank of pine purchased from Sandell Perkins Ltd., London and bore the shipping mark "+ TVA +" denoting that the wood was imported from Sweden. The plank was selected for its large proportion of heartwood, made visible by the heartwood staining reagent *o*-anisidine (Stalker, 1971).

Table 12 shows the average values of the block weight and density of the sets of blocks, and the MC, void volume, and maximum theoretical moisture content of the blocks. The nominal and actual soil MC are given together with the number and code number of blocks removed at each sample time. One set of Scots pine blocks were exposed to soil maintained at 35% MC until the 49th day, then the soil MC was increased to 45%. The heartwood blocks were maintained at 35% soil MC until the 147th day, when the soil MC was increased to 40%. The second set of sapwood blocks were maintained at 45% soil MC for 84 days before the soil was allowed to dry to 30% soil MC.

7.3 Results

Moisture content : Figure 50

The MC of Scots sapwood after 6 weeks at 35% soil MC was very similar to the 42 day sample values obtained in the previous experiment. A t-test showed no significant difference between the two sets of values. After the soil MC was increased at 7 weeks, the MC increased, particularly in the below ground zones and in the segments with exposed tangential faces. The MC

Table 12 Wood Block and Soil Data

Wood Species	: Scots pine	Soil Type	: Loam
Initial Block Wt.	: 9·42 ± 0·35 g	Initial Moist. Cont.	: 9·36 %
Calc. Oven Dry Wt.	: 8·61 g	Void Volume	: 61·72 %
Initial Dendity	: 0·502 g cm^{-3}	Max. Moist. Cont.	: 124·04 %

Soil Moisture Conditions

Nominal : 35% (Moist) 45 % (Very wet)

Calculated :

2	4	6	8	10	12	14	16	18	20	22	24	26	28 weeks
		35	37	42	45			45	45	45			

Code Number of Blocks Sampled at each Sample Time

2	4	6	8	10	12	14	16	18	20	22	24	26	28
		IB18	IE11	ID02	IE34			IC30	IB06	ID30			
		IC02	IC28	IB31	IC09			IE07	IC32	IE09			
		IE06	ID15	ID08	ID23			IE05	IE25	IE02			
		IE24	IB30	IC11									

Wood Species	: Scots pine Heartwood	Soil Type	: Loam
Initial Block Wt.	: 9·725 ± 0·22 g	Initial Moist. Cont.	: 8·86 %
Calc. Oven Dry Wt.	: 8·94 g	Void Volume	: 60·65 %
Initial Density	: 0·519 g cm^{-3}	Max. Moist. Cont.	: 119·84 %

Soil Moisture Conditions

Nominal : 35 % (Moist) 45 % (Very wet)

2	4	6	8	10	12	14	16	18	20	22	24	26	28 weeks
32	30	35	34	34	35		35	35	35	44	44	44	45

Code Number of Blocks Sampled at Each Sample Time

2	4	6	8	10	12	14	16	18	20	22	24	26	28
KA01	KA05	KA08	KA09	KA11	KA14		KA16	KB04	KB06	KB08	KB10	KB12	KB14
KA03	KA06	KA07	KA10	KA12	KA15		KA17	KB05	KB07	KB09	KB11	KB13	KB15
KA04				KA13									

Wood Species	: Scots pine	Soil Type	: Loam
Initial Block Wt.	: 9·796 ± 0·43 g	Initial Moist. Cont.	: 8·04 %
Calc. Oven Dry Wt.	: 9·10 g	Void Volume	: 60·80 %
Initial Density	: 0·522 g cm^{-3}	Max. Moist. Cont.	: 99·20 %

Soil Moisture Conditions

Nominal : 45 % (Very wet) 28 % (Dry)

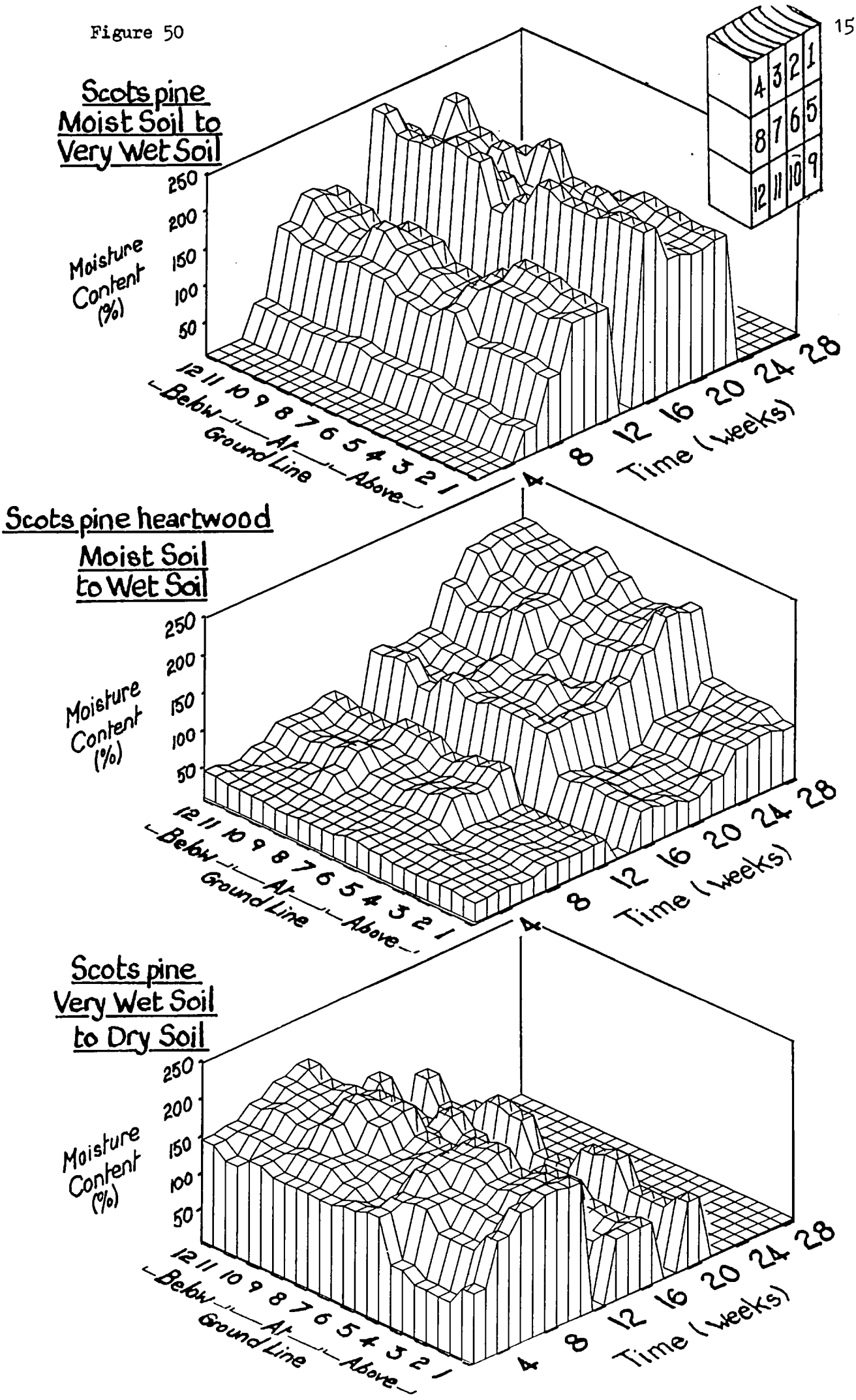
Calculated :

2	4	6	8	10	12	14	16	18	20	22	24	26	28 weeks
32	43	43	45	45	45	30	31		30				

Code Number of Blocks Sampled at each Sample Time

2	4	6	8	10	12	14	16	18	20	22	24	26	28
MB09	MB16	MB04	MC02	MB06	MC04	MC17	MC07		MC05				
MC09	MC15	MB08	MC20	MB03	MB17	MB20	MC01		MC03				
MB05	MC06	MB18	MB02		MC16								

Figure 50

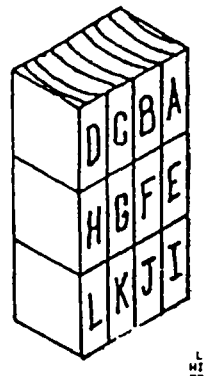

Scots pine
Moist Soil to
Very Wet Soil
Moisture Content (%)
250
200
150
100
50
12 11 10 9 8 7 6 5 4 3 2 1
Below Ground Line
At
Above
4 8 12 16 20 24 28
Time (weeks)
4 3 2 1
8 7 6 5
12 11 10 9
Scots pine heartwood
Moist Soil
to Wet Soil
Moisture Content (%)
250
200
150
100
50
12 11 10 9 8 7 6 5 4 3 2 1
Below Ground Line
At
Above
4 8 12 16 20 24 28
Time (weeks)
Scots pine
Very Wet Soil
to Dry Soil
Moisture Content (%)
250
200
150
100
50
12 11 10 9 8 7 6 5 4 3 2 1
Below Ground Line
At
Above
4 8 12 16 20 24 28
Time (weeks)

of the zone above ground was higher than at 6 weeks, (but lower than the buried zones) and the segments with exposed tangential faces were drier than the inner two segments. At 10 and 12 weeks, the rate of increase had levelled off and the above ground zone was wetter than those below ground. The effect of tangential penetration of water making the outer segments wetter than the inner segments, and the greater penetration from the outer tangential face was apparent at 10 weeks. Although the 16 week samples were wetter than the 70 and 84 day samples, the 18 and 20 week samples were at the same level of MC.

In the Scots pine heartwood exposed to 35% soil MC there was a distinct difference between the buried zones and the upper zone, particularly after 8 weeks. Also, the pattern of MC within the buried zones was opposite to that found in sapwood, the segment with the inner tangential face having the highest MC, followed by the adjacent inner segment and then the next, with the outer tangential segment being slightly wetter, as at 84 days. After 21 weeks, when the soil MC was increased from 35 to 45%, the deepest zone appeared to dry a little, while the ground line zone MC increased, and the increase in the above ground zone was quite distinct. Both buried zones increased their MC at subsequent sample times while the above ground zone MC increased only slightly. The large difference in MC between buried and exposed zones was maintained up to 28 weeks.

In the Scots pine sapwood blocks exposed to soil at 45% MC, the buried zones had reached 150% after only 2 weeks and their MC increased only slightly up to 10 weeks. The above ground zone was at 75-100% after 2 weeks, and increased MC with time. All the segments reached a similar MC at 10 weeks and after the soil began to dry out, the MC of all the segments decreased drastically.

Figure 51 shows the graphs of segment moisture content against time. In the sapwood exposed to moist soil the wood MC in all segments was around 50% after 6 weeks, similar to that found in the previous experiment in the same soil maintained at the same MC. After the soil was wet to 45%, the wood MC increased before levelling off at around 160%. The high value of MC recorded at 16 weeks may be an artifact of the exposure system and the way in which the soil MC was maintained. The weight of water lost by evaporation was replaced

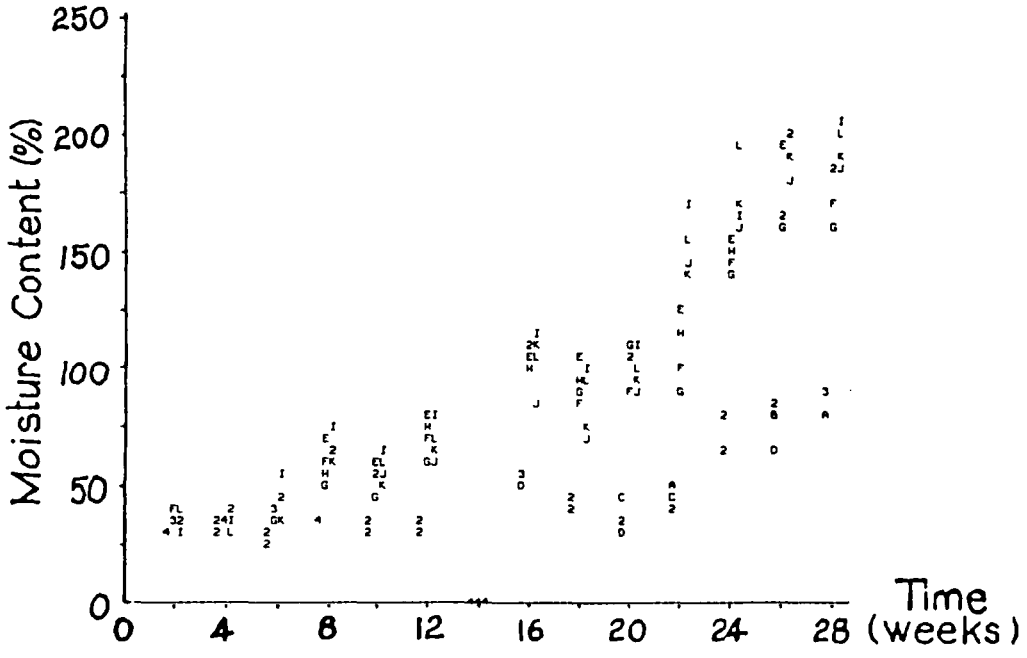


Scots pine

Moist Soil to Very Wet Soil

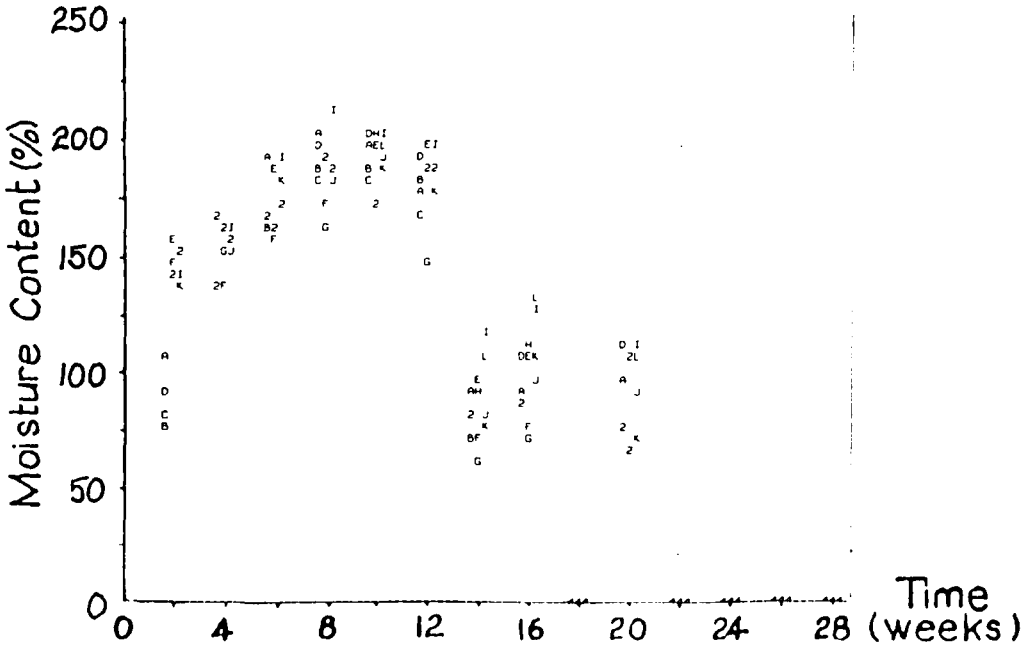
Scots pine heartwood

Moist Soil to Wet Soil



Scots pine

Very Wet Soil to Dry Soil



before the blocks to be sampled were removed. Perhaps the sampled blocks had taken up the applied water very rapidly, resulting in an artificially high and unrealistic MC in the wood.

In the heartwood the MC was around 30% at 2 weeks but increased slowly to 80% in the buried zones at 20 weeks. The above ground zone was very much drier at around 40%. The high values recorded at 16 weeks may be an artifact of the experimental method.After the soil was wet to 40% soil MC, the MC of all the segments increased, particularly in the buried zones, levelling off at around 170% below ground and 70% above ground.

In the sapwood exposed to very wet soil, the increase in wood MC was rapid, reaching 150% below ground and over 100% above ground after 2 weeks. At 4 weeks, all three zones were equally wet and the MC levelled off at around 180%. When the soil was allowed to dry, from 91 to 98 days, the sample at 14 weeks had a low MC in all segments (around 80%) which increased slightly at 16 weeks.

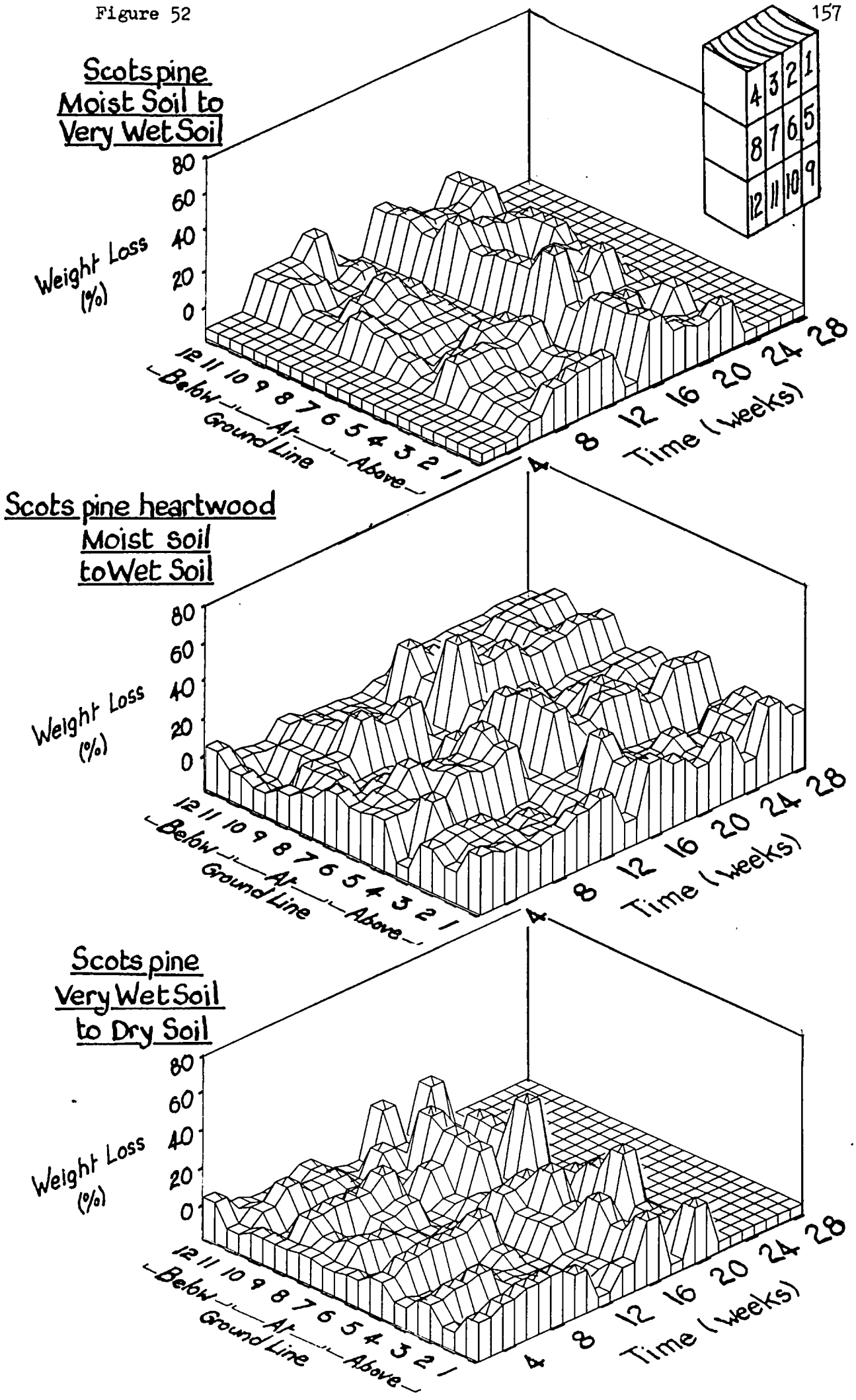
Weight loss : Figure 52

In the sapwood exposed to moist soil the overall weight loss was as observed in the previous experiment except that there were no initial weight gains. At 6 weeks, the first sample time, there was a distinct pattern of WL in each zone; decreasing values of WL from the outer to the inner tangential segment. At later time periods and in wet soil the inner tangential segment has the greatest WL although there was considerable overall variation. In the heartwood there was variation and no apparent trend of increasing WL with time although segments 4, 8 and 12 had lower and more consistent weight losses than the other segments.

In the sapwood exposed to very wet soil the overall pattern was of increasing WL in segments 1, 4 and 8 and the possibility that higher weight losses were recorded after the soil was dried than before, although the results were very variable and conclusions difficult to draw.

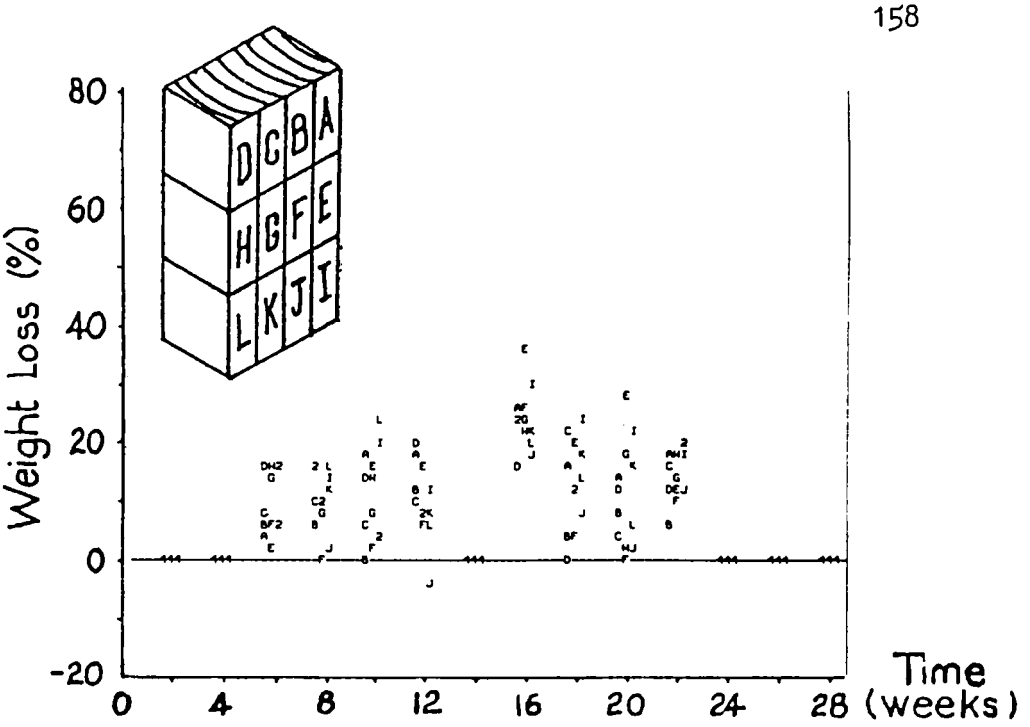
These trends were confirmed when the graphs of weight loss were compared (Figure 53). Variation was high, particularly in the Scots pine heartwood which looked uncolonised and undecayed even after 7 months in soil contact. The increase in WL at 112 days in the sapwood exposed to very wet, then dry, soil was quite distinct.

Figure 52


Scots pine
Moist Soil to
Very Wet Soil
Weight Loss
(%)
80
60
40
20
0
12 11 10 9 8 7 6 5 4 3 2 1
Below Ground Line
At
Above
4 8 12 16 20 24 28
Time (weeks)
4 3 2 1
8 7 6 5
12 11 10 9
Scots pine heartwood
Moist soil
to Wet Soil
Weight Loss
(%)
80
60
40
20
0
12 11 10 9 8 7 6 5 4 3 2 1
Below Ground Line
At
Above
4 8 12 16 20 24 28
Time (weeks)
Scots pine
Very Wet Soil
to Dry Soil
Weight Loss
(%)
80
60
40
20
0
12 11 10 9 8 7 6 5 4 3 2 1
Below Ground Line
At
Above
4 8 12 16 20 24 28
Time (weeks)

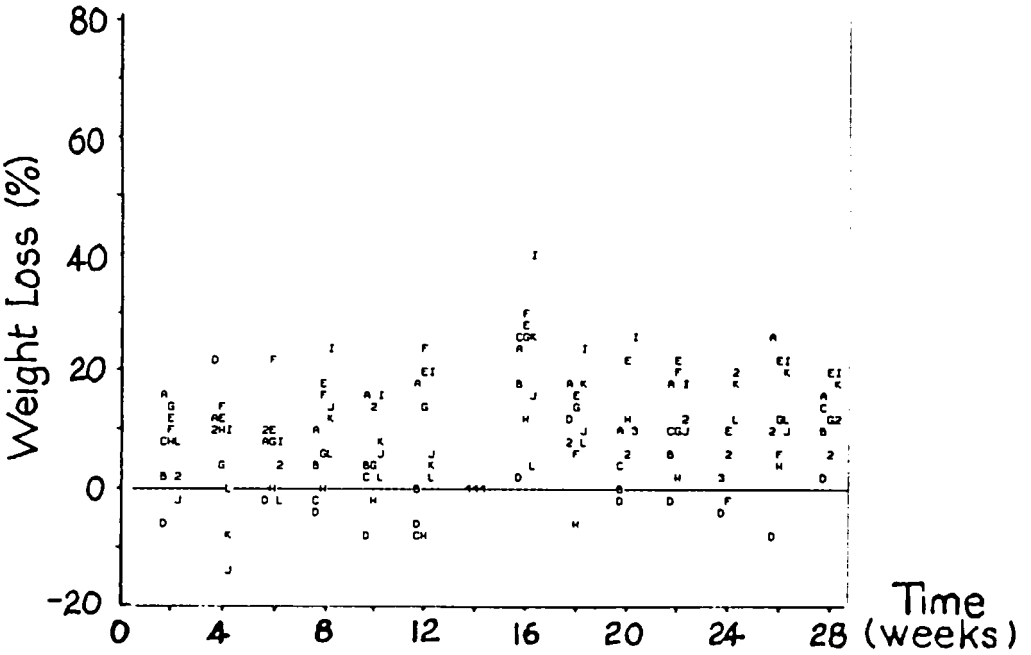
Scots pine

Moist Soil
to
Very Wet Soil



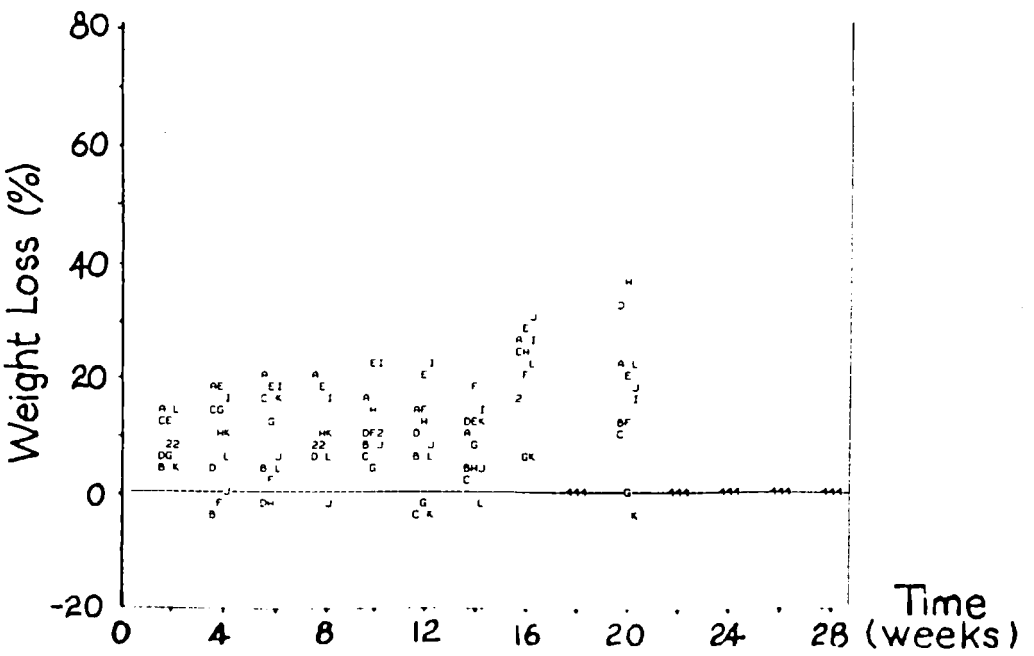
Scots pine heartwood

Moist Soil
to
Wet Soil



Scots pine

Very Wet Soil
to
Dry Soil



Acetylene reduction rate : Figure 54

As in the previous experiment, no AR activity was recorded in the sapwood blocks exposed to soil at 35% MC, and only after the soil was wet to 45% was any activity recorded. Activity increased in all segments with time, particularly in the above ground zone, up to 16 weeks, and then decreased.

In Scots pine heartwood blocks, no appreciable AR activity was found at any sample time either before or after the soil MC was increased.

In the sapwood blocks exposed to 45% soil MC some activity was detected at 2 weeks in the outer tangential segment of each zone. Activity was detected in all segments at 4 weeks and increased below ground at 6 weeks. By 10 weeks, most of the segments exhibited activity (some off scale in Figure 54), but after the soil MC was reduced, AR activity decreased.

The graphs of AR rate against time are shown in Figure 55. In the sapwood blocks in moist soil, there was no AR activity. After wetting to 45% the rate increased with time, notably in the 16 week sample which was wettest. Most activity was recorded in the above ground zone.

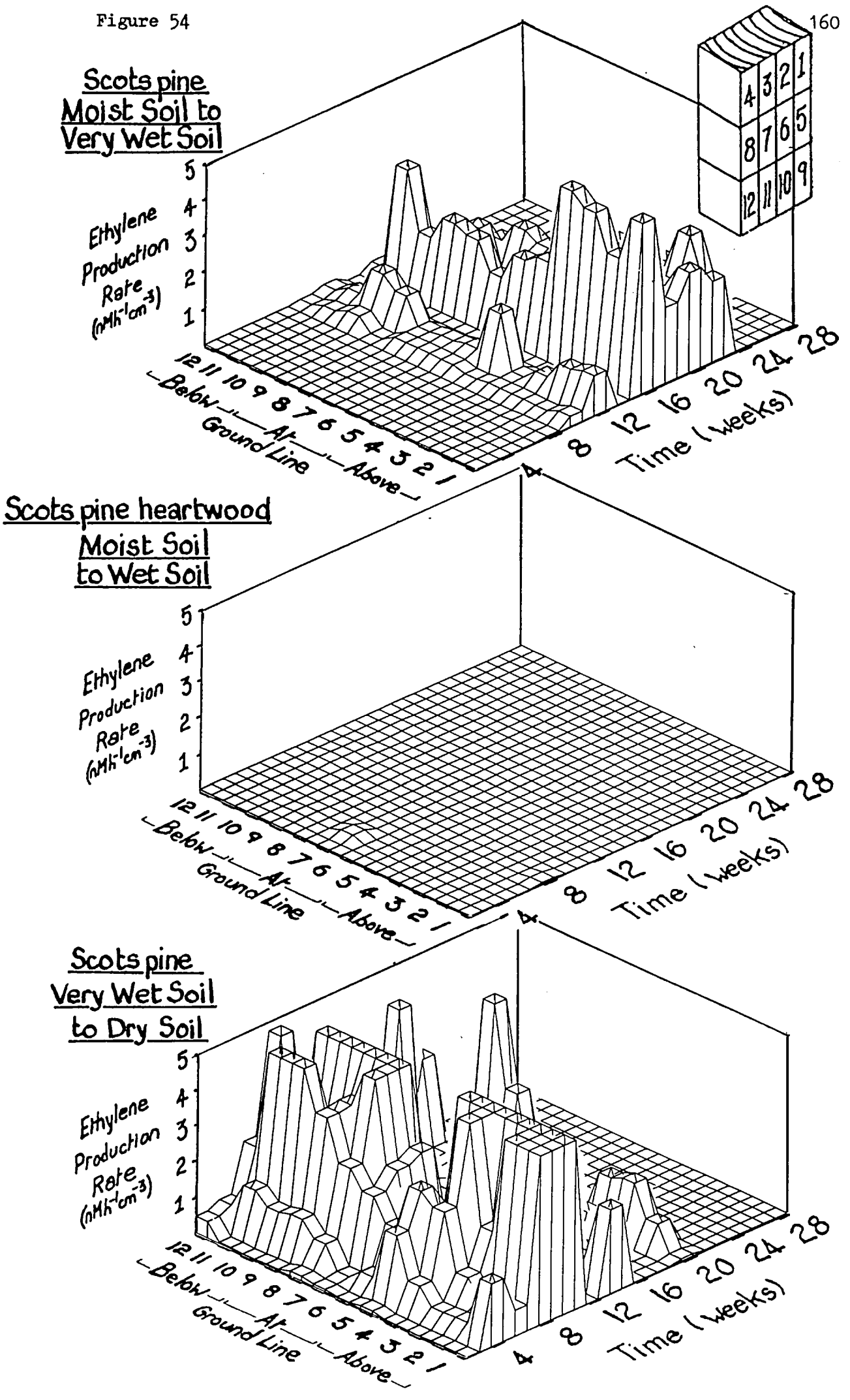
In the heartwood, only one segment showed any activity, either before or after the soil MC was increased.

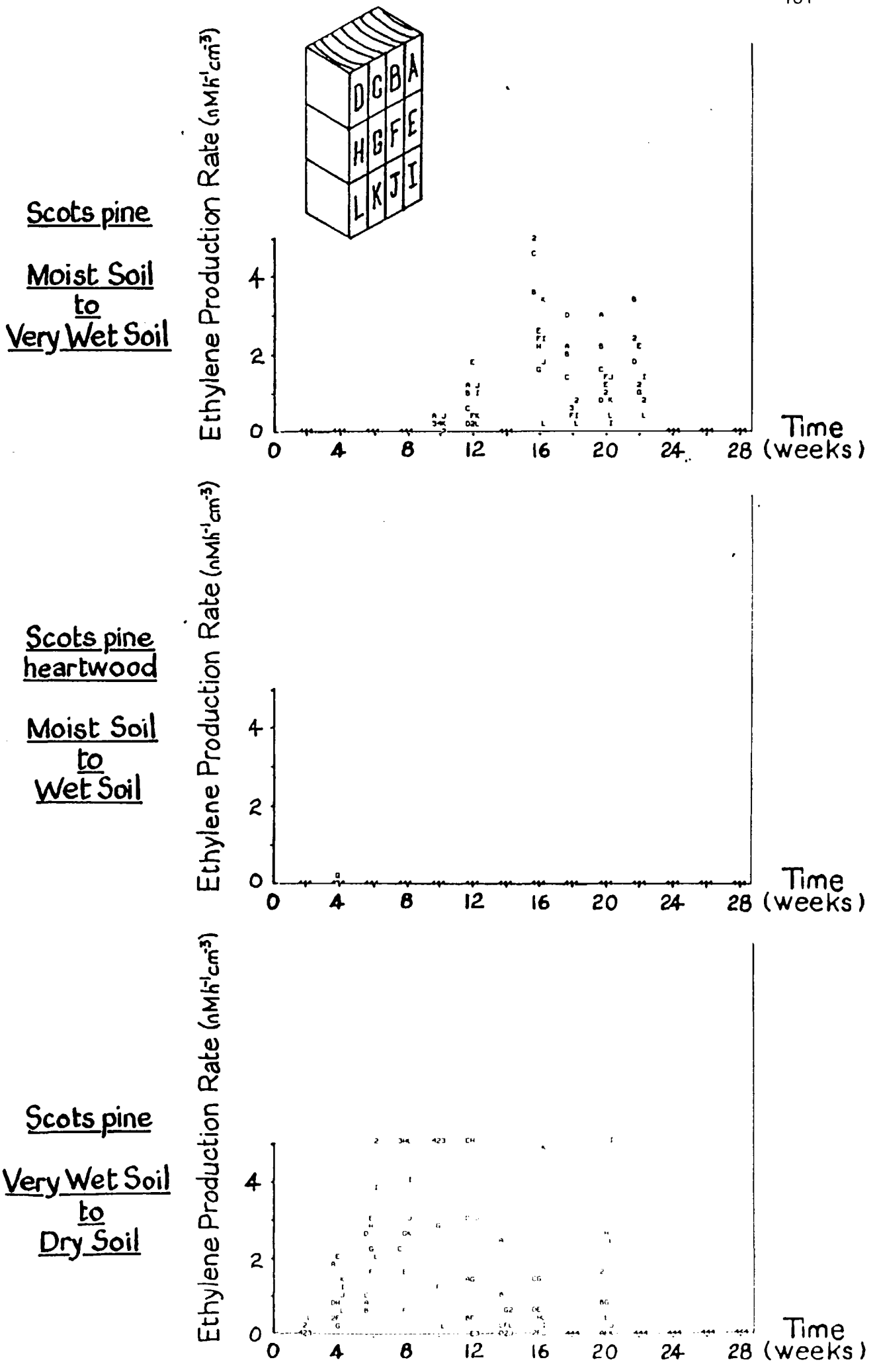
In the sapwood exposed to very wet soil, activity was detected at 14 days below ground and increased to a higher level than in the wetting experiment above, but decreased after the soil was allowed to dry out. The rate appeared to increase again with time in the drier soil.

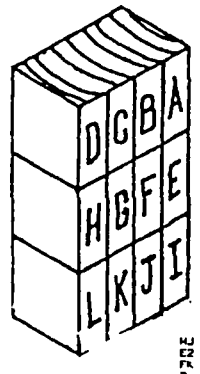
Water content : Figure 56

The water content of undecayed wood had a very similar pattern to moisture content and only the graphs of water content are shown in Figure 56. They show the two sets of sapwood blocks approaching the theoretical maximum water content of 57 % in very wet soil, while the heartwood reached only 20-40% after 12 weeks. After the soil MC surrounding the heartwood was increased to 40%, the WC of the buried zones increased to 60-80%, at the theoretical maximum of 57%.

Figure 54


Scots pine
Moist Soil to
Very Wet Soil
Ethylene
Production
Rate
(nMh⁻¹cm⁻³)
5
4
3
2
1
12 11 10 9 8 7 6 5 4 3 2 1
Below
At
Above
Ground Line
4
8
12
16
20
24
28
Time (weeks)
4 3 2 1
8 7 6 5
12 11 10 9
Scots pine heartwood
Moist Soil
to Wet Soil
Ethylene
Production
Rate
(nMh⁻¹cm⁻³)
5
4
3
2
1
12 11 10 9 8 7 6 5 4 3 2 1
Below
At
Above
Ground Line
4
8
12
16
20
24
28
Time (weeks)
Scots pine
Very Wet Soil
to Dry Soil
Ethylene
Production
Rate
(nMh⁻¹cm⁻³)
5
4
3
2
1
12 11 10 9 8 7 6 5 4 3 2 1
Below
At
Above
Ground Line
4
8
12
16
20
24
28
Time (weeks)


Scots pine
Moist Soil
to
Very Wet Soil
D C B A
H G F E
L K J I
Ethylene Production Rate (nMh⁻¹cm⁻³)
0
2
4
0 4 8 12 16 20 24 28
Time (weeks)
Scots pine
heartwood
Moist Soil
to
Wet Soil
Ethylene Production Rate (nMh⁻¹cm⁻³)
0
2
4
0 4 8 12 16 20 24 28
Time (weeks)
Scots pine
Very Wet Soil
to
Dry Soil
Ethylene Production Rate (nMh⁻¹cm⁻³)
0
2
4
0 4 8 12 16 20 24 28
Time (weeks)

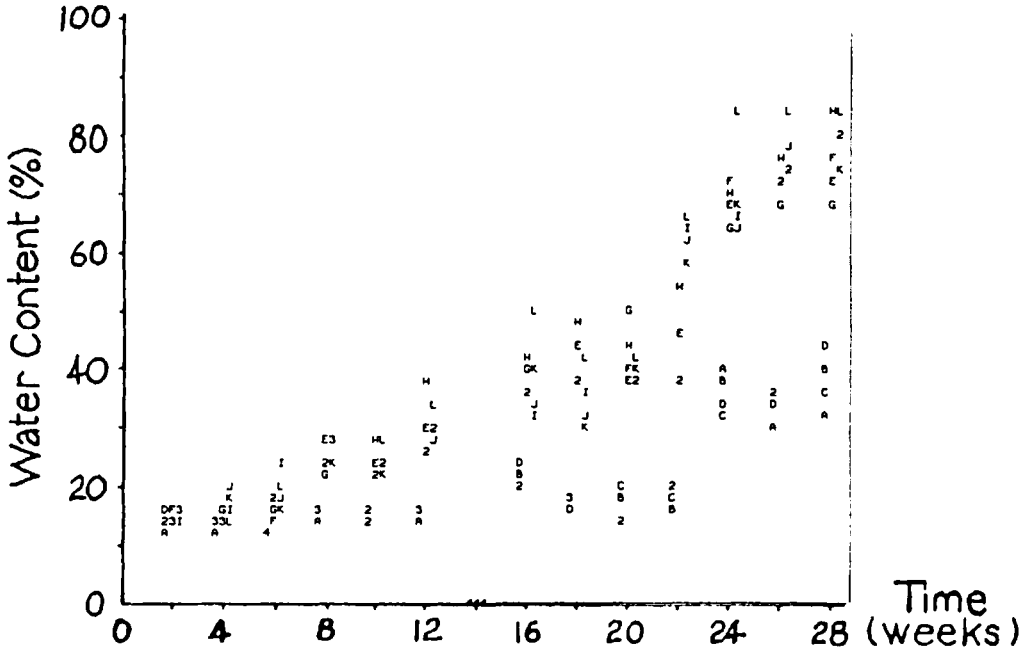


Scots pine

Moist Soil to Very Wet Soil

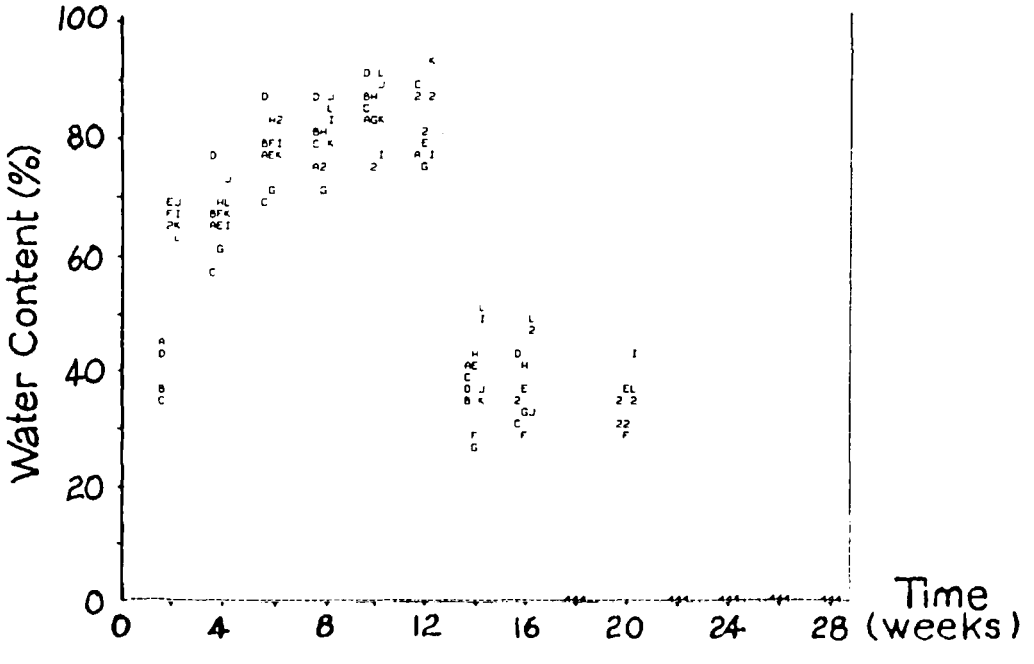
Scots pine heartwood

Moist Soil to Wet Soil



Scots pine

Very Wet Soil to Dry Soil



7.4 Discussion

The rate of water uptake exhibited by the sapwood and heartwood exposed to different soil moisture contents is shown in Table 13 together with the results from the previous experiment described in Section 6.

Table 13 Rate of water uptake from soil in Scots pine sapwood and heartwood.

Initial Soil Moisture Content	Final Soil Moisture Content	Time (weeks)	Rate of Water Uptake ($g\ d^{-1}$)
From Section 6 Scots pine sapwood			
25% Dry	25% Dry	2 - 10	0•009
35% Moist	35% Moist	2 - 10	0•034
40% Wet	40% Wet	2 - 10	0•127
25% Dry	50% Waterlogged	11 - 12	1•195
From Section 7 Scots pine sapwood			
35% Moist	35% Moist	0 - 6	0•052
35% Moist	45% Very Wet	7 - 8	0•847
45% Very Wet	45% Very Wet	0 - 2	0•679
45% Very Wet	30% Dry	13 - 14	-1•211
From Section 7 Scots pine heartwood			
35% Moist	35% Moist	2 - 10	0•021
35% Moist	35% Moist	10 - 20	0•031
35% Moist	40% Wet	21 - 22	0•243

In this series of experiments the rate of water uptake in moist soil was 0.052 gd^{-1}, compared to 0.034 in the previous series. (The former value was taken over 0-42 days exposure period while the latter was taken over 14-70 days). The former, higher value includes the initial wetting up phase from air dry to around 40%, while the latter, lower, value excludes this phase. The rate of uptake when the soil was wetted to 45% was 0.847 gd^{-1}, compared with 1.195 gd^{-1} in the previous experiment where the soil was wet to 50%. The value of 0.847 fits the exponential relationship in Figure 39 and shows that there is a relationship between the rate of water uptake by wood from soil (even above the water holding capacity of the soil) and soil moisture content, although the soil water availability is more relevant,

as explained in Section 6. The rate of uptake in air dry wood exposed to soil at 45% was 0.679 gd^{-1} which was lower than the rate when wood at around 40% MC was exposed to soil at 45% (0.847 gd^{-1}). However, the latter was calculated over 49-56 days, and excluding the initial wetting up phase, while the former was calculated over 0-14 days, a longer time period including the initial wetting up phase. This difference indicates the importance of initial wood MC in determining water uptake rate, in that air dry wood apparently has to reach f.s.p. before rapid uptake can occur, while wood at f.s.p. can take up water rapidly and immediately.

The effect of soil drying upon the wood MC was as dramatic as was wetting soil. After only 7 days with the lid removed, the bin system lost 421 g of water by evaporation, and the wood lost water at the rate of 1.211 gd^{-1}. This rate is remarkably similar to the maximal rate of uptake observed, implying that, under drying conditions, wood exposed to waterlogged soil could lose water as fast as it can be taken up and that over 1g of water per day could be passing through the wood. Such a flow of water through partially buried wood could be of major importance in timber decay. It could explain the movement of bacteria through wood and their occurrence above ground. It could assist the colonisation of wood by micro-organisms and also, and perhaps most intriguingly, carry nutrients from the soil into the wood. Perhaps this flow of water through wood caused by evaporation of water above ground into the air is an additional source of nitrogen for decay micro-organisms? The wood MC seems particularly sensitive to the "evaporating power" of the air, as the MC of the wood increased when the lid was replaced on the system, which presumably increased the RH of the air around the zone above ground. The high final MC of the wood at around 80% when exposed to soil at around 30% suggests that the wood is able to retain water better than soil and that the hysteresis effect can occur in wood in soil contact at a MC greater than f.s.p.

The Scots pine heartwood was less permeable than the sapwood with the rate of uptake over the first 70 days being 0.021 gd^{-1} and the average MC of the wood around 47%. But for 70 to 140 days, the MC increased to 89%, which was wetter than sapwood after 70 days, at a rate of 0.031 gd^{-1}. When this result is compared with the observation that in both this and the previous

experiment, the "final" MC or equilibrium moisture content of wood in soil appeared to increase at successive sample times, it would appear that equilibrium can take a long time to be achieved. Apparently the increase in EMC is not due to the amount of water available but to a slow change in the nature of the wood, which allows more water to be taken up as exposure continues. Because in the heartwood there was no visible decay, the implication is that the wood is not totally inert in soil contact, and that one of its physical properties, its ability to hold water, changes with time.

The effect of wetting the soil around the Scots pine heartwood was to increase the wood MC at a rate of water uptake of 0.243 gd^{-1}, which was faster than sapwood (0.127 gd^{-1}). However, the latter figure includes the initial wetting up phase so that in sapwood the rate of water uptake would be faster than heartwood if the sapwood was above f.s.p.

The results are consistent with the relationship between wood MC and soil water availability depicted in Figure 48. The lack of results for the period 0-14 days during the initial wetting up phase is regrettable, so that this part of the relationship is merely speculative. The other two phases have been confirmed in this experiment.

The inference from the drying experiment, that may be of major significance, is that there is a dynamic equilibrium between soil and wood, and that the MC in the wood merely represents a single instant in the achievement or adjustment of equilibrium, rather than a fixed, final, static equilibrium value. The equilibrium is affected by changes in soil, wood, water supply, evaporating power of the air and the effect of micro-organisms, so that it is far from a simple equilibrium.

The measured weight losses in the heartwood, which showed no visible sign of decay, indicated that the method of estimating loss is not sufficiently sensitive to monitor the effect of the presence and absence of nitrogen-fixing bacteria upon fungal decay, as the density and volume of the wood segments would need to be measured more accurately in order to detect minor differences between treatments. However, the technique is capable of detecting pronounced decay, as in hardwood in Section 5. In this experiment, in Scots pine sapwood, the presence of nitrogen fixing bacteria was not associated with major decay. In heartwood, there was no decay and nitrogen fixing bacteria were absent. Obviously

further work is necessary to evaluate the role of nitrogen fixing bacteria in timber decay in wet soil, where they occur most reliably and with greater activity, and in the field under more realistic, if less controlled, conditions. The exposure of hardwoods, particularly birch, to wet soil would be of interest, while the exposure of a different softwood, such as spruce could assist in the investigation of the effect of nitrogen fixing bacteria on timber decay.

7.5 Conclusion

The results are consistent with the relationship between soil and wood depicted in Figure 48 and described in Section 6. The inference from the drying experiment is that water could move through partially buried wood at a rate determined by the evaporative power of the air, and that a dynamic and sensitive equilibrium exists between wood MC, soil water and the relative humidity of the air. The bin exposure technique is adequate for the examination of the water balance of wood in soil contact but not of its decay rate. The results do reveal the lower relative permeability and greater durability of heartwood compared to sapwood.

8. THE EFFECT OF WET SOIL ON THE DECAY OF BIRCH AND SPRUCE

8.1 Introduction

The aim of this experiment was to investigate the MC, AR rate and decay of birch sapwood in moist and wet soils to allow comparison with the results obtained in dry soil and described in Section 5. The effect of wet soil upon MC, AR rate and decay in another softwood, spruce, a refractory timber, was to be compared with Scots pine exposed to wet soil, described in Section 6. Further information was to be collected on the relationship between wood and soil moisture in species other than Scots pine.

8.2 Materials and Methods

Table 14 gives the average value of the block weight and density of the sets of blocks, and the MC, void volume and maximum MC of the blocks. The nominal and calculated soil MC are given together with the number and code number of blocks removed at each sample time. Birch blocks were exposed to either soil maintained at 35% soil MC (moist) or at 45% (very wet). Spruce blocks were exposed to soil maintained at 45% (very wet).

8.3 Results - Moisture Content - Figure 57

In the birch blocks exposed to moist soil, by 4 weeks the usual pattern of MC distribution was apparent, with a distinct difference between zones below at and above the ground line, and with the outer segments in the zone at the ground line having a higher MC than the inner segments. After 8 weeks, in this zone, and from 4 weeks in the deepest zone, this pattern within the zones was not apparent, yet the pattern of MC within the segments was apparent again after 16 weeks.

In birch blocks exposed to wet soil, a high overall MC and the rapid increase of MC in all three zones was apparent. The zone below ground had the highest MC soonest, followed by the zone at the ground line and then the zone above ground. After 6 weeks the zones and segments had a very similar, high, MC.

In spruce blocks exposed to wet soil, at 2 weeks the pattern of MC distribution in all zones was distinct with the highest MC in the inner tangential segment, with decreasing MC towards the outer tangential segment. All three zones had a similar pattern of MC. After only 4 weeks all the zones had a similar, high MC.

Table 14 Wood Block and Soil Data

Wood Species : Scots pine
Soil Type : Loam
Initial Block Wt. : 12·962 ± 0·37 g
Initial Moist. Cont. : 9·19 %
Calc. Oven Dry Wt.: 11·87 g
Void Volume : 47·41 %
Initial Density : 0·691 g cm^{-3}
Max. Moist. Cont. : 100·71 %

Soil Moisture Conditions

Nominal : 35 % (Moist)

Calculated : 2	4	6	8	10	12	14	16	18	20	22	24	26	28 weeks
30	29	34	35	35	35		35	35	36	35			

Code Number of Blocks Sampled at each Sample Time

2	4	6	8	10	12	14	16	18	20	22	24	26	28
LB03	LC04	LC05	LB07	LC08	LB14		LB12	LC17	LC20	LB22			
LC03	LB05	LB06	LB08	LC10	LC12		LC14	LB18	LB21	LC22			
LB04				LC11	LC13		LC16	LC18	LC21	LB23			

Wood Species : Birch
Soil Type : Loam
Initial Block Wt. : 11·032 ± 0·49 g
Initial Moist. Cont. : 6·56 %
Calc. Oven Dry Wt.: 10·37 g
Void Volume : 56·59 %
Initial Density : 0·588 g cm^{-3}
Max. Moist. Cont. : 99·20 %

Soil Moisture Conditions

Nominal : 45% (Very wet)

Calculated : 2	4	6	8	10	12	14	16	18	20	22	24	26	28 weeks
36	40	42	45	45		44	45		43				

Code Number of Blocks Sampled at each Sample Time

2	4	6	8	10	12	14	16	18	20	22	24	26	28
NB27	NA33	NB03	NA30	NA14	NA04	NB31	NA08		NB32				
NA31	NB26	NA01	NB04	NA03	NB01	NA18	NA02		NA12				
NA10	NA06		NA05		NB30								
					NA15								

Wood Species : Spruce
Soil Type : Loam
Initial Block Wt. : 7·878 ± 0·39 g
Initial Moist. Cont. : 7·38 %
Calc. Oven Dry Wt.: 7·34 g
Void Volume : 68·70 %
Initial Density : 0·420 g cm^{-3}
Max. Moist. Cont. : 166·48 %

Soil Moisture Conditions

Nominal : 45 % (Very Wet)

Calculated : 2	4	6	8	10	12	14	16	18	20	22	24	26	28 weeks
35	39	43	44	44	45	44	45		45				

Code Number of Blocks Sampled at each Sample Time

2	4	6	8	10	12	14	16	18	20	22	24	26	28
PB34	PC09	PB02	PB27	PB22	PC16	PC05	PC19		PB21				
PC22	PC33	PC08	PB03	PB19	PB30	PB01	PC23		PB31				
PC31	PC14	PB23	PC10										

Figure 57

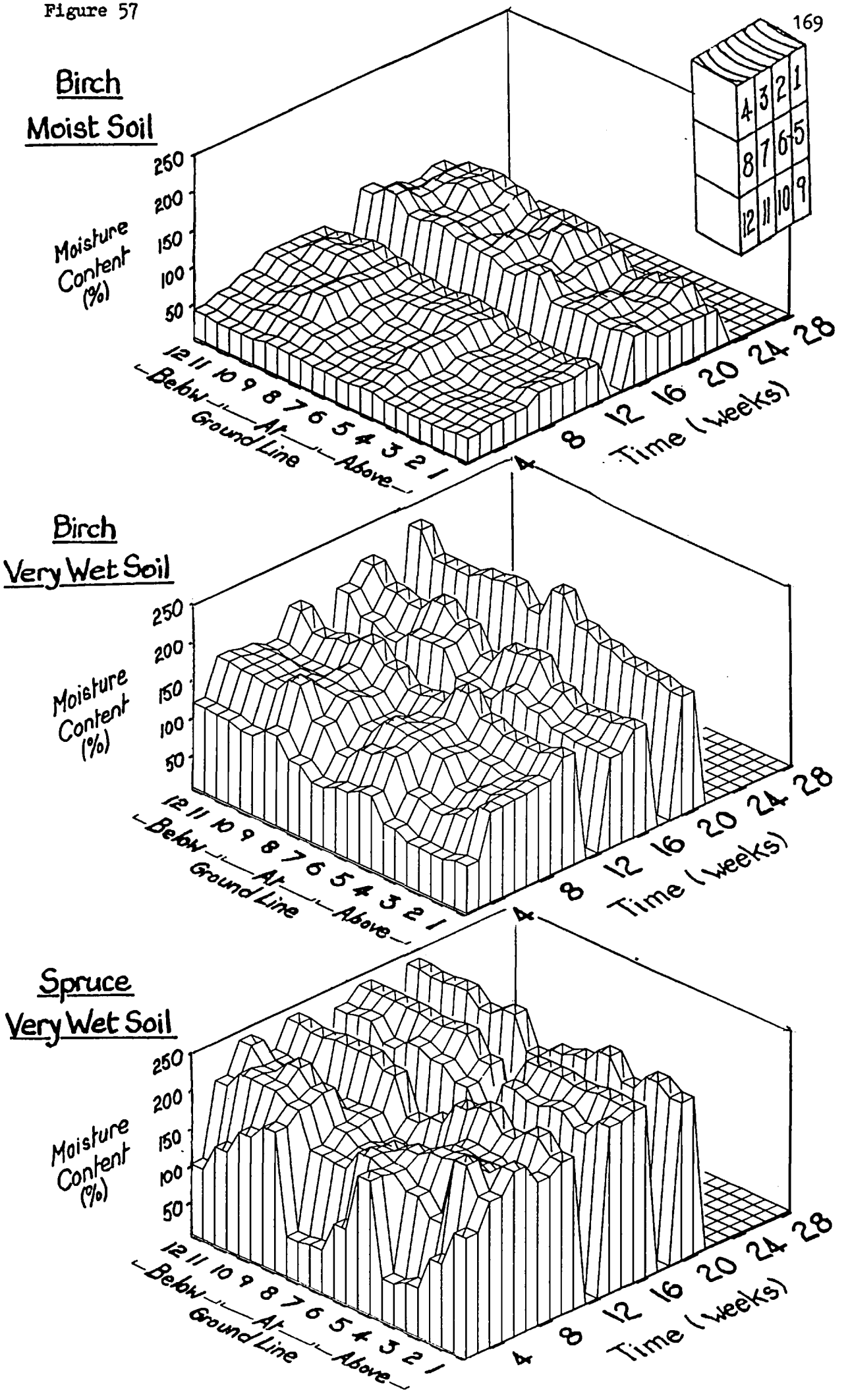

Birch
Moist Soil
Moisture Content (%)
250
200
150
100
50
12 11 10 9 8 7 6 5 4 3 2 1
Below Ground Line
At
Above
4 8 12 16 20 24 28
Time (weeks)
4 3 2 1
8 7 6 5
12 11 10 9
Birch
Very Wet Soil
Moisture Content (%)
250
200
150
100
50
12 11 10 9 8 7 6 5 4 3 2 1
Below Ground Line
At
Above
4 8 12 16 20 24 28
Time (weeks)
Spruce
Very Wet Soil
Moisture Content (%)
250
200
150
100
50
12 11 10 9 8 7 6 5 4 3 2 1
Below Ground Line
At
Above
4 8 12 16 20 24 28
Time (weeks)

Figure 58 shows the graphs of MC in the three systems. In the birch exposed to moist and wet soils the difference in the rate of increase of MC was apparent, as was the difference in final MC reached by the different segments and zones. The difference in MC between the zones was established at 2 weeks and was maintained throughout the period of exposure, although the zone above ground was usually considerably drier than the two buried zones, which had a similar MC.

In the spruce exposed to wet soil the rate of MC increase was very high, and the final MC reached by the segments was very high at around 250%. There was little difference between the zones, all having a similar MC, often with the ground line zone having the lowest MC of the three.

Weight loss - Figure 59

In the birch blocks exposed to moist soil the pattern of weight change, repeated in all 3 zones, suggested differences in density existed within each zone. These differences in weight loss were maintained throughout the exposure period, although each segment lost weight during exposure. In the blocks exposed to wet soil, the WL was very variable, although there appeared to be a slight change from weight gain to weight loss after 20 weeks exposure. In the spruce blocks in wet soil the weight gains after 14 days became weight losses after 20 weeks.

Figure 60 shows the weight loss graphs for the three systems. In the birch exposed to moist soil the increase of weight loss with time was apparent reaching around 20% after 22 weeks, considerably less than that observed in birch exposed to dry soil (Section 5). The above ground zone exhibited weight gain at 2 weeks which became a weight loss with time. Greatest decay was found in the below ground zone, and least in the zone above ground.

In the wet soil, WL was more variable, and weight gains were found up to 12 weeks. There appeared to be less decay in the wet soil than in moist soil. In the spruce exposed to wet soil, there appeared to be no decay, as the weight losses were maintained throughout the period of exposure with no indication of increasing weight loss with time.

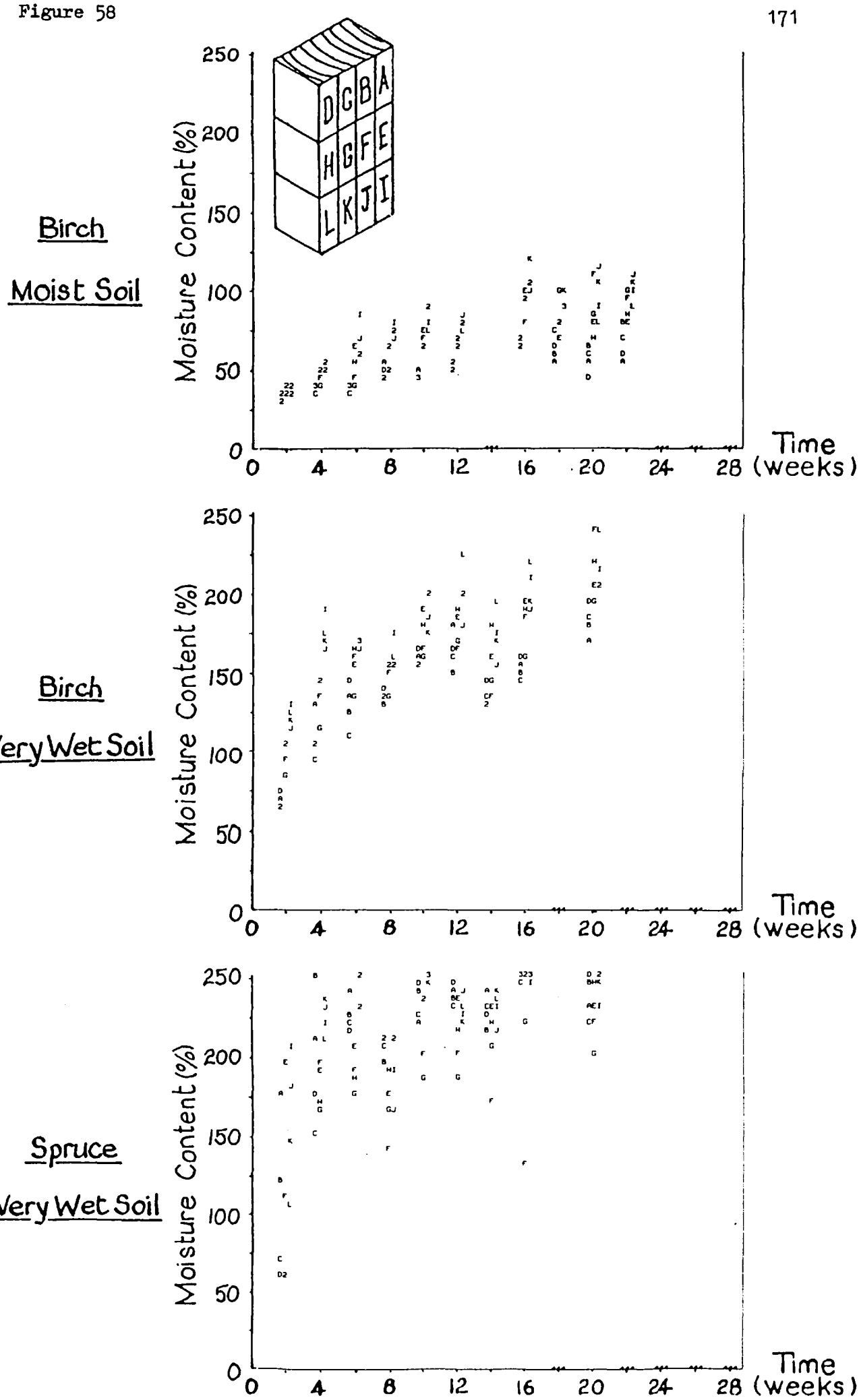

Birch
Moist Soil
Birch
Very Wet Soil
Spruce
Very Wet Soil
D C B A
H G F E
L K J I
Moisture Content (%)
250
200
150
100
50
0
0 4 8 12 16 20 24 28
Time (weeks)

Figure 59

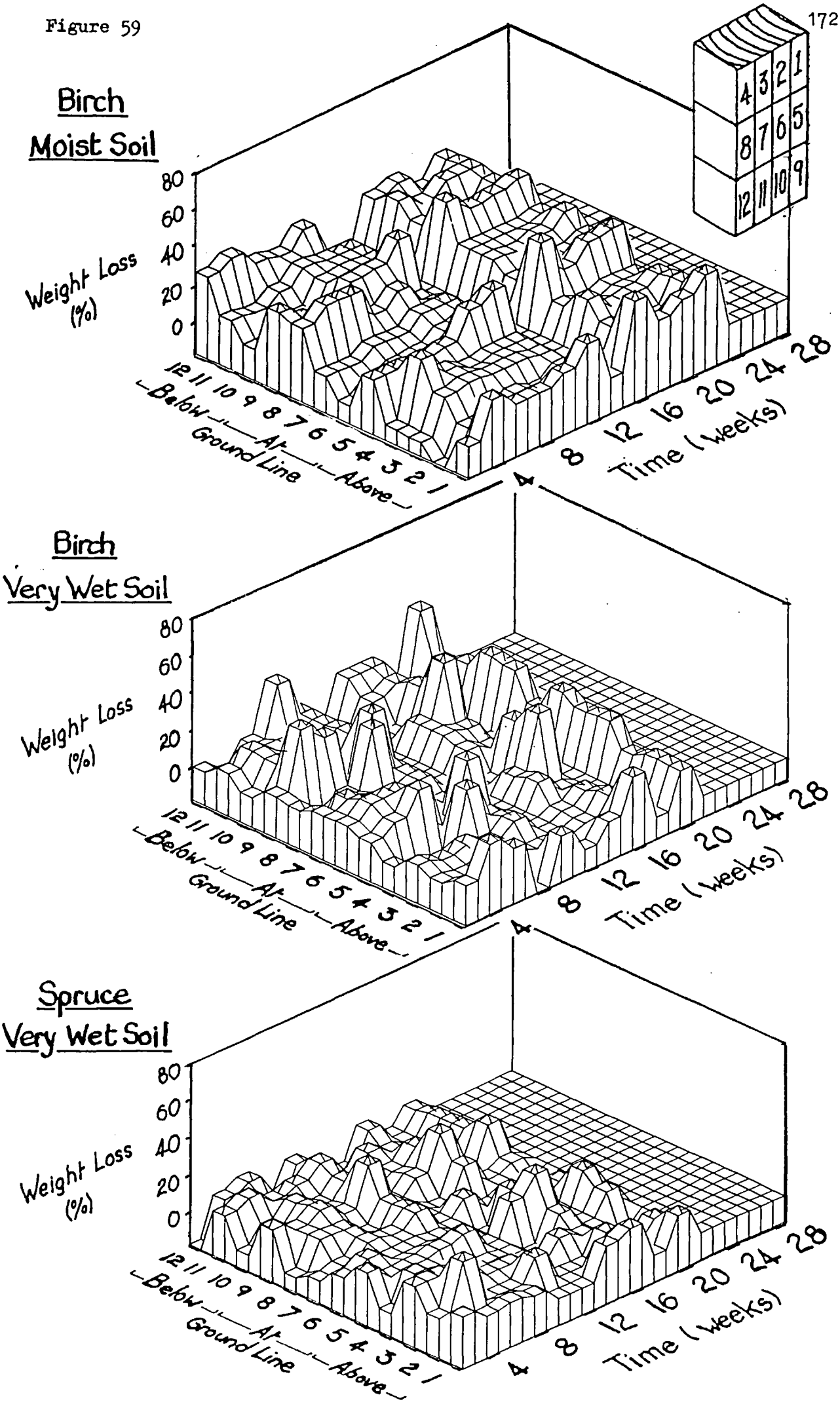
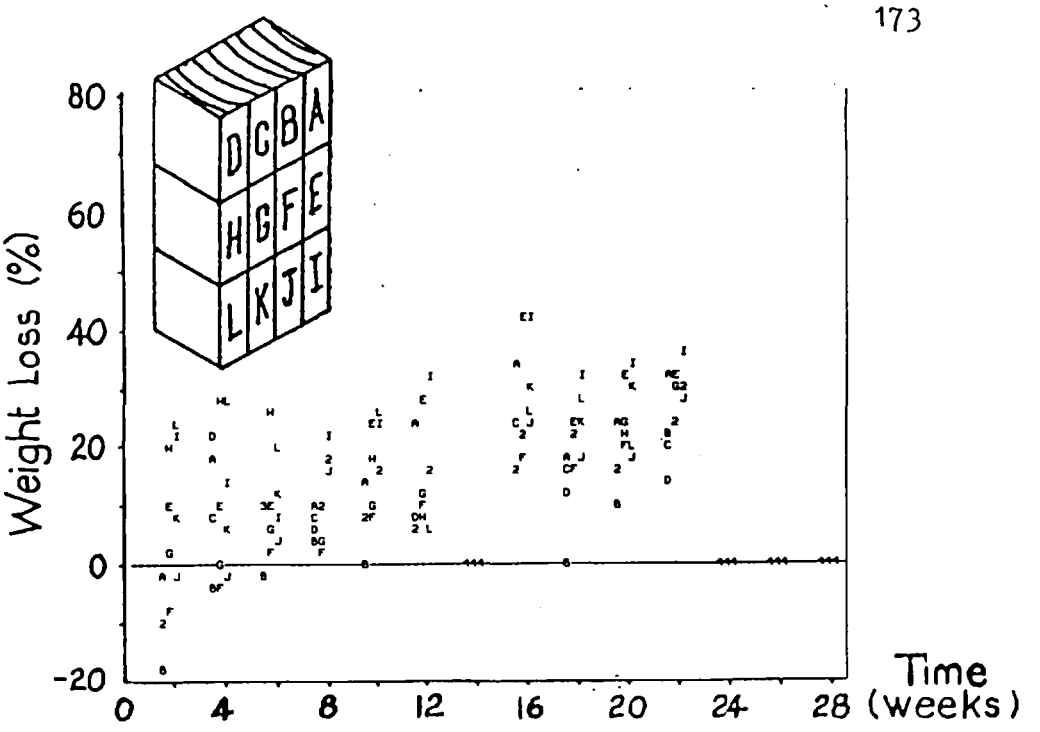
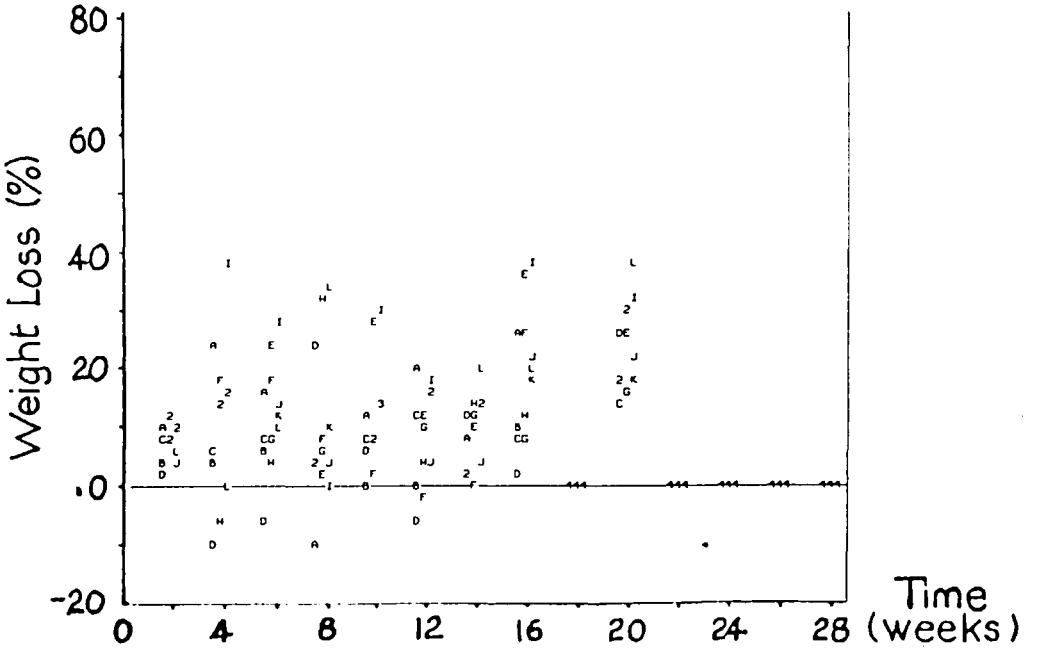

Birch
Moist Soil
Weight Loss (%)
80
60
40
20
0
12 11 10 9 8 7 6 5 4 3 2 1
Below Ground Line
At
Above
4 8 12 16 20 24 28
Time (weeks)
4 3 2 1
8 7 6 5
12 11 10 9
Birch
Very Wet Soil
Weight Loss (%)
80
60
40
20
0
12 11 10 9 8 7 6 5 4 3 2 1
Below Ground Line
At
Above
4 8 12 16 20 24 28
Time (weeks)
Spruce
Very Wet Soil
Weight Loss (%)
80
60
40
20
0
12 11 10 9 8 7 6 5 4 3 2 1
Below Ground Line
At
Above
4 8 12 16 20 24 28
Time (weeks)

Figure 60



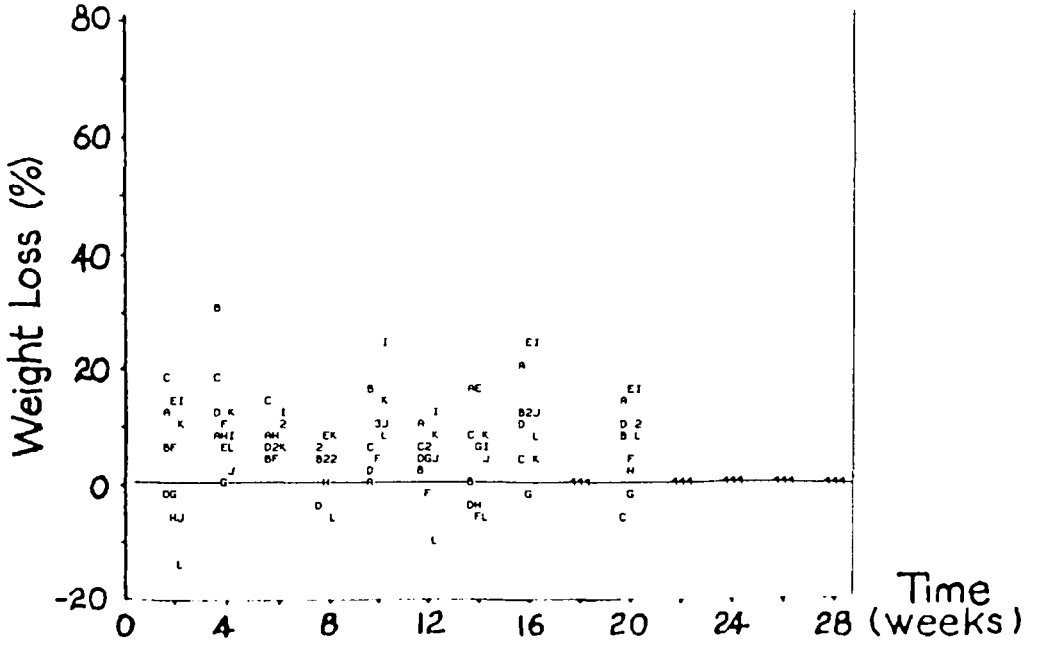
Birch

Moist Soil



Birch

Very Wet Soil



Spruce

Very Wet Soil

Acetylene Reduction - Figure 61

In the birch sapwood exposed to moist soil, only marginal AR activity was recorded, usually in below ground zones. In the birch exposed to wet soil, some activity was recorded after 2 weeks, and considerable activity at later times. The highest activity was recorded at 4 weeks in the segments having a high MC. At later times the activity was variable and found throughout the block.

In the spruce sapwood blocks exposed to wet soil, some activity was recorded at 6 weeks and rather more activity throughout the block at 10 weeks. At 14 weeks no activity was recorded above ground, unlike at 16 and 18 weeks where activity was found chiefly above ground. The rates were considerably less than those found in birch at the same soil MC.

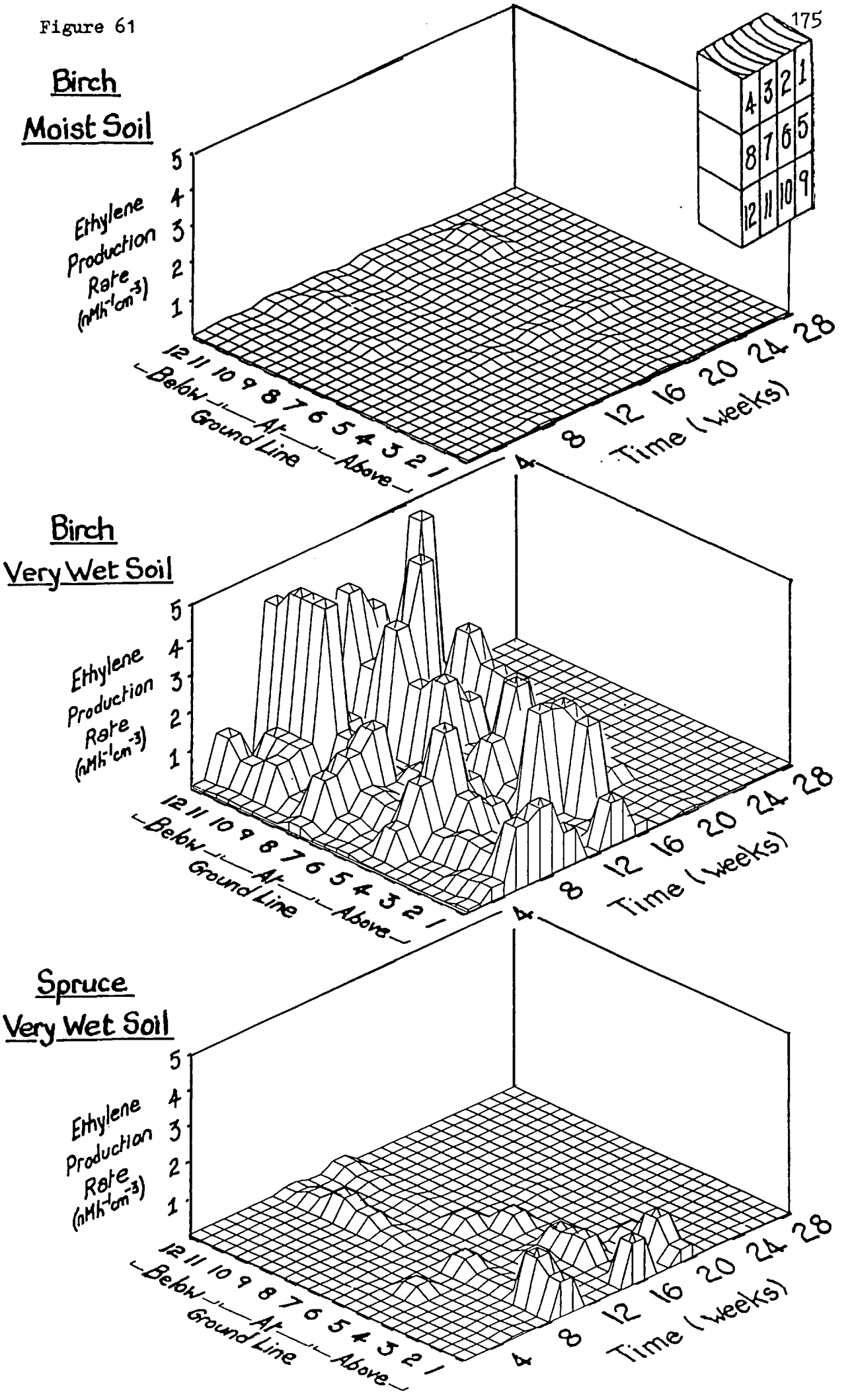
The graphs of AR rate against time are shown in Figure 62. In birch exposed to moist soil very little activity (around 0.1nMh^{-1}cm^{-3}) was recorded in the wood, and only in the buried zones. This contrasted strongly with the same wood exposed to wet soil where rates of up to 7.0 nMh^{-1}cm^{-3})were recorded. At most sample times after 2 weeks the rates observed in all segments exceeded 0.1 nMh^{-1}cm^{-3}, and were greatest below ground and least in the above ground zone.

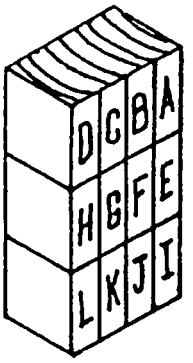
The activity in spruce exposed to wet soil was less than in birch and less than found in Scots pine at the same soil MC (see Figure 43). The highest rate (1.5 nMh^{-1}cm^{-3}) was found in the above ground zone at 10 weeks. The below ground zone had the least activity.

Water content - Figure 63

The graphs of water content against time show the gradual increase of WC in birch exposed to moist soil and contrasts with the rapid rise to 80-100% found in birch exposed to very wet soil. Spruce exhibited a rapid rise to 80-100% water content, a value in excess of its theoretical maximum water content (65 %).

Figure 61


Birch
Moist Soil
Ethylene Production Rate (nMh⁻¹cm⁻³)
5
4
3
2
1
12 11 10 9 8 7 6 5 4 3 2 1
Below
At
Above
Ground Line
4 8 12 16 20 24 28
Time (weeks)
4 3 2 1
8 7 6 5
12 11 10 9
Birch
Very Wet Soil
Ethylene Production Rate (nMh⁻¹cm⁻³)
5
4
3
2
1
12 11 10 9 8 7 6 5 4 3 2 1
Below
At
Above
Ground Line
4 8 12 16 20 24 28
Time (weeks)
Spruce
Very Wet Soil
Ethylene Production Rate (nMh⁻¹cm⁻³)
5
4
3
2
1
12 11 10 9 8 7 6 5 4 3 2 1
Below
At
Above
Ground Line
4 8 12 16 20 24 28
Time (weeks)

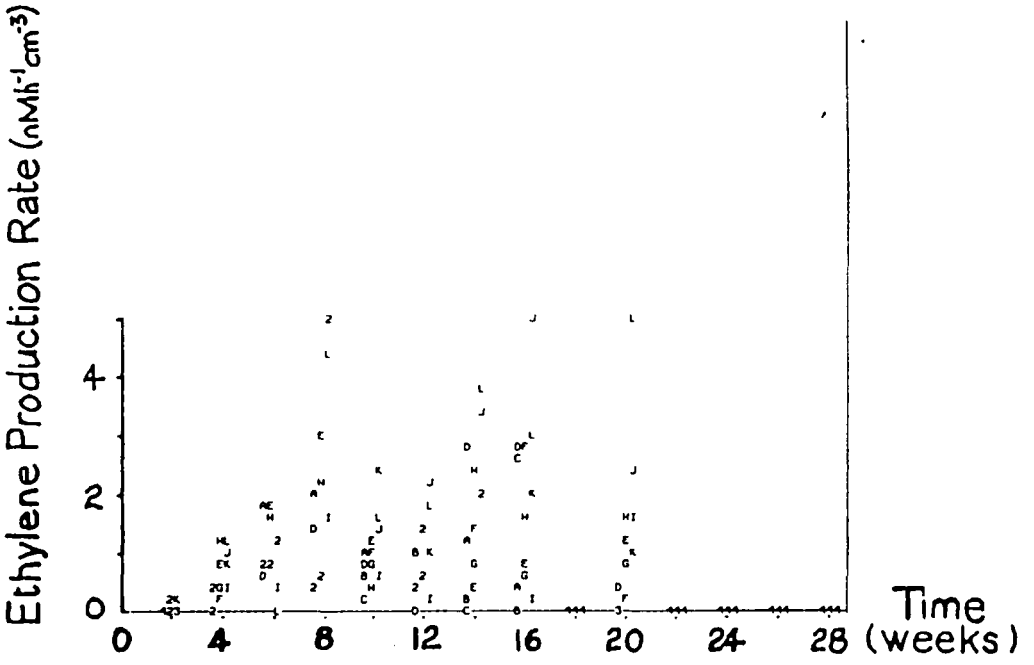


Birch

Moist Soil

Birch

Very Wet Soil



Spruce

Very Wet Soil

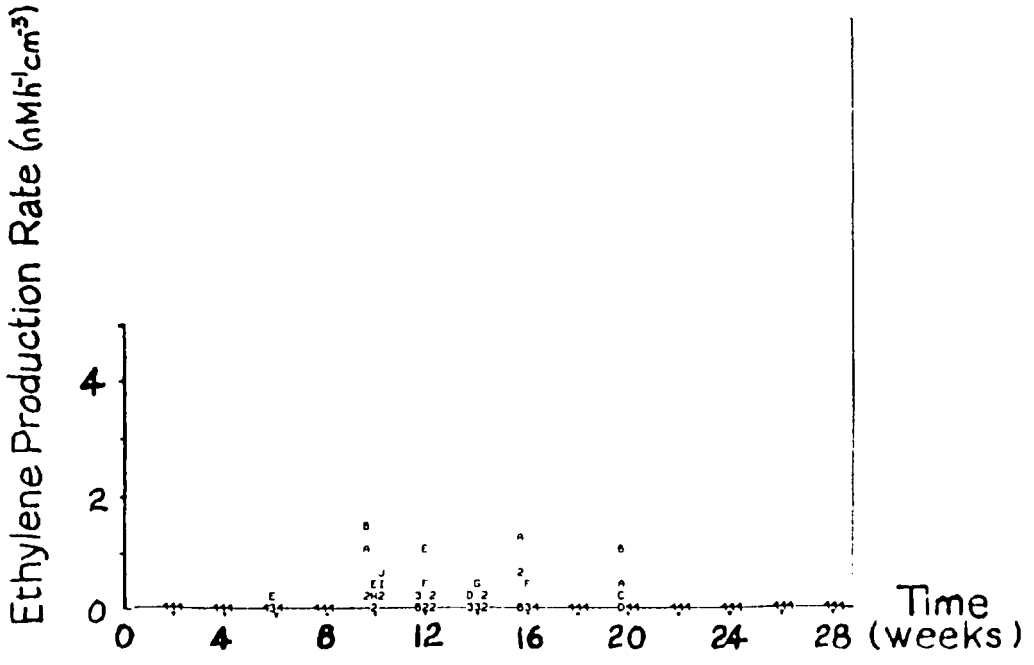
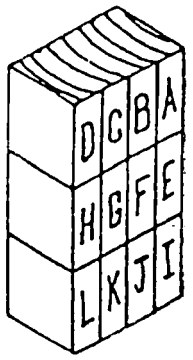


Figure 63

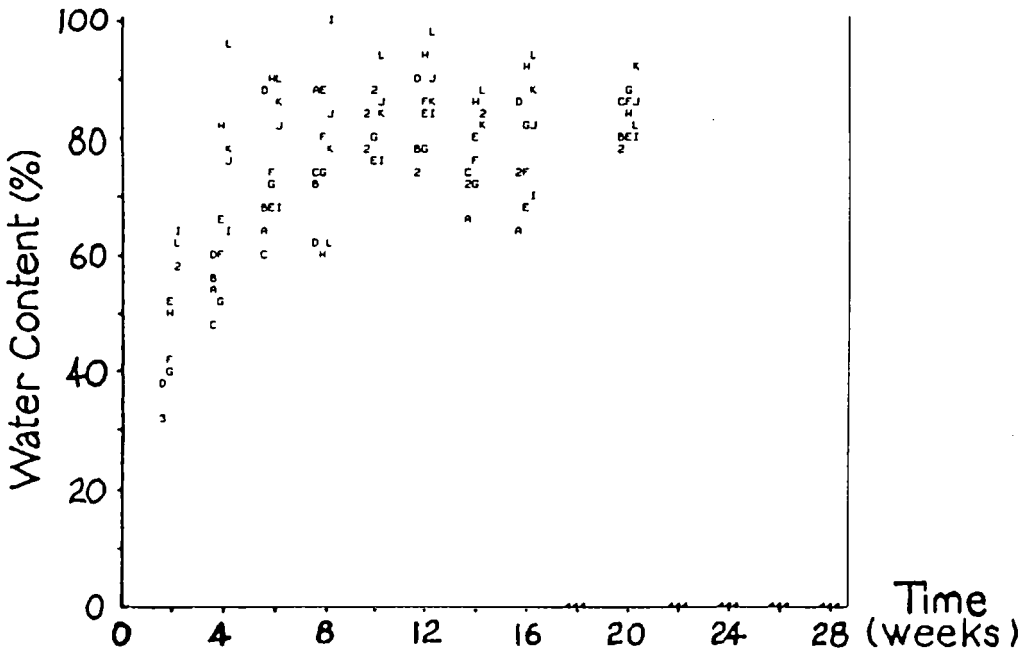


Birch

Moist Soil

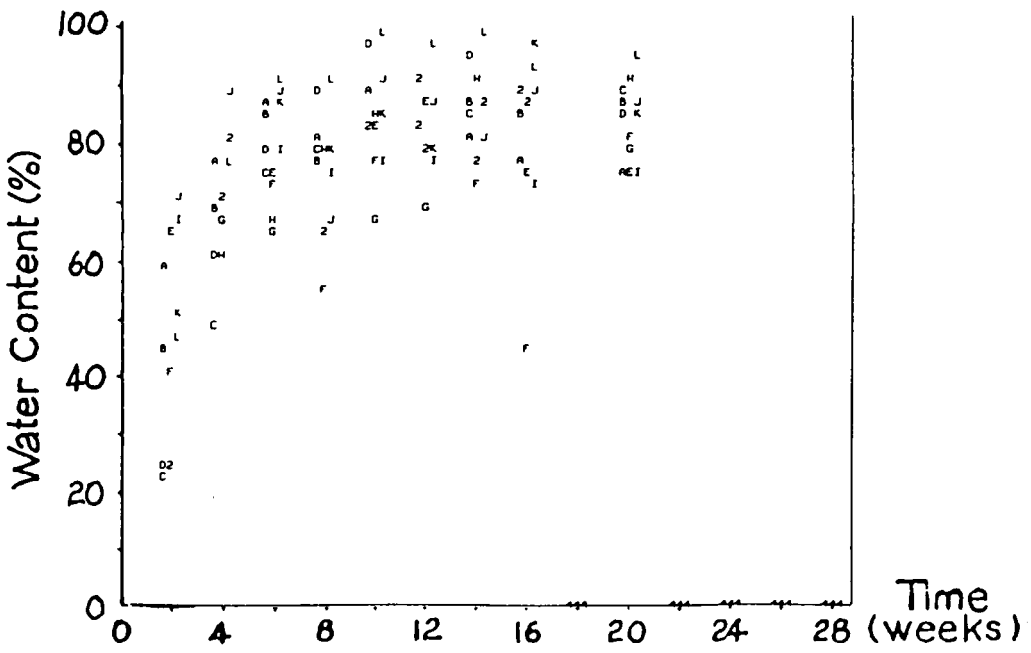
Birch

Very Wet Soil



Spruce

Very Wet Soil



8.4. Discussion

If the results for birch exposed to dry soil, described in Section 5, are compared with the results for birch in this experiment, Table 15, the interrelationship of soil moisture, wood moisture content and decay can be examined.

Table 15 Birch : Soil Moisture, Wood moisture content and Weight Loss.

Soil Moisture Content (%)	Weight Loss 10 weeks	Weight Loss 22 weeks	Final Moisture Content (10 weeks)	Rate of Moisture Content Change 2 - 10 weeks (% d^{-1})	Rate of Water Content Change 2 - 10 weeks (% d^{-1})	Final Moisture Content (22 weeks)	Rate of Moisture Content Change 10 - 22 weeks (% d^{-1})	Rate of Water Content Change 10 - 22 weeks (% d^{-1})
25 Dry		40•00	55•03	0•953	0•048	223•77	1•373	0•036
35 Moist	14•55	27•08	42•69	0•535	0•043	94•58	0•261	0•009
45 Very Wet	12•02	24•04	101•70	1•454	0•126	221•32	0•546	0•001

Weight loss was greatest in the dry soil reaching 40% after 22 weeks, with lower weight loss in moist soil (27.08%) and least in very wet soil (24.04%). The final MC of the birch blocks after 10 weeks in dry soil was higher than in moist soil and very high in the very wet soil, The implication of these results is that decay affects the precise relationship between wood moisture content and soil water availability described in Section 6 for Scots pine, where there was minimal weight loss.

Presumably, in the dry soil, the amount of available water in the soil was sufficient to allow the wood to reach a MC above that necessary for colonisation and decay to occur. As a consequence of decay, the amount of water in the block increased to around 55% after 10 weeks, partly due to the water produced from substrate utilisation, partly due to further uptake as the permeability of the wood was increased by ray and pit membrane breakdown and partly due to an increase of cell wall permeability due to fungal attack. From 10 to 22 weeks

decay continued and the MC of the wood increased further, so that the rate of MC change went from 0.953% d^{-1} over the first 10 weeks to 1.373% d^{-1} from 10 to 22 weeks. As explained in Section 3, MC is not a reliable measure of the water uptake of decaying wood so that when the rate of water uptake is compared up to, and after, 10 weeks, the rate of water uptake decreased as saturation of the decayed block approached.

In moist soil, where the amount of available water was higher than in dry soil, the MC of the wood presumably reached a higher level more rapidly than in dry soil, producing conditions in the wood which were not optimal for decay. In the absence of rapid decay, the wood MC increased less than in dry soil, reaching only 43% after 10 weeks, with a lower rate of water uptake than in dry soil. After 10 weeks the rate of water uptake decreased considerably as the block approached equilibrium with its surroundings, the final MC after 22 weeks being 95%.

In the very wet soil, with a considerable amount of water available, the wood rapidly took up water (0.125 gd^{-1}) reaching 101% MC after 10 weeks, presumably producing conditions in the wood which discouraged fungal attack. From 10 to 22 weeks the MC increased to 221% while the rate of uptake of water decreased considerably (0.001 gd^{-1}).

Obviously decay profoundly changes the properties of the wood, which complicates its water relations and affects the precise relationship between soil water, wood and wood MC derived for undecayed Scots pine. Although decay can itself result in a high MC in the wood, the imposition of the same high MC on undecayed wood discourages decay. However, soil water availability remains an important concept, as it can still be regarded as determining initial wood MC (which determines whether colonisation and decay can occur), and also the equilibrium wood MC as decay occurs.

The properties of decaying wood do not remain constant during exposure, so that the precise relationship described in Section 6 is adequate for undecayed wood or the initial stages of exposure, but if decay occurs, the wood changes and a different equilibrium must be established. This is illustrated by the MC of the wood exposed to soil exceeding the theoretical maximum MC of the wood. Wood can be regarded as in a dynamic state during soil exposure. Soil water availability, wood permeability, wood moisture content and susceptibility all affect each other and each affects decay rate. One implication of this is that if

decay can be regarded as a complex and dynamic process then the comparison of decay in different species with different treatments becomes particularly difficult i.e. the final MC at the end of an exposure period may not necessarily be a measure of MC throughout exposure, and may not be related to the critical MC which determines the onset of fungal attack. Similarly, final weight loss after a period of exposure may not be a valid comparison of decay rate in two species or treatments. Sampling at intervals throughout an exposure period is desirable, if not essential, to compare and contrast the rate of decay.

The mechanism of decay limitation in very wet soil may be related to the "wick action", where water flows through partially buried wood, acting as a wick, driven by the "evaporating power" of the air. The effect of wick action in dry soil, where presumably oxygen is dissolved in the soil water from air filled pores connected ultimately to the atmosphere, may be to supply oxygenated water to the wood. In very wet soil, where there are no air filled pores and the soil water is oxygen deficient, wick action may supply no oxygen. Some evidence for the aerobic/anaerobic nature of dry and very wet soils is produced by the occurrence of anaerobic nitrogen fixing bacteria in the wood, which do not occur in wood exposed to dry soil, but are common in wood exposed to very wet soil. The implication is that the conditions in very wet soil, and in wood exposed to very wet soil, are anaerobic, but aerobic in dry soil and wood exposed to dry soil. Although this work illustrates the possible interdependence of soil MC, wood MC, oxygen supply and decay, further work on the oxygen tension inside wood exposed to dry moist and very wet soils would be desirable.

The "response" of different wood species to the same soil MC is shown in Table 16, a comparison of water uptake in Scots pine, birch and spruce exposed to very wet soil.

Table 16 Comparison of water uptake rate in birch, spruce and Scots pine exposed to very wet soil.

	Uptake Rate ($g\ d^{-1}$)
Birch	0•126
Spruce	1•000
Scots pine	0•847

Spruce takes up water more quickly than Scots pine, while both softwoods are faster than birch. The implication of these results is in the treatment of timbers with preservatives, where Scots pine is classified as permeable while spruce is classified as only moderately permeable, despite their superficial anatomical similarity. These results indicate that the longitudinal flow of water through spruce is higher than in pine, so that perhaps spruce would be treated more effectively by longitudinal flow than by conventional tangential and radial penetration.

The occurrence of the highest AR rate in those segments above ground in spruce indicates that wick action was occurring, carrying the bacteria through the wood, resulting in their accumulation above ground. Alternatively the bacteria could be utilising nutrients in the water transported through the wood from the soil, which could also be accumulating above ground, producing the weight gain observed in above ground segments.

Nitrogen fixing bacteria appear not to be significant in the decay of birch and spruce, as considerable decay was found in birch in the absence of nitrogen fixing bacteria, while in the spruce and birch exposed to very wet soil and with considerable AR activity, little decay was recorded. Presumably, nitrogen was not limiting in birch in dry soil, and the conditions limited decay in spruce and birch in very wet soil. If the level of nutrients in the soil can affect the decay of wood when they are transported into the wood by wick action, it would be of interest to examine decay rate in a soil with a higher level of nitrogen fixing activity and a higher level of soil nitrogen.

The occurrence of greatest decay in the dry soil, with least decay in the moist and very wet soils may indicate that the organisms responsible for decay are adapted to dry soil conditions because these conditions are usual in the field. The examination of timber exposed in the field would allow the comparison of the laboratory soil exposure system to realistic exposure in the field and the investigation of the significance of nitrogen fixing bacteria to decay under realistic conditions.

8.5 Conclusions

The decay of wood in soil contact is complex and dynamic. It is dependent on the interaction between wood, soil, water and soil microorganisms. Wood moisture content is affected by decay and by soil MC, and decay is affected by soil MC and perhaps oxygen tension. Differences exist in the water relations of different wood species in soil contact, which may be of relevance in the performance and treatability of these species.

Any significance of nitrogen fixing bacteria to the decay of spruce and birch have not been detected in this experiment. The distribution of bacteria may be a measure of the occurrence of wick action and of the presence of anaerobic conditions in the soil and wood.

Further work is necessary on decay in different soils, and on decay rate and nitrogen fixing activity under realistic field exposure in order to assess the significance of nitrogen fixing bacteria to timber decay in soil contact.

9. THE EFFECT OF ANOTHER SOIL TYPE UPON BIRCH AND SCOTS PINE

9.1. Introduction

This experiment was to examine the AR rate and decay rate in birch and Scots pine blocks exposed to a soil with a high level of AR activity, implying a large population of active nitrogen-fixing organisms. Wet soil was used as this had been found to encourage the development and colonisation of wood by nitrogen fixing bacteria. To examine the effect of soil drying upon AR activity and decay, the soil was allowed to dry out.

9.2. Materials and Methods

The soil was a clay from the South Croydon area of London which had been used previously as an example of a fertile soil having nitrogen-fixing (AR) activity (J.W. Millbank pers. comm.). The soil was set up and maintained at 35% (moist) and the method of wood preparation and exposure was as described in Section 3. Table 17 gives the average value of the block weight and density of the sets of blocks, and the MC, void volume and maximum MC of the blocks. The nominal and calculated soil MC are given together with the number and code number of blocks removed at each sample time. After 119 days, the lids were left off both systems for one week, and were then replaced.

9.3. Moisture Content - Figure 64

In Scots pine after 4 weeks there was a distinct difference in the zones above and below ground although there was little difference between the zones after 8 weeks. When the soil was allowed to dry, the MC of the wood decreased dramatically. By 26 weeks there was once again a distinct difference between the zones.

In the birch blocks the difference in MC between the zones was distinct at 4 and 8 weeks but less distinct at 12 weeks, while at 16 weeks the above ground zone was wetter than the zones below ground. The outer tangential segments were usually wetter than the inner segments. After the dramatic reduction in wood MC after the soil had dried, the wood MC increased slightly, with the MC in all 12 segments being remarkably similar at 24 weeks.

The graphs of MC against time, Figure 65, shows the rapid increase

Table 17 Wood Block and Soil Data.

Wood Species	: Scots pine	Soil Type	: Clay
Initial Block Wt.	: 10•62 ± 0•24 g	Initial Moist. Cont.	: 8•48 %
Calc. Oven Dry Wt.	: 9•79 g	Void Volume	: 57•28 %
Initial Density	: 0•566 g cm^{-3}	Max. Moist. Cont.	: 104•20 %

Soil Moisture Conditions

Nominal : 35 % (Very Wet) 25 % (Moist)

Calculated :

2	4	6	8	10	12	14	16	18	20	22	24	26	28 weeks
	30		30		34		35	27	26		25		

Code Number of Blocks Sampled at each Sample Time

2	4	6	8	10	12	14	16	18	20	22	24	26	28 weeks
	JD29		JC26		JD34		JD31	JC15	JC16		JD32		
	JC21		JD26		JD22		JD27		JC14		JD21		
	JC16		JD35		JC30		JD23						
							JC35						
							JD24						

Wood Species	: Birch	Soil Type	: Clay
Initial Block Wt.	: 12•56 ± 0•38 g	Initial Moist. Cont.	: 7•47 %
Calc.Oven Dry Wt.	: 11•69 g	Void Volume	: 50•01 %
Initial Density	: 0•670 g cm^{-3}	Max. Moist. Cont.	: 77•45 %

Soil Moisture Conditions

Nominal : 35 % (Very Wet) 25 % (Moist)

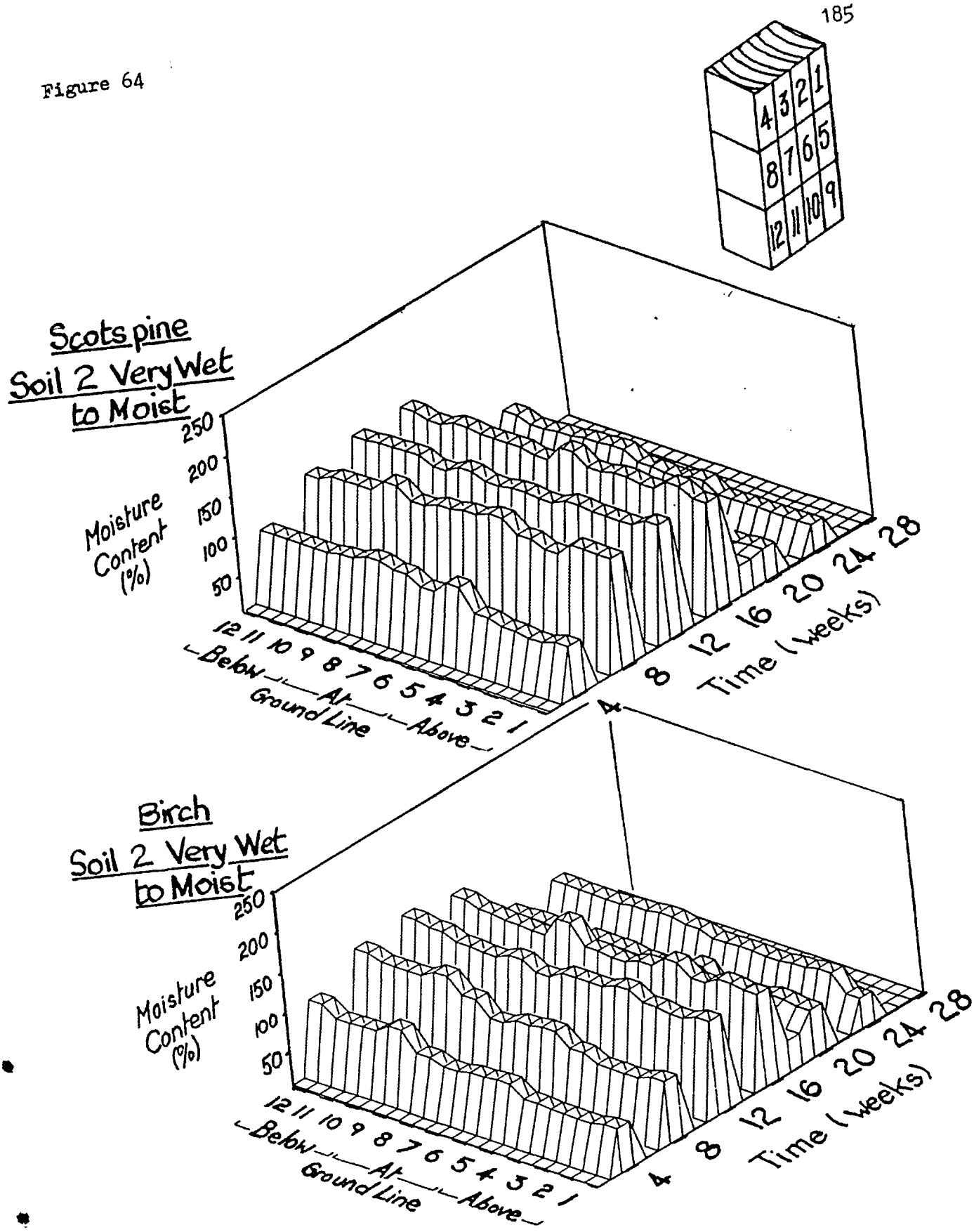
Calculated :

2	4	6	8	10	12	14	16	18	20	22	24	26	28 weeks
	31		31		32		35	20	24		25		

Code Number of Blocks Sampled at each Sample Time

2	4	6	8	10	12	14	16	18	20	22	24	26	28 weeks
	LD26		LD20		LE05		LE10	LE12	LE15		LE19		
	LE22		LE17		LD15		LE20		LE09		LE24		
	LE13		LE04		LD24		LE18						
							LE16						
							LD19						

Figure 64


4 3 2 1
8 7 6 5
12 11 10 9
Scots pine
Soil 2 Very Wet to Moist
Moisture Content (%)
250
200
150
100
50
12 11 10 9 8 7 6 5 4 3 2 1
Below
At
Above
Ground Line
4 8 12 16 20 24 28
Time (weeks)
Birch
Soil 2 Very Wet to Moist
Moisture Content (%)
250
200
150
100
50
12 11 10 9 8 7 6 5 4 3 2 1
Below
At
Above
Ground Line
4 8 12 16 20 24 28
Time (weeks)

in wood MC after exposure, reaching a plateau after only 8 weeks, presumably as the wood became saturated at around 130-150%. After the soil was allowed to dry, the MC of all 12 segments had decreased to 30-50% after only 7 days. The MC increased slightly when the lid was replaced, and a difference between buried and unburied zones was apparent at 24 weeks.

In the birch blocks, the maximum MC was reached more slowly, and was slightly lower than in Scots pine. When the soil dried out, the wood MC only decreased to 40-60% before the lid was replaced, and then the wood MC increased to 45-65%, which was maintained up to 24 weeks.

Weight Loss - Figure 66

In Scots pine, after 4 weeks, segments 1 and 3, 5 and 7, and 9 and 11 had weight losses, while the remaining 6 segments had weight gains, presumably due to density variation within the block. This variation in density, added to the natural variation to be expected in the decay of wood exposed to soil, obscured any increase in WL with time in the three-dimensional representations, although at 24 weeks the WL values were consistently higher than at any previous sample time.

In birch, the WL values were even more variable than in Scots pine although there were more segments exhibiting weight loss after 18 weeks when the soil was dry, than when the soil was wet.

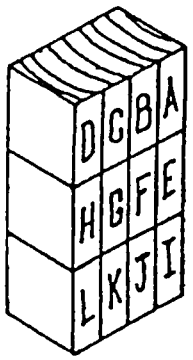
The graphs of weight loss against time, Figure 67, show that in pine there was no increase in WL in the wet soil, but in dry soil there was a slight increase in WL with time.

In birch there was a marginal increase in weight loss with time in the wet soil, but after the soil was allowed to dry, the weight loss increased noticeably with time.

Acetylene Reduction Rate - Figure 68

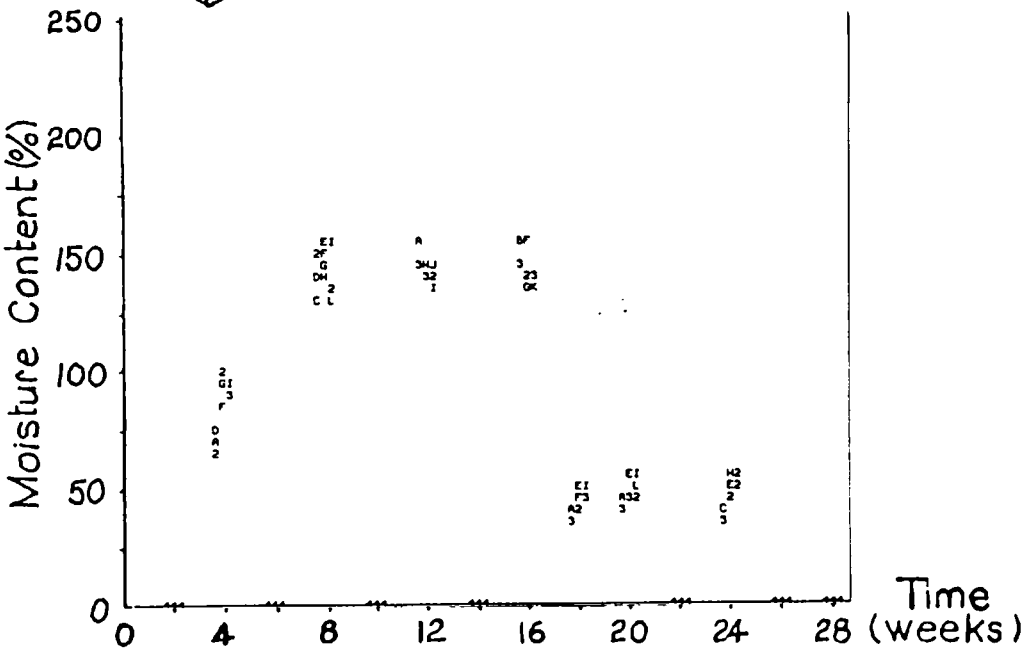
In Scots pine after 4 weeks, most activity was recorded in the below ground zones. In the deepest zone most activity was found in the outer segments, while in the zone at the ground line most activity was in the outer tangential segment. The segment immediately above this was the only segment in the above ground zone to exhibit AR activity, the inference being that the bacteria were moving longitudinally more easily

Figure 65



Scotspine

Soil 2
Very Wet
to
Moist



Birch

Soil 2
Very Wet
to
Moist

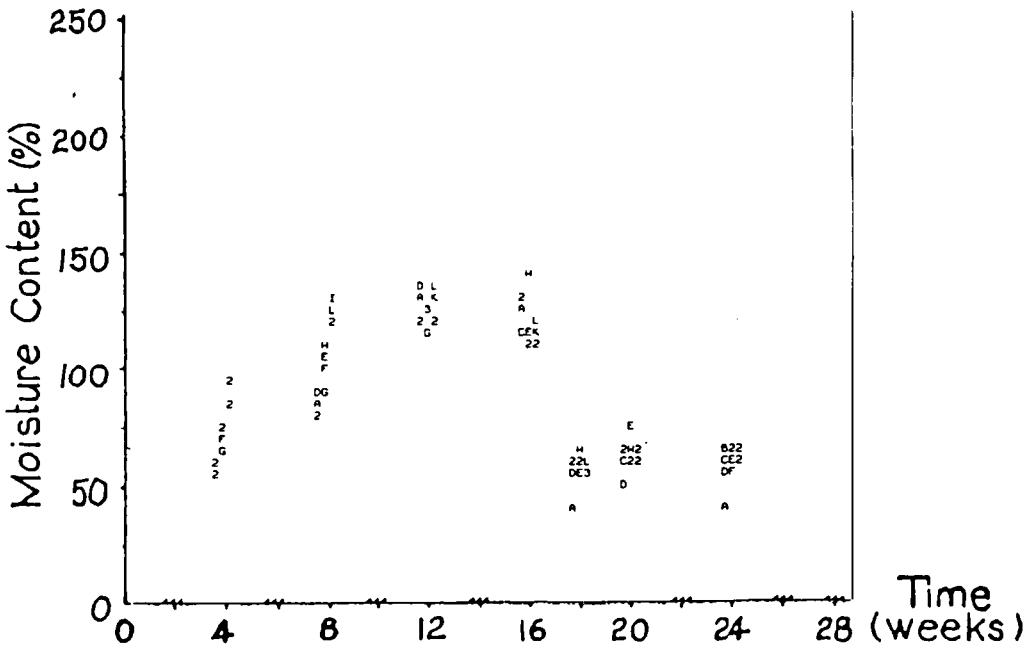


Figure 66

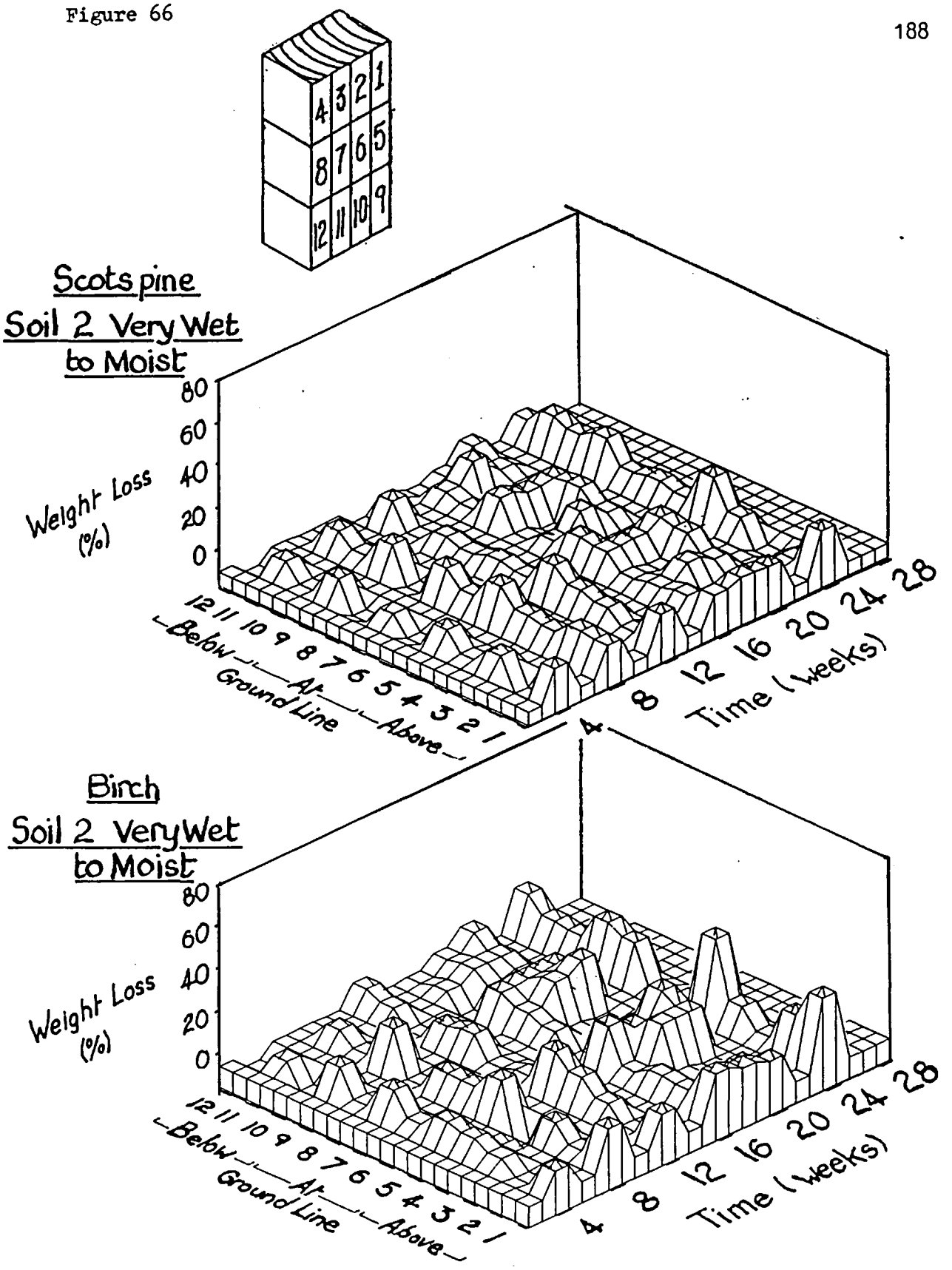
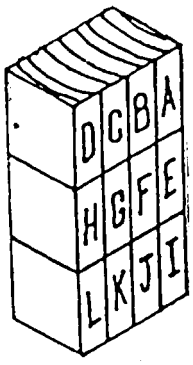
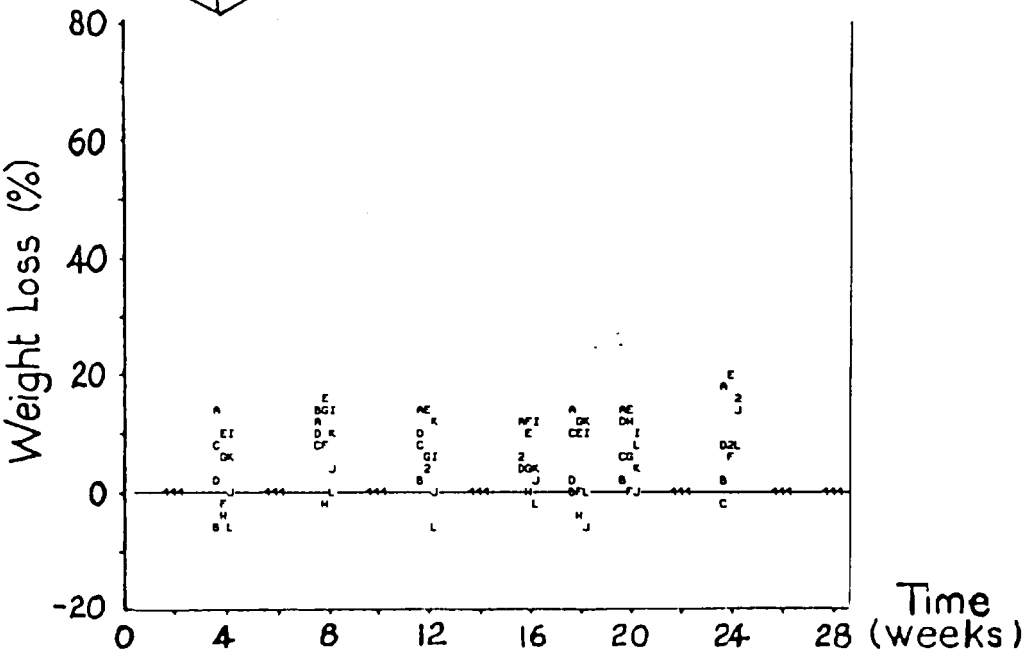

4 3 2 1
8 7 6 5
12 11 10 9
Scots pine
Soil 2 Very Wet to Moist
Weight Loss (%)
80
60
40
20
0
12 11 10 9 8 7 6 5 4 3 2 1
Below Ground Line
At
Above
4 8 12 16 20 24 28
Time (weeks)
Birch
Soil 2 Very Wet to Moist
Weight Loss (%)
80
60
40
20
0
12 11 10 9 8 7 6 5 4 3 2 1
Below Ground Line
At
Above
4 8 12 16 20 24 28
Time (weeks)

Figure 67



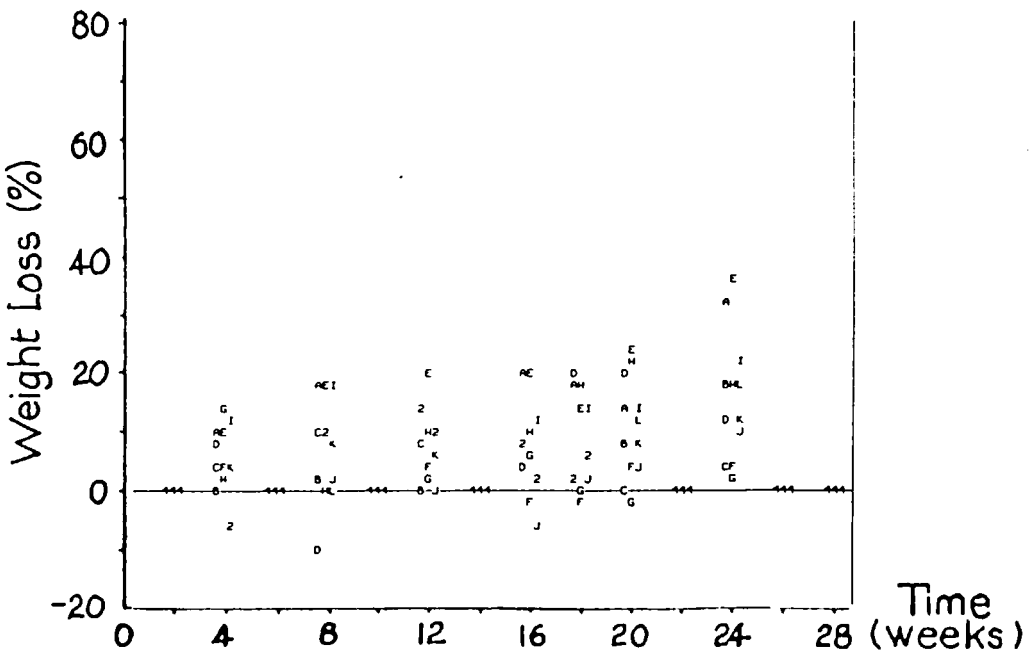
Scots pine

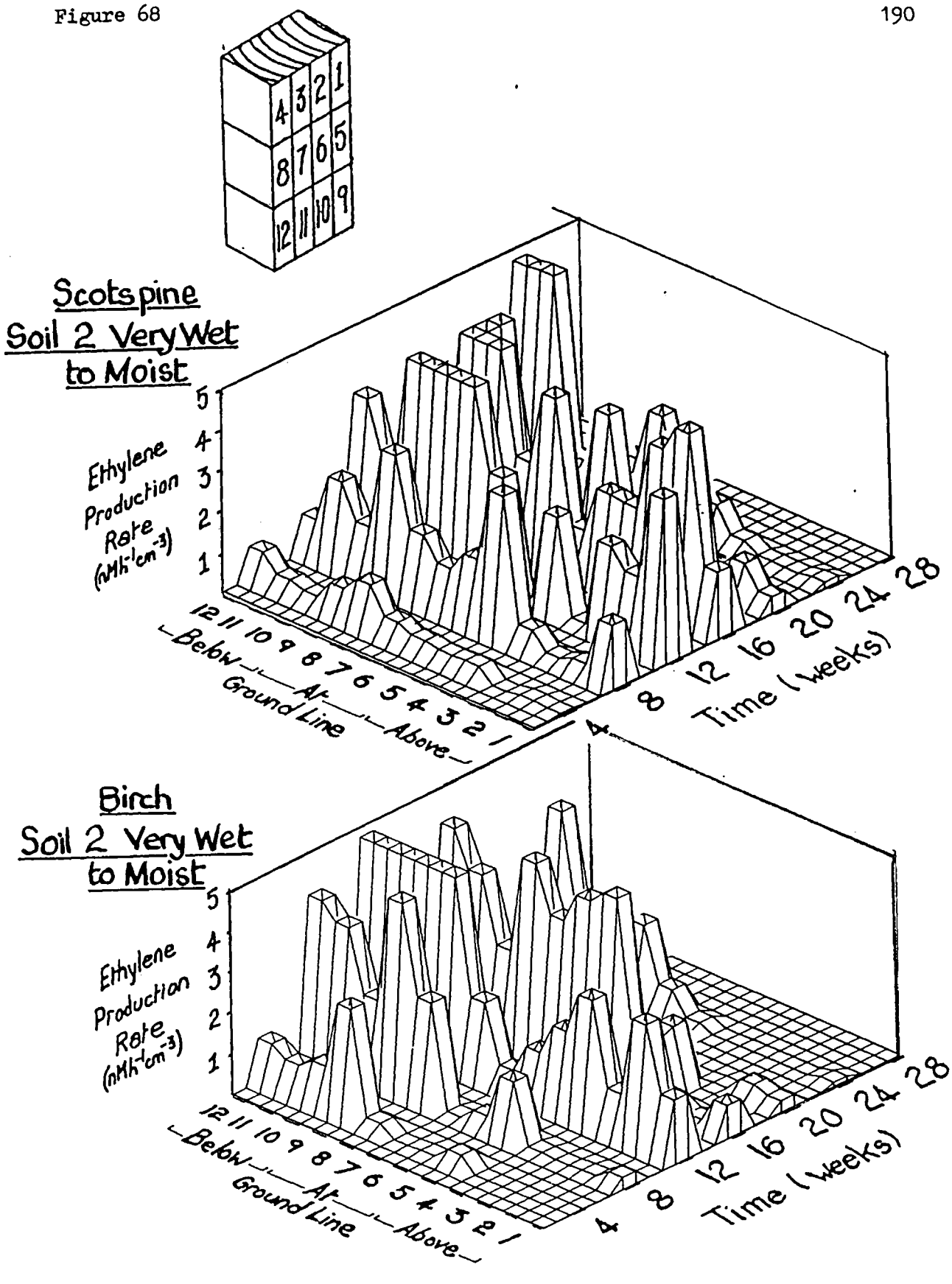
Soil 2
Very Wet
to
Moist



Birch

Soil 2
Very Wet
to
Moist




4 3 2 1
8 7 6 5
12 11 10 9
Scotspine
Soil 2 Very Wet to Moist
Ethylene Production Rate (nMh⁻¹cm⁻³)
5
4
3
2
1
12 11 10 9 8 7 6 5 4 3 2 1
Below Ground Line
At
Above
4 8 12 16 20 24 28
Time (weeks)
Birch
Soil 2 Very Wet to Moist
Ethylene Production Rate (nMh⁻¹cm⁻³)
5
4
3
2
1
12 11 10 9 8 7 6 5 4 3 2 1
Below Ground Line
At
Above
4 8 12 16 20 24 28
Time (weeks)

than radially, as found in Section 1.4. By 8 weeks, activity was recorded throughout the block. By 12 and 16 weeks activity was greater than 5 $nMh^{-1}cm^{-3}$ in some segments below ground and even above ground some of the highest values recorded in this series of experiments were found. After the soil was allowed to dry the AR rate in the above ground zone decreased dramatically, while some segments below ground still showed considerable activity.

In the birch blocks after 4 weeks there was activity in all the segments in the zone below ground, but only in the outer segments in the zone at the ground line, and no activity was recorded in the zone above ground. At 8 weeks, activity was recorded in all the segments, but with most activity in the inner tangential segment of each zone. At 12 weeks, activity was very high in the deepest zone and least in the ground line zone, decreasing slightly at 16 weeks. After the soil had dried, activity decreased drastically, particularly in the zone above ground.

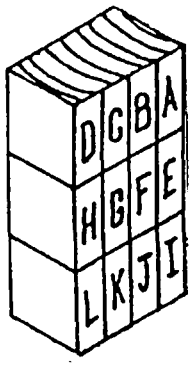
The graphs of AR against time, Figure 69, for Scots pine, show the increase of AR activity in all segments with time, reaching a maximum of 12.5 $nMh^{-1}cm^{-3}$ in segment 10 after 12 weeks. At 16 weeks all segments exhibited a high rate between 1.2 and 10. After the soil was dried, the rate decreased particularly above ground, but then increased slightly in the deepest zone up to 24 weeks.

In birch the AR activity increased more rapidly than in Scots pine, particularly below ground, reaching 14 $nMh^{-1}cm^{-3}$ after 16 weeks in segment 7. After the soil was dried the rate decreased dramatically, and as in Scots pine, increased once more at 20 and 24 weeks.

Water Content - Figure 70

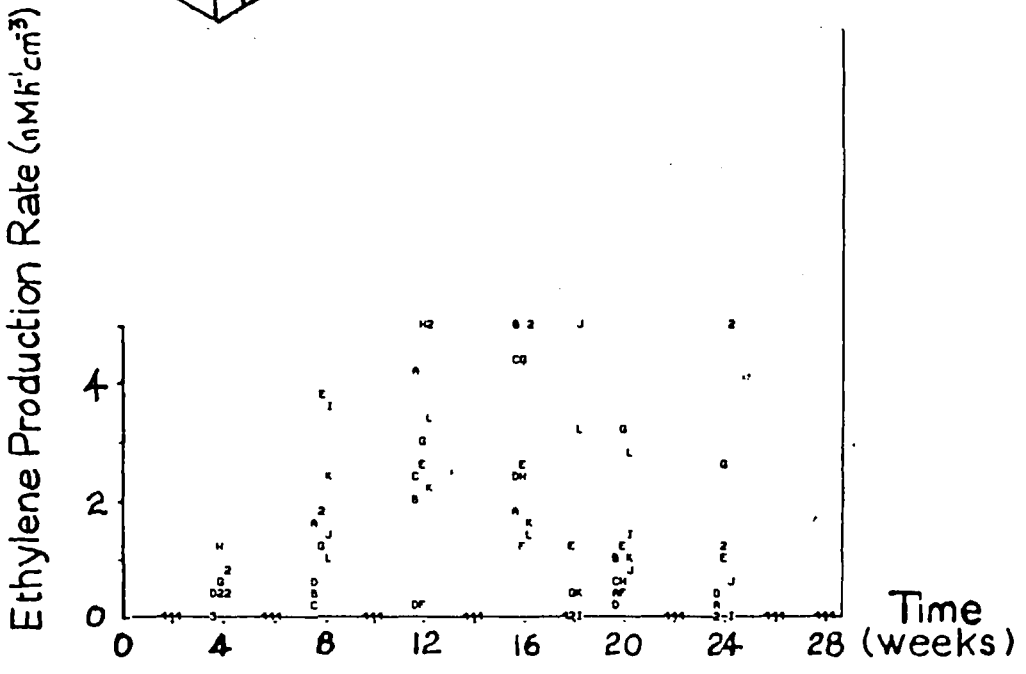
In Scots pine the difference in WC between the zones above ground and the zones below ground was apparent at 4 weeks, but not at later times, while the decrease in WC after the soil dried was obvious.

In birch the distinction between the zones was very noticeable at 4 weeks and was maintained up to 12 weeks, where the WC was very similar in all except the inner tangential segment of each zone. At 16 weeks this distribution was even more marked, while the drop in WC after the



Scots pine

Soil 2
Very Wet
to
Moist



Birch

Soil 2
Very Wet
to
Moist

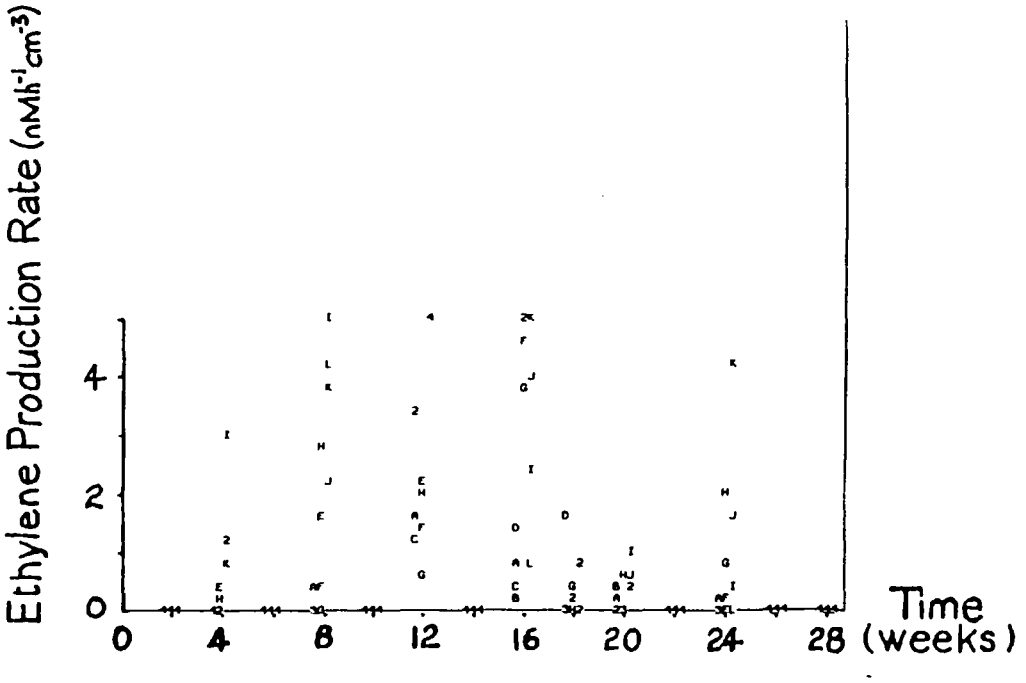
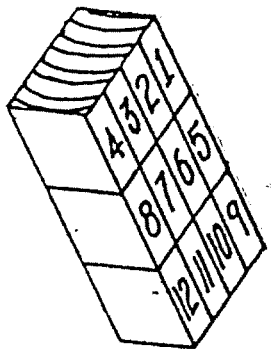
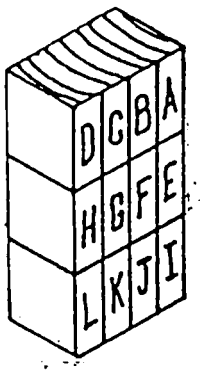


Figure 70


4 3 2 1
8 7 6 5
12 11 10 9

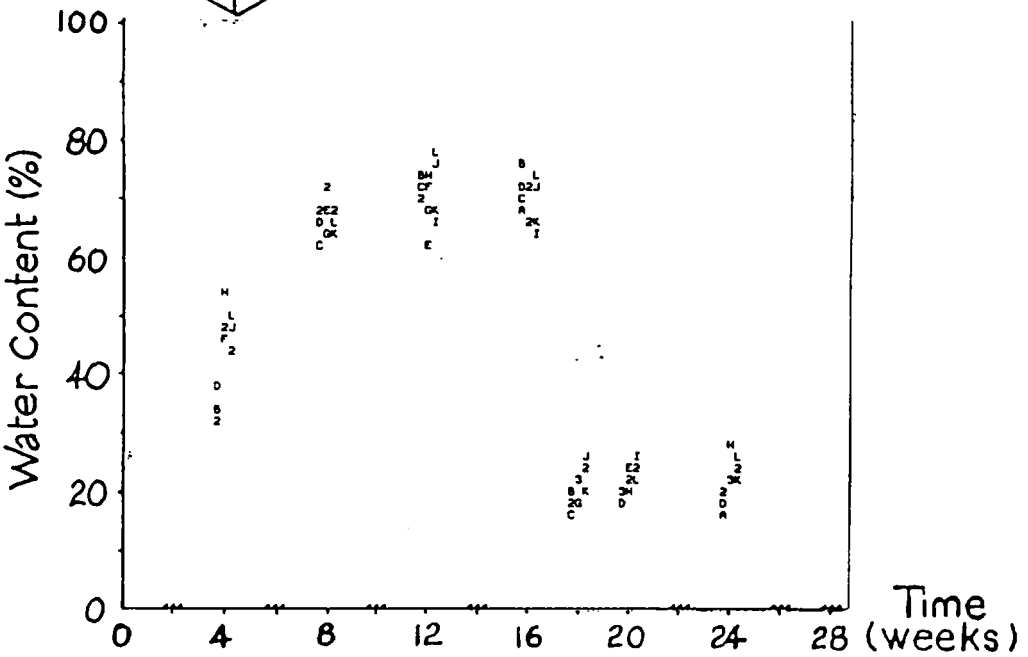
Scots pine
Soil 2 Very Wet to Moist
Water Content (%)
100
80
60
40
20
12 11 10 9 8 7 6 5 4 3 2 1
Below
At
Above
Ground Line
4 8 12 16 20 24 28
Time (weeks)
Birch
Soil 2 Very Wet to Moist
Water Content (%)
100
80
60
40
20
12 11 10 9 8 7 6 5 4 3 2 1
Below
At
Above
Ground Line
4 8 12 16 20 24 28
Time (weeks)

Figure 71



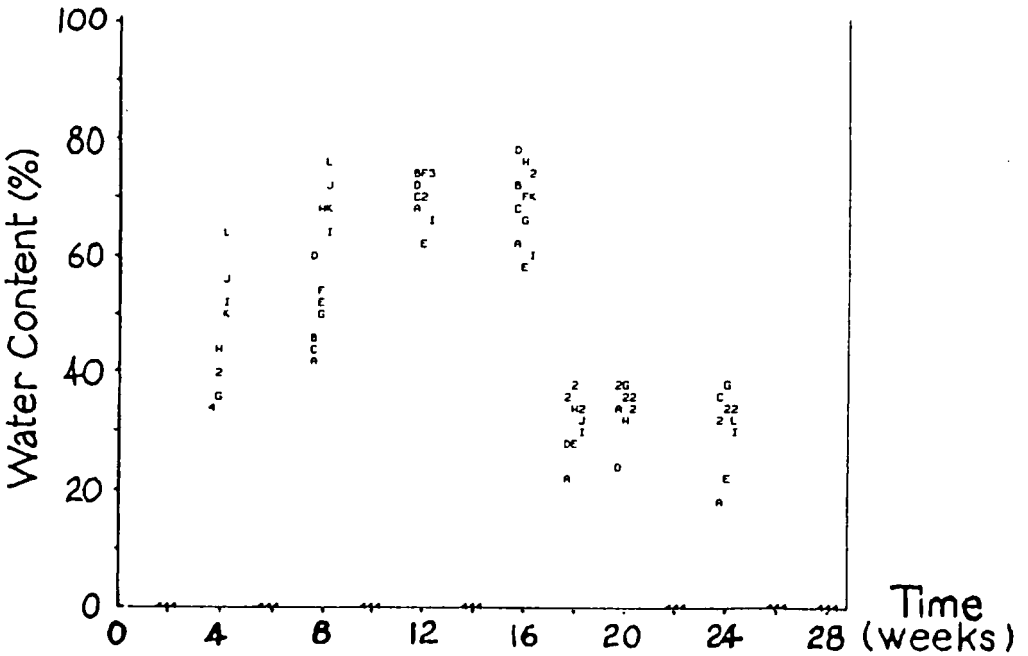
Scots pine

Soil 2
Very Wet
to
Moist



Birch

Soil 2
Very Wet
to
Moist



soil was dried was again obvious.

The graph of WC against time, Figure 71, shows the rapid increase in WC during the first 4 weeks in both species, although the WC of birch increased faster than Scots pine initially. In Scots pine the plateau was reached at around 70% in all 12 segments, while in birch the WC was more variable over a range from 59 to 78%. After drying, the WC of birch was higher at 25-35% than in Scots pine (18-25%), a difference that was maintained up to 24 weeks.

9.4. Discussion

Although the prime aim of this experiment was to examine the effect of a different soil type, with high AR activity, upon the decay of pine and birch, the value of soil water availability as a criterion of the MC of soil is immediately apparent. The brown loam soil used in the previous experiments was "wet" at 35% MC, while the clay used in this experiment at 35% MC was "waterlogged", and should be compared with the brown loam at 45% MC. Once again the familiar pattern of the moisture relations of wood and soil is apparent. Both species wetted up rapidly in the soil (with a considerable amount of available water) and rapidly approached saturation. Under these conditions, decay was minimal, while nitrogen fixing bacteria were encouraged and AR activity in the wood was high. The highest AR values recorded are presumably due to the large population of organisms in the soil which provided a large inoculum for the colonisation of the wood. After the soil was allowed to dry out, both species lost water rapidly by evaporation (pine faster than birch) until the lid was replaced and the R.H. around the exposed portion of the blocks increased, when the MC of the wood increased slightly. Under these conditions, AR activity decreased and it appeared that weight loss increased as decay began. Once again the close relationship between the wood and the water availability of its surroundings was revealed, together with the effect of soil conditions upon wood moisture content, AR activity, and decay. Wood decay remains a complex and dynamic process, and the wood is in a dynamic equilibrium with its surroundings, but it would appear that the general principles derived in one soil can be related to another soil, which is encouraging for the investigation of timber in soil contact.

In addition, this experiment provides some interesting observations which are of relevance to the concept of wick action in wood. A

difference in MC between buried and unburied zones was apparent initially, but then disappeared as all 12 segments reached the same MC. When the lid was removed from the system, the wet blocks rapidly lost water, particularly from the above ground zone and less from the zones below ground. The AR activity in the above ground zone stopped, while the buried zones continued to exhibit activity. Once the lid was replaced, the MC increased and AR activity returned to the zone above ground.

The implication of these results is that wick action occurs in the wood and is important in determining the MC of the wood. If during the wetting-up phase the rate of evaporation exceeds the rate of water supply, because the wood is relatively dry and not very permeable, then a marked difference in MC between the buried and unburied zones would occur. Once the zones below ground were wet, permeable and able to transport water, then the rate of supply would exceed the rate of evaporation and the entire block could become wet and the 12 segments would all have the same MC. If then the rate of evaporation exceeds the maximum rate of supply, such as when the lid is removed, the zone above ground loses water more rapidly than it can be supplied, and dries out, leaving the water in the buried zones relatively undisturbed, and allowing nitrogen fixing activity to continue. Once the lid is replaced and the rate of evaporation reduced, the rate of water supply can exceed the rate of evaporation once more and the zone above ground gets wetter, carrying organisms longitudinally from below to above ground. Presumably the water vapour leaving the wood and soil rapidly produces a high relative humidity around the wood which allows the wood MC to be maintained. The rate at which wetting-up and drying occurs in the wood depends on wood permeability, and as birch and pine have different permeabilities, it is hardly surprising that their rate of wetting and drying differs. This experiment provides further evidence for the existence of wick action and indicates that it may be very important in determining wood moisture content, the existence of a dynamic equilibrium between soil, water, wood MC and the relative humidity of the air, and be of significance in determining the decay of timber in soil contact.

The occurrence of high AR rates in both birch and pine indicates that it is the soil conditions, rather than the wood species, which determines the occurrence of nitrogen fixing bacteria in the wood. The

indication is that if the soil conditions are suitable, then nitrogen fixing bacteria can proliferate in the soil and colonise wood. However, as in the previous experiment, there is no obvious correlation between AR activity and weight loss, partly due to the extreme variability of both AR and weight loss.

From the results of this experiment it would seem unlikely that the presence of nitrogen fixing bacteria are a pre-requisite of decay, because these bacteria occur in the absence of decay and decay can occur in their absence, as in the previous experiments. However, there is the possibility that in fluctuating conditions, such as those artificially created in this experiment, which may occur under realistic conditions in the field, nitrogen fixing bacteria may be active in wood when the soil is very wet. On drying, the bacteria may die, releasing nitrogen into the wood which may be available to fungi which prefer the drier conditions. Fluctuating conditions may be of great significance in the decay of timber in soil contact, but have not been investigated, presumably because the monitoring of decay under controlled but fluctuating conditions in a system with a high level of inherent variability, is likely to be particularly difficult. Similarly, the use of constant conditions in the laboratory, although reducing variability, may be unrealistic as it eliminates the seemingly vital fluctuations which occur in the field. Obviously the comparison of decay in the laboratory and in the field would be of value in investigating the significance of fluctuations in soil moisture content and relative humidity.

It is encouraging that the principles evolved from one soil and one timber species can be extended to other species and another soil. The implication is that the relationship between soil water availability and wood moisture content could be applied generally to all situations in which wood is exposed to soil contact, if not to all situations in which timber is used.

9.5. Conclusion

This experiment, involving the exposure of Scots pine and birch to a waterlogged clay soil which was then allowed to dry out, has confirmed that a general relationship between soil water availability and wood moisture content exists and that it could be of significance in determining the MC of timber in service, which affects its susceptibility to decay.

The results imply that wick action was occurring in the wood, as the observations on changes in wood moisture content and acetylene reduction activity may be explained if wick action had occurred. The occurrence of high AR activity in the wood revealed the effect of a large population in the soil and the importance of the soil in determining both the conditions and the occurrence of organisms in the wood. From the results presented, there would appear to be no correlation between the occurrence of nitrogen fixing bacteria and decay in the wood. Nevertheless, both the water relations of wood and wick action are potentially of great significance in the decay of timber in soil contact.

10. SUMMARY AND DISCUSSION OF LABORATORY SOIL EXPOSURE EXPERIMENTS

The original aim of the series of experiments described in sections 1 to 9 was to determine the significance of nitrogen fixing organisms to timber decay in soil contact. From the results described it is apparent that the occurrence of nitrogen fixing bacteria in wood in soil contact is determined by soil conditions more than by timber species, in that both hardwoods and softwoods are colonised if the soil is very wet or waterlogged. There is no direct correlation of weight loss and decay with acetylene reduction rate, as there is decay in the absence of AR in dry soil, decay and AR in moist and wet soils, and minimal decay with maximal AR in very wet and waterlogged soils. Thus overall, on the basis of these results it would seem unlikely that the presence of nitrogen fixing bacteria is essential for the decay of timber in soil contact. When the rate of AR is converted to amounts of nitrogen fixed, assuming that the conversion is valid (see Section 2.4.6), and that the hourly rate is maintained for weeks and months, the increase in the nitrogen content of the wood may be only 0.004 % per day or 1.46 % per year. Obviously nitrogen fixing bacteria are a part of the decay ecosystem as they occur in wood exposed to wet, almost waterlogged, soil, but their significance depends upon how important to the decay organisms is an approximate 1 % increase in wood nitrogen content.

Nitrogen can increase decay as shown by the impregnation of wood with Abram's (1948) salts (Savory, 1954) which considerably increases the nitrogen content and promotes decay.
King, Oxley and Long (1976) found that differences of 100% in the nitrogen content of wood affected the amount of soft rot decay, and Merrill and Cowling (1966) found that similar differences in the natural nitrogen content of wood affected the amount of weight loss. Butcher (1975) found that the impregnation of veneers with solutions containing 0.25g/l nitrogen produced maximum soft rot attack. Thus the addition of nitrogen to wood increases fungal attack, and small additions of nitrogen could be

important for decay organisms, so that even the relatively small amount supplied by nitrogen fixing bacteria could be significant.

However, problems still exist in estimating the significance of these bacteria. From the results it appears that most decay was in dry soil, and most AR in wet soil, implying that decay and AR were mutually exclusive. Presumably, decay fungi are aerobic, being most active in oxygenated, dry soil, while the bacteria are anaerobic, being most active in oxygen depleted, wet soil. Thus in order for the nitrogen fixed by the bacteria to be available to the fungi, either the fixed nitrogen must be transported to the fungi from the bacteria,(which are active at different sites in the wood),or the conditions in the wood must fluctuate, allowing bacteria to be active in anaerobic, wet, conditions, and then fungi to be active when conditions are aerobic and drier. Although there is some evidence for an effect of this cyclic fluctuation in conditions upon decay from these experiments under artificially imposed conditions in the laboratory, the occurrence and effect of such fluctuations upon decay and AR can only realistically be investigated in the field (See Section 12).

Conceivably, nitrogen fixed by bacteria in the wood below ground could be transported to fungi active at the ground line, by wick action, although there is no evidence from these experiments that nitrogen fixing bacteria are confined to the zone below ground, and fungi to the ground line zone.

An additional problem is that the fixed nitrogen is incorporated into bacterial cells rather than being freely available in the water in the wood. Although cells may "leak" nitrogen, large amounts of nitrogen may only be available when the bacteria die. Of course, the nitrogenous products of bacterial death and those liberated by "leakage" must be assimilable and utilisable by the fungi for the products to be of significance in their nitrogen nutrition.

A third possibility is that the nitrogen fixing bacteria and fungi are "symbiotic" in the wood, with the fungi and bacteria living close together, with the fungi utilising the oxygen, reducing the oxygen concentration around the bacteria, and effectively creating an anaerobic environment. Line and Loutit (1973) describe some experiments

in which Clostridium and Pseudomonas species have this "symbiotic" relationship. N_o evidence is available from these experiments concerning this possibility.

Thus this series of experiments has shown that nitrogen fixing bacteria can occur in wood in soil under some conditions and that they may be capable of increasing the overall nitrogen content of the wood by around 1% per year. Further work would be required to determine whether this amount of fixed nitrogen is significant to the nitrogen economy of the decaying wood and specifically to the decay fungi.

The technique for the exposure of wood to soil contact under controlled conditions in the laboratory, described in Section 3, has been shown to be useful for the collection of data concerning the decay of wood in soil contact. The use of partially buried, rather than totally buried, wood, is potentially of great importance in the realistic simulation of ground contact exposure. The analysis of 3 separate zones has been important, although the two buried zones were broadly similar but often very different from the zone above ground. Information on the longitudinal penetration of both water and nitrogen fixing organisms has been obtained by sampling the three zones. The analysis of four segments within each zone has provided new information on the penetration of water and organisms into wood in soil contact, where the outer segments are more permeable and more susceptible to decay than the inner segments. No information on tangential penetration was obtained from this technique.

The cutting of the block into segments with knives was simple in theory but caused problems in practice. As the blocks did not split regularly, particularly at early sample times when dry and undecayed, the segments were not all the same volume. In addition, the density of the wood varied across one zone so that the results for weight loss (which depends on wood density and segment volume) and water content and AR rate (which depends on wood volume) were not always precise. The values of MC were precise as it is independent of wood volume and density but is affected by decay, and can give a misleading impression of the amount of water in the wood. These problems could have been overcome by measuring the volume of each segment after cutting each zone into segments but before each segment was sliced. Fortunately, the weight loss of the entire block could be calculated, providing a measure of the amount of decay.

The use of regularly spaced sample times has allowed the moisture content, water content and acetylene reduction rate to be monitored against time of exposure, allowing rates of water uptake and decay to be compared and contrasted. This would not have been possible if there had been only one sample time. It would appear that the rate of decay is controlled by the soil conditions in that very dry soil or very wet soil preclude decay, while intermediate levels of water allow decay but can affect decay rate.

The technique for the maintenance and control of soil MC, although rather tedious, has been shown to be vital for the investigation of the decay of timber in soil contact. Changes in soil moisture content, which may occur in the field, profoundly affect the MC of the wood exposed to soil, and the activity of organisms in the wood. Further work is required to examine the occurrence and significance of fluctuating soil moisture conditions in the field and their relevance to the laboratory exposure system. (See Section 12).

Overall, despite the complexity of the procedure and the inaccuracies and variability of the results, the measurement of moisture content, water content, weight loss and acetylene reduction rate in beech, birch, Scots pine and spruce at different soil MC and in two different soils has provided considerable information on the decay of timber in soil contact. However, in addition to the subjective description of the differences in performance of the four wood species, and of the effect of soil conditions upon decay, the results reveal the existence of some fundamental concepts which are potentially of great significance, as they are involved in the decay of timber in soil contact and have far-reaching implications for timber decay in general.

These major concepts are, that there is a relationship between soil MC and wood MC and that this relationship can be expressed as a mathematical model, that a dynamic equilibrium exists between the soil water and the water in the wood, and that wick action is occurring in wood in soil contact.

The relationship between wood MC and the relative humidity of the atmosphere to which it is exposed, is well documented (e.g. Stamm, 1964) and the significance of the relationship is well appreciated, as it affects dimensional change and the decay and the strength of the wood.

However, the existence of a similar, well-defined relationship above f.s.p., between the MC of the wood and the MC of its surroundings has not been investigated, or appreciated. Its significance lies in its importance in determining the MC of the wood, which affects the onset and development of decay, and in affecting wick action, which is discussed later. Obviously the performance of wood in soil contact is affected by the MC of the wood and the implication is that a knowledge of this relationship for either treated or untreated wood could assist in predicting the performance of timber in the field. The uptake of water by wood is of particular relevance to the treatment of timber with water-borne preservatives. This relationship and its significance is discussed in more detail in Section 11.

In addition, this quantitative approach to the relationship between wood and the moisture content of its surroundings, allows the first tentative step toward a mathematical model of timber decay in soil contact. There is sufficient information available from this series of experiments to allow a model to be constructed which quantifies the wetting-up of wood in soil contact and allows the effect of differences in wood permeability and of decay to be incorporated. This model is described in greater detail in Section 11. The ability to construct even this basic model is of great significance as it implies that a reasonable level of understanding of the moisture relationship of wood and soil has been reached.

The existence of a dynamic equilibrium between soil water, the water in the wood and the relative humidity of the atmosphere is significant. As the weather conditions surrounding the wood change and the soil conditions change, the wood exposed to these conditions will rapidly adjust as it remains in equilibrium with its surroundings. The effect of fluctuating moisture conditions in the wood upon the onset and development of decay has not been critically investigated, but the indication from this series of experiments is that fluctuating conditions profoundly affect the conditions inside the wood and the activity of organisms in the wood.

Perhaps the most important concept which has emerged from these experiments is that wick action is occurring in the wood exposed to soil contact. Its importance is that it is involved in maintaining the

dynamic equilibrium between wood and soil and air, and potentially in the transport of organisms, nutrients and oxygen from the soil into the wood. Further work would be required to examine the influence of wick action upon nutrient distribution in the wood and the effect of that distribution upon the distribution of organisms and their activity. Potentially wick action is of major significance and must be considered as a fundamental and vital process which occurs in wood in soil contact and could profoundly affect the performance of timber in ground contact.

Thus in addition to a quantification of nitrogen fixing activity in wood in soil contact, this series of experiments has provided an insight into the methods necessary to investigate timber decay in ground contact and introduced a number of concepts which extend our understanding of how timber decays in ground contact.

11. THE RELATIONSHIP BETWEEN WOOD MOISTURE CONTENT AND SOIL MOISTURE CONTENT

11.1 Introduction

The results from the experiments described in sections 4 to 9, and summarised in section 10 indicated that there was a relationship between the soil moisture content of wood and the moisture content of the soil to which it was exposed. This relationship appeared to determine the rate and amount of decay occurring in the wood as well as the occurrence and rate of activity of nitrogen fixing bacteria in the wood. As it seemed likely that this relationship was of fundamental importance to the performance of timber in soil contact and to the assessment of the significance of nitrogen fixing bacteria to the decay of wood, it was decided to examine this relationship further. There were two approaches.

The first was theoretical, in which the curves of moisture content against time obtained in sections 4 to 9 were analysed statistically and mathematically to attempt to derive a mathematical equation, or "model", which would describe, and also predict, the moisture content of wood after increasing periods of soil exposure. A model based on linear equations was examined first, and then a number of non-linear equations examined for their applicability. A Gompertz curve was chosen as being the most applicable and a least squares technique used to fit a curve to the data. The relevance of this model to the relationship between wood moisture content and soil moisture content are briefly discussed.

The second approach was experimental; to monitor the wood moisture content at different soil moisture contents. This involved the development of an artificial soil with more controllable and definable moisture retaining properties than natural soil. The concepts of moisture holding capacity, moisture content, capillarity and pore size, and water potential (which may be unfamiliar when applied to wood in soil contact) were of value in describing the relationship between soil moisture content and wood moisture content, so a brief introduction to these concepts has been included in this second approach.

The two approaches were then combined in an attempt to produce a coherent and general theory, summary, or model, of the relationship between wood moisture content and soil moisture content.

11.2 Theoretical Approach

11.2.1. Linear Analysis - Introduction

The simplest mathematical approach to the derivation of an equation or model from data is regression analysis. Analysis of variance applied to regression analysis allows the data to be examined for a linear increase in both variables, for the amount of deviation from the calculated straight line, and for the calculation of the confidence limits of the constants in the derived equation (Sokal and Rohlf,1969).

The aim was to use regression to derive an equation for the water content of a segment after increasing periods of exposure. Each segment would have slightly different constants in the equation, while the differences between the three soil moisture contents were expected to be large. The intention was to be able to predict the water content of a segment at any soil moisture content and after any period of exposure.

Materials and Methods

The results obtained from the exposure of Scots pine sapwood blocks to soil moisture contents of 25, 35 and 40% (Section 6) were selected. The water content values were chosen, rather than MC, as being most reliable, least variable, and of the most relevance to the relationship between wood and soil moisture content. The curve of water content against time of exposure appeared linear from 2 to 10 weeks and differed considerably at the three soil moisture contents. (Figure 72). The analysis of only segment 9 is shown in detail, although the other eleven segments were analysed similarly. A regression was performed on the data, using the programme described by Sokal and Rohlf (1969)(LINREG1, in Appendix 1), to obtain an equation for the line which best fitted the data points (Figure 73). This was tested by the analysis of variance and found to be significant at 35 and 40% soil moisture content, but not at 25%, due to the low rate of water uptake at this soil moisture content. Water uptake was probably non-linear at the beginning and end of the exposure period, but was linear from 2 to 10 weeks. The regression equations derived at each soil moisture content describe the relationship between the water content in segment nine and time of exposure.

Figure 72 Water content in Scots pine sapwood against time of exposure to soil at 25, 35 and 40 % moisture content.

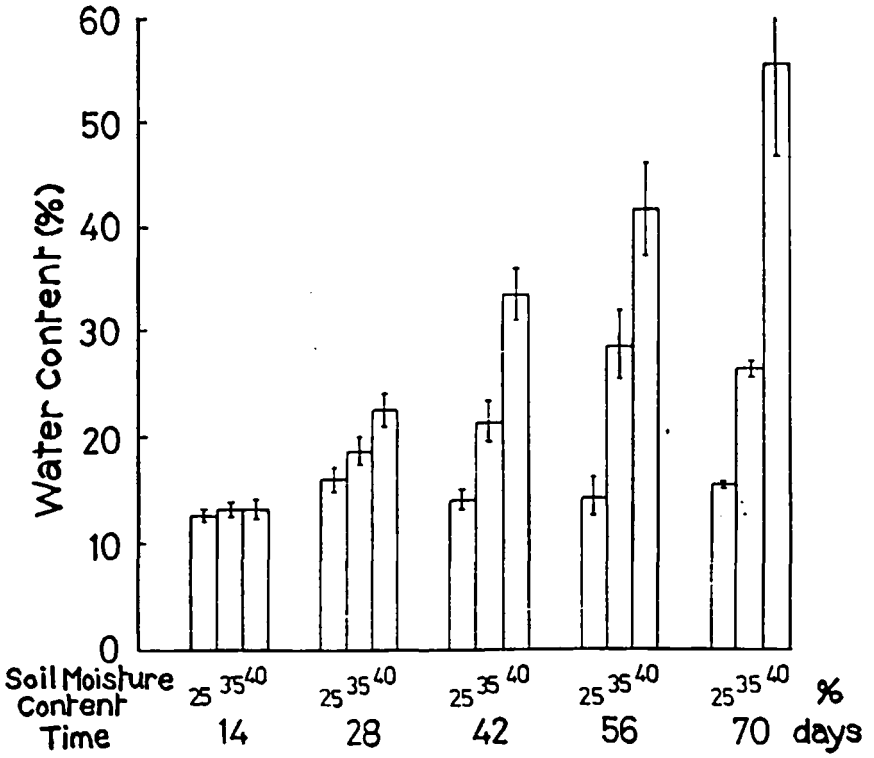
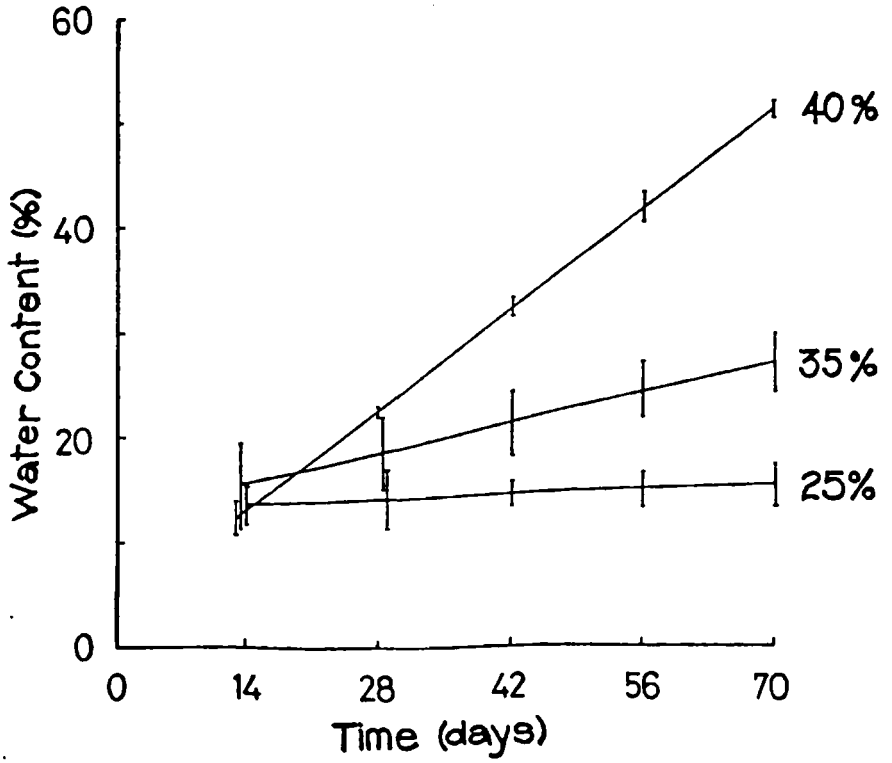


Figure 73 Water content in Scots pine sapwood against time of exposure to soil at 25, 35 and 40 % moisture content.



If the initial water content of the block = a %
and time of exposure = t days

then water content at 25% (nominal) = a + 0.031 t
35% = a + 0.206 t
40% = a + 0.700 t

Thus the value of the water content in segment nine at any time between 14 and 70 days at 25, 35 or 40% soil moisture content could be predicted by the substitution of the time of exposure in the appropriate equation.

The gradient of the line represents the rate of water uptake at different soil moisture contents. The gradient varies with the position of the segment within the block, so that the rate of uptake and the gradient is highest in the segment with the largest surface area exposed to the soil, and lowest in the adjacent, inner segments, decreasing toward the ground line and above it. The gradients for each segment at each soil moisture content are shown in Table 18.

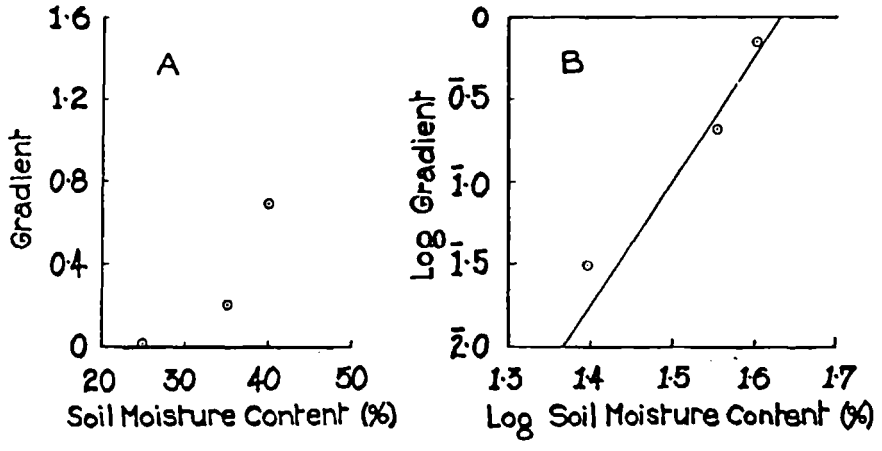
Table 18 Regression line gradient in the twelve segments of a block exposed to soil at different moisture contents.

	Dry Soil				Moist Soil				Wet Soil			
Segment	1	2	3	4	1	2	3	4	1	2	3	4
Gradient	•03	•03	•01	•05	•11	•07	•12	•16	•40	•43	•43	•55
Segment	5	6	7	8	5	6	7	8	5	6	7	8
Gradient	•07	•02	•05	•05	•19	•10	•10	•25	•63	•56	•45	•93
Segment	9	10	11	12	9	10	11	12	9	10	11	12
Gradient	•03	•01	•03	•05	•21	•13	•16	•26	•70	•69	•55	•95

If the gradient of the line is plotted against soil moisture content, e.g. in segment nine, Figure 74A, the relationship appears exponential, and a log transform of the values produced a straight line (Figure 74B), from which a further regression equation was derived. This equation described the relationship between the rate of water uptake and the soil moisture content for segment nine.

Figure 74 A : Gradient of the regression line of water content against time of exposure at each soil moisture content, against soil moisture content.

B : Log_{10} transformation of gradient against log_{10} soil moisture content.

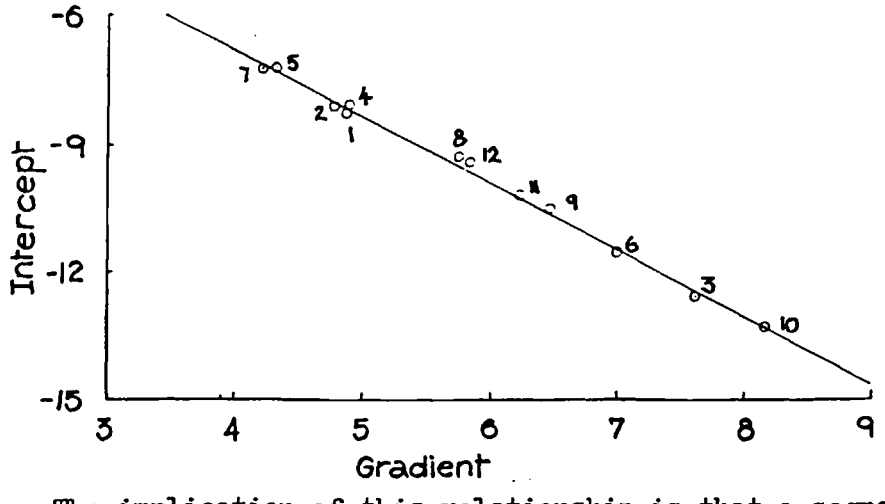


If the soil moisture content = s %

then Rate of water uptake at soil moisture content s = antilog (- 10.57 + 6.46 log s)

When the same procedure was applied to the other 11 segments, each equation allowed the rate of water uptake at any moisture content to be predicted, and given the soil moisture content and the time of exposure, the water content of a segment could be predicted. When the intercept and the gradient of each of the 12 equations were plotted against each other (Figure 75), a straight line was produced.

Figure 75 Intercept against gradient of regression line for log_{10} gradient against log_{10} soil moisture content, for segments 1 to 12.



The implication of this relationship is that a segment which has a low rate of uptake at low soil moisture content has a high rate of uptake at high soil moisture content, although it must be interpreted

with caution because each of the 12 points is derived from a straight line regression which was fitted to only three points. From a regression of the line in Figure 75,

If the gradient = m and the intercept = y
then $y = 0.574 - 1.563\ m$

Thus the relationship between the water content of a segment, the soil moisture content, and time, can be defined by a single index, here called i for each segment (Table 19).

Table 19 Index values relating soil moisture content and time of exposure for segments 1 to 12 of Scots pine sapwood blocks exposed to soil.

Segment Number	i value	Segment Numter	i value	Segment Number	i value
1	4.87	5	4.32	9	6.46
2	4.77	6	6.98	10	8.16
3	7.61	7	4.21	11	6.22
4	4.89	8	5.75	12	5.84

From this index value, the water content can be predicted in a segment at soil moisture contents between 25 and 40% and at any time from 14 to 70 days.

$$\text{Water content} = a + \text{antilog}\left[((-0.574 - 1.563\ i) + (\log s))\right]\ t$$

The accuracy of this predictive equation was tested. Values were calculated, using the equation, for the water content in each of the 12 segments of Scots pine after exposure to soil at 35% moisture content for 42 days.

These values were compared with those of the blocks removed from the soil at 35% MC after 6 weeks exposure, described in Section 7. A t-test of the observed and predicted values revealed that there was no significant difference between the values at the 0.05 probability level.

Discussion

The i values represent a combination of physical factors such

as the nature and extent of the wood surface exposed to soil contact, and permeability, and biological factors such as membrane breakdown or the degree of cell colonisation and degrade, all of which could affect the value of i. Even though the 12 i values are the summary of some 500 experimental points, there is insufficient information to determine why the i values differ in different segments.

However, the analysis has revealed a number of concepts. Firstly it has shown that reliable, reproducible, quantitative results can be obtained from a soil and wood system despite its notorious variability. Secondly it has shown that the relationship between soil, wood and water can be quantified, albeit empirically. Thirdly it has shown the severe limitation of empirical mathematical analysis; the final equation can only be used in a limited set of closely defined conditions. The analysis provides no fundamental understanding of the biological or physical difference in the index values or of the relationship between soil moisture, wood, water uptake and wood moisture content. The almost arbitary reduction of the data to a single empirical index value is unsatisfactory.

However, a less empirical analysis of the data, which incorporated the entire exposure period, the difference in rate of uptake at different soil moisture contents, and the difference between wood species would seem possible and desirable, if not essential, to understand, describe, and predict, the relationship between wood and soil moisture content.

Conclusion

The analysis has shown that a mathematical approach to the relationship between wood and soil moisture content is possible although the derived expression is only applicable to one soil, one wood species, a small range of soil moisture content and exposure times of only 2-10 weeks. A less empirical approach is required to improve the generality of the model and to improve understanding of the fundamental background to the relationship.

11.2.2. Trend analysis - Introduction

This section is a further analysis of the water content against time graph. The relationship was obviously non-linear at the beginning and end of the exposure period and a curve would seem more appropriate than a straight line. Deciding which curve to fit can be either arbitary, or by testing the accuracy with which a curve fits the data after transformation of one or both axes by regression analysis. In a technique described by Gregg, Hossell and Richardson (1964) any arbitary judgement is eliminated. Their method is primarily intended to fit a curve to data on commodity demand, allowing the future demand to be forecasted from the equation of the curve. Their technique allows the "goodness of fit" of a number of types of curve (see Table 20) to be determined, allowing the "best" curve to be selected. The values of the constants can then be calculated from the data. The technique also allows "smoothing" to allow for variability in the data. The aim of using this technique in this investigation was to derive an equation whose curve precisely describes the water content of a segment after any period of exposure to soil at any moisture content. It was hoped that differences in the curves between segments and between soil moisture contents could be quantified after inspection of the constants for each curve.

Method

The technique assumed that the data fitted the curve, so that the deviations of the data were due to random, short-term, or experimental, variation. The type of curve which best fitted the data was selected, and then the values of this type of curve which fitted the data were calculated. A computer program (FIT3PT in Appendix 1) was written to perform the analysis and tested on the example provided by Gregg, Hossell and Richardson. Briefly, it read the water content data and calculated moving averages over 5 sample times. Missing values were interpolated. The values shown in Table 20 were calculated and graphs plotted of the "slope characteristics" i.e. the slope of the moving average against sample time, slope divided by moving average against sample time, etc.). The curves were examined to determine the diagnostic shape. (Column 5, Table 20)

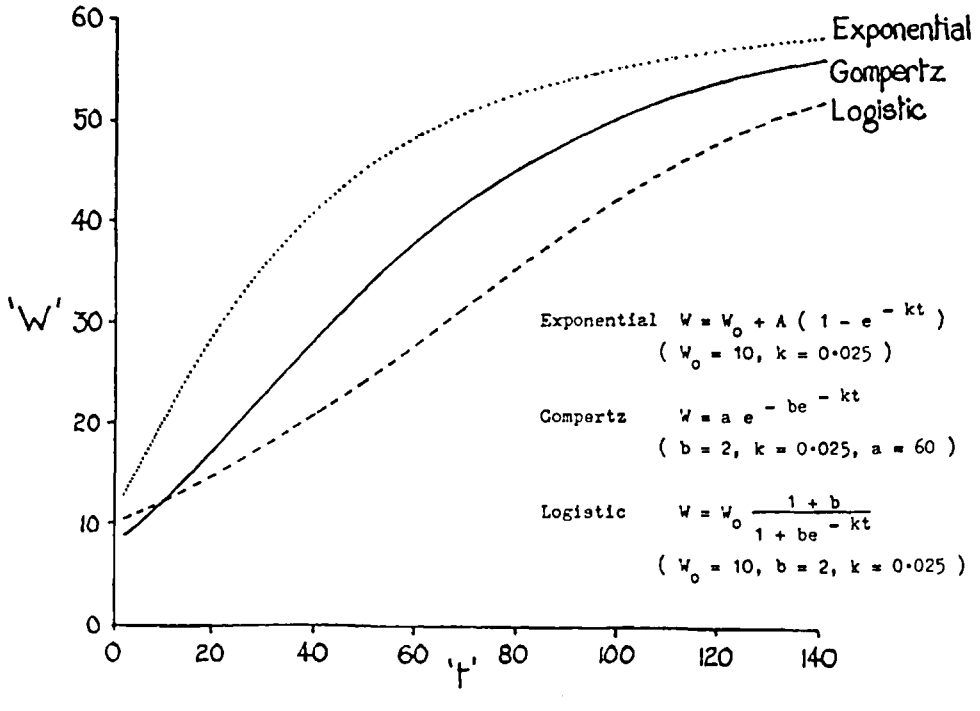
Table 20 Table of curves, their equations and constraints, with the values to be plotted out, and the diagnostic features of the resulting line.

Curve	Equation	Constants Constraints	Line to be plotted out	Diagnostic of plot line
Straight Line	$y = a + bx$	a,b	Slope	Horizontal
Parabola	$y = a + bx + c^2x$	a,b,c	Slope	At angle to Horizontal
Simple Exponential	$\text{Log } y = a + bx$	a,b	$\frac{\text{Slope}}{\text{Moving Average}}$	Horizontal
Logarithmic Parabola	$\text{Log } y = a + bx + cx^2$	a,b,c	$\frac{\text{Slope}}{\text{Moving Average}}$	At angle to Horizontal
Simple Modified Exponential	$y = a - br^x$	a,b $0<r<1$	Log Slope	Slope down to the right
Compertz	$\text{Log } y = a - br^x$	a,b $r<1$	$\text{Log}\left(\frac{\text{Slope}}{\text{Mov. Aver.}}\right)$	Slope down to the right
Logistic	$y = \frac{1}{a + br^x}$	a,b $r<1$	$\text{Log}\left(\frac{\text{Slope}}{\text{Mov. Aver.}}\right)^2$	Slope down to the right

Results

A representative set of data from each wood species, soil type and soil moisture content was analysed and the graphs plotted. The plots indicated that the curve of water content against time was a simple modified exponential, a Gompertz, or a logistic curve. These types of curve are shown in Figure 76.

Figure 76 Graphs of simple modified exponential, Compertz and logistic curves with the equations of the curves and values of the variables for each curve.



Discussion

The analysis of the water content against time curves indicates that the Gompertz type of curve best fits the data, although only that part of the S-shaped curve which increases from a value above zero towards a maximum value or plateau appears suitable. Both the logistic and exponential curves have an initial value on which the shape of the rest of the curve depends, while the Gompertz curve depends on the final value at the plateau. The experimental data suggests that the final value of water content is more important than the initial water content in the relationship between wood and soil moisture content. In addition, the Gompertz curve is linear initially, and would fit the linear part of the data analysed by regression in section 11.2.1. The rise to a maximum value could reflect the attainment of equilibrium between soil and wood, or the approach of saturation of the wood. The double exponential form of the equation with two constants allows a large family of curves to be specified, from a steep initial rise to almost linear, which could fit the experimental data obtained in very wet and dry soils respectively. The Gompertz curve thus appears to be the type of curve which should be fitted to the data although the technique of Gregg, Hossell and Richards would be complex and time consuming without the specially written computer programme.

Conclusion

The Gompertz type of curve was found to fit the experimental data on the increase of water content in wood after increasing periods of soil exposure. It would appear to be a reasonable model of the rate of water uptake by wood exposed to soil and worthy of further investigation as a model of the relationship between wood and soil moisture content.

11.2.3. Gompertz Curve Analysis - Introduction

A Gompertz curve of the form $y = ae^{-be^{-kx}}$ was found to fit experimental data on the increase of wood water content with time of soil exposure. The water content data was from 17 sets of soil contact exposures, described in sections 5 to 10, using beech, birch, Scots pine (sapwood and heartwood) and spruce in two different soils at five different soil moisture contents. The Gompertz curve which best fitted the data from each segment was determined with a computer programme using a least squares iterative method for the calculation of the curve parameters.

Method

The computer programme from the SHARE library (NLIN in Appendix 1) fitted a non-linear curve by using the Marquardt algorithm (Booth, Box, Muller and Peterson, 1959). The values of water content at each sample time were supplied to the programme together with initial guesses for the values of the parameters a, b and k in the equation. The programme calculated the predicted values from the initial guesses using the equation and compared the predicted with the observed by a least squares technique. The programme then changed the values of the parameters by a preselected amount and repeated the calculation and comparison. If the least square value was reduced, the process was repeated and continued until the least square value failed to decrease or the programme was halted.

Results

The values of the parameters in all 12 segments of each treatment were remarkably similar yet very different from the other exposure conditions or wood species. These parameters were pooled by the crude technique of averaging the values and the results are shown in Table 21. Each segment differed slightly in the shape of its water uptake against time curve, and in some segments the programme failed to find values of the parameters which fitted the data adequately. However in most cases the s.d. was small, reflecting the minor variation of the values for each segment and the similarity between segments when analysed by this technique. The deviation of the segments from the average value could be related to the amount of exposed surface and its position relative to the ground line, as discussed in Section 11.2.2.

Table 21 Average values of a, b, and k, with their standard deviation, in the Compertz equation, found by non-linear curve fitting.
(n = number of samples ; A, B, C, D and E in Scots pine refer to Figure 77, and A, B, C, and D in birch to Figure 78)

Wood	Soil	"a"	"b"	"k"	n	
Beech	Dry loam	19·81 ± 0·133	1·59 ± 0·004	0·31 ± 0·142	3	
S.Pine	Dry loam	15·27 ± 0·660	1·37 ± 0·047	0·16 ± 0·038	12	E
	Moist loam	32·98 ± 9·350	1·63 ± 0·178	0·05 ± 0·029	12	D
	Wet loam	52·62 ±11·542	2·09 ± 0·930	0·04 ± 0·004	8	C
	V.Wet loam	79·78 ± 5·480	2·76 ± 0·206	0·16 ± 0·061	12	A
	V.Wet loam	73·43 ± 6·470	1·41 ± 0·242	0·09 ± 0·018	12	B
	V.Wet clay	73·12 ± 6·243	2·39 ± 0·248	0·05 ± 0·024	12	
Birch	Dry loam	103·36 ±80·861	2·22 ± 0·605	0·03 ± 0·039	11	D
	Moist loam	38·69 ± 6·428	1·64 ± 0·419	0·06 ± 0·030	12	C
	V.Wet loam	83·16 ± 8·114	2·81 ± 0·463	0·11 ± 0·044	12	A
	V.Wet clay	73·38 ± 4·832	2·66 ± 0·188	0·06 ± 0·012	12	B
Spruce	VWet loam	82·49 ± 6·665	3·75 ± 1·317	0·15 ± 0·060	12	

When the curves for Scots pine were plotted out (Figure 77) the characteristic shape of the curves was apparent. The letters A to E are for the curves in Table 21, but no values are shown at less than 2 weeks because no data was available on the wood water content over this period of exposure. The shape of the curve in this initial phase is unknown. The second phase of almost linear uptake was followed by the third phase as the water content of the wood increased slowly with time. The final water content of the wood was related to the soil moisture content.

In birch (Figure 78) a similar pattern was observed although massive decay (60% weight loss) in the dry soil disturbed the pattern, and the linear phase of uptake continued throughout the time of exposure.

In Scots pine heartwood, even in moist soil, the water content increased linearly but very slowly, and no equation was derived for any of the segments. In the clay soil at around 45%, the parameters for Scots pine sapwood and birch were similar to those obtained at 50% soil

Figure 77 Compertz curves for Scots pine sapwood water content against time of exposure to soil in days, using values of a, b, and k from Table 21.

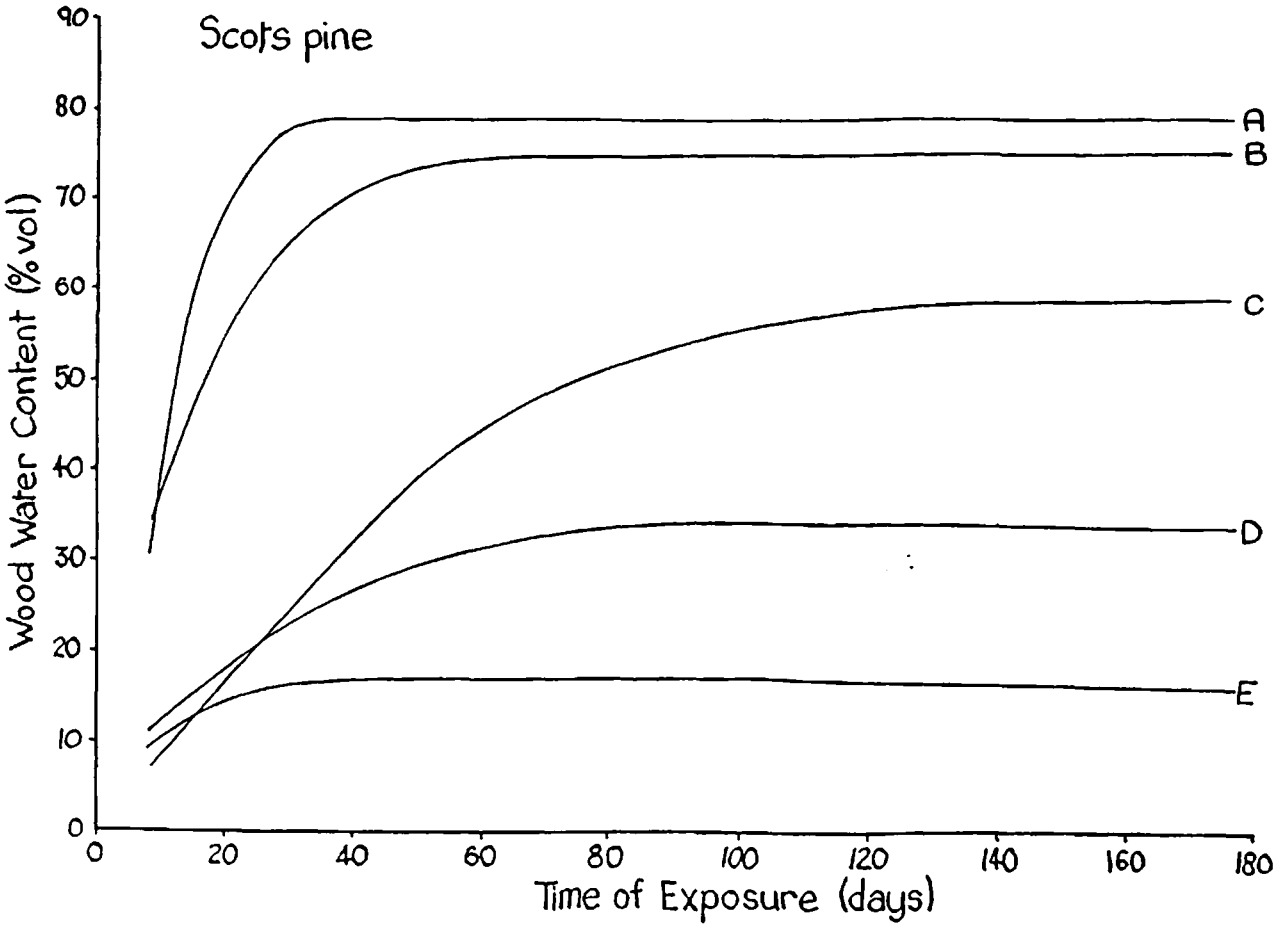
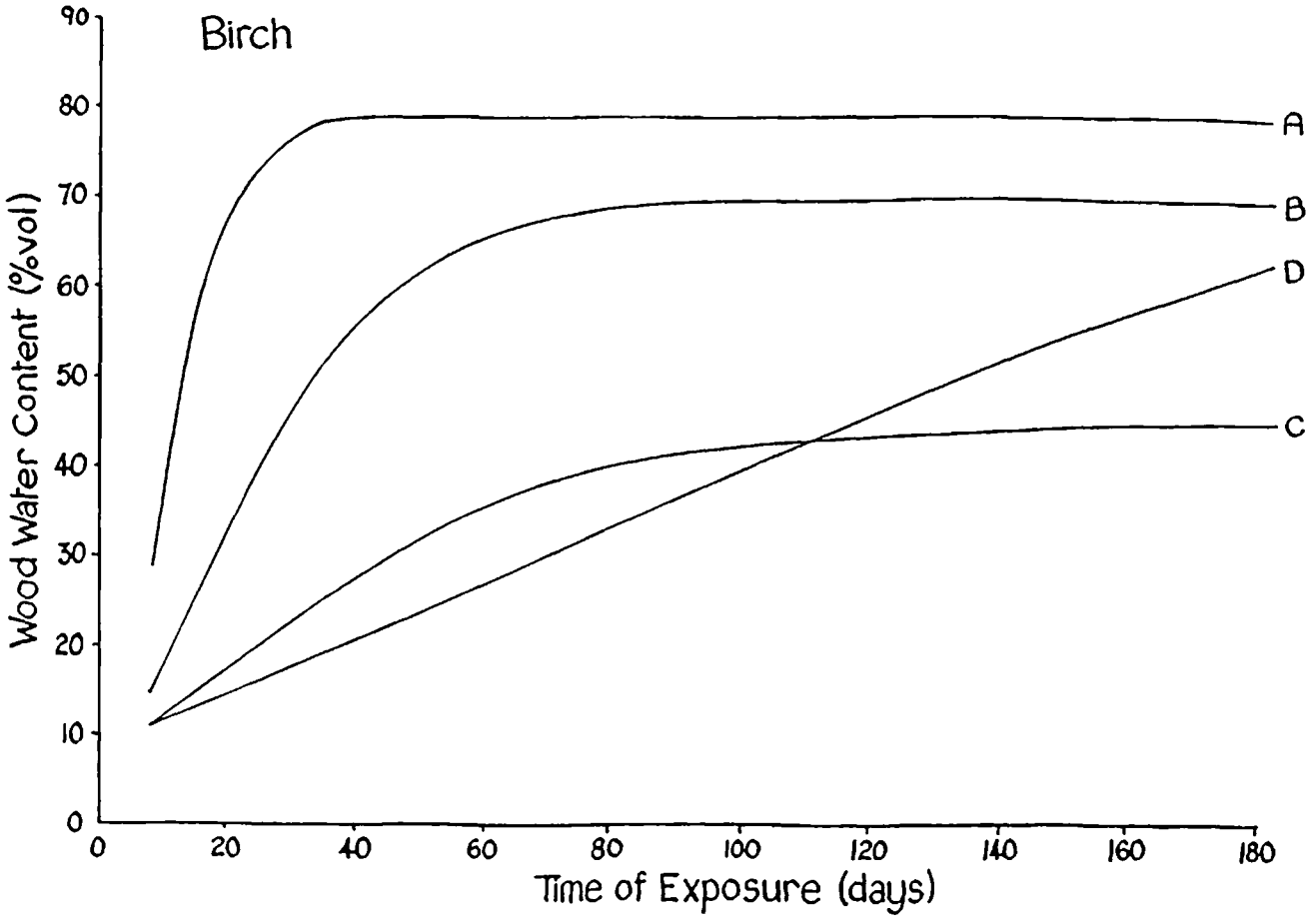


Figure 78 Compertz curves for birch sapwood water content against time of exposure to soil in days, using values of a, b, and k from Table 21.



moisture content in the loam.

Discussion

The curves of wood water content against time of exposure at different soil moisture contents show that moisture content has a considerable influence on the final moisture content of the wood and the rate at which that final value is attained. In wet soil (Curves A and B in Figures 77 and 78) the maximum water content was rapidly attained, while in moist soil the rate was less and the final value of water content was lower. In dry soil the rate at which equilibrium was attained was higher than in moist soil. Perhaps this was due to the faster movement and uptake of water vapour into the block exposed to dry soil compared to the slower rate of liquid water uptake into the block exposed to moist soil. In addition the amount of water transferred in the dry soil was less than the amount transferred in the moist soil and equilibrium was achieved faster in the dry soil. The influence of the wood's permeability cannot be excluded, as the heartwood apparently never reached equilibrium and the rate of water uptake was very low, presumably due to the impermeable nature of Scots pine heartwood compared to sapwood.

Perhaps the term "equilibrium" is inaccurate in the exposure of wood to soil. Both the data and the shape of the curves indicate that a final water content is rarely achieved after many weeks of exposure, and the water content increases indefinitely, albeit slowly. As the maximum theoretical void volume of the Scots pine blocks is around 60%, and their "final" water content was approaching 82%, the implication is that the void space of the wood increases during exposure. This may have been due to microbial attack, which obviously disturbed the rate of uptake and the "final" water content in birch (Curve D in Figure 78). The effect of decay is perhaps to increase the void volume by increasing wood permeability by the decay of the cell walls, allowing a greater water uptake than the theoretical, undecayed, uptake. Perhaps "equilibrium" between soil water and the water in wood is rapidly attained, but changes in the void volume and permeability of the wood disturb that equilibrium, which has to be regained by the transfer of water from soil to wood.

The differences in the final water content at different soil moisture contents were not linear (e.g. Curves A, B, C, D in Figure 77);

a small increase in soil moisture content between moist and wet soils results in a large increase in final water content, while a similar small increase between wet and waterlogged results in only a minor increase in final water content. In addition the curves are similar for Scots pine sapwood and birch at 45% in the clay soil and 50% in the loam. The implication of these results is that the soil moisture content determines the rate of water uptake and the final water content of the wood, although soil moisture content and wood moisture content are not linearly related. The inference is that another property of soil, such as its water availability or water potential determines the rate of uptake and the moisture content of the wood.

The non-linear analysis and calculation of curve parameters allows the prediction of an average wood water content from the data in Table 21, although the water content in a segment would be expected to differ slightly from the calculated value depending upon its position within the block and the area of contact with the soil. Soil moisture contents other than those used would need to be interpolated from Table 21 and Figures 77 and 78, while other wood species would have to be equated with either Scots pine or birch. The curves and their parameters are obviously limited in their application as a quantitative, predictive model of the relationship between wood moisture content and soil moisture content. They refer only to Scots pine and birch blocks exposed to one particular sandy loam. Nevertheless, the analysis could represent the first step in the development of a general model. The next step, discussed further in Section 11.4, would be to extend the general principles of this relationship to achieve a theory or model which applied to any wood species and to any soil.

Conclusion

The fitting of a non-linear Gompertz curve to the water content data has resulted in a series of curves for Scots pine and birch blocks exposed to soil at different moisture contents which relates wood water content to the length of exposure. Both the rate of water uptake and the final water content are influenced by the soil moisture content, although the overall pattern of water content increase differs in Scots pine and birch and is greatly affected by wood permeability and decay. Differences also occur in above and below ground zones and between segments within the same zone. The extension of the relationship which has been quantified by this analysis could lead to a more general model of the interaction between water, wood and soil.

11.2.4. Water and Soil : An Introduction

The general relationship between wood moisture content and the occurrence of decay i.e. that wood is not susceptible to fungal decay at a moisture content lower than 20% or when saturated, is often quoted in the literature, while different organisms and different wood species have different moisture content ranges over which decay is optimal (Liese and Ammer, 1964). Decay at lower moisture contents could be limited by the accessibility of the cell wall constituents to fungal enzymes (Cowling and Brown, 1969) and at high moisture contents by a low oxygen tension inside the water-saturated substrate (Griffin, 1968). Levy (1968) suggested that the distribution of fungi found in a decaying fence post was determined by the moisture content of the wood. Griffin (1972) considers a number of soil properties such as solute diffusion, soil aeration, pH, carbon dioxide and oxygen concentration, and osmotic potential, to be affected by soil water and to influence the activity of micro-organisms. Griffin (1977) concludes a review on the effect of water potential upon wood decay fungi with a plea for further work on the relationship between water and timber decay.

Obviously the interaction of soil, soil water, wood and wood decay is of major importance. However, as the concepts and terms used to describe this interaction are taken from soil physics, which may be unfamiliar, the next section is an introduction to some aspects of soil water physics which are relevant to timber decay.

An Introduction to Soil Water Physics

This aspect of soil water science has been investigated extensively because it is of major economic importance in agriculture. A more detailed description of how water and soil interact is given in Russell (1973) or Childs (1969). The individual solid particles of a soil vary greatly in size but are mainly microscopic and more or less closely packed together. Between the soil particles is a complex anastomosing series of voids or pores filled with either an aqueous solution or gas. The sizes of these particles and voids have a profound effect on the water in the soil and hence on the soil atmosphere. Soil water content may be expressed gravimetrically, volumetrically, or as a percentage of the amount of water when the soil is fully saturated. It is relatively easy to measure and is often quoted, although it is of little microbiological importance (Griffin, 1972). The water potential

of a soil is of more significance than its moisture content.

Water Potential

In a capillary the liquid column is under a tension T_1 given by

$$T_1 = \frac{2\sigma}{r} \cos \alpha_1$$

and the capillary rise by $$h_1 = \frac{2\sigma}{r\rho g_1}$$

where σ is the surface tension of the liquid, α_1 is the angle of contact, g_1 is the gravitational constant, ρ is the density of water, and r is the radius of the tube. Thus if water rises to a height h_1 in a capillary tube the air/water meniscus exerts a suction of h_1 cm on the water. Alternatively, a suction of h_1 cm water must be applied to the base of the tube to suck the water down to the same level as the water outside the tube, or a pressure of h_1 cm of water must be applied to the open top of the tube to achieve the same result. Suction and pressure are equivalent and can be expressed in the same units; dyn cm^{-2}, bars or cm water. The "intensity" or force with which water is held in a capillary tube can also be defined in terms of the reduction of its free energy below that of free water in bulk, and is measured in units of energy per unit of mass i.e. J kg^{-1}. Free energy reductions are commonly called "potentials" and can also be expressed in units of energy per unit volume. These potentials are equivalent to a pressure, head of water, or suction. Thus 10^6 dyn cm^{-2} = 1 bar = 100 J kg^{-1} = 1022 cm water = 0.987 atmospheres = 75 cm Hg.

A reduction in the free energy of water can also be caused by the dissolution of salts. This increases the osmotic potential of the water. A reduction in free energy or potential can also be caused by gravitational, pneumatic and osmotic pressure. As the range of free energies of the water in a soil between its wet and dry states is very large, Schofield (1935) suggested that the logarithm of the free energy, when expressed as a head in cm water, would be a more convenient unit than the free energy itself. This value is denoted by pF:

e.g. A free energy reduction of 1000cm water is equivalent to a pF of 3.0.

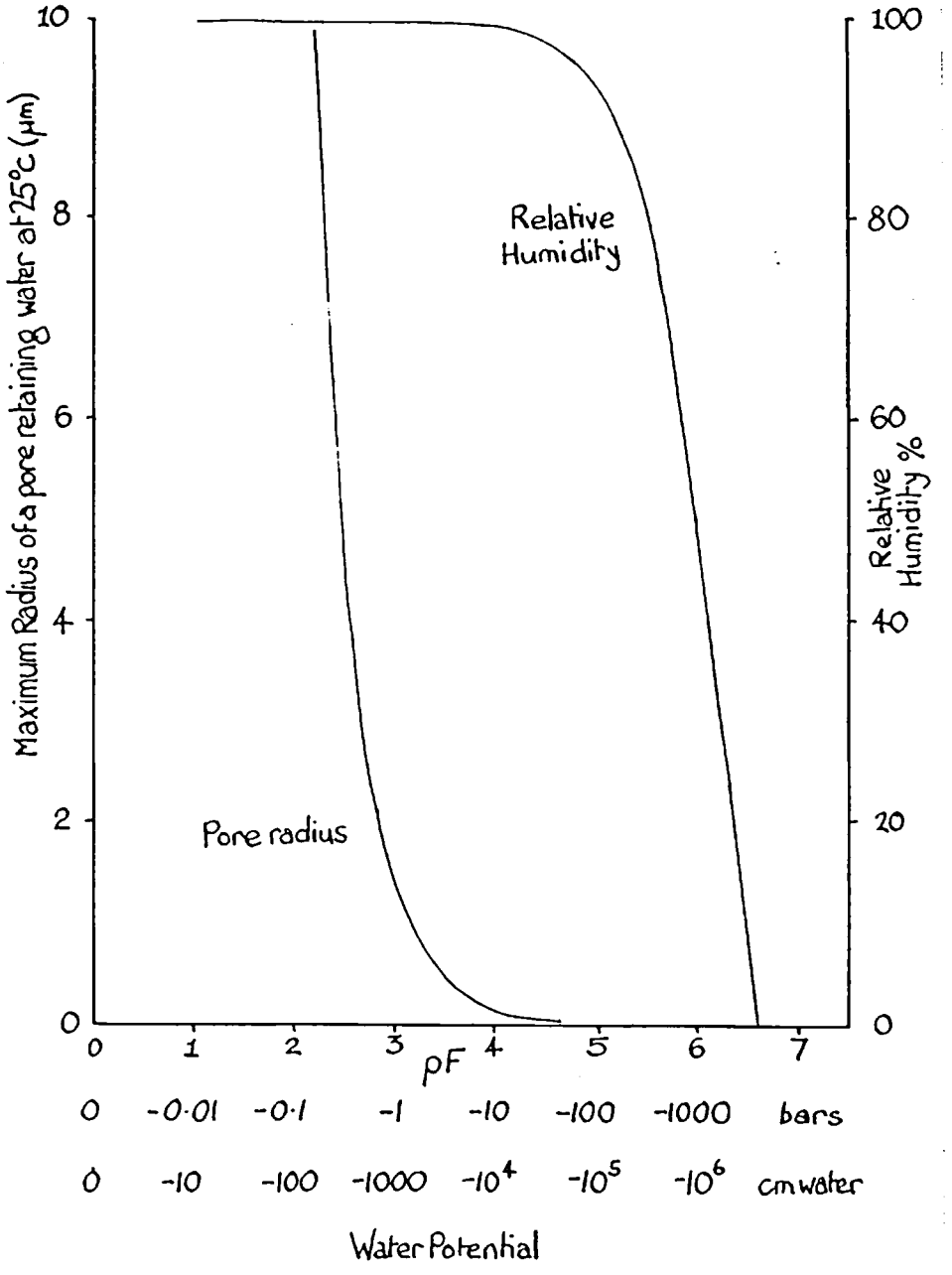
Thus suction, pressure, free energy reduction, water potential and pF are all interchangeable measures of the 'state' of water. In soil the water potential of the soil water is determined mainly by capillarity and the interaction of water/solid and water/air interfaces. This potential produced as a result of the interaction of the matrix with water is called the "matric" potential of the soil. The water potential of the soil is the sum of the matric, osmotic, pneumatic, hydraulic, and gravitational potentials.

The relative humidity (R.H.) of the vapour phase in equilibrium with a body of water is also an indirect measure of the potential of that water. In the absence of solutes, the potential is given by

$$\text{Potential (cm water)} = \frac{RT \ln \frac{p}{p_o}}{M_1 g} = 10844\ T \log \frac{p}{p_o}$$

where R is the gas constant, T is the temperature ($^{\circ}K$), ρ is the density of water at T°, M_1 is the molecular weight of water, p is the vapour pressure of water under the given conditions, and p_o the vapour pressure of a reference pool of water at T°. $\frac{p}{p_o}$ is equivalent to $\frac{R.H.}{100}$ and is called the "water activity". The relationship between RH at $25^{\circ}C$ and water potential is shown in Figure 79 where the water potential units of pF, bars and cm water are compared. It also shows the theoretical pore radius, calculated from $T_1 = \frac{2\sigma}{r} \cos \alpha_1$, plotted against water potential. This figure shows the relationship between the quantities which are of major importance in determining the matric potential of a soil. In reality, the capillaries and pores in soil are not regular, although a number of combinations of different pore sizes and shapes behave as if they were equivalent to regular pores of a single pore radius. The range of pore sizes and the number of each pore size is obviously large and variable in soil. It is this frequency distribution of pore size which determines the water retaining properties of soil. At a given soil moisture content, the water will be retained in pores of a particular size, and those smaller, by capillary forces. If there are many small pores in a soil, a large amount of water will be retained, requiring a large force to remove that water, giving the soil a high matric potential and low "water availability", with a high moisture content.

Figure 79 Maximum radius of a pore retaining water at 25°C, and Relative Humidity against water potential in units of pF, bars and cm water.



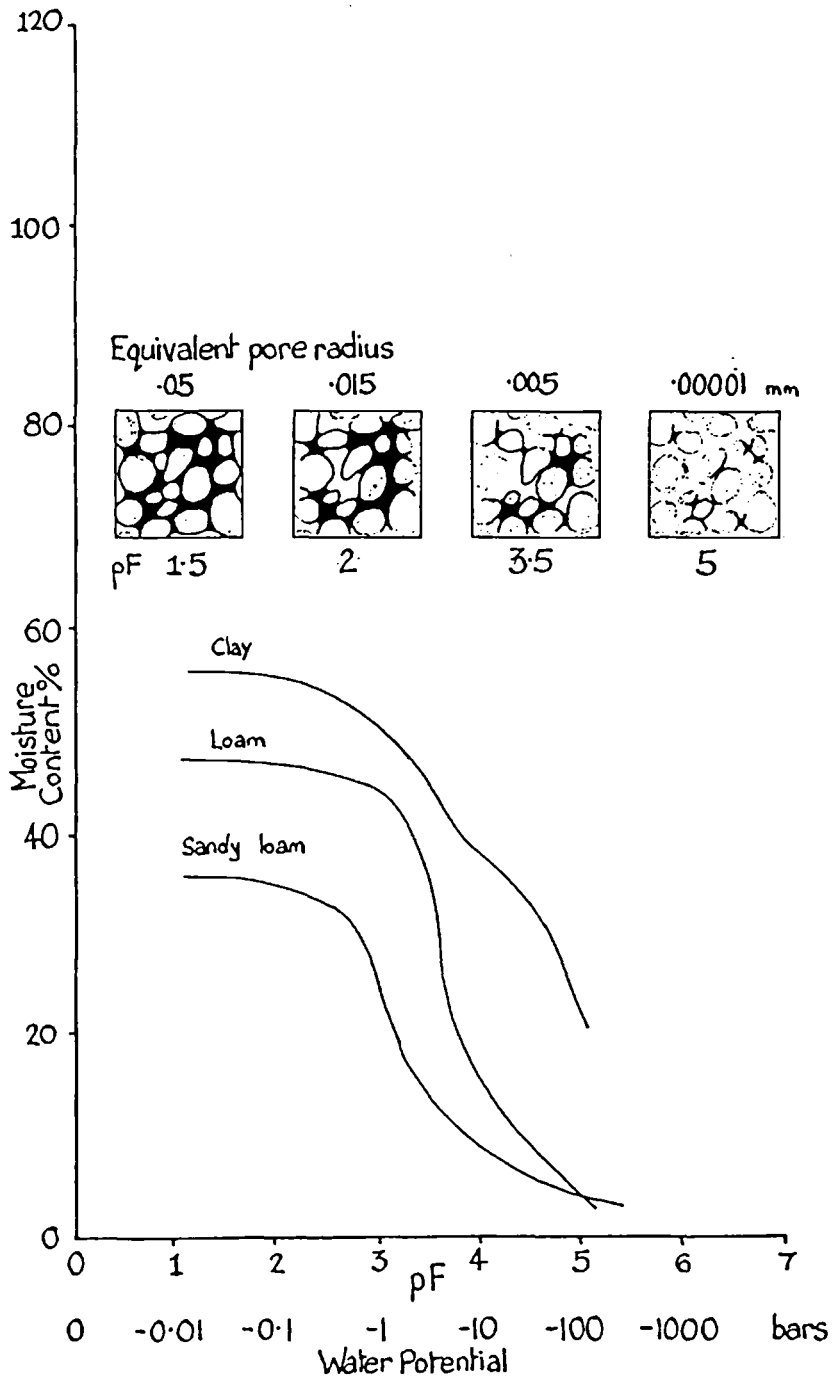
The relationship between matric potential and the water content of a soil varies with the frequency distribution of the various pore sizes and thus with soil type. The relationship is conveniently expressed in a graphical form called the "moisture characteristic". If a soil is subjected to known suction forces and the moisture content of the soil determined at each of those suctions, then the curve of moisture content against water potential forms the moisture characteristic of that soil, as shown in Figure 80 for a clay, a loam and a sandy loam. These curves are taken from Gardner (1960) quoted in Russell (1973).

The curves are the moisture characteristics of the three soil types and represent the moisture content of the three soils when they are in equilibrium with the applied suctions. The moisture characteristic represents the relationship between pore size, moisture content and the force with which water is retained in the soil. The three curves differ because the number and range of pore sizes in the soils is different. Although at a potential of -13 bars (pF 3.5) all the water will be retained in pores less than 0.005 mm radius in all three soils, because there are more pores of this size and smaller in the clay, the volume of water held in the clay will be higher, and its moisture content will be higher than the other soils at the same suction or water potential.

The moisture characteristic is a measure of the "water availability" of a soil. If all three soils are at the same moisture content, e.g. 30%, then the suction required to remove water will be around -1 bar for the sandy loam, and around -100 bars for the clay. The clay can be regarded as having less water "available" as it requires a larger force to remove water from it than from the sandy loam, which has more "available" water.

As suction and pore radius are related, it is possible to calculate the theoretical maximum radius of a pore retaining water at any water potential. The four inset diagrams of Figure 80 show a number of soil particles and a diagrammatic representation of the water distribution to illustrate the differences in soil moisture content and equivalent pore radius at different levels of water potential. At a pF of 1.5, the radius of the irregular pores containing water is

Figure 80 Moisture characteristics of a sandy loam, a loam and a clay, with diagrams of the water distribution at four pF values.



equivalent to a circular pore of 0.05 mm radius. As the suction is increased, water is withdrawn and the meniscus contracts to a pore radius in equilibrium with the applied suction. At a pF of 5, a suction of -100 bars, water is only retained in pores of 0.00001 mm radius. The effect of the boundary layer of water around the particles, the chemically bound water, and of soil shrinkage are ignored in this simple example. Obviously suctions are rarely applied to soil in vivo, but the concept is valuable in the description of how water is held in soil. In vivo, water is added to the soil by precipitation, or lost by evaporation, so that it is the soil moisture content which varies and affects the soil water potential, rather than vice versa. On wetting the water is taken up into the pores until at equilibrium all the water is retained in pores of an equivalent radius and the soil has a particular water potential corresponding to that radius. If water evaporates from the soil, water is removed from these pores, the meniscus retreats to a smaller pore radius, and the water potential increases. Hysteresis in soil arises because it requires less force to remove water from a wetted pore than to force water into a dry pore of the same radius. Thus the water potential of a soil at a given moisture content depends upon whether it is being wetted up or dried out.

A common value used to compare water in soils is their field holding capacity, broadly defined as the moisture content of a deep soil two days after all free water has left the soil surface and in the absence of evaporation. The field capacity of a soil is variable and closely dependent upon the history of the soil, so that the suction necessary to remove water from soil at its field capacity is within a range from about 50 to 350 cm water, i.e. a pF range of 1.7 to 2.7 (Russell, 1973).

Conclusion

The relevance of this introduction to soil water potential is that theoretically the moisture content of wood exposed to soil should be controlled by the water potential of the soil interacting with that of the wood. As the decay of wood is greatly influenced by its moisture content, the relationship between the MC of the wood and the soil water potential is likely to be of major significance in the decay of timber in soil contact. As wood in soil can be regarded as a series of interconnected capillaries and pores, in

contact with a series of interconnected soil capillaries and pores, then there should be interaction between the two series resulting in a redistribution of water until an equilibrium is achieved, and the water potential of the water in both wood and soil is the same. The equilibrium moisture content of the wood in the soil should be related to the water potential of the soil, and wood should have a moisture characteristic relating moisture content and water potential. The next two sections describe experiments to obtain the moisture characteristic of Scots pine and to investigate the relationship between wood moisture content and soil water potential.

11.3 Experimental Approach

11.3.1. Introduction

It was thought that further work on the relationship between soil water, wood water and the activity of organisms would be hampered by the use of natural soil, which was too variable, heterogeneous, uncontrollable and indefinable for use in a definitive investigation. The general requirement, for any research involving soil, is for a uniform, definable, reproducible soil substitute with adjustable and controlled pH, nutrient and water levels, and properties similar to those of natural soil.

Perhaps the most important property which affects wood exposed to soil is soil water availability. The principle expressed by Savory (1972) is that if wood is exposed to a range of soils, all at the same level of water availability, then at equilibrium the wood will be at the same moisture content in each soil.

A measure of the water availability of a soil is its water holding capacity, defined by Savory (1972), as the moisture content of a saturated soil after exposure to the suction exerted by a vacuum pump for 10 minutes. The moisture content of a soil at water holding capacity is likely to be lower than at its field capacity, and correspond to a pF of around 3 depending upon the efficiency of the pump used to exert the suction. Wood has an affinity for water and as it can be considered to exert a force to retain its water, it has a moisture characteristic, as shown partially in Figure 81. The values are taken from Rasmussen (1961) quoted in Siau (1971) and are the equilibrium moisture contents of wood at 18°C up to 99.9 per cent relative humidity, which corresponds to a pF of 5.16.

The implication is that if the moisture characteristic of wood were known, over the entire pF range, then it would be possible to predict the moisture content of wood exposed to soil at a certain pF value. If an artificial soil could be developed which had a variable pF, then any particular wood moisture content could be achieved by exposing the wood to the artificial soil with its pF adjusted to the appropriate value. Such a soil substitute would be of considerable value in the development of standardised laboratory test methods in which the soil is a reservoir of water, nutrients and organisms.

A number of artificial systems have been developed to replace soil as a reservoir of water, nutrients and organisms. Vermiculite has been used extensively, as for example by Kerner-Gang and Gersonde (1972) in the testing of soft rot fungi. Granular rockwool fibre has been used as a reservoir of water in the "mini-fungus-cellar" described by Hansen (1973).

In an examination of the relationship between water, soil, and fungi, Griffin (1963) used six grades of aluminium oxide grit as artificial soils. The particle size and pore size in each grade was relatively uniform and the water availability of each grit could be precisely controlled. Grit has also been used in an investigation of the decay of beechwood by soft rot fungi (D.J. Dickinson, personal communication), but because only a small amount of water (grit moisture content 2 per cent) is required to achieve 50 per cent in the wood, the system is susceptible to drying out.

At the other extreme is Perlite (Jackson, 1974), a siliceous mineral which is powdery when dry but can hold large amounts of water when wet, a property exploited in horticulture, where it is used as an artificial soil for the growth of plants.

The moisture characteristic of both grit and Perlite are shown in Figure 81. The values for grit are taken from Griffin (1963) and for Perlite from Jackson (1974).

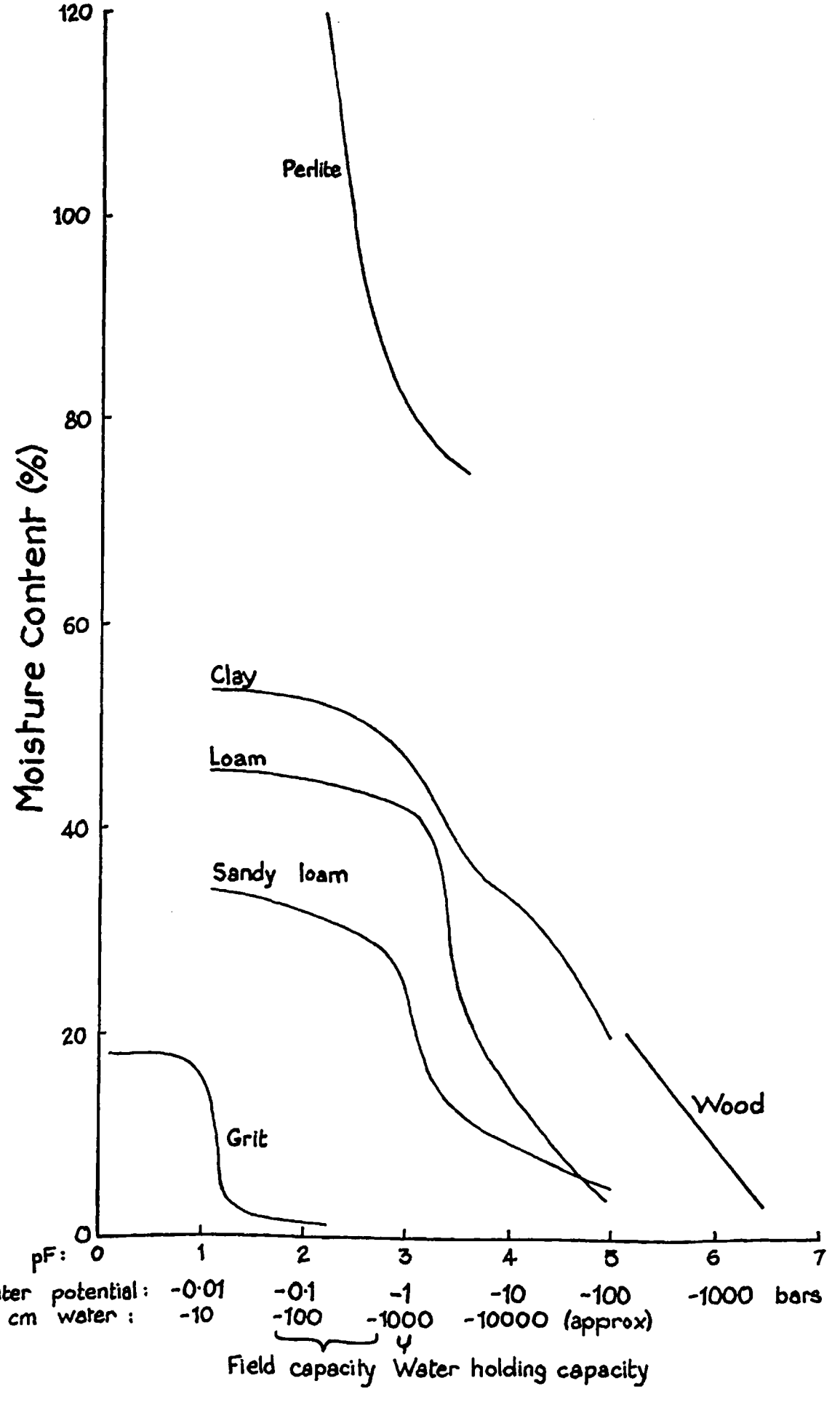
The hypothesis was that by mixing grit and Perlite an artificial soil with a variable but controllable moisture characteristic could be obtained. By exposing wood blocks to known pF's, and measuring wood MC after exposure, the moisture characteristic of the wood could be obtained.

Materials and Methods

Artificial Soil

The artificial soil was a mixture of aluminium oxide grit (TPX 30 Aloxite, Carborundum Co. Ltd., Manchester 17, UK) and Perlite (EUP 130, Johns-Manville Co. Ltd., 20 Albert Embankment, London, SE1). For the experiments involving the exposure of wood to mixtures of grit

Figure 81 Moisture characteristics of a clay, loam and sandy loam, grit, Perlite and wood.



and Perlite, a 200 g quantity of grit (bulk density approx. 2 g cm^{-3}) was weighed into a weighed glass jar, and the desired weight of Perlite (bulk density approx. 0.14 g cm^{-3}) added. Half the required weight of water was added and the components thoroughly mixed with a spatula before the remaining water was added to the uniform mixture. After the experiment, the two components were separated by pouring the mixture into two litres of water; the grit sank to the bottom and the Perlite floated; it was siphoned off. Both components were reused after careful washing.

Wood Exposure

Scots pine sapwood blocks 7.5 x 12.5 x 25 mm, with the tangential longitudinal face 7.5 x 25 mm were cut from edged, kiln-dried timber. Sixty blocks with no visible defect were oven-dried and weighed prior to being randomly assigned to one of ten screw-capped glass jars of approximately 350 ml capacity. The six blocks for each treatment were buried, the lids placed in position, and the jars incubated at 25°C for two weeks. The blocks were then removed, brushed free of adherent grit, weighed, oven-dried and reweighed. The moisture content of each block, based on its oven dry weight, was calculated. Despite the lack of sterile incubation, only two blocks were contaminated, and no weight loss was recorded. The same blocks were used in the three sets of experiments described below.

Water Holding Capacity Determination

The water holding capacity of the grit/Perlite mixtures was determined according to the method described by Savory (1971), involving 200 g samples supported on Whatman No. 4 filter paper in a Buchner funnel, but exposed to a vacuum for 5 minutes, rather than the 10 minutes specified.

pF Determination

pF was determined according to the method described by Fawcett and Collis-George (1967) involving the use of measurements of the moisture content of Whatman No. 42 filter paper exposed to soil for seven days at constant temperature. The pF of the soil could be determined from the calibration curve (provided in the paper) of filter paper moisture content against pF measured by the pressure plate technique.

Procedure

Wood blocks were exposed to a range of mixtures of grit and Perlite. Five ml of water were added to each vessel to achieve an initial moisture content of about 2.5 per cent. The same blocks and mixtures were used in a second experiment where the initial soil moisture content was about 5 per cent. The water holding capacity of a range of mixtures with 2.5 to 32.5 g Perlite added to 100 g grit was determined. The wood blocks were then exposed to mixtures having 19 to 21 g Perlite added to 100 g grit, and the water holding capacity of the mixtures determined at the end of the experiment.

Results

Table 22 shows the mean, and standard deviation of the mean, moisture content of the six Scots pine sapwood blocks, compared with the amount of Perlite in the Perlite/grit mixture of each treatment at 5 and 2.5 per cent moisture content.

Table 22 Scots pine sapwood moisture content at 2.5 % and 5.0 % moisture content in a range of grit and perlite mixtures.

g Perlite added to 100 g grit	Wood Block Moisture Content	
	Matrix Moisture Content	
	2.5 %	5.0 %
0.0	49.04 ± 28.49	86.60 ± 30.20
0.25	52.55 ± 26.02	100.24 ± 38.44
0.5	42.27 ± 23.74	93.54 ± 5.69
0.75	47.17 ± 13.17	86.07 ± 37.91
1.0	44.60 ± 18.80	83.75 ± 6.61
1.25	39.27 ± 19.95	71.25 ± 27.21
1.5	35.87 ± 8.39	70.84 ± 25.11
1.75	37.47 ± 13.21	86.37 ± 4.36
2.0	37.10 ± 15.56	67.57 ± 5.72
2.5	33.03 ± 8.70	67.61 ± 1.95

There is considerable variation within treatments which masks any difference between the treatments, however there is a tendency towards lower mean moisture contents combined with a lessening of the variation with higher amounts of Perlite in the mixture. This can be explained in terms of the action of the wood and mixture upon the water present in the closed system. When dry wood is exposed to the wet mixture, the wood absorbs water, although the uptake is limited to the available water in the system. The blocks take up this water, until at equilibrium, the affinity of the matrix for the water equals the affinity of the wood for the water, and presumably this is the condition when the measurements are taken. Where the matrix is entirely grit,

most of the water in the system is in the wood blocks, but not equally distributed among the blocks because of their different uptake rates. This difference in uptake rate, presumably due to differences in block permeability or the degree of contact between wood and matrix, combined with only a limited amount of available water for uptake, produces large differences in moisture content in the blocks, and a large standard deviation is recorded. The effect of adding Perlite is to reduce the amount of available water, which lowers the overall moisture content of the blocks, and also to increase the homogeneity of the matrix, so that uptake rates are more equal, the block moisture contents are similar, and a smaller standard deviation is recorded.

The water holding capacity of the mixtures (determined by the vacuum method) was less than 5 per cent, whereas soil taken from the Imperial College Field Station at Silwood was found to have a water holding capacity of 28 per cent. To produce a mixture having a water holding capacity similar to natural soil, further quantities of Perlite were added and the water holding capacity of the mixtures determined. Figure 82 shows the relationship between the amount of Perlite added to 100 g grit and the water holding capacity of the mixture.

From the regression equation:

$$y = 1.45 + 1.27\ x\ (r^2 = 0.9627,\ n = 31)$$

were calculated the water holding capacities of mixtures containing 19, 20, 21 and 22 g of Perlite added to 100 g grit. The mixtures were made up by adding dry Perlite to wet mixtures of Perlite and grit, and dry wood blocks were exposed to these mixtures moistened to 35 per cent moisture content. The water holding capacity of these mixtures was determined after the blocks were removed. The results (Table 23) show no correlation between the proportions of the mixture or theoretical water holding capacity and wood moisture content.

Table 23 Scots pine sapwood moisture content in a range of grit and Perlite mixtures, and the calculated and measured water holding capacities of the mixtures.

g Perlite added to 100 g grit	Block moisture content at 35% soil moisture content	Water holding capacity	
		Calculated	Measured
19	105·71 ± 9·39	25·51	26·51
20	82·50 ± 10·90	26·78	30·12
21	76·66 ± 6·75	28·04	31·02
21	77·95 ± 3·80	28·04	30·35
22	86·35 ± 13·16	29·31	29·48

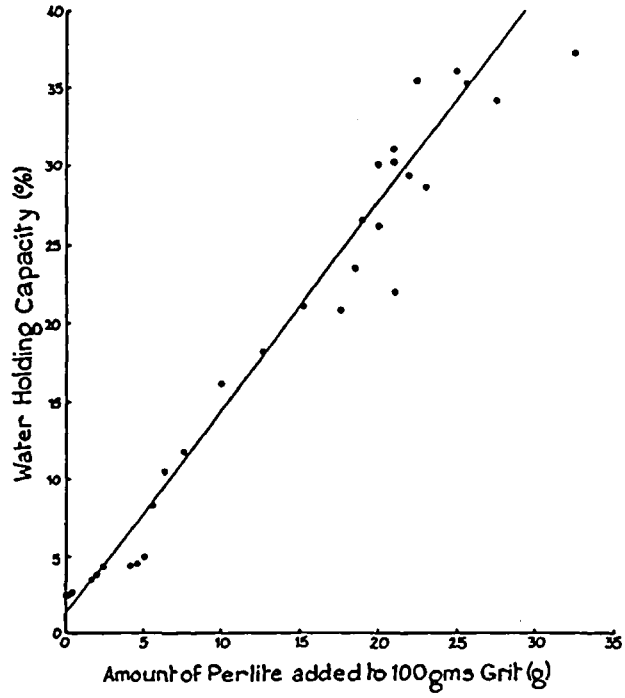


Figure 82 Water holding capacity of grit and Perlite mixtures against the amount of Perlite added to 100 g grit.

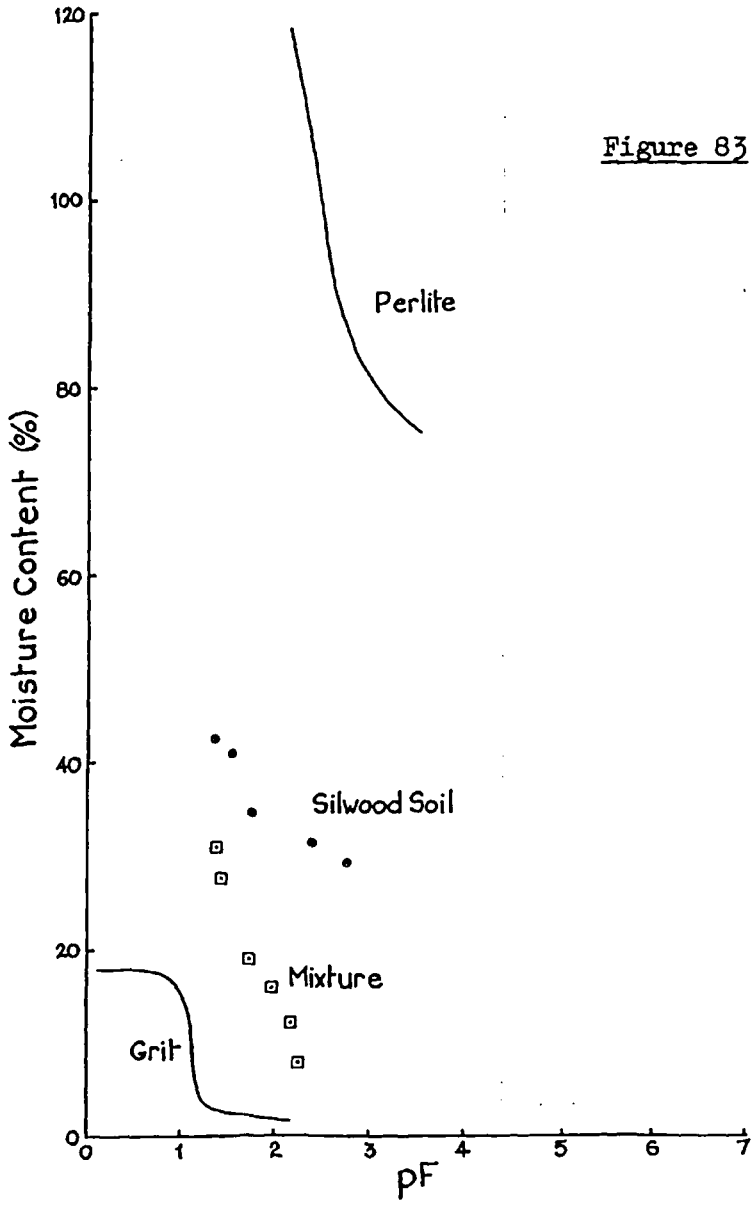


Figure 83 Moisture characteristics of grit, Perlite, Silwood soil and a grit and Perlite mixture.

Discussion

There is no correlation between measured water holding capacity and wood moisture content. The explanation is that the addition of dry Perlite to wet mixtures did not achieve the desired water holding capacity, and implies that care must be exercised in handling the mixtures in order to achieve their maximum uniformity and reliability.

The moisture characteristic of a Perlite/grit mixture (17 g Perlite added to 83 g grit), and of a sample of soil from Imperial College Field Station were determined using the filter paper technique. The results, with the curves for grit and Perlite for comparison, are shown in Figure 83. The moisture characteristic of the mixture is intermediate between the grit and Perlite, but more like grit than soil.

Conclusion

The further development of the mixture is desirable as it seems likely that the mixture could be improved to achieve a moisture characteristic resembling soil. It has the advantages of reproducibility and homogeneity as well as having the potential to adjust the level of water, pH, nutrients and organisms, properties which are essential in an artificial medium used for the testing of timber.

11.3.2. Water potential and wood moisture content - 2

Introduction

The aim of this experiment was to examine the relationship between the moisture content of wood and the water potential of its surroundings. Blocks of Scots pine sapwood were to be buried in a grit/Perlite artificial soil matrix at different moisture contents and the water potential of the matrix and wood moisture content determined after incubation.

Materials and Methods

Artificial Soil

The artificial soil matrix was a mixture of aluminium oxide grit (TPX30 Aloxite) and Perlite (EUP 130). To each of 20 weighed screw topped glass jars of approximately 350 ml capacity was added 83 g of dry grit and 17 g of Perlite. Four jars at each moisture content (15, 25, 30, 35 and 40%) was achieved by the addition of distilled water to the matrix, which was then thoroughly mixed. The lids were replaced and the jars were allowed to equilibrate for one week at 25°C before use.

Water Potential Determination

The water potential of the matrix was determined according to the method described by Fawcett and Collis-George (1967). One piece of weighed Whatman No.42 Filter Paper 30 x 20 mm was buried in each jar, recovered at the end of the exposure period, weighed and oven dried. The moisture content was calculated and the water potential of the matrix determined from the calibration curve of filter paper moisture content against water potential given in the paper.

Wood Exposure

Scots pine sapwood blocks 7.5 x 12.5 x 25 mm with the tangential longitudinal face 7.5 x 25 mm were cut from edged, kiln-dried timber. 120 blocks with no visible defect were oven-dried and weighed before being randomly assigned to one of the 20 jars. The six blocks in each jar were buried with the filter paper, the lids attached, and the jars incubated at 25°C.

Sampling Procedure

After 3 days, and 2, 3 and 4 weeks, the blocks were removed from one jar at each moisture content. They were brushed to remove adherent grit, weighed, oven-dried and reweighed. The moisture content of each block, based on its oven dry weight, was calculated. The moisture content of the matrix in the jar was also determined together with the moisture content of the filter paper, from which the water potential of the matrix was estimated.

Results

Figure 84A shows the moisture content of the matrix plotted against the water potential of the matrix measured by the filter paper technique, in pF units. There is a linear relationship between the two quantities.

Figure 84B shows the moisture content of the wood plotted against the water potential of the matrix. These points define the moisture characteristic for Scots pine sapwood blocks over the pF range 1 to 2.4. The variability is due to variation in the wood blocks, presumably due mainly to differences in void volume and permeability.

Discussion

There is a linear relationship apparent between the moisture content of the matrix and the water potential of the matrix, which implies that the water potential is controllable and reproducible. The inference is that the water potential of the matrix could be changed by varying its moisture content, which would then determine the moisture content of the wood exposed to it. The capability thus exists of varying the "water availability" of the matrix, allowing the effect of water potential upon decay to be investigated. Although it requires careful mixing and a period of equilibration before use, the matrix of grit and Perlite could be the basis of a reproducible and definable artificial soil which could replace sand and vermiculite as a test medium for "soil" exposure in the laboratory. It combines the uniformity of sand and vermiculite while eliminating the possibility of drying out which occurs in sand, and of waterlogging which can occur in vermiculite.

Figure 84A Moisture content of a grit/perlite mixture against water potential measured using the filter paper technique.

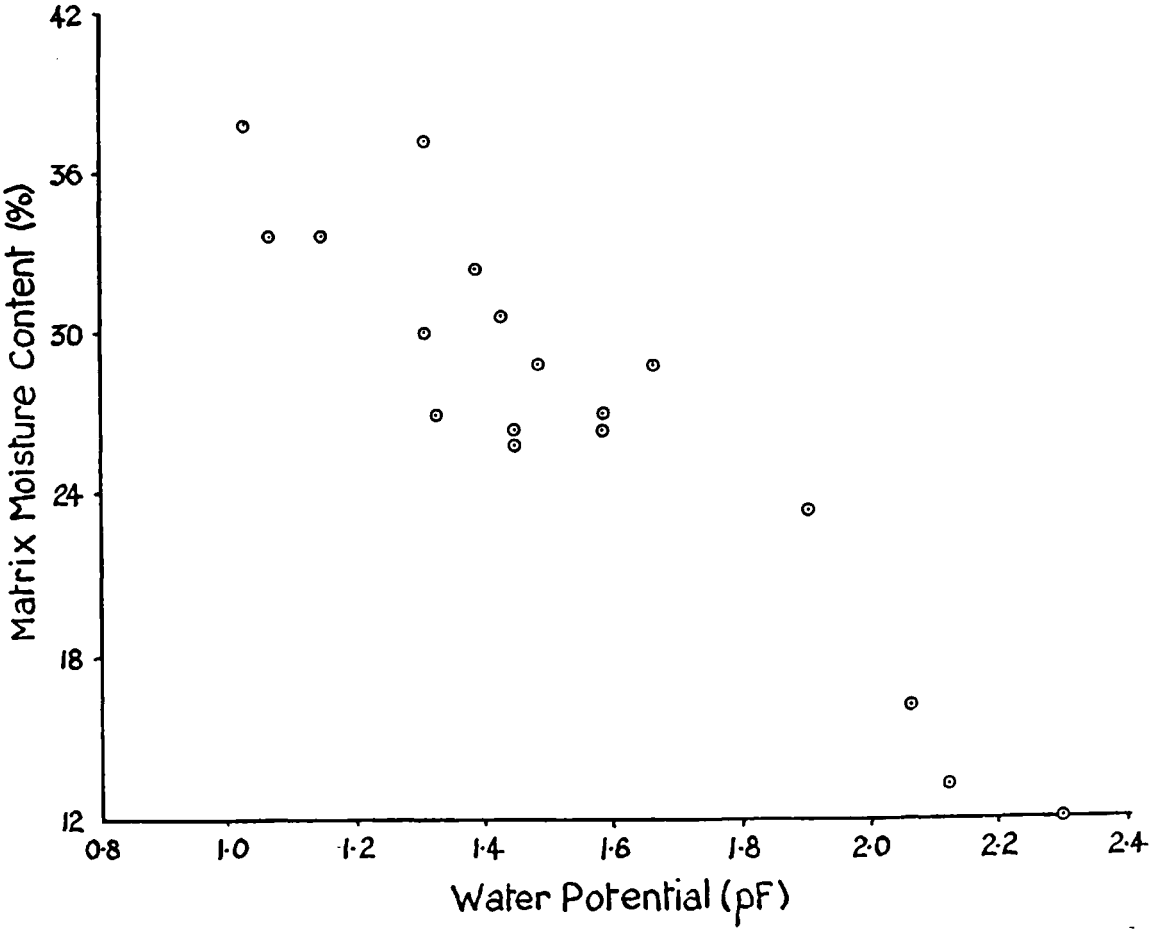
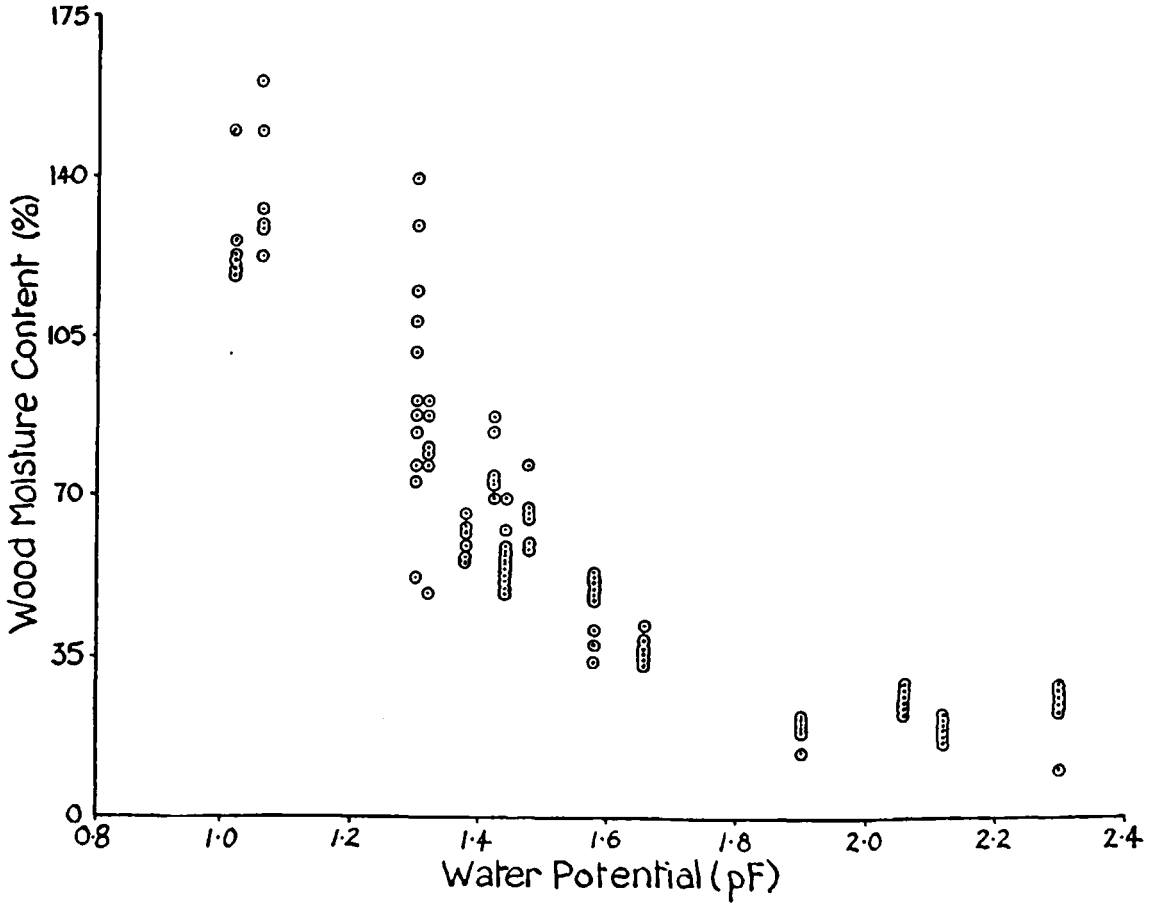


Figure 84B Moisture content of Scots pine sapwood against water potential of the grit/perlite mixture.



The existence of a moisture characteristic for Scots pine sapwood blocks is potentially highly significant to timber decay and wood preservation. This relationship between the moisture content of the wood and the water potential of its surroundings, at all levels from very wet to very dry is both novel and important. The inference is that the moisture content of the wood is controlled by the water potential, even above the water holding capacity of the matrix, and a particular moisture content in wood could be achieved by its exposure to a matrix of defined water potential. This could only previously be achieved by trial and error.

The existence of the relationship also implies that wood can be regarded as a network of interconnecting pores and capillaries of definable size and number as found in soil and discussed in Section 11.2.4. The pores must vary enormously in size, from those within the cell wall, to the pit apertures and torus pores, to the lumina of tracheids, vessels or fibres. The frequency distribution of the pore sizes and thus the shape of the moisture characteristic must depend upon the nature of the cell wall, the type and form of wall pitting and the size, frequency and arrangement of fibres, vessels or tracheids. Any variation between different species and even between different trees could be reflected in a slightly different moisture characteristic. This curve represents a summary of the anatomy of the wood and could relate anatomy to water uptake and permeability, or vice versa, which is of prime interest in timber preservation. Potentially, wood, a biological material, with its inherent variability, could be defined in physical terms, and the properties of the wood, particularly with regard to water, could be quantitative, rather than merely empirical.

Conclusion

There is a relationship between wood moisture content and the water potential of its surroundings even above the water holding capacity of the matrix and the f.s.p. of the wood. The grit/Perlite matrix is potentially useful as a reproducible, definable and controllable matrix for test purposes as an alternative to soil burial. The existence of a moisture characteristic for wood, relating wood moisture content and

water potential is of major significance in understanding the performance of timber in service and the preservation of timber. Its significance is further discussed in the next section.

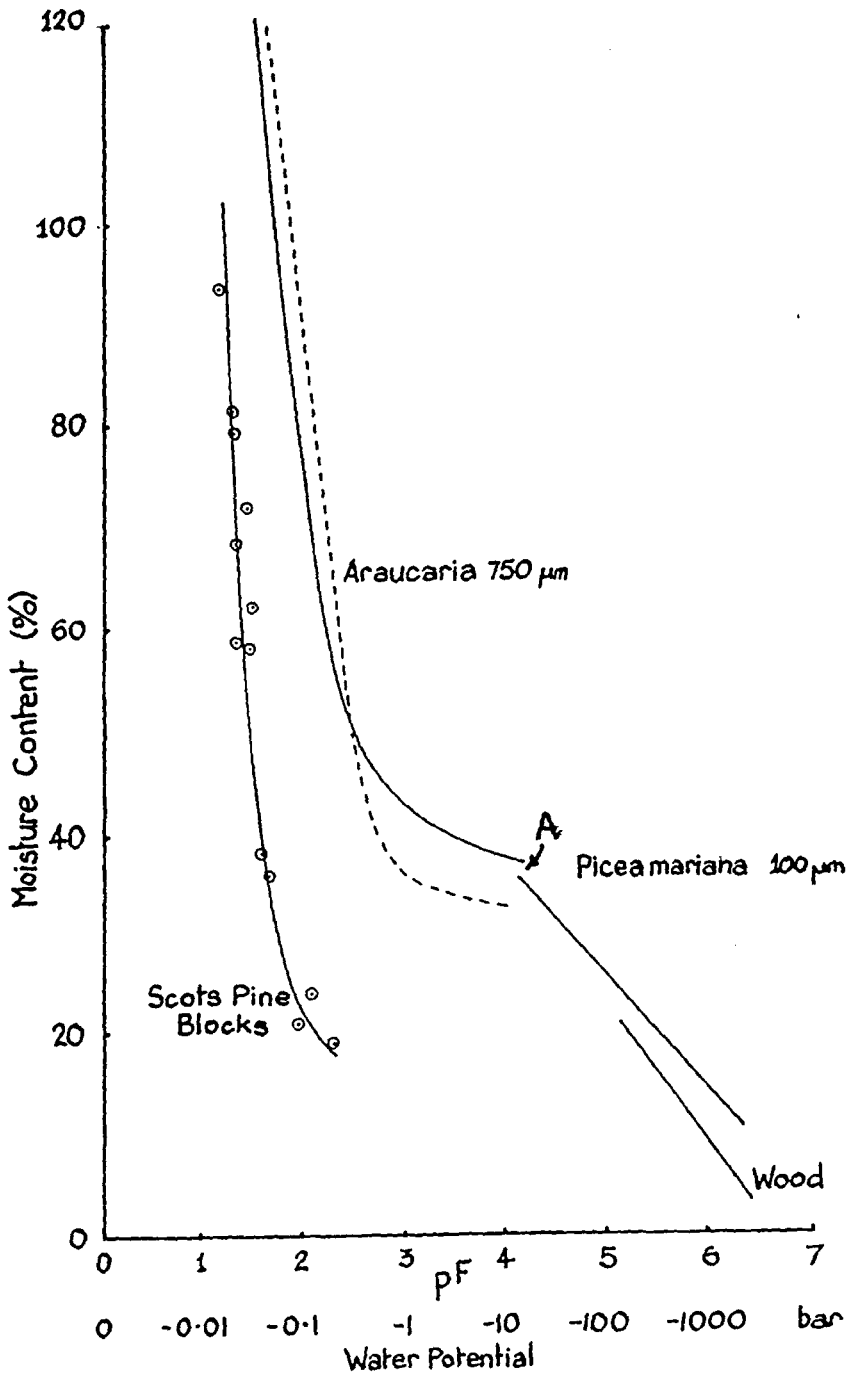
11.4. Discussion and Summary

The existence of a moisture characteristic for wood is of fundamental significance. Figure 85 shows the moisture characteristic for Scots pine sapwood obtained from the experiment described in Section 11.3. The values for Araucaria and Picea mariana are taken from Griffin (1977) and are the moisture contents of thin sections after exposure to a range of suction pressures, converted to pF units. The values for "wood" are taken from Rasmussen (1961) quoted in Siau (1971) and are the equilibrium moisture contents of wood at 18°C up to 99.9% R.H. The overall similarity between the curves is striking, while the difference in the position of the curves could be explained by differences in size and species of the samples. There would appear to be an inflection point at A on the Picea curve which occurs at around 35% and may represent the fibre saturation point of the wood, where a profound change occurs in the properties of wood with regard to water.

The fundamental importance of the wood moisture characteristic and of water potential is in the understanding of the relationship between soil, soil water, wood, wood moisture content and the relative humidity of the air. The concept of water potential can be used to explain why wood gets wet in wet soil, and dries out in dry soil or when exposed to the air. The wood moisture characteristic quantifies these changes in moisture content and could allow the moisture content of wood to be predicted, given the water potential to which it is exposed.

For example, if dry wood, having a high water potential is placed in wet soil, which has a low water potential, then the wood will take up water until the potential of soil and wood are equal and a state of equilibrium is reached. Now, if wet wood, at low water potential is exposed to dry air, having a high water potential, then the wood should lose water to the air until the potential of wood and air are equal and a state of equilibrium is reached. The implication of the concept of water potential is that the moisture content of the wood is determined by the water potential of its surroundings, whether it is the matric potential of the soil or the R.H. of the air. Wood exposed to a particular environment at a particular water potential will attain

Figure 85 Moisture characteristics of Scots pine sapwood blocks, *Picea mariana* and *Araucaria* sections, and 'wood'.

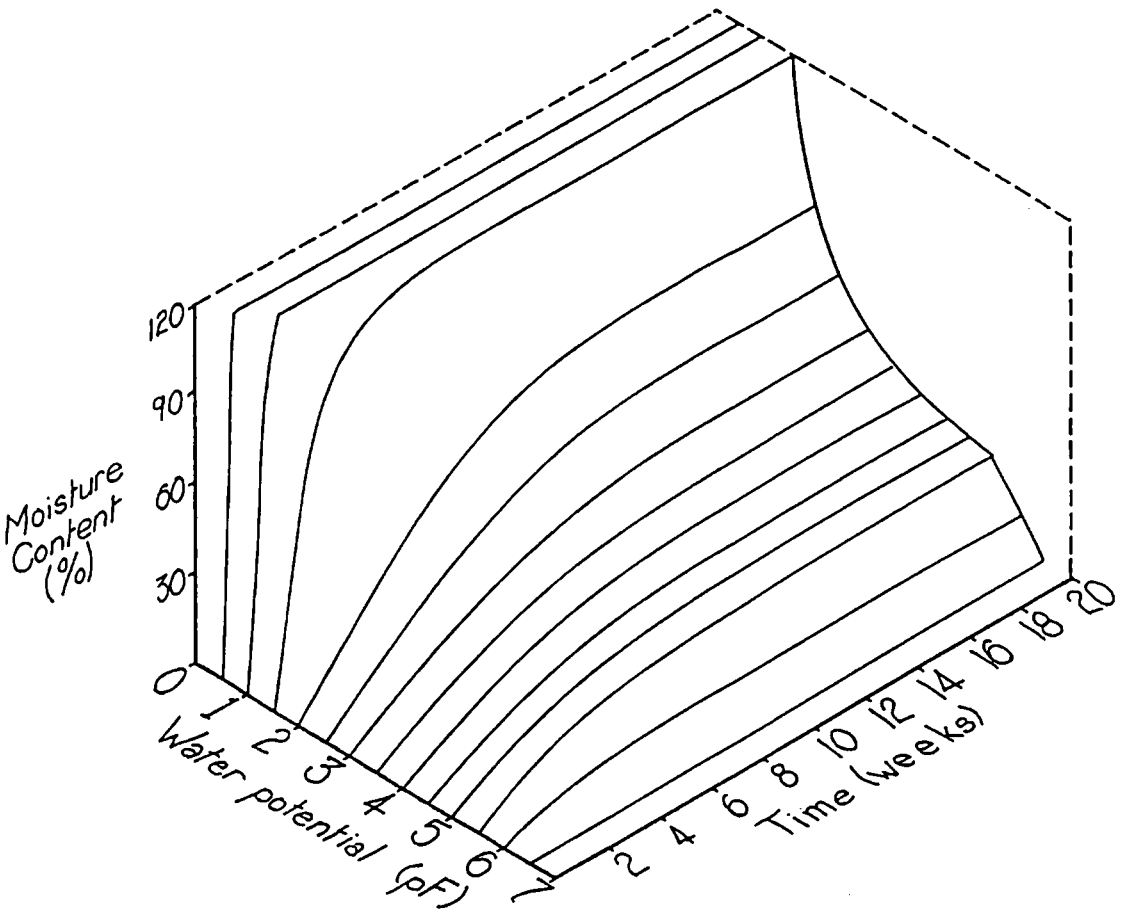


equilibrium with that potential at a moisture content specific to that wood. However, the moisture characteristic alone cannot fully explain the water relations of wood. It is a "static" relationship which represents the final equilibrium condition, which may take a long time to achieve and perhaps never be reached if rapid changes in the environmental water potential occur. The permeability of the wood must be taken into account because it determines the rate at which water is taken up and lost from the wood, and thus the time taken to reach equilibrium.

Some information on the rate of water uptake and the time taken to reach equilibrium can be obtained from the experiments described in Sections 5 to 10, involving small blocks of different wood species exposed to different soil moisture conditions. These results were analysed mathematically in Section 11.2. These results are summarised in Figure 86 where the moisture content of Scots pine sapwood is plotted against water potential in pF units and the time of exposure to soil in weeks. The curves of water content against time have been taken from Figure 77 in Section 11.2.3 and converted to values of moisture content.

At low water potential (pF 1.5) the uptake of water from soil is rapid and the equilibrium moisture content of around 120% is reached after about 4 weeks. At a pF of 3, the rate of uptake is lower and equilibrium is reached at 50% after about 8 weeks. At a high water potential (pF5) in dry soil, the time to reach an equilibrium of 30% is only 4 weeks, while at pF 6 equilibrium is reached in only 2 weeks. The time taken to reach equilibrium must be a combination of the permeability of the wood, the potential difference between the soil and the wood, and the rate of transfer of water from soil to wood. Thus at high soil pF the potential difference is low, little water must be transferred from soil to wood and the equilibrium is reached quickly. At low pF, the potential difference between wet soil and dry wood is high and equilibrium is reached quickly. A less permeable timber, such as birch, would theoretically have a lower rate of uptake throughout the water potential range, but would have a similarly shaped moisture characteristic at equilibrium. The curve of final equilibrium moisture content against water potential at 20 weeks is the moisture characteristic.

Figure 86 Moisture content of Scots pine sapwood against the water potential of its surroundings, and the time of exposure.



This series of curves could be very significant in the investigation of the water relations of timber in soil contact. If moisture content can be predicted from a knowledge of the moisture characteristic, of wood permeability, time of exposure and water potential, then the moisture characteristic must be regarded as a fundamental and important property of wood. However, the moisture characteristic is a measure of the amount of water contained in the capillaries and pores of the wood at each potential. Perhaps an even more important concept is of wood as a series of interconnecting capillaries and pores. They may behave in a complex manner, and vary enormously from sample to sample and species to species, but they must obey physical laws which can be discovered. The implication is that the water relations of wood could be described in quantitative terms i.e. modelled mathematically (see Footnote). The approach described here could be the first step in a mathematical description of the water relations of wood in soil contact. If the effect of the network of capillaries and pores in wood upon the wood's permeability could be understood, quantified, and predicted, then perhaps the performance of wood, when treated with preservatives and exposed to soil, could be further improved.

Conclusion

The application of the moisture characteristic and the concept of water potential has proved very valuable in summarising the relationship between soil, wood and water. The dual theoretical and experimental approaches have been combined to achieve a hypothesis of the relationship between soil and water which must be tested to examine its relevance, reliability and generality.

Footnote : The capillary and pore network of wood has been extensively investigated by Comstock, Petty, Bolton and others and is reviewed by Siau (1983). Permeability of wood has also been reviewed by Banks (1975).

12. FIELD EXPERIMENT

12.1. Introduction

The results from Sections 4 to 9, summarised in Section 10, showed that a field experiment was necessary to compare the results obtained from laboratory soil exposure with realistic field exposure. It was considered desirable to monitor the moisture content, AR rate and decay in a hardwood and a softwood, untreated and treated with a commercial timber preservative after increasing periods of exposure.

The wood species selected were birch and Scots pine, as in the laboratory experiments, and the block size 200 x 30 x 50 mm, with the 300 x 200 face tangential, to resemble the laboratory exposure blocks. The stakes were to be exposed in the field trial site near the Old Farm at Silwood Park, Ascot, where a field trial of fence posts had been installed in 1958, and was a well-known and well-used site for the study of timber decay in ground contact. The stakes were to be partially buried so that one third was above ground, reflecting the laboratory exposure system, and sampled at one month intervals.

The stakes were to be sampled at three levels; below, at and above ground, and the slices cut into 12 segments, so that the two central segments had not been in soil contact and could be used to monitor penetration of the wood by micro-organisms. The moisture content, AR rate, volume, water content and weight loss of each segment was to be measured. It was intended that this experiment would allow the relevance and realism of the laboratory system to be assessed in comparison to realistic field exposure and also to assess the occurrence, activity and significance of nitrogen fixing bacteria to the decay of timber in soil contact.

12.2. Materials and Methods

Stake preparation

Scots pine and birch planks from the same source as the blocks in Sections 4 to 9 were cut into stakes 200 x 30 x 50 mm, with the 30 x 200 face tangential. Thirty stakes of each species with no major visible defect (knots, heartwood, shakes) were numbered and randomly assigned to be treated or untreated.

The stakes to be treated were despatched to Hickson's Timber Products Research Laboratory, Castleford, Yorkshire where they were

weighed, treated to refusal with Tanalith C (CT106) at a concentration of 3.32%, and weighed again. After 2 weeks fixation and air drying they were returned. Seven of each species were randomly selected and leached by vacuum impregnation in distilled water followed by immersion in distilled water for 3 weeks with the water changed weekly. They were then allowed to air dry.

The net dry salt retention of the Scots pine stakes was $24.81 \pm 1.58\ (15)\ kgm^{-3}$. The birch stakes had an average retention of $24.10 \pm 1.22\ (15)\ kgm^{-3}$.

Exposure

The stakes were installed in the Old Farm Site of the Imperial College Field Station, Sunninghill, Ascot, Berkshire on 1st December 1975. Holes were excavated at 60cm spacings with a sheet metal corer that was hammered into the soil and then removed, which left a rectangular hole 130mm deep into which a stake was inserted. The surrounding soil was pressed firmly into contact with the wood. A soil sample was retained for moisture content and water potential measurement in the laboratory.

When the stakes were sampled, the untreated stakes were always more difficult to withdraw from the soil and they always had more soil adhering to the zone below ground.

Sampling

At monthly intervals the stakes to be sampled (See Table 24) had their ground line marked, were uprooted, cleaned of adherent soil and placed in separate plastic bags. The air and soil temperature was recorded with a thermometer and a soil sample taken for later measurement of its moisture content and water potential by the filter paper method of Fawcett and Collis-George (1967). The stakes were transported to the laboratory as quickly as possible.

They were unwrapped, weighed, marked out, and sawn into slices with a circular saw (See Figure 87). The slices were weighed and their lengths measured. Slices 4, 7 and 10 were then chopped, using the

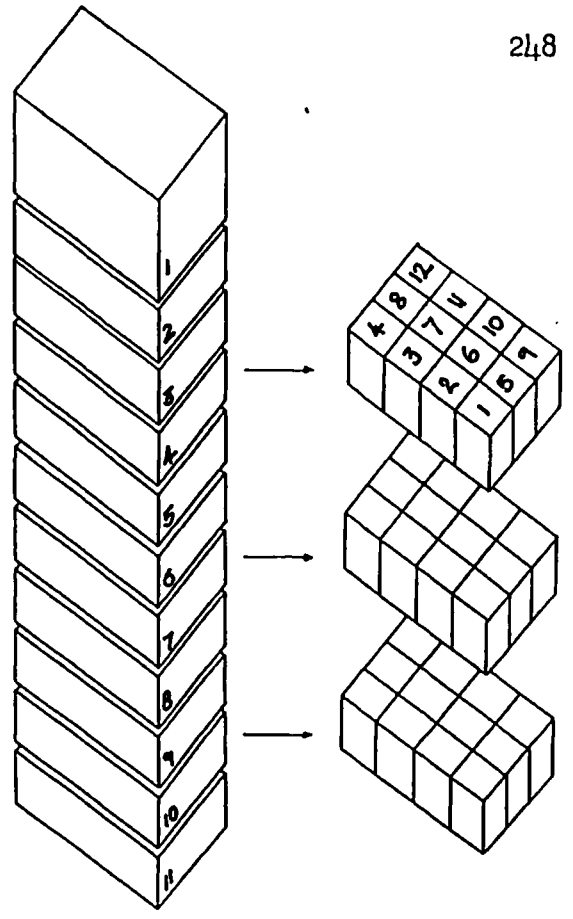


Figure 87 Sampling of field stakes : Slices 1 to 11. Slices 4, 7 and 10 subsampled to produce 12 segments from each slice.

apparatus described in Section 3, into 12 segments. Although it was originally intended that the ground line was level with slice 4, this was not always achieved. The dimensions of each segment, in tangential and radial directions, was recorded. Each segment was then chopped into 11 slices and the slices transferred to a McCartney bottle. Their AR rate, moisture content, water content and weight loss was then determined as described in Section 3.

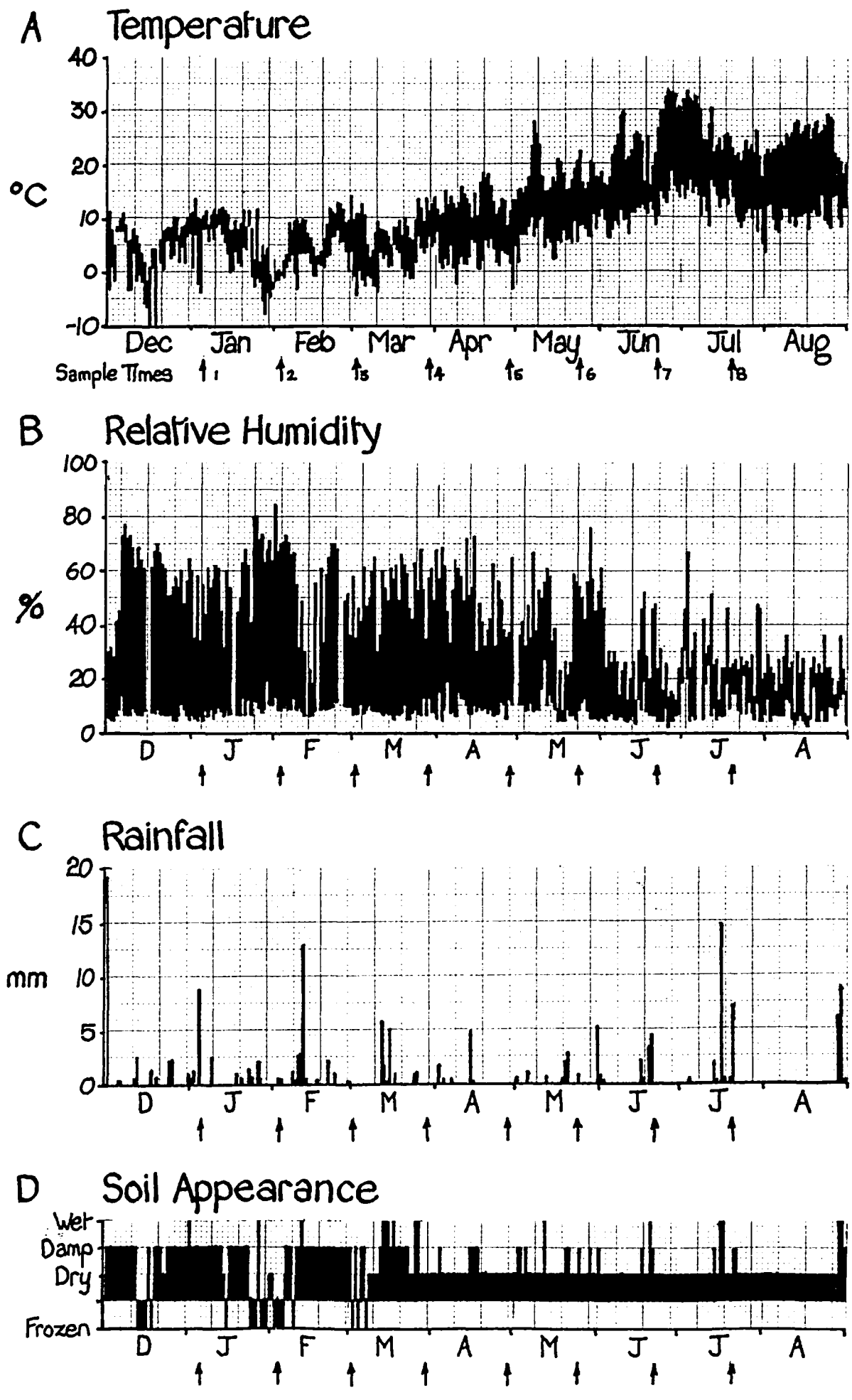
At one sample time (3 Feb) there was snow on the ground in places. Snow also occurred on the tops of the Scots pine but not the birch stakes. On wet and rainy days (e.g. 25 May) the sides of the untreated stakes showed a distinct wet zone above ground which was as wide as the stakes at the ground line and tapered to a point which was 10 to 40mm above the ground line.

12.3 Results

Weather

The weather record for the Imperial College Field Station, Sunninghill, Berkshire is shown in Figure 88. The Old Farm Exposure Site was approximately one mile from the weather station.

Figure 88 Weather record during the field exposure period.



Temperature

The maximum and minimum temperature recorded on each day of exposure are shown in Figure 88A. The "average" temperature was between 0°C and 10°C from December to March, the first 4 months of Exposure, with three periods of below zero temperatures. From April to June the temperature increased until the average was around 20°C with three periods of high temperatures between 25 and 30°C. At the end of June (between the 6 and 7 month samples) the temperature reached over 30°C. The temperature dropped during July to the last sample time in mid-August.

Relative Humidity

The maximum and minimum relative humidity recorded on each day of exposure are shown in Figure 88B. In December, January and February the R.H. fluctuated between 70 and 95%, with several occasions when the R.H. dropped to 40-60%. which coincided with cold periods (e.g. late January). In March, April and May the R.H. fluctuated between 40 and 95% and by June and July the R.H. fluctuated between 20 and 95%, with the low R.H. coinciding with periods of high temperature.

Rainfall

The amount of rain (mm) falling on each day of exposure is shown in Figure 88C. The highest daily rainfall was recorded on the day the stakes were installed, and high rainfall occurred on three further occasions in January, February and July. There were periods of no rainfall in mid January, early March, late April and early June and particularly from late June to early July.

Soil Conditions

The state of the soil on each day of exposure is plotted in Figure 88D. The soil conditions were described as "frozen", "dry", "damp" or "wet". Throughout December, January, February and March the soil was described as dry and sometimes frozen, only rarely as wet or dry. After March the soil was more often dry with occasional periods when it was wet or damp. Not surprisingly, the state of the soil corresponded to the rainfall and temperature charts, where low

temperature resulted in frozen ground, and rainfall is followed by the occurrence of wet soil. In June and July the soil only remained wet for a day or two after rainfall, indicating how rapidly the soil dried out in the warm weather. Dry soil corresponded to high temperature, low R.H. and no rainfall.

The days on which stakes were removed for analysis are also shown in Figure 88. The weather record on the sampling day is shown in Table 25 together with the temperatures measured at the exposure site, the soil moisture content determined by the oven-drying of samples and the pF determined by the filter paper method.

Table 24 Code numbers of field stakes sampled at each sample time.

Sample Time (months)	Untreated	Treated	Treated & Leached	Untreated	Treated	Treated & Leached
1	DE7 CD4	CA1	EA6	GI9 GJ3	GJ6	GH8
2	DE1 DC5	DC7	CA6	GI5 GG10	GH6	GI3
3	CD6 DC4	CD1	DE5	GI8 HA4	HC8	HA6
4	CD2 DC1	DE2	CA9	GH7 GG2	HA5	HC2
5	EA1 DC8	CA8	EA3	GH2 HA2	GH3	HC3
6	CD9 DC2	EA4	CD5	GG4 GJ5	GG6	GH4
7	DE8	CD8		HC4	GI7	
8	DE4	CA4	CA5	GH9	GI2	HC6

Table 25 Weather record of the Field Station and Exposure Site on each day of sampling.

	Field Station Readings								Exposure Site Readings							
	Temperature (°C)			Rel Hum (%)			Rainfall (mm)				Soil Moisture Content (%)			Soil Temp (°C)			Air (°C)		
Sample Time	Max	Min	Mean	Max	Min	Mean	-2 days	-1 day	Sample day	Soil Appearance	Mean	Std Dev	n	Mean	Std Dev	n		Filter Paper Moist Cont % pF	Soil Sample Moist Cont %
1 6 Jan	11·25	7·75	9·2	89	76	84	0	0	0	Damp	43·07	1·02	8					24 4·5	11
2 3 Feb	0·00	-0·75	-1·3	96	86	94	0	0	0·5	Snow	47·54	1·73	6					78 2·4	27
3 2 Mar	10·50	-4·50	3·0	96	48	79	0·2	0	0	Frzn	51·47	2·25	6	2·75	0·64	4	9·5	73 2·5	26
4 30 Mar	12·50	4·75	9·0	90	59	74	0	0	0	Dry	55·48	1·17	6	6·40	0·52	5	12–18	59 2·7	27
5 28 Apr	11·25	1·50	5·8	92	38	62	0	0	0	Dry	47·13	1·69	6	7·15	0·22	5	12·5	98 2·1	24
6 25 May	16·75	8·25	11·5	92	40	79	0	0·7	0	Damp	40·40	1·51	6	11·40	0·42	5		96 2·1	24
7 22 Jun	26·50	10·25	18·8	92	45	72	4·5	0	0	Dry	24·88	1·21	6					43 2·9	20
8 20 Jul	23·00	13·75	15·8	93	55	83	0	0·3	7·1	Dry	25·09	1·21	6					48 2·8	

Scots Pine Moisture Content (Figure 89)

After only one month's exposure the moisture content was 60 to 80% in both replicate stakes. There was a distinct gradient of moisture content from the deepest segment to the ground line. The slices above ground were of a similar, low, moisture content except for the topmost slice, which generally had a higher MC. The overall MC of the slices increased up to the fourth month of exposure and then decreased at the next three sample times.

The CCA treated Scots pine stakes were drier than the untreated stakes, and reached only 40 to 50% after 1 month. The deepest slice was wettest, but there was no gradient of MC decrease to the ground line. There was a distinct and steep gradient from immediately below to immediately above the ground line, with the slices above ground having an MC of only 25%. Once again the stakes increased in MC to the fourth sample time, and then dried slightly before increasing in MC at the eighth sample time.

Weight Loss (Figure 90)

The weight loss results, like those for the blocks described in Sections 4 to 9, were variable. In the untreated stakes of Scots pine, there was no increase of weight loss with time of exposure, and no pattern of weight loss within a stake, except that the derived values for weight loss in segments 4, 7 and 10 were often different to the rest of the slices from a stake.

The overall high level of weight loss in the CCA treated stakes was an artifact of the method of calculating the weight loss (See Footnote) If the values at each sample time are compared to the one month sample, then there was little weight loss in any slice, except those above ground at 6 and 8 months. These stakes had been chewed by rabbits or squirrels and a considerable amount of wood removed.

Footnote : The calculations did not allow for leaching of extractives and other materials from the stakes during treatment and subsequent leaching.

Figure 89 Moisture content in stakes taken from field exposure after 1 to 8 months.

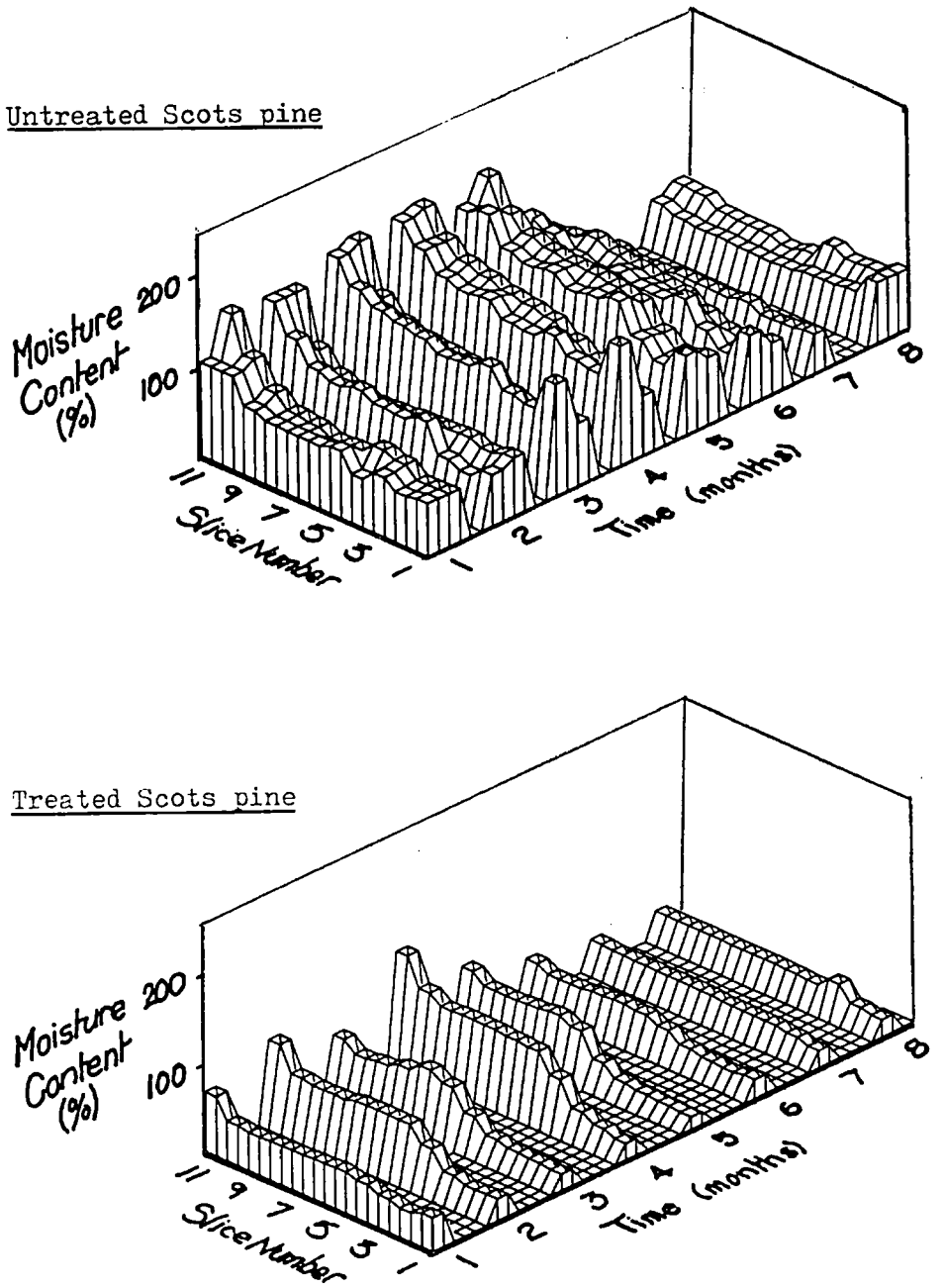
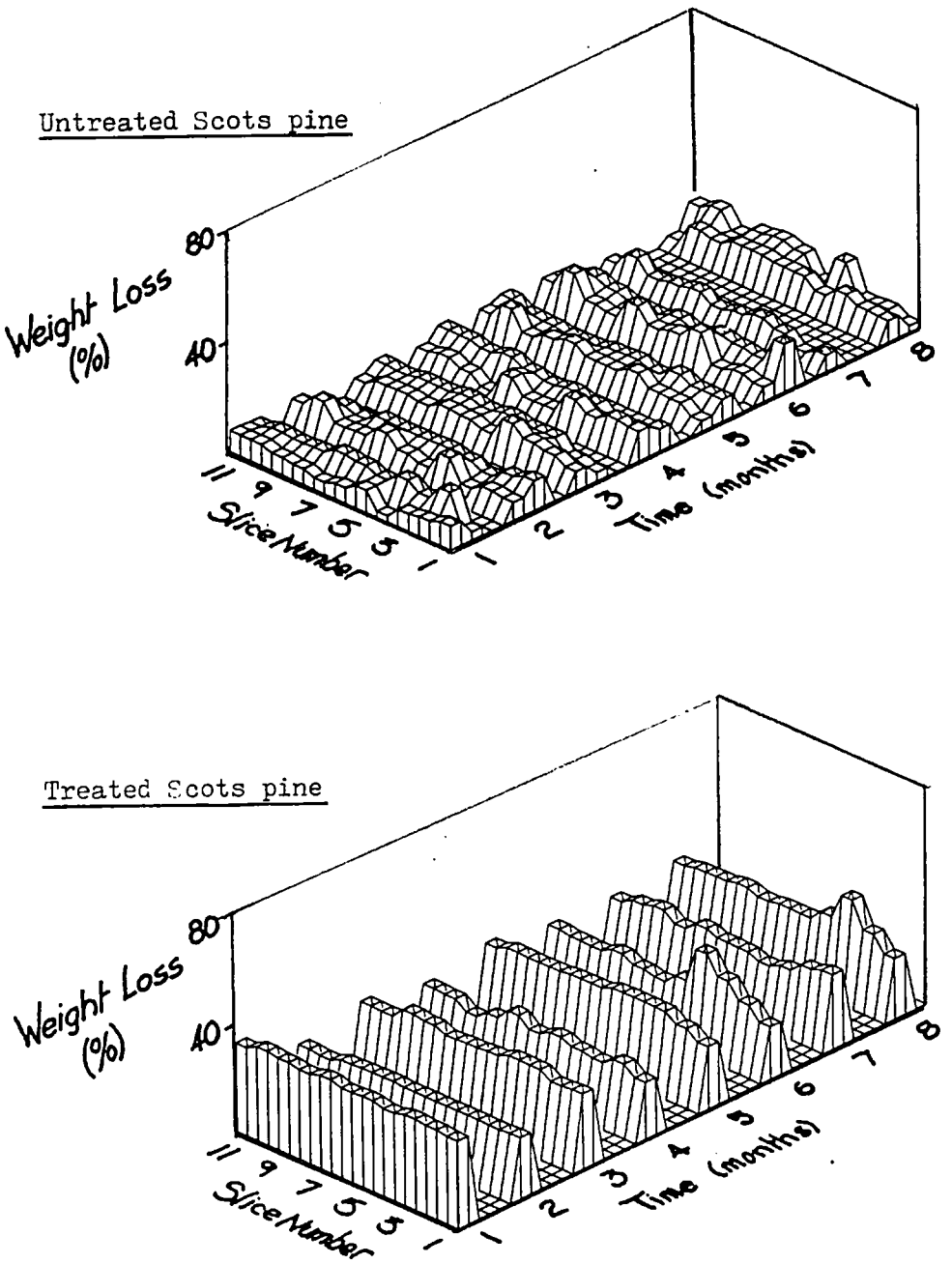


Figure 90 Weight loss in stakes taken from field exposure after 1 to 8 months.



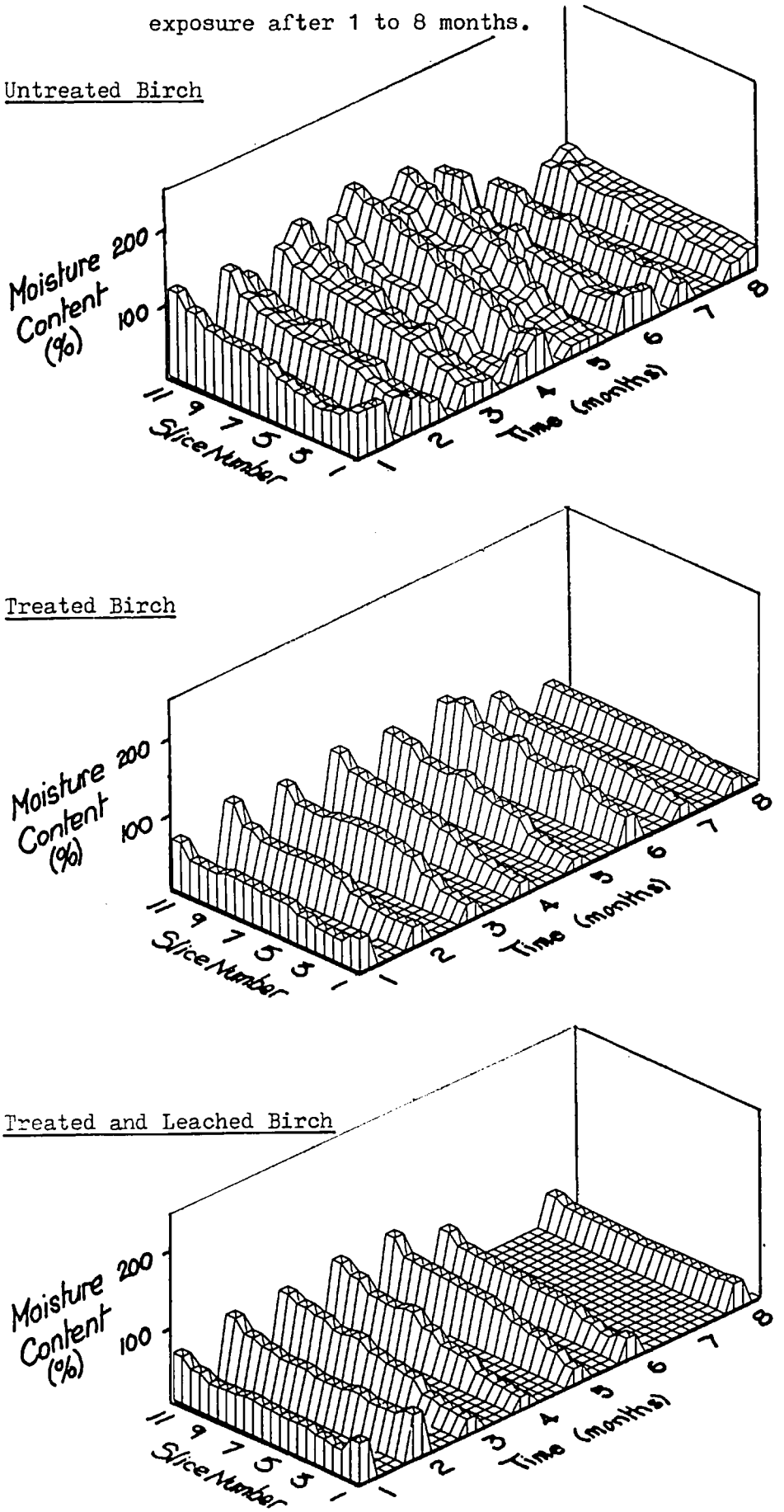
Birch Moisture Content (Figure 91)

The untreated birch stakes showed a double gradient of moisture content from below to above ground: there was one gradient from the high MC of the deepest slice to the ground line, and a second, steeper curve from the ground line to above ground. At most sample times the topmost slice was wetter than the others above ground. The two replicate stakes had generally similar moisture contents although some discrepancies did occur, e.g. at 4 months. The slices above ground at 5 months were very dry at around 10% while those below ground had a high MC of around 80%. The slices were much drier at the 6 and 7 month samples, and had an almost linear gradient of MC at 8 months.

In the CCA treated, unleached blocks, the MC was generally lower than in untreated stakes. The deepest slice was again wettest, while the MC of the remaining buried slices was remarkably similar. There was a steep gradient of MC from the ground line to above ground, with the exposed slices being generally very dry. In the stakes sampled at 3 months the slices near the ground line were wetter than those deeper. Once again the stakes were drier at 6, 7 and 8 months.

In the CCA treated, leached blocks the pattern was very similar to the treated unleached stakes, although there was no gradient of MC above the ground line at 1 and 2 months. The leached stakes often had a higher MC than the treated unleached stakes. No stake was sampled at 7 months.

Figure 91 Moisture content in Birch stakes taken from field exposure after 1 to 8 months.



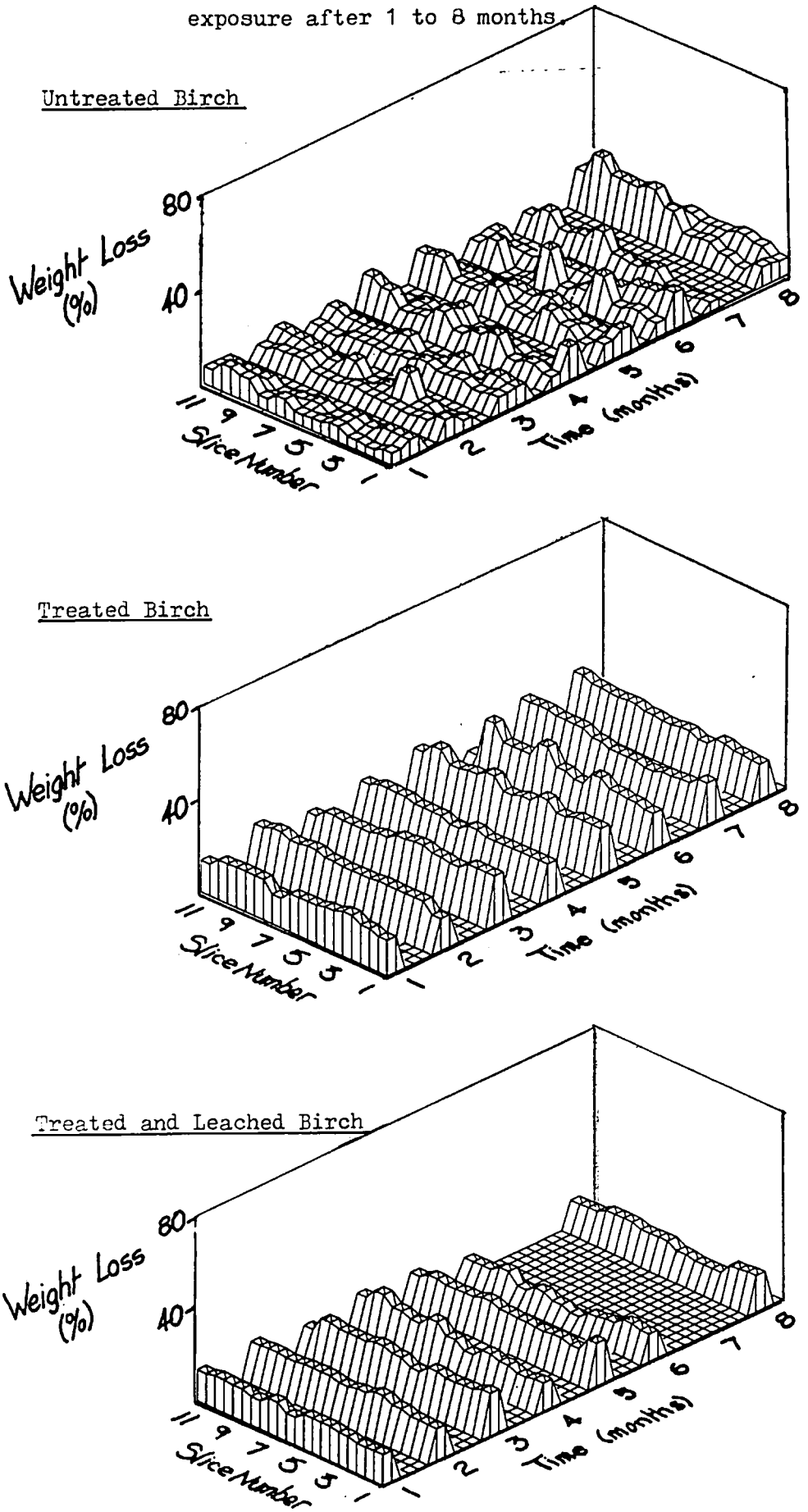
Birch Weight Loss (Figure 92)

The effect of calculating the overall weight loss of slices 4, 7 and 10 from the weights of their segments is particularly clear in Figure 92, where the WL values of these slices is often abnormal when compared to the other slices. In the untreated stakes there appeared to be an increase in WL with time, shown particularly after 8 months in one stake. At 6 months the weight loss was higher above ground, while it was lowest in this zone at 7 months.

In the treated, unleached stakes there was no increase of WL with time or of higher WL in certain zones of the stakes, other than in slices 4, 7 and 10. At 7 months there was a weight gain rather than a weight loss in the slices just below ground.

In the treated leached stakes the pattern of WL was the same as the treated unleached stakes. The high WL at 5 and 8 months was due to the loss of wood by animal rather than fungal attack. Once again there was a weight gain in the segments just above the ground line at 8 months.

Figure 92 Weight loss in Birch stakes taken from field exposure after 1 to 8 months.



Scots Pine Segment Moisture Content and AR Rate Figure 93

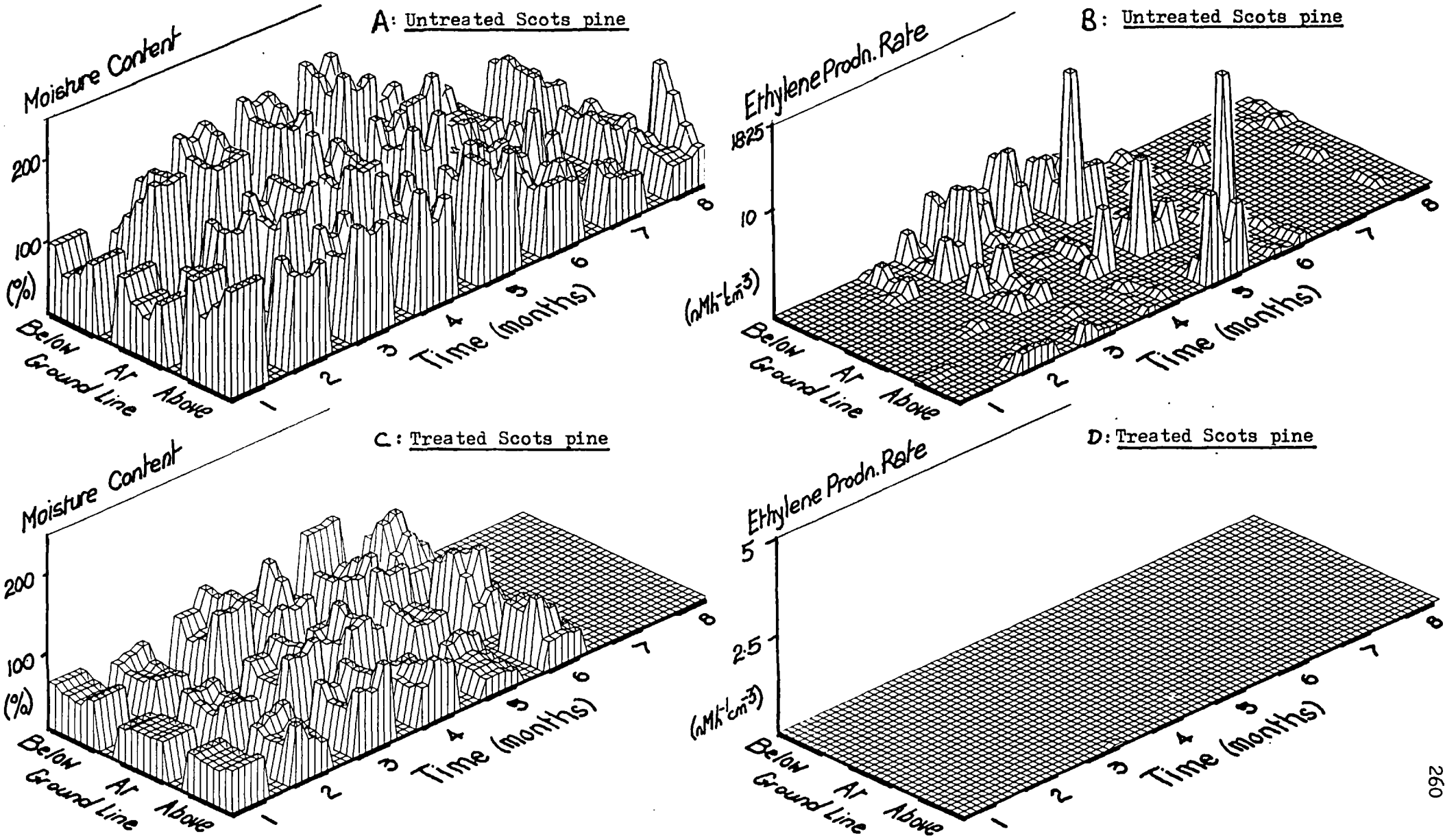
Figure 93A shows the MC in the segments cut from slices 4, 7 and 10 (above, at, and below the ground line respectively) of untreated Scots pine stakes. Stakes were removed from the ground at monthly intervals up to 8 months. Figure 93B shows the AR rate recorded in the same segments. Figures 93C and D show MC and AR in the Scots pine stakes which had been treated and leached. In the 1 month samples, each slice was cut into only four segments, but at the other sample times each slice was cut into 12 segments. The three segments nearest the right hand axis in each set of 12 represent the outer tangential face of the stake, and the two segments in the centre of the set had no surface exposed to soil.

The MC of the slices increased only marginally from 1 to 5 months, often with the corner segments with two faces exposed to soil at the highest MC. The central two segments usually had the lowest MC of the segments in a slice. The MC in the three slices was very similar and did not show the large differences in MC between slices above, at, and below the ground line which were found in the small blocks used in the laboratory experiments. At six months, the MC of all segments decreased, and decreased further at 7 months.

The AR rate in the same segments (Figure 93B) appeared to be correlated with the MC of the segment. There was little activity in any slice at 1 month, but it occurred in all the slices measured at 2 months. At 3 and 4 months most activity was recorded in the slice below ground, and least in the slice above ground. At 5 months high activity was recorded in all the slices, with the highest rate in the zone above ground (18.25 $nMh^{-1}cm^{-3}$). At 6 months the rate was very much reduced, and was recorded in only one segment at 7 months. There was little activity at 8 months.

In the treated, leached stakes, the MC of the wood was considerably less than in the untreated stakes. At 4 months the highest MC was only around 107%, while the untreated stakes at the same sample time had a MC of around 160%. At 5 months the slice below ground was distinctly wetter than the zone at the ground line,

Figure 93 Moisture content and Ethylene production rate in Scots pine stakes after 1 to 8 months exposure in the field.



while the zone above ground was driest. No stakes were sampled at 7 and 8 months.

AR activity was only recorded in one segment above ground after 3 months exposure of the treated and leached Scots pine stakes.

Birch Segment Moisture Content and AR Rate Figure 94

Figure 94A shows the MC in the segments cut from slices 4, 7 and 10 (above, at, and below the ground line respectively) of untreated birch stakes. Stakes were removed from the ground at monthly intervals. Figure 93C and 93D show the MC and AR in segments of treated unleached birch in months 1 to 4, and in segments of treated, leached birch for months 5 and 6. No stakes were sampled in months 7 and 8.

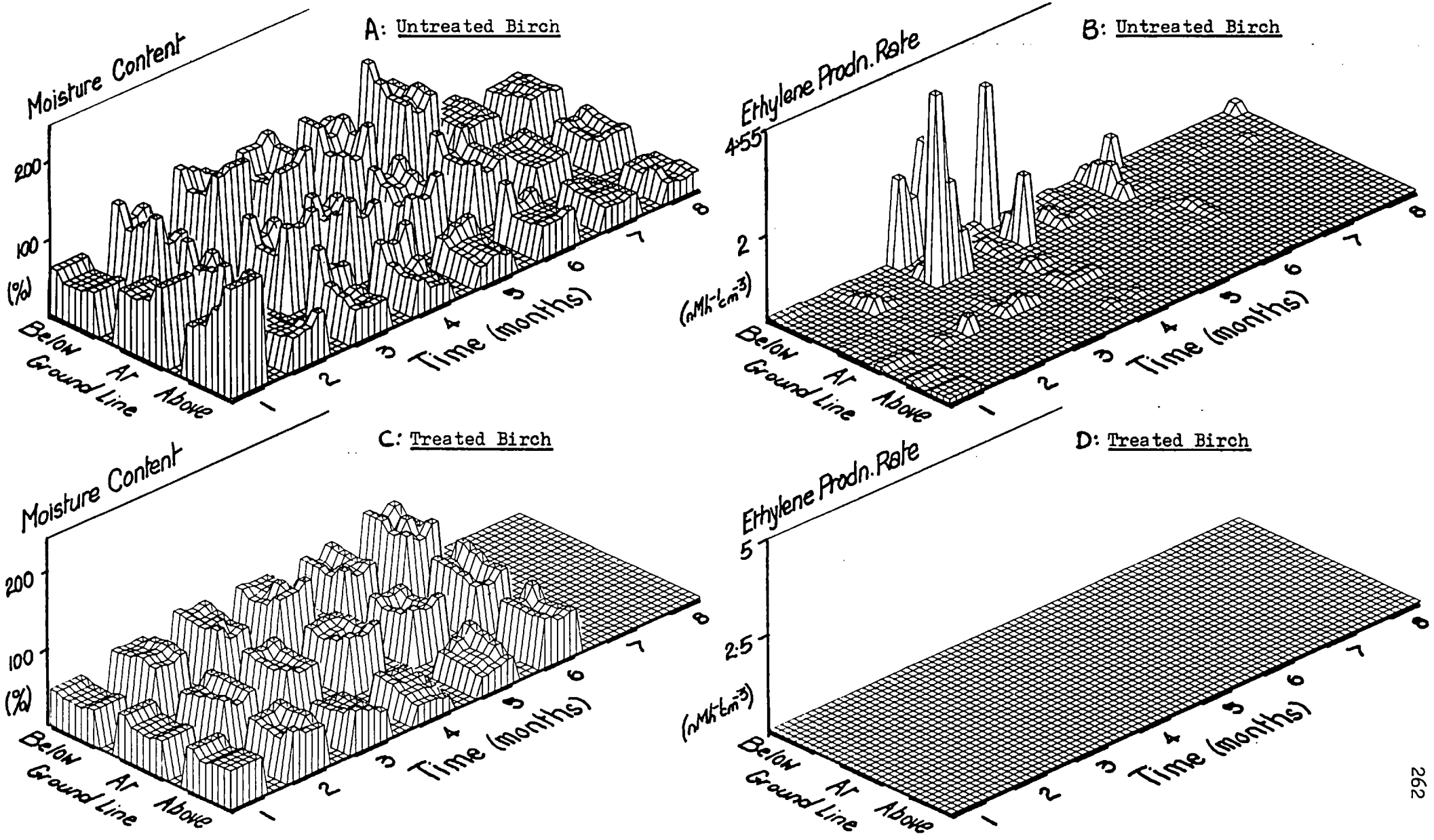
The MC in the untreated birch was highest above ground at one month, but from 2 to 6 months, the deepest zone was the wettest. The MC above ground was around 40%. The corner segments were often wetter than the others in a slice, while the central segments had the lowest MC. The MC of the zones below ground decreased in months 7 and 8, but only slightly decreased in the zone above ground.

The AR rate at 1 month was highest above ground, while at later sample times, highest activity was recorded in the zone below ground, and very little activity in the zone above ground. Highest activity was recorded after 3 months (4.55 $nMh^{-1}cm^{-3}$), which was considerably lower than the maximum rate measured in untreated Scots pine. As in Scots pine, the AR activity decreased drastically at 7 and 8 months.

The MC of the treated stakes was, like the pine, very much lower than in the untreated stakes. The MC of the segments in all 3 slices was similar at 2 months, but had developed the usual pattern of higher MC below ground, and lowest MC above ground, at 3 to 5 months.

No AR activity was recorded in any segment from the treated birch, at any sample time.

Figure 94 Moisture content and Ethylene production rate in Birch stakes after 1 to 8 months exposure in the field.



12.4. Discussion

The initial aim of the field experiment was to compare the laboratory results with those obtained in the field. The correlation appears remarkably good although it was fortuitous that the field conditions replicated the drying regime of the Scots pine blocks in Section 7. In both laboratory and field a decrease in soil MC and in wood MC was accompanied by a reduction in AR activity. The laboratory incubation temperature of 25°C was rather higher than the temperatures experienced in the field, but hopefully the high temperature only accelerated the colonisation and decay rather than changing the type of colonising organisms. The range of soil MC investigated also appeared to reflect the conditions experienced in the field where the soil fluctuated between dry and waterlogged depending upon the prevailing weather conditions. The MC of the Scots pine and birch in the field, in the slices and segments cut from each slice was very similar to the MC achieved in the laboratory under similar soil moisture conditions. The MC of the stakes in the field was related to the amount of rainfall in the three days prior to sampling. There was no relationship between R.H., soil MC and wood MC, presumably because the R.H. fluctuated widely over a 24 hour period. As there is considerable data on the MC of wood exposed to atmospheres of differing R.H., it is likely that the exposed areas of the stakes did respond to R.H. However as the equilibration was

likely to take some time, and the R.H. can vary considerably over a small area depending upon the local conditions, then it seems unlikely that the MC of the few samples taken at monthly intervals would correlate with the maximum and minimum R.H. on the sample day.

The partial burial of the stakes, as used in the laboratory experiments, reflecting typical end uses such as fence posts and telegraph poles would appear to be essential to achieve realistic conditions in the wood. The MC in the zones below at and above the ground line were distinctly different with a curve of MC from the deepest slice to the ground line and from the ground line to the penultimate slice above ground. The uppermost slice was often wetter than those below, presumably due to condensation, dew, or greater uptake into the shakes and splits present in the exposed horizontal transverse face.

The significance of the curve of MC with depth below ground and above ground is that it implies that the wood was in equilibrium with the soil surrounding it, and that the slices above ground were in equilibrium with the air. Intermediate slices are at an intermediate equilibrium value. The mechanism of achieving equilibrium may well be wick action. The wet zones visible on the exposed surfaces of the stakes on wet days, which rapidly disappeared if the day became drier, warmer and windier suggests that water is evaporating from the exposed portion of the stake and replaced by uptake from soil below ground. This would create a movement of water through the stake by wick action. The inference is that if wood in soil contains moving water and that the wood rapidly adjusts to the prevailing conditions, then as the conditions change daily, if not hourly, then fluctuating conditions must occur in the wood. There are a number of implications. The moisture conditions at any point in the stake must be the result of a dynamic equilibrium between soil water, wood MC and the air temperature, humidity and wind speed. Depending upon how rapidly the wood responds to a change in weather conditions, and the results of section 7 would indicate that it was very rapid, then the measured MC in the wood could be a result of an equilibrium achieved on the day of sampling or a few days prior to sampling. In addition the MC recorded merely measures the amount of water present at the sample time and is not a measure of the amount of water passing through the stake by wick action. Thus the monthly

samples do not show a progressive change of wood MC as the stakes got wetter over a period of months, but are the result of short term rapid changes of wood MC which are continuously occurring in the field. Any long term effect of decay to increase MC, as observed in the laboratory experiments, is masked by short term fluctuations caused by changes in the environment. These environmental fluctuations determine wood MC and affect the conditions in the wood, and may profoundly influence decay. If the wood is constantly wetting and drying and responding to environmental changes then the activity of the decay fungi, which would be affected by such fluctuations, must also fluctuate. This is illustrated by the effect of a decrease in soil moisture and wood MC upon the AR activity of nitrogen fixing bacteria. Decay fungi could be similarly affected by changes in wood moisture conditions.

The wider implication is that decay in the field is not necessarily a slow, methodical succession of events inside the protected and isolated environment of the wood, but a dynamic process where the conditions change constantly, the organisms respond to those changes and the soil, wood and weather interact continually. If adverse conditions arise, and the decay fungi die, then recolonisation might be necessary. If conditions favour one species or group of fungi, then it might colonise and decay the wood very rapidly while favourable conditions prevail. The implications of this dynamic concept is that although wood in soil contact will eventually decay, it is the rate which is profoundly affected by wood species, weather and climate. Although the service life of timber must be related to climate and weather, considerable work would be necessary to investigate any correlation.

One of the most intriguing observations in this experiment was the occurrence of snow on the Scots pine and its absence from birch. It could be related to a difference in permeability, heat conduction or albedo of the two species, but no further investigation was performed or any satisfactory explanation found.

The occurrence of AR activity in all 3 zones could indicate that the stakes were perhaps too short and that all 3 zones constituted the ground line zone of Levy (1968), and that no "anaerobic zone" occurred in these stakes. However the analysis of the 12 segments cut from the slices did reveal a difference in activity in different zones of the wood. The wettest segments, with two faces exposed to the soil, had the highest AR rate, followed by the slightly drier segments with only one exposed

face, and the central two segments which were usually drier than the others in a slice, and had no exposed faces, and the lowest AR rate. The implication is that the water carries the organisms into the wood as it penetrates, or the motile bacteria swim in the water as it penetrates, and the distribution of AR reflects the distribution of water in the stake. The occurrence of high MC and AR rates above ground shows that the wood can be wet above ground, and that fluctuating conditions can cause the MC to change dramatically. The zone of wet wood above ground can change and the effective ground line separating "wet" wood and "dry" wood must move up and down the stake depending upon the prevailing conditions. The effect of this fluctuation in the ground line upon decay fungi which are most active at and just below the ground line, is unknown.

The effect of the preservative was spectacular. The treated stakes were very much drier than the untreated stakes, and no AR activity was found in any segment of any slice at any sample time in any treated stake. There was also no weight loss other than that due to rabbit attack. From the work of Sorkhoh (1976) and Clubbe (1980) it would seem likely that CCA treated birch would be colonised by fungi after 6-8 months exposure, and the inference is that the presence of nitrogen fixing bateria is not an essential precursor of fungal and particularly soft rot, attack of CCA treated hardwoods. The effect of preservatives upon the adherence of soil to the wood was particularly noticeable. Perhaps wood colonising fungi bind the soil particles around the stake to each other and the soil surface, or perhaps soil animals, moving through the soil, grazing on fungal hyphae, mix and bind the soil to the wood surface. Presumably the preservative discourages massive colonisation and the binding of soil by the hyphae as well as repelling soil animals. The inference is that an active population of soil fungi and animals surround thestake and ultimately bring the stake into intimate contact with the soil, so that the wood becomes part of the soil. The preservative prevents this association as it enters the soil from the stake by ion-exchange, diffusion and leaching. Perhaps this "sterilisation" effect, and the initial water repellent nature of the treated wood are part of the mechanism of action of the preservative. Presumably the sterilisation effect could be overcome, particularly by heavy metal tolerant organisms, and once the wood is wet, colonisation could begin, leading to decay and failure.

The occurrence of activity in Scots pine and birch, with greater activity in the Scots pine, and at rates equivalent to those found in the laboratory does indicate that the laboratory conditions realistically simulated those in the field and that the laboratory results could be extrapolated to field exposure. The implication from this field experiment, admittedly only over 8 months, is that AR is not particularly significant in timber decay. The activity of nitrogen fixing bacteria is relatively low in untreated timber (see sections 2 to 9), even under ideal conditions that may only occur rarely in the field, and was completely eliminated by the use of a CCA type preservative.

12.5 Conclusions

The conclusion from this field experiment involving the exposure of untreated and CCA-treated Scots pine and birch stakes for 8 months, is that the occurrence of nitrogen fixing bacteria in decaying wood remains an interesting but unimportant source of nitrogen for timber decay fungi. They could be of benefit under some soil conditions, and in localised areas within the wood, but are not a vital precursor of fungal attack. They are completely eliminated from CCA treated Scots pine and birch for the 8 month exposure period. The results suggest that the conditions in the system developed and used in the laboratory are realistic and that the laboratory results could be extrapolated to the field. The field results reveal the equilibrium between the fluctuating conditions of the environment and those of the wood. The stakes exposed to soil contact must be in a dynamic equilibrium with its surroundings, and confirms the concepts developed from the laboratory experiments.

13. DISCUSSION

The preliminary investigations of Aho, Seidler, Evans and Raju (1974), Cornaby and Waide (1973), Sharp and Millbank (1973), and Sharp (1975), showed that nitrogen fixing bacteria occurred in decaying wood and that they could be an important source of nitrogen for wood decay fungi. In the preliminary experiments of this investigation, AR was found to be a reliable measure of the occurrence of nitrogen fixing bacteria in wood in soil contact although there was no direct relationship found between AR and ^{15}N incorporation. It was found that AR analysis should only be carried out on samples which are incubated at constant temperature, at the lowest possible oxygen tension, and are of a similar volume and surface area. Small samples (less than 1g) could be assayed reliably over a 24 hour period but there was considerable variability in activity between replicates. The refined technique, using a modified gas chromatograph, allowed the rapid, sensitive and accurate analysis of AR rate in samples taken from wood exposed to soil contact.

The combination of Kjeldahl digestion, Markham distillation and mass spectrometry using the ^{15}N exposure and analytical technique described by Hitch (1975) was shown to be capable of both accurate and precise measurements of ^{15}N incorporation. The necessity for the calibration of ^{15}N against AR was illustrated in the results of Section 2.4.7 , where the theoretical ratio of 3 was only approached in two out of 70 samples. Despite the variability in the observed ratio, the crude conclusion was that an AR rate of less than $1nMh^{-1}g^{-1}$ was equivalent to approximately 0.0004 µg nitrogen fixed per day, that $1\text{-}10nMh^{-1}g^{-1}$ was equivalent to 0.02, and greater than $10nMh^{-1}g^{-1}$ was equivalent to approximately 0.04 µg nitrogen fixed per day. The occurrence of AR activity in wood was shown to be evidence for the presence of nitrogen fixing bacteria. Having obtained this information on the application of AR analysis to wood from soil contact, and an idea of the relationship, albeit crude, between AR and nitrogen fixation, then it was possible to answer some of the questions in the Introduction which arose from Sharp's work (1974).

The preliminary experiments involving the AR analysis of segments cut from partially sealed blocks exposed to soil showed that nitrogen fixing bacteria were capable of penetrating greater than 10mm into wood in soil contact and that they were not merely surface

colonisers. The occurrence of AR activity in blocks with a tangential, radial or transverse face exposed showed that penetration was not limited to one face, although penetration was fastest in longitudinal and radial directions and was thought to accompany water ingress into the block. Activity was often highest and occurred most rapidly in the outer tangential segments, which would have been nearer the cambium in the living tree. The MC of this zone was also higher than that of the inner segments illustrating the correlation between high moisture content and high AR activity. The preliminary finding that hardwoods were not colonised, while softwoods were, was later refuted when the MC of the wood was found to be crucial. No colonisation occurred in Douglas fir and beech which were drier than Scots pine and in which AR activity was found. If the MC was high, greater than 50% in beech and 100% in Scots pine, then colonisation occurred. If the soil and wood dried out, activity ceased.

The major factor affecting the occurrence of the bacteria was found to be soil moisture content and there was no major difference between the two soils used. The effect of temperature was as expected, a reduction in rate at low temperature and an increase in rate with increase in temperature. The effect of oxygen tension upon AR activity was less clear and indicated that the AR assay had to be performed under uniform, anaerobic conditions.

The combined experimental evidence suggests that nitrogen fixing bacteria are not a prerequisite for the decay of timber in soil contact. Decay occurred, particularly in birch and beech, in the absence of nitrogen fixing bacteria and of AR activity. Relatively high rates of AR activity in Scots pine failed to increase decay. Perhaps if blocks which had exhibited activity had been subjected to conventional weight loss tests, then any beneficial effect of the presence of the bacteria in the wood could have been assessed. However the absence of AR activity in stakes treated with a CCA preservative and exposed in the field, suggests that nitrogen fixing bacteria are not of major importance in the early colonisation of preservative treated hardwoods by fungi, and particularly soft rot fungi.

The development of a soil exposure system using partially buried, orientated blocks 50 x 25 x 15 mm in soil maintained at constant MC by weighing and water addition was of major importance in this investigation. It allowed the exposure of the wood to controlled conditions of soil MC for periods up to 28 weeks. Beech, birch, Scots pine sapwood and heartwood and spruce were exposed to 5 levels of soil MC and 2 different soils, with the soil MC being maintained, increased or decreased. The AR rate, MC, weight loss and a novel measure of the amount of water in the wood that was independent of weight loss (water content) could be measured on the same sample. Samples were taken from below, at or above the ground line and from four positions within each zone. Although the cutting could have been more accurate, which would have lessened the variability in the weight loss results, the use of 2 to 5 replicates at each fortnightly sample time allowed a more detailed, quantitative and comprehensive picture of the relationship of AR, decay, wood MC and soil MC to be developed than had been obtainable previously.

The chosen soil moisture contents and the fluctuations which were imposed in the laboratory were similar to those which occurred naturally in the field. The response of the wood, whether of small blocks in the laboratory, or of similarly proportioned but larger stakes in the field, was similar, and implied that field conditions could be reproduced in the laboratory and that laboratory results could be extrapolated to the field.

The use of the computer proved essential to the investigation, and was used for the calculation and display of the results, as well as in the statistical evaluation of the data and further mathematical analysis. Despite the amount of time spent in programming the computer and in punching data onto cards, the use of the computer proved to be both a fast and accurate method of data processing. The quantitative approach to the investigation of nitrogen fixing bacteria in soil contact is novel in that computing techniques have not previously been applied to the investigation of nitrogen fixing bacteria, or of timber decay.

The soil exposure technique and the quantitative approach could be of major significance in the investigation of timber decay and have allowed an assessment to be made of the significance of nitrogen fixing bacteria to timber decay.

Nitrogen fixing bacteria are likely to occur in wood not treated with preservative and exposed to soil contact. They appear to follow the ingress of water into the wood, and become established in the zones of a stake which wet up most rapidly. The highest activity occurs in wood exposed to soil at a low water potential and which is very wet. No information was obtained on whether relatively high rates of AR corresponded to the presence of large numbers of relatively inactive bacteria, or relatively few, very active bacteria. Their occurrence is not essential for the decay of hardwoods, but there was some evidence that their presence marginally enhanced decay in Scots pine. However they have a disjunct distribution in the wood and their activity is low compared to other free-living nitrogen fixing bacteria and other nitrogen fixing systems. Even at maximal rate, maintained continuously for one year, they are only likely to increase the nitrogen content of wood by around 5%. The field experiment showed that conditions did not remain constant in wood in service and that optimal conditions of temperature, moisture content, pH, nutrient supply etc. would not exist for very long. The conclusion is that nitrogen fixing bacteria are unlikely to be of major significance in the nitrogen budget of decaying wood. However, they may be of minor significance where, under the right conditions of soil and wood moisture content they could exhibit relatively high rates of nitrogen fixation. This high rate of activity in localised areas of the wood could be of significance to timber decay fungi although further work would be necessary to examine how much of the fixed nitrogen was made available to the decay fungi and how significant that amount of nitrogen was to the activity of the fungi. The seemingly paradoxical relationship between aerobic fungi and anaerobic bacteria could be resolved by the occurrence of fluctuating conditions in the wood. The change from wet to dry conditions in the wood, observed in the field and reproduced in the laboratory, indicate that the bacteria could be active in wet anaerobic conditions,

while the fungi were active in aerobic, dry conditions, and utilise the nitrogen fixed by the bacteria. The bacteria could utilise carbon sources liberated by the fungi during their periods of activity.

In addition to being able to quantify the significance of nitrogen fixing bacteria to timber decay, the laboratory exposure system allowed the relationship between soil MC and wood MC to be investigated in more detail than attempted previously. This relationship is of crucial importance because it affects the performance of timber in service, both untreated, and treated with commercial timber preservatives.

The uptake of water by wood in soil contact appears to have three phases. Up to fibre saturation point at around 30% MC, the uptake of water from soil is controlled by the wood and limited by the permeability of the timber which determines the rate at which water can enter the dry wood. The wood's permeability is a function of its anatomy and must be related to the hydrophobic nature of the dry cell wall , the small capillaries in the wall, the nature and state of the bordered pits, and the pitting of the rays (see p.245) Above 30% MC, in the second phase, the MC attained by the wood is an equilibrium value depending upon the available water in the soil and the permeability of the wood. The third phase is characterised by a levelling off of the rate of uptake as the wood and soil approach a final equilibrium where the wood reaches a maximum MC for that wood species in that soil at its particular soil MC.

The relationship was investigated quantitatively using two complementary approaches, one theoretical, and one experimental. The application of regression and curve fitting to the data collected in the laboratory revealed that the second and third phases could be fitted to a non-linear Gompertz curve. The analysis showed that a statistical and mathematical analysis could be applied to systems which involved soil and wood which had previously been regarded as too variable for mathematical analysis. The ability to reduce a mass of data to a single family of equations which accurately described the relationship between wood moisture content, soil MC and time indicates the potential of the laboratory exposure

system, a quantitative approach, and access to a computer, for the investigation of timber decay. The theoretical approach applied to only Scots pine and birch suggested that an even more general relationship existed between the wood MC and the MC of its surroundings.

The experimental approach confirmed the existence of such a relationship, and the use of water potential as a measure of the water availability of the surroundings proved particularly valuable in describing the relationship. In addition the approach necessitated the development of a novel artificial soil in which the water potential, MC, nutrient level and organism complement could be controlled. It may have considerable potential in the testing of wood in soil contact where a reproducible soil matrix is desirable.

The two approaches could be combined to produce a hypothetical relationship between water potential, wood moisture content and time of exposure, which fitted the data obtained in the laboratory exposure system, were consistent with the results from the artificial soil experiments and incorporated the results from the curve fitting exercise. Its ability to incorporate the effect of decay, which disturbs the relationship, perhaps indicates the generality of the relationship and its potential in the understanding of wood decay in soil contact. The existence of a "moisture characteristic" for wood is particularly interesting as it can relate wood MC to relative humidity, and also to the MC of the soil, two relationships which were previously unconnected. It also allows the relationships to be quantified, which could allow the MC of wood exposed to any soil to be predicted. The data obtained in this series of experiments is not suitable as a test of the hypothetical relationship, which needs to be rigorously tested by monitoring the MC of wood exposed to soils at different water potential and how the moisture characterisitc of a number of wood species differ. The next step could be the prediction of the moisture characteristic of a wood from its anatomy alone (see Footnote), and then to predict its moisture uptake properties from its characteristic. The possibility of predicting a timber's moisture uptake characteristics, such as waterborne preservative uptake, or water uptake in service, is particularly

Footnote: This approach has been reviewed by Siau (1983) and Banks (1975).

attractive as it would be the basis of a model for predicting timber decay and service life. Perhaps the most important concept which would have to be modelled, and whose significance has been revealed by these investigations, is that wood in soil contact is in a dynamic equilibrium with its surroundings. The conditions in the wood must change rapidly as the wood responds to changes in the surrounding environmental conditions. The effect of these fluctuating conditions upon the type and rate of fungal attack is unknown and would be difficult to investigate but it would seem to require a similar combination of a quantitative approach, a realistic, controllable, laboratory exposure system and access to a computer to perform the necessary statistical and mathematical analysis, a combination which has been used in these investigations.

A second important concept, whose involvement in timber decay has only been indicated by these experiments and which may be of major significance, is wick action. This was investigated by Becker and Zycha (1958) who found that it did not bring about a redistribution of preservatives that were applied in bandages to the outside of poles in ground contact. Levy (1968) suggested that water uptake occurred below ground and evaporation above ground. The flow of water from below ground to above ground, at a rate presumably dependent upon the rate of evaporation above ground and the rate of uptake below ground could be very significant in the decay of timber in soil contact. It could be the mechanism which controls the MC of the wood exposed to soil contact and for the attainment of equilibrium between the wood and its surroundings. If the rate of uptake and loss of water from the blocks exposed in the laboratory occurred in the field, then the field stakes must rapidly respond to changes in the moisture content of soil or air. The visible wet zone just above the ground line perhaps represents the upper limit of wet wood (i.e. greater than f.s.p.) above ground, and is the boundary between liquid water movement and vapour diffusion through the wood. Thus wood in the field is not only in a dynamic equilibrium with its surroundings, it contains water moving through the wood at a rate which is dependent upon those surrounding conditions. Thus MC, which is a static measure of the water content is unsatisfactory because it is not a measure of the <u>rate</u> of water

movement. Two samples of wood could have the same MC, yet the rate of water flow through them could be very different. The concept of wick action and its occurrence in partially buried wood in soil contact has a number of implications.

If wick action occurs in wood, in soil contact, then any soluble substances in the soil water, like salts, nitrogen compounds, oxygen, could be taken up by the wood and transported through the wood and delivered to the ground line zone. At and just above the ground line where the water evaporated, any soluble substances would be deposited, perhaps creating a nutrient rich zone around the ground line which is the zone most susceptible to fungal attack. Perhaps the major source of nitrogen for the decay fungi is the soil water itself, which is transported through the wood by wick action? Perhaps wick action can also explain the occurrence of nitrogen fixing bacteria above ground and the increase in weight found in above ground segments in the laboratory trials, with both bacteria and soluble salts being deposited as the water evaporated. Wick action becomes crucial in the relationship between soil water, water potential, wood MC and the R.H. of the atmosphere because the rate of flow must depend on the relationship. Obviously any model of timber decay in soil contact would have to take wick action into account and further work would be necessary to examine the rate of wick action in different wood species under different soil moisture and environmental conditions.

The significance of wick action is not confined to soil contact alone. In window joinery, if water enters at a joint, it could be transported through the wood and then evaporate, albeit slowly, through a paint or varnish film. It would seem reasonable that wood, which is highly adapted for the transport of water from roots to leaves in the living tree, continues to conduct water when in service, and that the same driving force of transpiration (water evaporation) drives wick action. These investigations merely illustrate the existence of the process, but the full significance to timber decay has yet to be determined.

Potentially, given the information on water uptake rate with time, the relationship between wood MC and water potential, if the rate of diffusion of water vapour through wood at different MC

could be determined, then perhaps the rate of wick action driving the flow of water through wood could be predicted. The results presented in this investigation are the first step in the mathematical modelling of wick action and perhaps of a model of the performance of timber in soil contact.

Perhaps the most intriguing aspect of the occurrence of wick action is its possible involvement in supplying nitrogen to the decay fungi in wood. If nitrogenous nutrients, perhaps nitrate, were available in soil, then they could be transported into the wood by wick action and deposited around the ground line, where the decay fungi have been found to be most active (Levy, 1968). Obviously optimal conditions must occur in this zone, as it is the usual zone of maximal attack, and of failure. It would indeed be interesting if wick action were responsible for achieving this optimal blend of nutrient supply, waste products removal, oxygen tension, and moisture content. Of course, wick action is unlikely to be the sole source of nitrogen, as it is extremely likely that fungi are able to translocate nitrogen compounds and other nutrients from the soil into the decaying wood (King and Waite, 1978).

Thus although this investigation was originally intended to examine the role and significance of nitrogen fixing bacteria to timber decay in soil contact, it was soon recognised that water had a profound effect on nitrogen fixing bacteria, that these bacteria were not very significant in timber decay and that water was a more important factor. The concept of water potential and the existence of a moisture characteristic for wood are potentially of great significance, as are the concepts of a dynamic equilibrium between wood and its surroundings, and wick action. The combined laboratory and field experiments and their theoretical analysis represent a novel, quantitative approach to the investigation of timber decay in soil contact.

APPENDIX

Introduction

This appendix consists of a brief description and listing of the computer programmes used, together with a sample of typical input and output.

In the course of the experimental work, it became obvious that a considerable amount of data was being collected and that the use of a computer would be essential to analyse the data rapidly and accurately. Computing facilities were easily available in the Imperial College Computer centre, where the equipment consisted of a CDC Cyber 174 and a CDC 6500, with associated disc files, teletypewriters, visual display units, printers, card readers and graphics terminals. FORTRAN IV was the most widely used programming language using the University of Minnesota MNF5 complier and the Centre provided a number of programme packages for statistical analysis and graphics. The Centre provided a number of courses on the programming and use of the computer, and a Programme Advisory Service.

Once the capability of the computer for data anlysis had been recognised, it was used routinely for the calculation and display of experimental results as graphs, histograms, density maps and tables on the line printer, and as graphs and 3-dimensional displays generated on microfilm from the Tektronics 4014 graphics terminal.

Method

Experimental data was usually recorded on a standardised form, such as that shown in Figure 18. The results from a few sets of data were calculated by hand, and a FORTRAN programme written to perform the same calculations. The programme and data were transferred to punched cards and run on the CDC 6600. The programme was debugged and the results checked for accuracy. When the programme ran satisfactorily, the remaining data was punched and the programme run routinely on the CDC 6600 at the University of London Computer Centre via the Imperial College network.

Each programme usually consists of a main routine which calls a number of subroutines. Each subroutine is responsible for one function, such as reading in data, calculating results, writing out data, drawing a histogram, density map or graph, or storing data on file. The subroutines could, with minor modifications, be transferred to other programmes.

ARDATA

Program ARDATA generates "ready reckoner" tables to ease the analysis of the pen recorder charts from the acetylene reduction assay, described in section 2.3.4. The baseline position in chart recorder units is read off the first table; e.g. if the baseline is at 51 units on an attenuation of x10, then the baseline is actually at 51.0 chart recorder units.

DIVS	x10	x20	x50	x100	x200	x500	1000	2000	5000
50.5	50.5	25.3	10.1	5.1	2.5	1.0	.5	.3	.1
51.0	51.0	25.5	10.2	5.1	2.5	1.0	.5	.3	.1
51.5	51.5	25.8	10.3	5.2	2.6	1.0	.5	.3	.1
52.0	52.0	26.0	10.4	5.2	2.6	1.0	.5	.3	.1
52.5	52.5	26.3	10.5	5.3	2.6	1.0	.5	.3	.1
53.0	53.0	26.5	10.6	5.3	2.7	1.1	.5	.3	.1
53.5	53.5	26.8	10.7	5.3	2.7	1.1	.5	.3	.1
54.0	54.0	27.0	10.8	5.4	2.7	1.1	.5	.3	.1
54.5	54.5	27.3	10.9	5.4	2.7	1.1	.5	.3	.1
55.0	55.0	27.5	11.0	5.5	2.8	1.1	.6	.3	.1
55.5	55.5	27.8	11.1	5.6	2.8	1.1	.6	.3	.1
56.0	56.0	28.0	11.2	5.6	2.8	1.1	.6	.3	.1
56.5	56.5	28.3	11.3	5.7	2.8	1.1	.6	.3	.1
57.0	57.0	28.5	11.4	5.7	2.8	1.1	.6	.3	.1
57.5	57.5	28.8	11.5	5.8	2.9	1.1	.6	.3	.1
58.0	58.0	29.0	11.6	5.8	2.9	1.2	.6	.3	.1
58.5	58.5	29.3	11.7	5.8	2.9	1.2	.6	.3	.1
59.0	59.0	29.5	11.8	5.9	3.0	1.2	.6	.3	.1
59.5	59.5	29.8	11.9	5.9	3.0	1.2	.6	.3	.1
60.0	60.0	30.0	12.0	6.0	3.0	1.2	.6	.3	.1

The ethylene peak is read from the chart record, and the number of chart recorder units read from the second table; e.g. if the peak height is 59 on an attenuation of x100, then this is equivalent to 5900 chart recorder units.

DIVS	x10	x20	x50	x100	x200	x500	x1000	x2000	x5000
50.5	505.0	1010.0	2525.0	5050.0	10100.0	25250.0	50500.0	101000.0	252500.0
51.0	510.0	1020.0	2550.0	5100.0	10200.0	25500.0	51000.0	102000.0	255000.0
51.5	515.0	1030.0	2575.0	5150.0	10300.0	25750.0	51500.0	103000.0	257500.0
52.0	520.0	1040.0	2600.0	5200.0	10400.0	26000.0	52000.0	104000.0	260000.0
52.5	525.0	1050.0	2625.0	5250.0	10500.0	26250.0	52500.0	105000.0	262500.0
53.0	530.0	1060.0	2650.0	5300.0	10600.0	26500.0	53000.0	106000.0	265000.0
53.5	535.0	1070.0	2675.0	5350.0	10700.0	26750.0	53500.0	107000.0	267500.0
54.0	540.0	1080.0	2700.0	5400.0	10800.0	27000.0	54000.0	108000.0	270000.0
54.5	545.0	1090.0	2725.0	5450.0	10900.0	27250.0	54500.0	109000.0	272500.0
55.0	550.0	1100.0	2750.0	5500.0	11000.0	27500.0	55000.0	110000.0	275000.0
55.5	555.0	1110.0	2775.0	5550.0	11100.0	27750.0	55500.0	111000.0	277500.0
56.0	560.0	1120.0	2800.0	5600.0	11200.0	28000.0	56000.0	112000.0	280000.0
56.5	565.0	1130.0	2825.0	5650.0	11300.0	28250.0	56500.0	113000.0	282500.0
57.0	570.0	1140.0	2850.0	5700.0	11400.0	28500.0	57000.0	114000.0	285000.0
57.5	575.0	1150.0	2875.0	5750.0	11500.0	28750.0	57500.0	115000.0	287500.0
58.0	580.0	1160.0	2900.0	5800.0	11600.0	29000.0	58000.0	116000.0	290000.0
58.5	585.0	1170.0	2925.0	5850.0	11700.0	29250.0	58500.0	117000.0	292500.0
59.0	590.0	1180.0	2950.0	5900.0	11800.0	29500.0	59000.0	118000.0	295000.0
59.5	595.0	1190.0	2975.0	5950.0	11900.0	29750.0	59500.0	119000.0	297500.0
60.0	600.0	1200.0	3000.0	6000.0	12000.0	30000.0	60000.0	120000.0	300000.0

The units of ethylene produced by the sample is this value (5900) minus the baseline (51.0), giving 5849 units.

ARDATA Listing

```
      PROGRAM ARDATA(INPUT,OUTPUT,TAPE5=INPUT,TAPE6=OUTPUT)
      DV = 0.0
      WRITE (6, 1000)
1000  FORMAT (1HO)
      DO 1001 K = 1, 10
      WRITE(6,1002)
1002  FORMAT ( 1HO, 3X, 4HDIVS, 3X, 3HX10, 2X, 3HX20, 2X, 3HX50, 1X,
     2 4HX100, 1X, 4HX200, 1X, 4HX500, 1X, 4H1000, 1X, 4H2000, 1X,
     3 4H5000, 3X, 4HDIVS, 3X, 3HX10, 4X, 3HX20, 4X, 3HX50, 4X, 4HX100,
     4 4X, 4HX200, 4X, 4HX500, 4X, 5HX1000, 4X, 5HX2000, 4X, 5HX5000)
      DO 1003 N = 1, 20
      DV= DV + 0.5
      B1= DV * 1.0

      B2= DV * 0.5
      B3= DV * 0.2
      B4= DV * 0.1
      B5= DV * 0.05
      B6= DV * 0.02
      B7= DV * 0.01
      B8= DV * 0.005
      B9= DV * 0.002
      P1 = DV * 10.0
      P2 = DV * 20.0
      P3 = DV * 50.0
      P4 = DV * 100.0
      P5 = DV * 200.0
      P6 = DV * 500.0
      P7 = DV * 1000.0
      P8 = DV * 2000.0
      P9 = DV * 5000.0
      WRITE(6,1004) DV, B1, B2, B3, B4, B5, B6, B7, B8, B9, DV, P1, P2,
     2  P3, P4, P5, P6, P7, P8, P9
1004  FORMAT (2X, 2(F6.1,1X), 8(F4.1,1X), 2X, F6.1, 1X, 3(F6.1,1X),
     2  3(F7.1,1X), 4(F8.1,1X))

1003  CONTINUE
1001  CONTINUE
      STOP
      END
```

ARDATA Output

Examples of the tables generated by ARDATA are shown in the text on the previous page.

TIGROT

TIGROT calculates the moisture content, weight loss, acetylene reduction rate and water content of the segments from the blocks exposed to soil contact and described in Sections 5 to 9. It writes out the results in tabular form, and then calculates the mean of each of the four values in each segment and zone of the replicate blocks. The results are then written out and displayed as a 'density map'.

The main programme sets the size of the memory stores for the variables in the DIMENSION statement, and defines those variables which are to be available to the subroutines in the COMMON statement. It then calls the subroutines.

BOTIN reads in the empty weight and volume of the 144 bottles used in the acetylene reduction assay. INDAT reads in the number of replicate blocks (usually 2 to 5) and the number of segments in each block (usually 12). The data is then read in, in the same format as shown in Figure 18, where each line of the form corresponds to a punched card, and each column of the form corresponds to one of the 80 columns on the card. WDVAL calculates the moisture content, weight loss and water content for each segment, and ARVAL calculates the acetylene reduction rate. The results are printed out by DATOT and the averages calculated by AVBLK, which calls the subroutines SEGFL (which averages the replicate segments), and ZONAV (which averages replicate zones). The mean, standard deviation etc. are calculated by AVRAGE which is taken from Davies (1971). The results are then printed out as a density map, where the value of moisture content, weight loss, acetylene reduction rate or water content is converted to a value from 1 to 20 and printed to produce a scale diagram of the side view of the block.

```
      PROGRAM TIGROT(INPUT,OUTPUT,TAPE5=INPUT,TAPE6=OUTPUT)
C COMPUTES MEAN ETC. OF MOISTURE CONTENT, WEIGHT LOSS, ACETYLENE
C REDUCTION IN EACH SEGMENT OF THE SAMPLED BLOCKS
      DIMENSION EMPB(160),FACB(160),ROW(60),SYMBOL(25),SPEC(6),NBIN(6),
     1BLKN(6),IBLKP(6),ISAMT(6),IDATE(6),WINI(6),WREM(6),STDA(6),STDB(6
     2),WLSGAV(12),WCPCAV(12)   ,M(12,6),WETB(12,6),DRYB(12,6),TIUN(12,6
     3),TOUN(12,6),TITM(12,6),TOTM(12,6),SLGT(12,6),WETW(12,6),DRYW(12,
     46),WATC(12,6),CONM(12,6),WSEG(12,6),WLSEG(12,6),NSEG(12,6),UNITT(
     512,6),TIME(12,6),RTCM(12,6),CONMAV(12),RTCMAV(12),WCPCV(12,6)
      COMMON      EMPB,FACB,MD,SYMBOL,SPEC,NBIN,BLKN,IBLKP,ISAMT,IDATE,
     1WINI,WREM,STDA,STDB,NB,NS,WCPCV    ,M,WETB,DRYB,TIUN,TOUN,TITM,
     2TOTM,SLGT,WETW,DRYW,WATC,CONM,WSEG,WLSEG,NSEG,UNITT,TIME,RTCM,
     3    WCPCAV,CONMAV,WLSGAV,RTCMAV,ROW
      CALL BOTIN
 1011 CALL INDAT
      IF(NB.EQ.0)GO TO 1010
      CALL WDVAL
      CALL ARVAL
      CALL DATOT
      CALL AVBLK
      CALL WAPER
      GO TO 1011
 1010 STOP
      END

      SUBROUTINE BOTIN
C READS IN BOTTLE NO., VOL., FACTOR
      DIMENSION EMPB(160),FACB(160),ROW(60),SYMBOL(25),SPEC(6),NBIN(6),
     1BLKN(6),IBLKP(6),ISAMT(6),IDATE(6),WINI(6),WREM(6),STDA(6),STDB(6
     2),WLSGAV(12),WCPCAV(12)   ,M(12,6),WETB(12,6),DRYB(12,6),TIUN(12,6
     3),TOUN(12,6),TITM(12,6),TOTM(12,6),SLGT(12,6),WETW(12,6),DRYW(12,
     46),WATC(12,6),CONM(12,6),WSEG(12,6),WLSEG(12,6),NSEG(12,6),UNITT(
     512,6),TIME(12,6),RTCM(12,6),CONMAV(12),RTCMAV(12),WCPCV(12,6)
      COMMON      EMPB,FACB,MD,SYMBOL,SPEC,NBIN,BLKN,IBLKP,ISAMT,IDATE,
     1WINI,WREM,STDA,STDB,NB,NS,WCPCV    ,M,WETB,DRYB,TIUN,TOUN,TITM,
     2TOTM,SLGT,WETW,DRYW,WATC,CONM,WSEG,WLSEG,NSEG,UNITT,TIME,RTCM,
     3    WCPCAV,CONMAV,WLSGAV,RTCMAV,ROW
      NM = 40
      READ(5,1110)(MDA, EMPB(MDA), FACB(MDA), MDB, EMPB(MDB), FACB(MDB),
     2 MDC, EMPB(MDC), FACB(MDC), MDD, EMPB(MDD), FACB(MDD), MM=1,NM)
 1110 FORMAT (4 ( I3, 2F6.2, 4X))
      READ(5,101)(SYMBOL(I),I=1,25)
  101 FORMAT(25A2)
      RETURN
      END

      SUBROUTINE INDAT
      DIMENSION EMPB(160),FACB(160),ROW(60),SYMBOL(25),SPEC(6),NBIN(6),
     1BLKN(6),IBLKP(6),ISAMT(6),IDATE(6),WINI(6),WREM(6),STDA(6),STDB(6
     2),WLSGAV(12),WCPCAV(12)   ,M(12,6),WETB(12,6),DRYB(12,6),TIUN(12,6
     3),TOUN(12,6),TITM(12,6),TOTM(12,6),SLGT(12,6),WETW(12,6),DRYW(12,
     46),WATC(12,6),CONM(12,6),WSEG(12,6),WLSEG(12,6),NSEG(12,6),UNITT(
     512,6),TIME(12,6),RTCM(12,6),CONMAV(12),RTCMAV(12),WCPCV(12,6)
      COMMON      EMPB,FACB,MD,SYMBOL,SPEC,NBIN,BLKN,IBLKP,ISAMT,IDATE,
     1WINI,WREM,STDA,STDB,NB,NS,WCPCV    ,M,WETB,DRYB,TIUN,TOUN,TITM,
     2TOTM,SLGT,WETW,DRYW,WATC,CONM,WSEG,WLSEG,NSEG,UNITT,TIME,RTCM,
     3    WCPCAV,CONMAV,WLSGAV,RTCMAV,ROW
C INDAT READS IN DATA FOR WHOLE BLOCK,THEN SEGMENTS
      READ(5,1210)NB,NS
 1210 FORMAT(I2,1X,I2)
      IF(NB.EQ.0) RETURN
      DO 1213 L=1,NB
      READ(5,1211)SPEC(L),NBIN(L),BLKN(L),IBLKP(L),ISAMT(L),IDATE(L),
     2WINI(L),WREM(L),STDA(L),STDB(L)
 1211 FORMAT(A5,2X,I2,2X,A4,2X,I2,2X,I3,2X,I6,2X,2(F5.2,2X),2(F6.1,2X))
      DO 1213 K=1,NS
      READ(5,1212)NSEG(K,L),M(K,L),WETB(K,L),DRYB(K,L),TIUN(K,L),
     2TOUN(K,L),TITM(K,L),TOTM(K,L),SLGT(K,L)
 1212 FORMAT(1X,I2,1X,I3,2F7.2,2F10.1,2F7.2,F6.1)
 1213 CONTINUE
      RETURN
      END

      SUBROUTINE WDVAL
      DIMENSION EMPB(160),FACB(160),ROW(60),SYMBOL(25),SPEC(6),NBIN(6),
     1BLKN(6),IBLKP(6),ISAMT(6),IDATE(6),WINI(6),WREM(6),STDA(6),STDB(6
     2),WLSGAV(12),WCPCAV(12)   ,M(12,6),WETB(12,6),DRYB(12,6),TIUN(12,6
     3),TOUN(12,6),TITM(12,6),TOTM(12,6),SLGT(12,6),WETW(12,6),DRYW(12,
     46),WATC(12,6),CONM(12,6),WSEG(12,6),WLSEG(12,6),NSEG(12,6),UNITT(
     512,6),TIME(12,6),RTCM(12,6),CONMAV(12),RTCMAV(12),WCPCV(12,6)
      COMMON      EMPB,FACB,MD,SYMBOL,SPEC,NBIN,BLKN,IBLKP,ISAMT,IDATE,
     1WINI,WREM,STDA,STDB,NB,NS,WCPCV    ,M,WETB,DRYB,TIUN,TOUN,TITM,
     2TOTM,SLGT,WETW,DRYW,WATC,CONM,WSEG,WLSEG,NSEG,UNITT,TIME,RTCM,
     3    WCPCAV,CONMAV,WLSGAV,RTCMAV,ROW
      DO 1313 L=1,NB
      DO 1313 K=1,NS
C SHORT CIRCUIT IF NO BOTTLES WEIGHED
      IF(M(K,L).EQ.0) GO TO 1310
C WOOD WTS. FROM BOTTLE WEIGHTS
      MP=M(K,L)
      WETW(K,L)=WETB(K,L)-EMPB(MP)
      DRYW(K,L)=DRYB(K,L)-EMPB(MP)
      GO TO 1311
 1310 WETW(K,L)=WETB(K,L)
      DRYW(K,L)=DRYB(K,L)
 1311 WATC(K,L)=WETW(K,L)-DRYW(K,L)
      CONM(K,L)=WATC(K,L)*100.0/DRYW(K,L)
      WSEG(K,L)=WINI(L)*SLGT(K,L)/200.0
      WLSEG(K,L)=(WSEG(K,L)-DRYW(K,L))*100.0/WSEG(K,L)
CNEGATIVE WT. LOSS IS WEIGHT INCREASE
      WCPCV(K,L) = (WATC(K,L) * 100.0) / ((SLGT(K,L) / 10.0) * 0.9375)
 1313 CONTINUE
      RETURN
      END

      SUBROUTINE ARVAL
      DIMENSION EMPB(160),FACB(160),ROW(60),SYMBOL(25),SPEC(6),NBIN(6),
     1BLKN(6),IBLKP(6),ISAMT(6),IDATE(6),WINI(6),WREM(6),STDA(6),STDB(6
     2),WLSGAV(12),WCPCAV(12)   ,M(12,6),WETB(12,6),DRYB(12,6),TIUN(12,6
     3),TOUN(12,6),TITM(12,6),TOTM(12,6),SLGT(12,6),WETW(12,6),DRYW(12,
     46),WATC(12,6),CONM(12,6),WSEG(12,6),WLSEG(12,6),NSEG(12,6),UNITT(
     512,6),TIME(12,6),RTCM(12,6),CONMAV(12),RTCMAV(12),WCPCV(12,6)
      COMMON      EMPB,FACB,MD,SYMBOL,SPEC,NBIN,BLKN,IBLKP,ISAMT,IDATE,
     1WINI,WREM,STDA,STDB,NB,NS,WCPCV    ,M,WETB,DRYB,TIUN,TOUN,TITM,
     2TOTM,SLGT,WETW,DRYW,WATC,CONM,WSEG,WLSEG,NSEG,UNITT,TIME,RTCM,
     3    WCPCAV,CONMAV,WLSGAV,RTCMAV,ROW
      DO 1412 L= 1,NB
      DO 1412 K= 1,NS
      MP=M(K,L)
C SHORT CIRCUIT IF NO ACET. REDN VALUES
      IF(TITM(K,L).LT.1.0) RTCM(K,L) =  1000.0
      IF (TITM(K,L) .LT. 1.0 ) GO TO 1412
```

```
      UNITT(K,L)=TIUN(K,L)-TOUN(K,L)
      TIME(K,L)=TITM(K,L)-TOTM(K,L)
C FINDS ODD/EVEN FOR STANDARDS
      I=MP/2
      D=I
      F=MP
      E=F/2.0
      IF ( E - D .GT. 0.0 ) GO TO 1410
      RTCM(K,L) = (((UNITT(K,L) / TIME(K,L) ) * FACB(MP) ) / STDA(L) )
     2 / ((SLGT(K,L) / 10.0 ) * 0.9375)
      GO TO 1412
 1410 RTCM(K,L) = (((UNITT(K,L) / TIME(K,L) ) * FACB(MP) ) / STDB(L) )
     2 / ((SLGT(K,L) / 10.0 ) * 0.9375)
 1412 CONTINUE
      RETURN
      END

      SUBROUTINE AVBLK
      DIMENSION SPEC(12),FACB(168),RCW(6),CONM(6,12),SEGL(12),NBIN(12),
     1ISAMT(6),IDATE(6),ISAMT(6),IDATE(6),NBIN(6),WHBIN(6),STDA(6),STDB(6
     2),WLSGAV(12),WCPCAV(12)  ,NS(12,6),WETT(12,6),CONM(12,6),TIUN(12,6
     3),TOUN(12,6),TITM(12,6),TOTM(12,6),SLGT(12,6),WETW(12,6),RCWW(12,
     4L),WATC(12,6),CONM(12,6),WSEG(12,6),WLSEG(12,6),WSEG(12,6),UNITT(
     512,6),TIME(12,6),RTCM(12,6),WCPCV(12,6),CONMAV(12),RTCMAV(12),WCPCV(12,6)
      COMMON      SPEC,FACB,MP,SYMBOL,SEGL,NBIN,ISAMT,IDATE,
     1NBIN,WHBIN,STDA,STDB,NB,NS,WCPCV  ,CONM,WETT,DRYT,TIUN,TOUN,TITM,
     2TOTM,SLGT,WETW,DRYW,WATC,RCWW,WSEG,WLSEG,WSEG,UNITT,TIME,RTCM,
     3  WCPCAV,CONMAV,WLSGAV,RTCMAV,RCW
      L = 1
      WRITE(6,1620)SPEC(L),NBIN(L),ISAMT(L),IDATE(L)
 1620 FORMAT(1H1,4HWOOD,1X,A5,3X,3HBIN,1X,I2,3X,11HSAMPLE TIME,1X,I3,
     26X,4HDATE,1X,I6)
      WRITE (6,1617)
 1617 FORMAT (1X,/15HSEGMENT RESULTS,/)
      WRITE (6,1612)
 1612 FORMAT (3X,6HM.CONT)
      WRITE(6,1611)
 1611 FORMAT (1X,  26X,4HMEAN,4X,9HPLUS SERR,2X,10HMINUS SERR,3X,
     2 9HSTAND ERR,3X,9HSTAND DEV,3X,8HVARIANCE,4X,9HCOEFF VAR,3X,2H DF)
      CALL SEGFL (CONM,NB,NS,CONMAV)
      WRITE (6,1613)
 1613 FORMAT(3X,5HWLSEG)
      WRITE (6,1611)
      CALL SEGFL (WLSEG,NB,NS,WLSGAV)
      WRITE(6,1614)
 1614 FORMAT (3X,7HRT.CM.)
      WRITE (6,1611)
      CALL SEGFL (RTCM,NB,NS,RTCMAV)
      WRITE (6,1615)
 1615 FORMAT (3X,5HWCPCV)
      WRITE (6,1611)
      CALL SEGFL (WCPCV,NB,NS,WCPCAV)
      WRITE(6,1620)SPEC(L),NBIN(L),ISAMT(L),IDATE(L)
      WRITE (6,1616)
 1616 FORMAT (1X,/ 13HZONE AVERAGES,/)
      WRITE(6,1612)
      WRITE (6,1611)
      CALL ZONAV(CONM,NB,NS)
      WRITE(6,1613)
      WRITE(6,1611)
      CALL ZONAV (WLSEG,NB,NS)
      WRITE(6,1614)
      WRITE(6,1611)
      CALL ZONAV (RTCM,NB,NS)
      WRITE (6,1615)
      WRITE (6,1611)
      CALL ZONAV (WCPCV,NB,NS)
      RETURN
      END

      SUBROUTINE SEGFL (ABC,NB,NS,DEF)
      DIMENSION X(20),ABC(12,6),DEF (12)
      DO 1598 K=1,NS
      I  = 0
      SUM = 0.0

      DO 1597 L=1,NB
      I=I+1
      X(I)=ABC(K,L)
 1597 CONTINUE
      CALL AVRAGE(X,I,XBAR)
      DEF(K)=XBAR
 1598 CONTINUE
      RETURN
      END

      SUBROUTINE ZONAV (GHI,NB,NS)
      DIMENSION X(20) , GHI(12,6)
      IK = 1
 1699 I = 0
      KI = IK + 3
      DO 1697 K = IK,KI
      DO 1697 L = 1 ,NB
      I = I +1
      X(I) = GHI(K,L)
 1697 CONTINUE
      CALL AVRAGE (X,I,XBAR)
      IK = KI + 1
      IF (IK.NE. NS +1 ) GO TO 1699
      RETURN
      END

      SUBROUTINE AVRAGE (X,NTMS,XAV )
      DIMENSION X(20)
      N = NDF = 0
      SM= SSQ= XBAR= XPLUS= XMINUS= SERR= SDEV= VAR= COEFF= XAV= 0.0
      DO 1712 I=1,NTMS
      IF ( X(I) .GT. 999.999) GO TO 1712
      SM=SM+X(I)
      SSQ=SSQ + X(I) * X(I)
      N=N+1
 1712 CONTINUE
      IF ( SM .GT. -1.0E-10 .AND. SM .LT. 1.0E-10) GO TO 1714
      AN=N
      XBAR = SM/AN
      XAV = SM / AN
      IF ( N .EQ. 1 )  GO TO 1714
      VAR = (SSQ - SM * XBAR) / ( AN - 1.0)
      SDEV = SQRT (VAR)
      COEFF = (SDEV / XBAR)* 100.0
      SERR = SDEV / SQRT (AN)
      NDF = N-1
```

```
      XPLUS = XBAR + SERR
      XMINUS = XBAR - SERR
      WRITE (6,1711) XBAR,XPLUS,XMINUS,SERR,SDEV,VAR,COEFF,NDF
 1711 FORMAT(2X,7(F10.2,2X),I3)
      RETURN
      END
      SUBROUTINE DATOT
      DIMENSION [illegible]
      COMMON [illegible]
      DO 1518 L=1,NB
      BWWSM = 0.0
      BWDSM = 0.0
      BWESM = 0.0
      WRITE(6,1543)
 1543 FORMAT (1H1)
      DO 1511 MJ=1,3
      WRITE(6,1510)SPEC(L),NBIN(L),ISAMT(L),BLKN(L),IBLKP(L),IDATE(L)
 1510 FORMAT(1X,' 4HWOOD.1X,A5,3X,3HBIN.1X,I2,3X,11HSAMPLE TIME.1X,I3,
     23X,5HBLOCK.1X,A4,3X,4HPOSN.1X,I2,3X,4HDATE,1X,I6)
 1511 CONTINUE
      WRITE(6,1523)
 1523 FORMAT ( )
      WRITE (6,1512)
 1512 FORMAT(2X,4HBNUM,2X,4HEMPB,2X,4HFACB,2X,4HWETB,2X,4HDRYB,3X,
     24HTIUN,5X,4HTOUN,2X,4HTITH,2X,4HTOTM,1X,4HSLGT)
      DO 1514 K=1,NS
      MP=M(K,L)
      IF ( M(K,L) .LT. 1 ) MP = 159
      WRITE(6,1513)M(K,L),EMPB(MP),FACB(MP),WETB(K,L),DRYB(K,L),
     2TIUN(K,L),TOUN(K,L),TITM(K,L),TOTM(K,L),SLGT(K,L)
 1513 FORMAT(3X,I3,1X,4(F5.2,1X),2(F7.1,1X),2(F5.2,1X),F4.1)
 1514 CONTINUE
      WRITE(6,1523)
      WRITE(6,1515)
 1515 FORMAT (1X,3HSEG,1X,5HWETWT,1X,5HDRYWT,2X,5HUNITS,3X,4HTIME,1X,
     2 5HWATER,2X,5HWCPCV,4X,5HMCONT,3X,5HWLSEG,3X,6HRATECM)
      DO 1517 K=1,NS
      WRITE (6,1516) NSEG(K,L), WETW(K,L), DRYW(K,L), UNITT(K,L),
     2 TIME(K,L), WATC(K,L), WCPCV(K,L), CONM(K,L), WLSEG(K,L),
     3 RTCM(K,L)
 1516 FORMAT(2X,I2,1X,2(F5.2,1X),F7.1,1X,2(F5.2,1X),2(F6.2,3X),F6.2,3X,
     2 F9.4)
 1517 CONTINUE
      WRITE (6,1525)
 1525 FORMAT (1X,/    13HBLOCK RESULTS,/)
      DO 1528 K=1,NS
      MP=M(K,L)
      IF ( M(K,L) .LT. 1 ) MP =159
      BWWSM=BWWSM+WETB(K,L)
      BWDSM=BWDSM+DRYB(K,L)
      BWESM=BWESM+EMPB(MP)
 1528 CONTINUE
      RATIO = 50.0 / 46.0
      WCAEV = (BWWSM-BWDSM)* RATIO
      DWEB =(BWDSM- BWESM) * RATIO
      WLSPC =((WINI(L) -  DWEB  ) / WINI(L)) * 100.0
      WCOREM = WREM(L) - DWEB
      CMOREM =(WCOREM / DWEB) * 100.0
      WRITE (6,1526)
 1526 FORMAT (1X,5HBLOCK,1X,5HBWWSM,2X,5HBWDSM,2X,5HBWESM,3X,4HWINI,
     2 2X,4HWREM,1X,5HWCAEV,2X,4HDWEB,1X,5HWLSPC,1X,5HWCORM,1X,
     3 5HMCORM)
      WRITE (6,1527) BLKN(L), BWWSM, BWDSM, BWESM, WINI(L), WREM(L),
     2 WCAEV, DWEB, WLSPC, WCOREM, CMOREM
 1527 FORMAT(1X,A4,2X,3(F6.2,1X),6(F5.2,1X),F6.2)
      WRITE (6,1529)
 1529 FORMAT (1X,/  1X,12HZONE RESULTS)
      WRITE (6,1530)
 1530 FORMAT (11X,/ 16HMOISTURE CONTENT)
      WRITE(6,1611)
 1611 FORMAT (1X,  26X,4HMEAN,4X,9HPLUS SERR,2X,10HMINUS SERR,3X,
     2 9HSTAND ERR,2X,9HSTAND DEV,3X,8HVARIANCE,4X,9HCOEFF VAR,3X,2HDF)
      CALL ZONER (CONM,L)
      WRITE(6,1535)
 1535 FORMAT (11X,/ 11HWEIGHT LOSS)
      WRITE(6,1611)
      CALL ZONER (WLSEG,L)
      WRITE (6,1536)
 1536 FORMAT (11X,/ 18HACET REDN RATE CM3)
      WRITE(6,1611)
      CALL ZONER (RTCM,L)
      WRITE (6,1537)
 1537 FORMAT (11X,/ 18HWAT CONT PER C VOL)
      WRITE(6,1611)
      CALL ZONER (WCACV,L)
 1518 CONTINUE
      RETURN
      END
      SUBROUTINE ZONER(XYZ,L)
      DIMENSION  X(20),XYZ(12,6)
      IK = 1
 1598 IN = 0
      KI = IK + 3
      DO 1599 K = IK,KI
      IN = IN + 1
      X(IN) = XYZ(K,L)
 1599 CONTINUE
      CALL AVRAGE(X,IN,XBAR)
      IK = KI + 1
      IF (IK .NE. 13) GO TO 1598
      RETURN
      END
      SUBROUTINE MAPER
      DIMENSION [illegible]
      COMMON [illegible]
```

```
      SUBROUTINE DENSITY(NB,NS,NBIN,ISAMT,IBLKW,IBLKP,IDATE,ITIME,SLGT,
     *  WCPCV,CONMAV,WLSGAV,RTCMAV,CONM,WLSEG,RTCM,WCPCV)
      INTO=2
      IFS = 3
      INTA=2
      DO 2003 NO= 1, 60
 2003 ROW(NO)  = SYMBOL(23)
      INGP=4
      WRITE(6,1917)
 1917 FORMAT (14H1 DENSITY MAPS.//)
      WRITE(6,1920)
 1920 FORMAT (1HQ)
      DO 1900 L=1,NB
      IK=1
      DO 1909 JM = 1,3
      WRITE(6,1510)SPEC(L),NBIN(L),ISAMT(L),BLKW(L),IBLKP(L),IDATE(L)
 1510 FORMAT(1X,4HWOOD,1X,A5,3X,3HBIN,1X,I2,3X,11HSAMPLE TIME,1X,I3,
     23X,5HBLOCK,1X,A4,3X,4HPOSN,1X,I2,3X,4HDATE,1X,I6)
 1909 CONTINUE
      WRITE(6,1910)
 1910 FORMAT(1HO,7X,16HMOISTURE CONTENT,12X,15HWEIGHT LOSS SEG,12X,
     1 19HACET REDN RATE CM 3,10X,18HWAT CONT PER C VOL)
 1908 KI=IK+3
      N=1
      DO 1902 K=IK,KI
      IJ=CONM(K,L)/10.0 + 1.0
      IF(IJ.GT.20)IJ=21
      IF(IJ.LT.0)IJ=22
 1902 Y(K,L,N)=SYMBOL(IJ)
      N=2
      DO 1903 K=IK,KI
      IJ=WLSEG(K,L)/5.0 + 1.0
      IF(IJ.GT.20)IJ=21
      IF ( WLSEG(K,L) .LT. -0.00001 ) IJ = 22
 1903 Y(K,L,N) = SYMBOL(IJ)
      N=3
      DO 1904 K=IK,KI
      IF ( RTCM(K,L) .LT. 0.001 )  RTCM(K,L) = 0.0011
      IF (RTCM(K,L) .GT. 999.999) IJ = 25
      IF (RTCM(K,L) .GT. 999.999) GO TO 1904
      IJ=(ALOG10(RTCM(K,L)*1000.0))*4.0 + 1.0
      IF(IJ.GT.20)IJ=21
 1904 Y(K,L,N)=SYMBOL(IJ)
      N = 4
      DO 1918 K = IK,KI
      IJ = WCPCV (K,L) / 5.0 + 1.0
      IF (IJ .GT. 20) IJ = 21
      IF ( IJ .LT. 0) IJ = 22
      Y (K,L,N) = SYMBOL (IJ)
 1918 LGT = SLGT (K,L) - 6.0
      CALL LINER(INTO,IFS,INGP,SYMBOL,ROW,Y,L,IK,KI,INTA)
      DO 1905 IJK=1,LGT
      WRITE (6,1906)  (ROW(NO), NO = 1, 60)
 1906 FORMAT (1X,60A2)
 1905 CONTINUE
      WRITE(6,1907)
 1907 FORMAT ( )
      IK=KI+1
      IF(L.EQ.6.AND.IK.EQ.13)GO TO 1914
      IF(IK.EQ.13)GO TO 1900
      GO TO 1908
 1900 CONTINUE
      L=NB
      WRITE (6,2231)
 2231 FORMAT (1X,////,8HAVERAGES,/////)
      DO 1915 JM=1,3
      WRITE(6,1916)SPEC(L),NBIN(L),ISAMT(L),IDATE(L)
 1916 FORMAT(1X,4HWOOD,1X,A5,3X,3HBIN,1X,I2,3X,11HSAMPLE TIME,1X,I3,
     26X,4HDATE,1X,I6)
 1915 CONTINUE
      WRITE(6,1910)
      L=6
      DO 2230 K=1,NS
      CONM(K,L)=CONMAV(K)
      WLSEG(K,L)=WLSGAV(K)
      RTCM(K,L)=RTCMAV(K)
      WCPCV(K,L) = WCPCAV (K)
      SLGT(K,L)=15.0
 2230 CONTINUE
      IK=1
      GO TO 1908
 1914 WRITE (6,1919)
 1919 FORMAT (1HR)
      RETURN
      END

      SUBROUTINE LINER(INTO,IFS,INGP,SYMBOL,ROW,Y,L,IK,KI,INTA)
      DIMENSION SYMBOL(25),ROW(80),Y(12,6,4)
      IB=1
      IC=0
      CALL PLOTER(IB,INTA,IC,ROW,SYMBOL(23))
      DO 2001 N=1,INGP
      DO 2002 K=IK,KI
      SYM=Y(K,L,N)
      IB=IC+1
      CALL PLOTER(IB,IFS,IC,ROW,SYM)
 2002 CONTINUE
      IB=IC+1
      CALL PLOTER(IB,INTO,IC,ROW,SYMBOL(23))
 2001 CONTINUE
      RETURN
      END

      SUBROUTINE PLOTER(IB,IFS,IC,ROW,SYM)
      DIMENSION ROW(80)
      IC=(IB+IFS)-1
      DO 2000 ID=IB,IC
 2000 ROW(IC)=SYM
      RETURN
      END
```

TIGROT Input

Card 1 - 40 : Code number of bottles, Empty bottle weight, Volume factor. (160 bottles, 40 cards)

e.g. 001_41•98_55•32____002_44•43_53•16____003_42•41_55•34____004_44•05_52•88

Card 41 : Symbols for density map

e.g. 1 2 3 4 5 6 7 8 9101112131415161718192O++--____..

Card 42 : Number of replicate blocks (usually 2 to 5), Number of segments per block (usually 12)

e.g. 02_12

Card 43 : Data for block, as in Figure 18 ; Species code, Soil container code, Block number, Position of block, Sample time in days, Block initial weight, Block weight on sampling, Ethylene standard for Column A, and Column B, of gas chromatograph.

e.g. SPINE__09__IE16__18__014__300975__08•11__10•49__6100•0__8900•0

Card 44 - 55 : Data for segments, as in Figure 18 ; Segment code number, Bottle number, Wt of bottle + wood wet, Wt. of bottle + oven dried wood, Units of ethylene at time 0, Units of ethylene at time 1, Time 1 in hours, Time 0 in hours, Length of segment in mm.

e.g. _01_097_42•80_42•62__220•0_____220•0_____07•86__01•00__17•0

Card 56 : Data for block, as Card 43 above.

Card 57 - 68 : Data for segments, as 44 - 55 above.

Card 69 : Cards in same format as 42 onwards continue data processing. If 00_00, program stops.

e.g. 00_00

TIGROT Output

WOOD SPINE BIN 9 SAMPLE TIME 14 BLOCK IE16 POSN 18 DATE 300975
WOOD SPINE BIN 9 SAMPLE TIME 14 BLOCK IE16 POSN 18 DATE 300975
WOOD SPINE BIN 9 SAMPLE TIME 14 BLOCK IE16 POSN 18 DATE 300975

BNUM	BMPB	FACB	WETB	DRYB	TIUN	TOUN	TITM	TOTM	SLGT
97	42.02	54.78	42.80	42.62	220.0	220.0	7.86	1.00	17.0
98	41.97	56.74	42.90	42.68	100.0	100.0	7.88	1.00	17.0
99	44.14	53.32	44.98	44.70	0	0	0	1.01	17.0
100	43.99	52.64	44.58	44.45	0	145.0	0	1.01	17.0
101	43.14	54.32	43.78	43.63	210.0	210.0	7.91	1.05	14.0
102	43.20	53.10	43.90	43.80	135.0	135.0	7.93	1.05	14.0
103	42.62	55.66	43.21	43.01	0	0	0	1.05	14.0
104	42.92	54.64	43.48	43.34	0	0	0	1.06	14.0
105	41.88	57.52	42.55	42.40	215.0	215.0	7.95	1.06	15.0
106	42.36	55.22	43.21	43.00	140.0	140.0	7.98	1.08	15.0
107	42.54	54.12	43.36	43.14	0	0	0	1.08	15.0
108	43.43	53.14	44.28	44.12	0	0	0	1.08	15.0

SEG	WETWT	DRYWT	UNITS	TIME	WATER	WCPCV	MCONT	WLSEG	RATECM
1	.78	.60	0	6.86	.18	11.29	30.00	12.96	0
2	.92	.71	0	6.88	.21	13.19	29.58	-3.00	0
3	.84	.65	8000.0	23.55	.19	11.92	29.23	5.71	1000.0000
4	.59	.46	12325.0	23.58	.13	8.16	28.26	33.27	1000.0000
5	.65	.49	0	6.86	.16	12.19	32.65	13.69	0
6	.79	.60	0	6.88	.19	14.48	31.67	-5.69	0
7	.79	.59	1500.0	23.62	.20	15.24	33.90	-3.93	1000.0000
8	.56	.42	1185.0	23.66	.14	10.67	33.33	26.02	1000.0000
9	.67	.52	0	6.89	.15	10.67	28.85	14.51	0
10	.84	.65	0	6.90	.21	14.93	32.31	-6.86	0
11	.82	.62	1850.0	23.67	.20	14.22	32.26	-1.93	1000.0000
12	.65	.49	845.0	23.69	.14	11.38	32.65	19.44	1000.0000

```
BLOCK RESULTS

 BLOCK BWWSH  BWDSH   BWESM    WINI  WREM VCAEV  DWEB WLSPC WCORM MCORM
 IE16  523.12 521.00 514.20  8.11 10.49  2.30  7.39  8.86  3.10  41.92

 ZONE RESULTS

MOISTURE CONTENT
                           MEAN   PLUS SERR  MINUS SERR  STAND ERR  STAND DEV  VARIANCE  COEFF VAR  DF
                         29.267     29.638     28.897       .371       .741      .549      2.532    3
                         32.888     33.368     32.408       .480       .960      .922      2.920    3
                         31.516     32.411     30.622       .894      1.789     3.199      5.676    3

WEIGHT LOSS
                           MEAN   PLUS SERR  MINUS SERR  STAND ERR  STAND DEV  VARIANCE  COEFF VAR  DF
                         12.236     19.969      4.503      7.733     15.466   239.196    126.395    3
                          7.522     15.081      -.038      7.559     15.119   224.577    201.005    3
                          6.289     12.620      -.043      6.332     12.664   160.374    201.381    3

ACET REDN RATE CM3
                           MEAN   PLUS SERR  MINUS SERR  STAND ERR  STAND DEV  VARIANCE  COEFF VAR  DF
                              0          0          0          0          0         0          0    0
                              0          0          0          0          0         0          0    0
                              0          0          0          0          0         0          0    0

WAT CONT PER C VOL
                           MEAN   PLUS SERR  MINUS SERR  STAND ERR  STAND DEV  VARIANCE  COEFF VAR  DF
                         11.137     12.205     10.070      1.068      2.135     4.560     19.174    3
                         13.143     14.192     12.094      1.049      2.098     4.402     15.964    3
                         12.800     13.847     11.753      1.047      2.093     4.383     16.356    3

 WOOD SPINE   BIN  9   SAMPLE TIME  14      DATE 300975

SEGMENT RESULTS

   M.CONT
                           MEAN   PLUS SERR  MINUS SERR  STAND ERR  STAND DEV  VARIANCE  COEFF VAR  DF
                         30.389     31.379     29.399       .990      1.979     3.918      6.513    3
                         30.550     31.026     30.074       .476       .952      .905      3.115    3
                         28.966     29.641     28.291       .675      1.351     1.824      4.663    3
                         28.557     29.225     27.890       .668      1.336     1.784      4.677    3
                         36.371     37.773     34.970      1.402      2.803     7.857      7.707    3
                         33.247     34.585     31.910      1.338      2.675     7.156      8.046    3
                         35.148     36.598     33.698      1.450      2.900     8.408      8.250    3
                         34.677     36.024     33.329      1.348      2.695     7.264      7.772    3
                         35.962     39.175     32.748      3.213      6.427    41.306     17.872    3
                         32.737     33.731     31.744       .994      1.987     3.949      6.070    3
                         34.627     35.529     33.725       .902      1.804     3.253      5.209    3
                         33.387     34.314     32.461       .927      1.853     3.435      5.551    3

   WLSEG
                           MEAN   PLUS SERR  MINUS SERR  STAND ERR  STAND DEV  VARIANCE  COEFF VAR  DF
                         13.266     15.065     11.468      1.798      3.597    12.937     27.113    3
                         -2.265      1.287     -5.818      3.552      7.105    50.478   -313.654    3
                          5.482      9.596      1.368      4.114      8.229    67.710    150.099    3
                         24.709     28.017     21.402      3.308      6.615    43.759     26.771    3
                         13.793     14.802     12.784      1.009      2.018     4.071     14.628    3
                         -7.988     -6.366     -9.610      1.622      3.244    10.526    -40.616    3
                          2.070      4.291      -.152      2.221      4.443    19.737    214.641    3
                         26.019     26.454     25.583       .436       .871      .759      3.348    3
                         16.626     20.027     13.225      3.401      6.803    46.278     40.916    3
                         -8.868     -6.419    -11.317      2.449      4.899    23.996    -55.239    3
                         -3.466       .671     -7.603      4.137      8.275    68.471   -238.738    3
                         28.462     32.064     24.860      3.602      7.204    51.898     25.311    3

   RT.CM.3
                           MEAN   PLUS SERR  MINUS SERR  STAND ERR  STAND DEV  VARIANCE  COEFF VAR  DF
                              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          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
                              0          0          0          0          0         0          0    0
                              0          0          0          0          0         0          0    0
                              0          0          0          0          0         0          0    0

   WCPCV
                           MEAN   PLUS SERR  MINUS SERR  STAND ERR  STAND DEV  VARIANCE  COEFF VAR  DF
                         11.712     12.610     10.815       .898      1.796     3.224     15.331    3
                         13.783     14.174     13.392       .391       .783      .613      5.680    3
                         12.047     12.089     12.005       .042       .084      .007       .694    3
                          9.506     10.041      8.971       .535      1.070     1.146     11.261    3
                         13.892     14.770     13.014       .878      1.756     3.085     12.643    3
                         15.886     16.786     14.985       .900      1.801     3.243     11.336    3
                         15.187     15.792     14.583       .605      1.209     1.462      7.961    3
                         11.378     12.146     10.610       .768      1.536     2.360     13.502    3
                         13.167     14.162     12.171       .995      1.991     3.963     15.119    3
                         15.733     16.275     15.192       .542      1.083     1.173      6.885    3
                         15.889     17.060     14.718      1.171      2.341     5.481     14.735    3
                         10.511     10.840     10.182       .329       .658      .433      6.257    3
```

WOOD SPINE BIN 9 SAMPLE TIME 14 DATE 300975

ZONE AVERAGES

M.CONT

	MEAN	PLUS SERR	MINUS SERR	STAND ERR	STAND DEV	VARIANCE	COEFF VAR	DF
	29.616	30.010	29.221	.394	1.578	2.491	5.328	15
	34.841	35.544	34.178	.683	2.733	7.471	7.841	15
WLSEG	34.178	35.045	33.312	.866	3.466	12.011	10.140	15

	MEAN	PLUS SERR	MINUS SERR	STAND ERR	STAND DEV	VARIANCE	COEFF VAR	DF
	10.298	13.267	7.330	2.968	11.874	140.982	115.299	15
	8.473	11.926	5.121	3.353	13.411	179.848	158.269	15
RT.CM.3	8.189	12.376	4.001	4.187	16.749	280.516	204.538	15

	MEAN	PLUS SERR	MINUS SERR	STAND ERR	STAND DEV	VARIANCE	COEFF VAR	DF
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
WCPCV	0	0	0	0	0	0	0	0

	MEAN	PLUS SERR	MINUS SERR	STAND ERR	STAND DEV	VARIANCE	COEFF VAR	DF
	11.762	12.228	11.297	.465	1.862	3.467	15.830	15
	14.046	14.655	13.517	.569	2.277	5.183	16.169	15
	13.825	14.503	13.147	.678	2.713	7.361	19.625	15

WOOD SPINE BIN 9 SAMPLE TIME 14 BLOCK IE16 POSN 1A DATE 300975
WOOD SPINE BIN 9 SAMPLE TIME 14 BLOCK IE16 POSN 1A DATE 300975
WOOD SPINE BIN 9 SAMPLE TIME 14 BLOCK IE16 POSN 1A DATE 300975

MOISTURE CONTENT

```
3 3 3 2 2 2 2 2 2 2 2 2
3 3 3 2 2 2 2 2 2 2 2 2
3 3 3 2 2 2 2 2 2 2 2 2
3 3 3 2 2 2 2 2 2 2 2 2
3 3 3 2 2 2 2 2 2 2 2 2
3 3 3 2 2 2 2 2 2 2 2 2
3 3 3 2 2 2 2 2 2 2 2 2
3 3 3 2 2 2 2 2 2 2 2 2
3 3 3 2 2 2 2 2 2 2 2 2
3 3 3 2 2 2 2 2 2 2 2 2

3 3 3 3 3 3 3 3 3 3 3 3
3 3 3 3 3 3 3 3 3 3 3 3
3 3 3 3 3 3 3 3 3 3 3 3
3 3 3 3 3 3 3 3 3 3 3 3
3 3 3 3 3 3 3 3 3 3 3 3
3 3 3 3 3 3 3 3 3 3 3 3
3 3 3 3 3 3 3 3 3 3 3 3
3 3 3 3 3 3 3 3 3 3 3 3

2 2 2 3 3 3 3 3 3 3 3 3
2 2 2 3 3 3 3 3 3 3 3 3
2 2 2 3 3 3 3 3 3 3 3 3
2 2 2 3 3 3 3 3 3 3 3 3
2 2 2 3 3 3 3 3 3 3 3 3
2 2 2 3 3 3 3 3 3 3 3 3
2 2 2 3 3 3 3 3 3 3 3 3
2 2 2 3 3 3 3 3 3 3 3 3
```

WEIGHT LOSS SEG

```
2 2 2 - - - 1 1 1 6 6 6
2 2 2 - - - 1 1 1 6 6 6
2 2 2 - - - 1 1 1 6 6 6
2 2 2 - - - 1 1 1 6 6 6
2 2 2 - - - 1 1 1 6 6 6
2 2 2 - - - 1 1 1 6 6 6
2 2 2 - - - 1 1 1 6 6 6
2 2 2 - - - 1 1 1 6 6 6
2 2 2 - - - 1 1 1 6 6 6
2 2 2 - - - 1 1 1 6 6 6

2 2 2 - - - - - - 5 5 5
2 2 2 - - - - - - 5 5 5
2 2 2 - - - - - - 5 5 5
2 2 2 - - - - - - 5 5 5
2 2 2 - - - - - - 5 5 5
2 2 2 - - - - - - 5 5 5
2 2 2 - - - - - - 5 5 5
2 2 2 - - - - - - 5 5 5

2 2 2 - - - - - - 3 3 3
2 2 2 - - - - - - 3 3 3
2 2 2 - - - - - - 3 3 3
2 2 2 - - - - - - 3 3 3
2 2 2 - - - - - - 3 3 3
2 2 2 - - - - - - 3 3 3
2 2 2 - - - - - - 3 3 3
2 2 2 - - - - - - 3 3 3
```

ACET REDN RATE CM 3

```
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 . . . . . .
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 . . . . . .
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 . . . . . .

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 . . . . . .
0 0 0 0 0 0 . . . . . .
0 0 0 0 0 0 . . . . . .
```

WAT CONT PER C VOL

```
2 2 2 2 2 2 2 2 2 1 1 1
2 2 2 2 2 2 2 2 2 1 1 1
2 2 2 2 2 2 2 2 2 1 1 1
2 2 2 2 2 2 2 2 2 1 1 1
2 2 2 2 2 2 2 2 2 1 1 1
2 2 2 2 2 2 2 2 2 1 1 1
2 2 2 2 2 2 2 2 2 1 1 1
2 2 2 2 2 2 2 2 2 1 1 1
2 2 2 2 2 2 2 2 2 1 1 1
2 2 2 2 2 2 2 2 2 1 1 1

2 2 2 2 2 2 3 3 3 2 2 2
2 2 2 2 2 2 3 3 3 2 2 2
2 2 2 2 2 2 3 3 3 2 2 2
2 2 2 2 2 2 3 3 3 2 2 2
2 2 2 2 2 2 3 3 3 2 2 2
2 2 2 2 2 2 3 3 3 2 2 2
2 2 2 2 2 2 3 3 3 2 2 2
2 2 2 2 2 2 3 3 3 2 2 2

2 2 2 2 2 2 2 2 2 2 2 2
2 2 2 2 2 2 2 2 2 2 2 2
2 2 2 2 2 2 2 2 2 2 2 2
2 2 2 2 2 2 2 2 2 2 2 2
2 2 2 2 2 2 2 2 2 2 2 2
2 2 2 2 2 2 2 2 2 2 2 2
2 2 2 2 2 2 2 2 2 2 2 2
2 2 2 2 2 2 2 2 2 2 2 2
```

WOOD SPINE BIN 9 SAMPLE TIME 14 DATE 300975
WOOD SPINE BIN 9 SAMPLE TIME 14 DATE 300975
WOOD SPINE BIN 9 SAMPLE TIME 14 DATE 300975

MOISTURE CONTENT

```
3 3 3 3 3 3 2 2 2 2 2 2
3 3 3 3 3 3 2 2 2 2 2 2
3 3 3 3 3 3 2 2 2 2 2 2
3 3 3 3 3 3 2 2 2 2 2 2
3 3 3 3 3 3 2 2 2 2 2 2
3 3 3 3 3 3 2 2 2 2 2 2
3 3 3 3 3 3 2 2 2 2 2 2
3 3 3 3 3 3 2 2 2 2 2 2
3 3 3 3 3 3 2 2 2 2 2 2
3 3 3 3 3 3 2 2 2 2 2 2

3 3 3 3 3 3 3 3 3 3 3 3
3 3 3 3 3 3 3 3 3 3 3 3
3 3 3 3 3 3 3 3 3 3 3 3
3 3 3 3 3 3 3 3 3 3 3 3
3 3 3 3 3 3 3 3 3 3 3 3
3 3 3 3 3 3 3 3 3 3 3 3
3 3 3 3 3 3 3 3 3 3 3 3
3 3 3 3 3 3 3 3 3 3 3 3

3 3 3 3 3 3 3 3 3 3 3 3
3 3 3 3 3 3 3 3 3 3 3 3
3 3 3 3 3 3 3 3 3 3 3 3
3 3 3 3 3 3 3 3 3 3 3 3
3 3 3 3 3 3 3 3 3 3 3 3
3 3 3 3 3 3 3 3 3 3 3 3
3 3 3 3 3 3 3 3 3 3 3 3
3 3 3 3 3 3 3 3 3 3 3 3
```

WEIGHT LOSS SEG

```
2 2 2 - - - 1 1 1 4 4 4
2 2 2 - - - 1 1 1 4 4 4
2 2 2 - - - 1 1 1 4 4 4
2 2 2 - - - 1 1 1 4 4 4
2 2 2 - - - 1 1 1 4 4 4
2 2 2 - - - 1 1 1 4 4 4
2 2 2 - - - 1 1 1 4 4 4
2 2 2 - - - 1 1 1 4 4 4
2 2 2 - - - 1 1 1 4 4 4
2 2 2 - - - 1 1 1 4 4 4

2 2 2 - - - 0 0 0 5 5 5
2 2 2 - - - 0 0 0 5 5 5
2 2 2 - - - 0 0 0 5 5 5
2 2 2 - - - 0 0 0 5 5 5
2 2 2 - - - 0 0 0 5 5 5
2 2 2 - - - 0 0 0 5 5 5
2 2 2 - - - 0 0 0 5 5 5
2 2 2 - - - 0 0 0 5 5 5

3 3 3 - - - - - - 5 5 5
3 3 3 - - - - - - 5 5 5
3 3 3 - - - - - - 5 5 5
3 3 3 - - - - - - 5 5 5
3 3 3 - - - - - - 5 5 5
3 3 3 - - - - - - 5 5 5
3 3 3 - - - - - - 5 5 5
3 3 3 - - - - - - 5 5 5
```

ACET REDN RATE CM 3

```
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
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
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
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
0 0 0 0 0 0 0 0 0 0 0 0
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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
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
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
```

WAT CONT PER C VOL

```
2 2 2 2 2 2 2 2 2 1 1 1
2 2 2 2 2 2 2 2 2 1 1 1
2 2 2 2 2 2 2 2 2 1 1 1
2 2 2 2 2 2 2 2 2 1 1 1
2 2 2 2 2 2 2 2 2 1 1 1
2 2 2 2 2 2 2 2 2 1 1 1
2 2 2 2 2 2 2 2 2 1 1 1
2 2 2 2 2 2 2 2 2 1 1 1
2 2 2 2 2 2 2 2 2 1 1 1
2 2 2 2 2 2 2 2 2 1 1 1

2 2 2 3 3 3 3 3 3 2 2 2
2 2 2 3 3 3 3 3 3 2 2 2
2 2 2 3 3 3 3 3 3 2 2 2
2 2 2 3 3 3 3 3 3 2 2 2
2 2 2 3 3 3 3 3 3 2 2 2
2 2 2 3 3 3 3 3 3 2 2 2
2 2 2 3 3 3 3 3 3 2 2 2
2 2 2 3 3 3 3 3 3 2 2 2

2 2 2 3 3 3 3 3 3 2 2 2
2 2 2 3 3 3 3 3 3 2 2 2
2 2 2 3 3 3 3 3 3 2 2 2
2 2 2 3 3 3 3 3 3 2 2 2
2 2 2 3 3 3 3 3 3 2 2 2
2 2 2 3 3 3 3 3 3 2 2 2
2 2 2 3 3 3 3 3 3 2 2 2
2 2 2 3 3 3 3 3 3 2 2 2
```

HISTGRF

This programme calculates the moisture content, weight loss, water content and acetylene reduction rate in segments taken from blocks exposed to soil, as does TIGROT. It then plots histograms and graphs of the results on the line printer.

The main programme calls BOTIN, INDAT, WDVAL, ARVAL and AVBLK, as in TIGROT. Subroutine STORGE transfers the results into a three dimensional array for access by the plotting routines.

HISTR plots a histogram of MC, WL, AR and WC in each of the 12 segments in the blocks at each sample time. It also plots the standard error. It calls GRAMR, PLUSERR and WLGMR. GRAFER 1 calls AVPLOT which sets up an array of values which are first plotted against sample time. The same values are then plotted so that the graphs of MC, WL, AR and WC are all on the same, appropriate, scale. GRAFER 2 calls ARRER which sets up arrays of MC, WL, AR and WC and plots each against the others. Both GRAFER 1 and GRAFER 2 use a modified GRAFIT graph plotting subroutine from the Imperial College Programme Library.

The subroutines common to TIGROT and HISTGRF are not listed. The input to HISTGRF is identical to that of TIGROT, so that the same deck of data cards could be used with both programmes.

HISTGRF could produce graphs on the line printer and on microfilm, from which the graphs in section 2 to 9 were produced. Values for MC, WL, AR and WC in segments 1 to 12 were plotted as the characters A to L. Segments 1 to 4 (from the zone above ground) were plotted as A to D on one vertical line, segments 5 to 8 (from the zone at the ground line) were plotted as E to H on a second line, and the segments 9 to 12 (from the zone below ground) plotted as I to L on a third line. If more than one value occurred on the same point, the number of points was plotted.

To generate the three-dimensional diagrams used in sections 2 to 9, the average MC, WL, AR and WC in each segment at each sample time, was transferred to a two-dimensional array and stored on permanent file. This file was accessed by the MATMAP programme of the Imperial College Programme Library using a Tektronics 4014 graphics terminal. The pictures could be generated on the terminal and then on microfilm, from which the pictures in section 2 to 9 were produced. Time of exposure in weeks is shown on the righthand axis, and segment number and zone on the left-hand axis. The values of MC, WL, AR and WC are plotted vertically.

```
      PROGRAM HISTGRF (INPUT,OUTPUT,TAPE5=INPUT,TAPE6=OUTPUT)
      DIMENSION    SPEC(6), IBLKP(6), WETB(12,6), WETW(12,6), STDA(6),
     2 SYMBOL(25), NBIN(6), ISAMT(6), DRYB(12,6), DRYW(12,6), STDB(6),
     3 CONMAV(12), BLKN(6), IDATE(6), TIUN(12,6), WATC(12,6), WINI(6),
     4 WLSGAV(12), WREM(6),  M(12,6), TOUN(12,6), CONM(12,6), ROW(60),
     5 RTCMAV(12),   VCONM(14,6,12),  TITM(12,6), WSEG(12,6), NBL(12),
     6 WCPCAV(12),   VRTCM(14,6,12),  TOTM(12,6), NSEG(12,6), EMPB(160),
     7 CMSERR(12),  VWCPCV(14,6,12), WCPCV(12,6), SLGT(12,6), FACB(160),
     8 WLSERR(12),  VWLSEG(14,6,12), WLSEG(12,6), TIME(12,6), ICMSR(12),
     9 RTSERR(12),   HISTG(100)     , UNITT(12,6), RTCM(12,6), IWLSR(12),
     A WCSERR(12),   TITLE(80)      , CMARR(1000),RTARR(1000), IRTSR(12),
     B                IVAL(12)      , WLARR(1000),WCARR(1000), IWCSR(12)
           CALL BOTIN ( EMPB, FACB, VCONM, VWLSEG, VRTCM, VWCPCV)
1011       CALL INDAT ( NB, NS, SPEC, NBIN, BLKN, IBLKP, ISAMT, IDATE,
     2  WINI, WREM, STDA, STDB, NSEG, M, WETB, DRYB, TIUN, TOUN, TITM,
     3  TOTM, SLGT )
       IF ( NB .EQ. 0 ) GO TO 1010
           CALL WDVAL ( NB, NS, M, WETB, DRYB, EMPB, SLGT, WETW, DRYW,
     2  WATC, CONM, WSEG, WLSEG, WCPCV, WINI )
           CALL ARVAL ( NB, NS, M, TITM, TIUN, TOUN, TOTM, FACB, STDA,
     2  STDB, UNITT, TIME, RTCM, SLGT )
           CALL AVBLK ( NB, NS, CONM, CONMAV, WLSEG, WLSGAV, RTCM,
     2 RTCMAV, WCPCV, WCPCAV, CMSERR, RTSERR, WLSERR, WCSERR)
           CALL STORGE ( NB, NS, ISAMT, CONM, VCONM, WLSEG, VWLSEG,
     2  RTCM, VRTCM, WCPCV, VWCPCV, CONMAV, WLSGAV, RTCMAV, WCPCAV )
       CALL HISTR        (SPEC, NBIN, ISAMT, IDATE, CONMAV, WLSGAV, NS,
     2  RTCMAV, WCPCAV, HISTG, IVAL, CMSERR, RTSERR, WLSERR, WCSERR)
       GO TO 1011
1010       CALL GRAFER1 ( NOSAMT, NBL, VCONM, VWLSEG, VRTCM, VWCPCV )
           CALL GRAFER2 ( NOSAMT, NBL, VCONM, VWLSEG, VRTCM, VWCPCV )
       STOP
       END


      SUBROUTINE BOTIN ( EMPB, FACB, VCONM, VWLSEG, VRTCM, VWCPCV)
      DIMENSION VCONM(14,6,12), VRTCM(14,6,12), VWCPCV(14,6,12),
     2  VWLSEG(14,6,12), FACB(160), EMPB(160)
C     READS IN BOTTLE NUMBER, VOLUME, FACTOR
      NM = 6
      READ(5,1110)(MDA, EMPB(MDA), FACB(MDA), MDB, EMPB(MDB), FACB(MDB),
     2 MDC, EMPB(MDC), FACB(MDC), MDD, EMPB(MDD), FACB(MDD), MM=1,NM)
1110 FORMAT (4 ( I3, 2F6.2, 4X))
      VALUE = 0.0
      DO 4002 IT = 1, 14
      DO 4002 II = 1, 6
      DO 4002 IK = 1, 12
      VCONM       ( IT, II, IK ) = VALUE
      VWLSEG      ( IT, II, IK ) = VALUE
      VRTCM       ( IT, II, IK ) = VALUE
      VWCPCV      ( IT, II, IK ) = VALUE
4002 CONTINUE
      RETURN
      END


      SUBROUTINE INDAT ( NB, NS, SPEC, NBIN, BLKN, IBLKP, ISAMT, IDATE,
     2  WINI, WREM, STDA, STDB, NSEG, M, WETB, DRYB, TIUN, TOUN, TITM,
     3  TOTM, SLGT )
C     INDAT READS IN HEADING DATA THEN SEGMENT DATA
      DIMENSION SPEC(6), IBLKP(6), WETB(12,6), STDA(6), NBIN(6),
     2  ISAMT(6), DRYB(12,6), STDB(6), BLKN(6), IDATE(6), TIUN(12,6),
     3  WINI(6), TOUN(12,6), TITM(12,6),TOTM(12,6), NSEG(12,6),
     4  SLGT(12,6), M(12,6), WREM(6)
      READ(5,1210) NB, NS
1210 FORMAT ( I2, 1X, I2 )
      IF ( NB .EQ. 0 ) RETURN
      DO 1211 L = 1, NB
      READ(5,1211)SPEC(L),NBIN(L),BLKN(L),IBLKP(L),ISAMT(L),IDATE(L),
     2WINI(L),WREM(L),STDA(L),STDB(L)
1211 FORMAT ( A6, 2X, I2, 2X, A4, 2X, I2, 2X, I3, 2X, I6, 2X,
     2  2 ( F5.2, 2X), 2( F6.1, 2X))
      DO 1213 K=1,NS
      READ(5,1212)NSEG(K,L),M(K,L),WETB(K,L),DRYB(K,L),TIUN(K,L),
     2TOUN(K,L),TITM(K,L),TOTM(K,L),SLGT(K,L)
1212 FORMAT ( 1X, I2, 1X, I3, 2F7.2, 2F10.1, 2F7.2, F6.1)
1213 CONTINUE
1214 CONTINUE
      RETURN
      END


      SUBROUTINE WDVAL ( NB, NS, M, WETB, DRYB, EMPB, SLGT, WETW, DRYW,
     2  WATC, CONM, WSEG, WLSEG, WCPCV , WINI )
      DIMENSION WETB(12,6), WETW(12,6), DRYB(12,6), DRYW(12,6),
     2  WATC(12,6), CONM(12,6), WSEG(12,6), WINI(6),    EMPB(160),
     2  SLGT(12,6), WLSEG(12,6), WCPCV(12,6), M(12,6)
      DO 1313 L=1,NB
      DO 1313 K=1,NS
C     SHORT CIRCUIT IF NO BOTTLES WEIGHED
      IF(M(K,L).EQ.0) GO TO 1314
      NB=M(K,L)
```

```
      WETW(K,L)=WETW(K,L)-EMPR(MP)
      DRYW(K,L)=DRYW(K,L)-EMPR(MP)
      GO TO 1311
 1310 WETW(K,L)=WETW(K,L)
      DRYW(K,L)=DRYW(K,L)
 1311 WATC(K,L)=WETW(K,L)-DRYW(K,L)
      CONM(K,L)=WATC(K,L)*100.0/DRYW(K,L)
      WSEG(K,L) = WTH(L) * SLGT(K,L) / 200.0
      WLSEG(K,L)=(WSEG(K,L)-DRYW(K,L))*100.0/WSEG(K,L)
C NEGATIVE WT. LOSS IS WEIGHT INCREASE
      WCPCV(K,L) = ( WATC(K,L) * 100.0 ) / (( SLGT(K,L) / 100.0 )**0.9375)
 1312 CONTINUE
      RETURN
      END


      SUBROUTINE ARVAL ( NB, NS, M, TITM, TIUN, TOUN, TOTM, FACB, STDA,
     2  STDB, UNITT, TIME, RTCM, SLGT )
      DIMENSION  STDA(6), STDB(6), TIUN(12,6), RTCM(12,6), TOUN(12,6),
     2  TITM(12,6), TOTM(12,6), SLGT(12,6), TIME(12,6), FACB(160),
     3  UNITT(12,6), M(12,6)
      DO 1412 L= 1,NB
      DO 1412 K= 1,NS
      MP=M(K,L)
C SHORT CIRCUIT IF NO RECD. READ VALUES
      IF(TITM(K,L).LT.1.0) RTCM(K,L) =  1000.0
      IF (TITM(K,L) .LT. 1.0 ) GO TO 1412
      UNITT(K,L)=TITM(K,L)-TOUN(K,L)
      TIME(K,L)=TITM(K,L)-TOTM(K,L)
C FINDS ODD/EVEN FOR STANDARDS
      I=MP/2
      J=I
      F=MP
      G=F/2.
      IF ( F - G .GT. 0.0 ) GO TO 1410
      RTCM(K,L) = (( UNITT(K,L) / TIME(K,L) ) * FACB(MP)) / STDA(L))
     2  / (( SLGT(K,L) / 100.0 ) ** 0.9375 )
      GO TO 1412
 1410 RTCM(K,L) = (( UNITT(K,L) / TIME(K,L) ) * FACB(MP)) / STDB(L))
     2  / (( SLGT(K,L) / 100.0 ) ** 0.9375 )
 1412 CONTINUE
      RETURN
      END


      SUBROUTINE AVBLK ( NB, NS, CONM, CONMAV, WLSEG, WLSGAV, RTCM,
     2 RTCMAV, WCPCV, WCPCAV, CMSERR, RTSERR, WLSERR, WCSERR)
      DIMENSION  CONMAV(12), WLSGAV(12), RTCM(12,6), CONM(12,6),
     2  RTCMAV(12), WCPCAV(12), WLSEG(12,6), WCPCV(12,6), CMSERR(12),
     3  WLSERR(12), RTSERR(12), WCSERR(12)
      CALL SEGFL  ( CONM, NB, NS, CONMAV, CMSERR )
      CALL SEGFL  ( WLSEG, NB, NS, WLSGAV, WLSERR )
       CALL SEGFL  ( RTCM, NB, NS, RTCMAV, RTSERR )
      CALL SEGFL  ( WCPCV, NB, NS, WCPCAV, WCSERR )
      RETURN
      END


      SUBROUTINE SEGFL ( AGC, NB, NS, DEF, GHI )
      DIMENSION X(20), ABC(12,6), DEF(12), GHI(12)
      DO 1598 K = 1, NS
      I = 0
      SUM = 0.0
      DO 1597 L = 1, NB
      I = I + 1
      X(I) = ABC(K,L)
 1597 CONTINUE
      CALL AVRAGE ( X, I, XBAR, XERR )
      DEF(K) = XBAR
      GHI(K) = XERR
 1598 CONTINUE
      RETURN
      END


      SUBROUTINE AVRAGE ( X, NTMS, XAV, XPLUS )
      DIMENSION X(20)
      N = 0
        XPLUS = SM = XAV = SSQ = 0.0
      DO 1712 I = 1, NTMS
      IF ( X(I) .GT. 999.999 ) GO TO 1712
      SM = SM + X(I)
      SSQ = SSQ + X(I) * X(I)
      N = N + 1
 1712 CONTINUE
      IF ( SM .GT. -1.0E-10 .AND. SM .LT. 1.0E-10 ) GO TO 1714
      AN = N
      XAV = SM / AN
      IF ( N .EQ. 1 ) GO TO 1714
      XPLUS = XAV + ((SQRT((SSQ-SM*XAV) / (AN-1.0))) / SQRT(AN))
      IF ( XPLUS.LT. -50.0 .OR. XPLUS .GT. 250.0 ) XPLUS = 100.0
 1714 RETURN
      END
```

```
      SUBROUTINE STORGE ( NB, NS, ISAMT, CONM, VCONM, WLSEG, VWLSEG,
     2  RTCM, VRTCM, WCPCV, VWCPCV, CONMAV, WLSGAV, RTCMAV, WCPCAV )
      DIMENSION  ISAMT(6), CONMAV(12), WLSGAV(12), RTCM(12,6),WCPCV(12,6
     2),CONM(12,6), RTCMAV(12), VCONM(14,6,12),WCPCAV(12), VRTCM
     3  (14,6,12), VWCPCV(14,6,12), VWLSEG(14,6,12), WLSEG(12,6)
      IT = ISAMT(1) / 14
      DO 4000 IL = 1, NB
      DO 4000 IK = 1, NS
      VCONM(IT,IL,IK) = CONM(IK,IL)
      VWLSEG(IT,IL,IK) = WLSEG(IK,IL)
      IF (RTCM(IK,IL) .GT. 999.999 ) RTCM(IK,IL) = -0.005
      VRTCM(IT,IL,IK) = RTCM(IK,IL)
      VWCPCV(IT,IL,IK) = WCPCV (IK,IL)
4000  CONTINUE
      IL = 6
      DO 4001 IK = 1, NS
      VCONM(IT,IL,IK) = CONMAV(IK)
      VWLSEG(IT,IL,IK) = WLSGAV(IK)
      VRTCM (IT,IL,IK) = RTCMAV(IK)
      VWCPCV(IT,IL,IK) = WCPCAV(IK)
4001  CONTINUE
      RETURN
      END


      SUBROUTINE HISTR  (SPEC, NBIN, ISAMT, IDATE, CONMAV, WLSGAV, NS,
     2  RTCMAV, WCPCAV, HISTG, IVAL, CMSERR, RTSERR, WLSERR, WCSERR)
      DIMENSION SPEC(6), NBIN(6), ISAMT(6), IDATE(6), CONMAV(12),
     2  WLSGAV(12), RTCMAV(12), WCPCAV(12), HISTG(100), IVAL(12),
     3  CMSERR(12), WLSERR(12), RTSERR(12), WCSERR(12), ICMSR(12)
     4  ,IWLSR(12), IRTSR(12), IWCSR(12)
      L = 1
      WRITE(6,1620)SPEC(L),NBIN(L),ISAMT(L),IDATE(L)
1620  FORMAT(1H1,4HWOOD,1X,A5,3X,3HBIN,1X,I2,3X,11HSAMPLE TIME,1X,I3,
     26X,4HDATE,1X,I6)
      WRITE (6,2401)
2401  FORMAT (1X,/1X,16HMOISTURE CONTENT)
      WRITE ( 6,2402)
2402  FORMAT(1X,30X,1H0,9X,2H25,8X,2H50,8X,  2H75, 8X, 3H100, 7X, 3H125,
     2          7X, 3H150, 7X, 3H175, 7X, 3H200, 7X, 3H225, 8X, 3H250)
      DO 2403 K = 1, NS
      IF ( CONMAV(K) .GT. 250.0 ) CONMAV(K) = 250.0
      IVAL (K) = CONMAV(K) / 2.5 + 1.0
      ICMSR (K) = CMSERR(K) / 2.5 + 1.0
2403  CONTINUE
      CALL GRAMR ( IVAL, NS, HISTG, ICMSR)
      WRITE ( 6,2411)
2411  FORMAT (1X,/,1X,42HWATER CONTENT AS PERCENTAGE OF WOOD VOLUME)
      WRITE (6,2415)
2415  FORMAT(1X, 30X, 1H0, 9X, 2H10, 8X, 2H20, 8X, 2H30, 8X, 2H40, 8X,
     2  2H50, 8X, 2H60, 8X, 2H70, 8X, 2H80, 8X, 2H90, 8X, 3H100)
      DO 2413 K = 1, NS
      IVAL(K) = WCPCAV(K)  + 1.0
      IWCSR(K) = WCSERR(K) + 1.0
2413  CONTINUE
      CALL GRAMR ( IVAL, NS, HISTG, IWCSR)
      WRITE ( 6,2408)
2408  FORMAT (1X,/,1X,34HACETYLENE REDUCTION RATE NM/HR/CM3)
      WRITE ( 6,2409)
2409  FORMAT(31X, 4H.001, 2X, 4H.002, 4X, 4H.005, 2X, 3H.01, 3X, 3H.02,
     2 5X, 3H.05, 3X, 2H.1, 4X, 2H.2, 6X, 2H.5,4X, 1H1, 5X, 1H2, 7X,
     3 1H5, 5X, 2H10, 4X, 2H20, 6X, 2H50, 4X, 3H100)
      DO 2410 K = 1, NS
      IF ( RTCMAV(K) .LT. 0.001 ) RTCMAV(K) = 0.0011
      IF(RTSERR(K) .LT. 0.001) RTSERR(K) = 0.0011
      IVAL (K) =(ALOG10 ( RTCMAV(K) * 1000.0) * 20.0) + 1.0
      IRTSR(K) = (ALOG10(RTSERR(K) * 1000.0) * 20.0) + 1.0
2410  CONTINUE
      CALL GRAMR ( IVAL, NS, HISTG, IRTSR)
      WRITE (6,2405)
2405  FORMAT(1X,/13HWEIGHT CHANGE)
      WRITE(6,2414)
2414  FORMAT(1X,10X,4(1H+),11HWEIGHT GAIN,5(1H+),5(1H-),
     1    4(11HWEIGHT LOSS,9(1H-)))
      WRITE(6,2406)
2406  FORMAT  (1X, 10X,2H20, 8X, 2H10, 7X, 2H 0, 9X, 2H10, 8X, 2H20, 8X,
     2       2H30, 8X, 2H40, 8X, 2H50, 8X, 2H60, 8X, 2H70, 8X, 2H80)
      DO 2407 K = 1, NS
      IVAL(K) = WLSGAV(K)
2407  CONTINUE
      CALL WLGMR (IVAL, WLSERR)
      RETURN
      END


      SUBROUTINE GRAMR ( IHGM, NV, HISTG, ISERR)
      DIMENSION IHGM(12), HISTG(100), ISERR(12)
      DO 2500 K = 1,NV
      DO 2404 KB = 1,100
      HISTG (KB) = 1H
2404  CONTINUE
```

```
      IERROR = ISERR(K)
      CALL PLUSERR( HISTG, IERROR)
      HISTG(IERROR) = 1H
      NCH =IHGM(K)
      IF ( NCH .LT. 1 )  NCH = 1
      IF ( NCH .GT. 100 )  NCH = 100
      DO 2502 KI = 1, NCH
       HISTG (KI) = 1HX
2502  CONTINUE
      WRITE(6,2503) (HISTG(KZ), KZ = 1, 100)
2503  FORMAT(1H+, 30X, 100A1)
2500  CONTINUE
      RETURN
      END


      SUBROUTINE PLUSERR ( HLINE,IERR)
      DIMENSION HLINE (100)
      IF ( IERR .GT. 100) IERR = 100
      IF (IERR .LT. 1 ) IERR = 1
      HLINE(IERR) = 1HI
      WRITE(6,2901) ( HLINE(KY), KY = 1, 100)
2901  FORMAT(31X, 100A1)
      RETURN
       END


       SUBROUTINE WLGMR ( IHGM, RERR)
      DIMENSION IHGM(12), ALINE(100), RERR(12)
      DO 2700 NV = 1, 12
      DO 2702 KJ = 1,100
      ALINE(KJ) = 1H
2702  CONTINUE
      IF ( IHGM(NV) .LT. -20) IHGM(NV) = - 20
      IF ( IHGM(NV) .GT.  80 ) IHGM(NV) = 80
      IF ( IHGM(NV) .LE. -1 ) GO TO 2801
      N3 = 21 + IHGM(NV)
      DO 2602 K = 21, N3
      ALINE(K) = 1HX
2602  CONTINUE
      GO TO 2802
2801  N2 = 21 + IHGM(NV)
      DO 2601 K = N2 , 20
       ALINE(K) = 1HX
2601  CONTINUE
2802  WRITE (6,2201)(ALINE(NCH), NCH = 1, 100 )
2201  FORMAT ( 11X, 100A1)
      IERROR  = RERR(NV)
      IF ( IERROR .LT. -20) IERROR = -20
      IF ( IERROR .GT. 80 ) IERROR = 80
      IF ( IERROR .LE. -1) GO TO 2803
      ALINE ( IERROR + 21 ) = 1HI
      GO TO 2904
2903  IERRA = 21 - IABS(IERROR)
       ALINE (IERRA) = 1HI
2904  WRITE(6,2805) (ALINE(NCHS), NCHS = 1, 100)
2805  FORMAT (1H+, 10X, 100A1)
2700  CONTINUE
      RETURN
      END


      SUBROUTINE GRAFER1 ( NOSAMT, NBL, VCONM, VWLSEG, VRTCM, VWCPCV )
      DIMENSION VCONM(14,6,12), VRTCM(14,6,12), VWCPCV(14,6,12),
     2  VWLSEG(14,6,12), NBL(14)
      READ (5,4202) NOSAMT
4202  FORMAT (I2)
      READ(5,4203)(NBL(IST),IST= 1, NOSAMT)
4203  FORMAT (14(I2,1X))
      CALL AVPLOT ( VCONM, NOSAMT, 1)
      CALL AVPLOT(VWLSEG, NOSAMT, 2)
      CALL AVPLOT(VRTCM, NOSAMT, 3)
      CALL AVPLOT(VWCPCV, NOSAMT, 4)
      RETURN
      END


      SUBROUTINE AVPLOT ( AVARAY, NOSAM , N )
       DIMENSION AVARAY(14,6,12), X(10000), Y(10000)
      NPT = 0
      I = 0
      IL = 6
      IKL = 1
6000  IKM = IKL + 3
      NPT = NPT + 1
      DO 6002 IK = IKL, IKM
      DO 6004 IT = 1, NOSAM
      I = I + 1
      Y(I) = AVARAY(IT,IL,IK)
      IF ( NPT .EQ. 1 ) X(I) = ( IT * 7 ) - 2
      IF ( NPT .EQ. 2 ) X(I) = ( IT * 7 ) - 1
      IF ( NPT .EQ. 3 ) X(I) = ( IT * 7 )
```

```
      6004 CONTINUE
      6002 CONTINUE
           IKL = IKM + 1
           IF(IKL .NE. 13) GO TO 6000
           I = I + 1
           X(I) = 100.0
           Y(I) = 0.0
            CALL GRAFIT( X, Y, I, 50, NOSAM )
           IF ( N .EQ. 1 ) Y(I) = 250.0
           IF ( N .EQ. 2 ) Y(I) = 80.0
           IF ( N .EQ. 3 ) Y(I) = 10.0
           IF ( N .EQ. 4 ) Y(I) = 100.0
           CALL GRAFIT ( X, Y, I, 50,NOSAM )
           RETURN
           END


           SUBROUTINE ARRER(VARR,RARR,NV, NBL,     NOSAM )
           DIMENSION VARR(14,6,12), RARR(1000), NBL(14)
           I = 0
           DO 6005 IK = 1, 12
           DO 6005 IT = 1, NOSAM
           NB = NBL(IT)
           DO 6005 IL = 1, NB
           I = I +1
           RARR(I) = VARR(IT,IL,IK)
      6005 CONTINUE
           NV = I
           RETURN
           END


           SUBROUTINE GRAFER2 ( NOSAMT, NBL, VCONM, VWLSEG, VRTCM, VWCPCV )
           DIMENSION VCONM(14,6,12), VWLSEG(14,6,12), VRTCM(14,6,12),
          2  VWCPCV(14,6,12), NBL(14), CMARR(1000), WLARR(1000),
          2  RTARR(1000), WCARR(1000)
           NBLOCK = 0
           CALL ARRER(VCONM, CMARR, IV,NBL,     NOSAMT)
           CALL ARRER(VWLSEG, WLARR, IV, NBL,     NOSAMT)
           CALL ARRER(VRTCM, RTARR, IV, NBL,     NOSAMT)
           CALL ARRER(VWCPCV,WCARR,IV, NBL,     NOSAMT)
           DO 6006 IT = 1, NOSAMT
           NBLOCK = NBLOCK + NBL(IT)
      6006 CONTINUE
           CALL GRAFIT ( CMARR, WLARR, IV, 50, NBLOCK )
           CALL GRAFIT ( CMARR, RTARR, IV,100, NBLOCK )
           CALL GRAFIT ( CMARR, WCARR, IV, 50, NBLOCK )
           CALL GRAFIT ( WLARR, RTARR, IV, 100, NBLOCK )
           CALL GRAFIT ( WCARR, WLARR, IV, 50, NBLOCK )
           CALL GRAFIT ( WCARR, RTARR, IV, 100, NBLOCK )
           RETURN
           END


           SUBROUTINE GRAFIT ( X, Y, NN, IHEAD, NGRP )
           DIMENSION X(NN),Y(NN),PLOT(101),NTENS(12),NHUNDS(11) , CHAR(15)
           DIMENSION CXAXIS(4), CNUM(9), IPLTCT(101)
           DATA CI/1HI/, CBL/1H /, CX/1HX/, CMINUS/1H-/, KBL/1H /, KONE/1H1/,
          1  (CXAXIS(J),J=1,4)/5H98765,5H43210,5H12345,5H67890/,
          2(CHAR(JK), JK = 1, 15)  /1HD, 1HE, 1HF, 1HG, 1HH, 1HI, 1HJ, 1HK,
          3                        1HL, 1HM, 1HN, 1HO, 1HP, 1HQ, 1HR/,
          4(CNUM(JL),JL=1,9)  /1H1, 1H2, 1H3, 1H4, 1H5, 1H6, 1H7, 1H8, 1H+/
           REAL INVXINT,INVYINT
           N = NN
            TYINTS = IABS ( IHEAD / 10 ) * 10
           IF (TYINTS .LT. 50.) TYINTS = 50.0
           CALL GRAFIX ( X, N, XINT, NXOR, 100.0 )
           CALL GRAFIX ( Y, N, YINT, NYOR, TYINTS)
           IF ( IHEAD .GT. 0 ) GO TO 4
           WRITE (6,2) XINT, YINT
         2 FORMAT ( 1X, ///, 30X, 13H X INTERVAL =, F8.4, 10X,
          2  13H Y INTERVAL =, F8.4)
           GO TO 1
         4 READ(5,5) ( PLOT(J), J = 1,80)
         5 FORMAT (80A1)
           WRITE (6,6) XINT, (PLOT(J), J = 1,80), NN
         6 FORMAT (13H1X INTERVAL =,E9.2,15X,80A1,5X,I4,1X,6HPOINTS)
           DO 7 J= 1,80
           IF ( PLOT(J) .NE. CBL) PLOT(J) = CMINUS
         7 CONTINUE
           WRITE(6,8) YINT, (PLOT(J), J = 1,80) , NGRP
         8 FORMAT (13H Y INTERVAL =,E9.2,15X,80A1,5X,I4,1X,6HGROUPS/1X)
        10 NLINES = TYINTS + 1.1
           DO 40 LINE = 1, NLINES
           CBLK = CBL
           NAXIS = NYOR + NLINES - LINE
           IF ( NAXIS .EQ. 0 ) CBLK = CMINUS
           DO 15 J = 1,101
           IPLTCT(J) = 0
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      15  PLOT(J) = CBLK
          IAXIS = 1 - NXOR
          IF ( IAXIS .GE. 1 .AND. IAXIS .LE. 101) PLOT(IAXIS) = C1
          INVXINT = 1.0 / XINT
          INVYINT = 1.0 / YINT
          DO 25 J = 1,N
          XJAY = X(J)
          YJAY = Y(J)
          SING = 0.0
          IF (YJAY .GT. 0.0) SING = 0.5
          IF (YJAY .LT. 0.0) SING = -0.5
          IO = IFIX ( YJAY * INVYINT + SING )
          IF ( IO .NE. NAXIS ) GO TO 25
          SIG  = 0.0
          IF ( XJAY .LT. 0.0) SIG  = -.5
          IF ( XJAY .GT. 0.0 ) SIG  = .5
          M = IFIX ( XJAY * INVXINT + SIG ) + IAXIS
          IPLTCT(M) = IPLTCT(M) + 1
          JK = ( ( J *NGRP ) - 1 ) / NGRP
          IF ( JK .GT. 15 ) JK = 15
          IF ( PLOT(M) .NE. CBLK ) GO TO 70
          PLOT(M) = CHAR(JK)
          GO TO 25
      70  MCNT = IPLTCT(M)
          IF ( MCNT .GE. 9 ) MCNT = 9
          PLOT(M) = CNUM(MCNT)
      25  CONTINUE
          YAXIS = FLOAT (NAXIS) * YINT
          NAX = IABS ( NAXIS - ( NAXIS/ 10) * 10)
          IF ( NAX .EQ. 0 ) WRITE (6,30) YAXIS,NAXIS,PLOT
      30  FORMAT ( 1H*,1PE15.2,I5,3X,101A1)
          IF( NAX .NE. 0 ) WRITE(6,35) NAX, PLOT
      35  FORMAT( 1H*,15X,I5,3X,101A1)
      40  CONTINUE
          DO 43 J = 1,10
          PLOT ( 2* J - 1 ) = CXAXIS(3)
      43  PLOT (2*J)= CXAXIS(4)
          IF ( NXOR .GE. 0) GO TO 47
          NREV = - NXOR / 10
          IF ( NREV .GT. 10 ) NREV =  10
          DO 45 J = 1 ,NREV
          PLOT (2* J-1) = CXAXIS(1)
      45  PLOT (2*J) = CXAXIS(2)
      47  WRITE(6,48) (PLOT(J), J= 1,20)
      48  FORMAT(1H*/1H*,23X,1H0,20A5)
          NTENS(1) = NXOR / 10
          PLOT(1) = FLOAT(NXOR) + XINT
          DO 60 K = 1,11
          NTENS(K+1) = NTENS(K) + 1
          PLOT(K+1) = PLOT(K) + 10.0 * XINT
          NHUNDS (K) = IABS( NTENS(K) / 10)
      60  NTENS(K) = NTENS(K) - (NTENS(K) / 10) * 10
          NXVALO = - NXOR / 10 + 1
          IF(NXVALO .LE. 11 .AND. NXVALO .GE.1) PLOT(NXVALO) = 0.0
          WRITE(6,62) (NTENS(K), K= 1,11)
      62  FORMAT(1H*,14X,11(8X,I2))
          IF(IABS(NXOR+50) .GE. 150) WRITE (6,62) NHUNDS
          DO 63 K = 1,11
          IF ( NHUNDS(K) .EQ. 0 ) NHUNDS(K) = KBL
           IF(NHUNDS(K) .EQ. 1) NHUNDS(K) = KONE
      63  CONTINUE
          IF( IABS(NXOR+50) .LT. 150) WRITE(6,64) NHUNDS
      64  FORMAT(1H*,14X,11(9X,A1))
          WRITE(6,66) (PLOT(K),K = 2,10,2)
      66  FORMAT(1H*,18X,1P5E20.2)
          WRITE(6,68) (PLOT(K),K = 1,11,2)
      68  FORMAT(1H*,8X,1P6E20.2)
          RETURN
          END


          SUBROUTINE GRAFIX (A,N,AIN , NOR, TINTS)
          DIMENSION A(N), PREF(14)
          DATA(PREF(J), J= 1,14) / 1.0,  1.2 , 1.5 , 1.8 , 2.  , 2.5 , 3.0,
         1  3.5 , 4.0 , 6.0 , 6.0 , 7.0 , 8.0 , 10.0 /
          ANIT = AIN
          AMAX = A(1)
          AMIN = A(1)
          DO 11 J = 2, N
           IF ( A(J) .GT. AMAX) AMAX = A(J)
          IF ( A(J) . LT. AMIN) AMIN = A (J)
      11  CONTINUE
          IF ( AMAX .LE. AMIN ) GO TO 20
          ANIT = ( AMAX - AMIN) / TINTS
          IPTEN = ALOG10 (ANIT)
           IF ( ANIT .LT. 1.0) IPTEN = IPTEN - 1
          ANIT = ANIT / 10.0 ** IPTEN
          DO 15 J = 1,13
          IF( PREF(J+1) .GT. ANIT) GO TO 16
      15  CONTINUE
          J = 14
```

```
      16  ANIT = PREF(J) * 10.0 ** IPTEN
          SIG  = -.46
          IF ( AMIN .LT. 0.0 ) SIG  = -.96
          IF ( AMIN .GT. 0.0) SIG  = .04
          NOR = IFIX ( 0.1 * AMIN / ANIT * SIG  ) * 10
          AIN = ANIT
          IF ( AMAX / ANIT - FLOAT (NOR) .LT. TINTS * 0.5) RETURN
          J = J + 1
          IF ( J .LE. 14) GO TO 16
          J = J - 14
          IPTEN = IPTEN + 1
          GO TO 16
      20  DO 25 J = 1, N
      25   A(J) = J
          WRITE(6,30) AMAX
      30  FORMAT(63H-***2.5** ALL ELEMENTS OF X OR Y ARRAY HAVE SAME
     1  VALUE,1PE12.4,45H SO ELEMENTS REPLACED BY OWN SUBSCRIPTS)
          GO TO 10
          END
```

LINREG1

This programme performs linear regressions on MC, WL, AR or WC data from the segments of the blocks which were exposed to soil contact and described in sections 2 to 9.

The main programme calls the subroutines BOTIN, INDAT, WDVAL, and ARVAL which perform the same functions as in TIGROT and HISTGRF. The subroutine STORGE sets up three-dimensional arrays of MC, WL, AR and WC. The dimensions are the 14 possible times, the 6 replicate blocks, and the 12 segments in each block. These arrays are accessed and the linear regression carried out using subroutines modified from the programme published by Sokal and Rohlf (1969).

Subroutine REGRN reads in a title card, the number of sample times, and the number of blocks sampled at each sample time. It reads in a code which selects MC, WL, AR or WC for regression analysis and the sample times to be used in the regression. The number of segments to be analysed is read in, together with the code numbers of those segments. Transformation of the data can be selected. REGRN then calls ARRYFL to set up the x and y arrays for the regression and then calls LINREGA with the x and y arrays, the transformation codes, a t-table, and the confidence level. LINREGA calls INPUTA (which transforms the data, if required, using the function TRANSG) and then performs the regression using analysis of variance. It prints out a conventional 'Anova' table, and returns control to REGRN. REGRN calls REGCOMP which calculates values for an 'a posteriori' test of the equality of the regression lines. SIGTST is called to print a list of the tests to be performed to assess the statistical significance of the results.

```
      PROGRAM LINREG1 (INPUT,OUTPUT,TAPE5=INPUT,TAPE6=OUTPUT)
      DIMENSION    SPEC(6), IBLKP(6), WETB(12,6), WETW(12,6), STDA(6),
     2             NBIN(6), ISAMT(6), DRYB(12,6), DRYW(12,6), STDB(6),
     3             BLKN(6), IDATE(6), TIUN(12,6), WATC(12,6), WINI(6),
     4             WREM(6),  M(12,6), TOUN(12,6), CONM(12,6), ROW(60),
     5               VCONM(14,6,12),  TITM(12,6), WSEG(12,6), NBL(12),
     6               VRTCM(14,6,12),  TOTM(12,6), NSEG(12,6), EMPB(160),
     7              VWCPCV(14,6,12), WCPCV(12,6), SLGT(12,6), FACB(160),
     8              VWLSEG(14,6,12), WLSEG(12,6), TIME(12,6),
     9               TITLE(80),       UNITT(12,6), RTCM(12,6)
           CALL BOTIN ( EMPB, FACB, VCONM, VWLSEG, VRTCM, VWCPCV)
1011       CALL INDAT ( NB, NS, SPEC, NBIN, BLKN, IBLKP, ISAMT, IDATE,
     2  WINI, WREM, STDA, STDB, NSEG, M, WETB, DRYB, TIUN, TOUN, TITM,
     3  TOTM, SLGT )
      IF ( NB .EQ. 0 ) GO TO 1010
           CALL WDVAL ( NB, NS, M, WETB, DRYB, EMPB, SLGT, WETW, DRYW,
     2  WATC, CONM, WSEG, WLSEG, WCPCV, WINI )
           CALL ARVAL ( NB, NS, M, TITM, TIUN, TOUN, TOTM, FACB, STDA,
     2  STDB, UNITT, TIME, RTCM, SLGT )
           CALL STORGE ( NB, NS, ISAMT, CONM, VCONM, WLSEG, VWLSEG,
     2  RTCM, VRTCM, WCPCV, VWCPCV  )
      GO TO 1011
1010  CALL REGRN ( VCONM, VWLSEG, VRTCM, VWCPCV )
      STOP
      END

      SUBROUTINE REGRN ( VCONM, VWLSEG, VRTCM, VWCPCV )
      DIMENSION VCONM (14,6,12), VWLSEG(14,6,12), VRTCM(14,6,12),
     2 VWCPCV(14,6,12), IXVAL(14), ISEG(12), ARX(14), NBL(14),
     3 ARY(100), TITLE(80), TAT5(30),ASUMX2(12), ASUMXY(12),ASSEXP(12)
     4 , ASSUMEX(12), TLINE(80)
      DATA (TAT5(L), L =1, 30 ) /  12.71, 4.303, 3.182, 2.776, 2.571,
     2 2.447, 2.365, 2.306, 2.262, 2.228, 2.201, 2.179, 2.160, 2.145,
     3 2.131, 2.120, 2.110, 2.101, 2.093, 2.086, 2.080, 2.074, 2.069,
     4 2.064, 2.060, 2.056, 2.052, 2.048, 2.045, 2.042 /
      READ (5,9000) NOSAMT, ( NBL(IZ), IZ = 1, NOSAMT)
9000  FORMAT ( I2, 1X, 14(I2, 1X) )
C.....READS NO. SAMPLE TIMES, THEN NO. BLOCKS SAMPLED AT EACH TIME
C.....E.G. 05 04 04 04 03 03 03
      READ(5,9003) (TITLE(J) , J = 1, 80)
9003  FORMAT ( 80A1)
C.....READS IN TITLE CARD--PARAMETER---SEG NO.---SAMPLE TIMES
      READ (5,9007) INUM, NOXVAL, ( IXVAL(IN), IN = 1, NOXVAL )
9007  FORMAT ( I2, 1X, I2, 1X, 14(I3,1X) )
C.....READS IN A CODE NUMBER, THEN NO. OF X-VALUES,THEN ACTUAL X-VALUES
C.....E.G. 01 06 014 028 042 056 084 112
C.....  1=MOISTURE CONT  2=WEIGHT LOSS  3=ASET REDN RATE  4=WATER CONTENT
C.....  5=RETURN
      IF ( INUM .EQ. 5 ) GO TO 9012
      READ(5,9008) ISEGNO, ( ISEG(IY), IY = 1, ISEGNO )
9008  FORMAT ( I2, 1X, 12 ( I2, 1X ) )
C.....READS IN NO. OF SEGS, THEN IDENTIFYING NOS. OF SEGMENTS
C.....E.G. 09 01 02 03 03 04 05 06 07 08 09
      DO 9009 IX = 1, NOXVAL
      ARX(IX) = IXVAL(IX)
9009  CONTINUE
      READ (5,9013) ICODE, ITRANX, ITRANY
9013  FORMAT ( 3 ( I2, 1X ) )
C.....READS IN CODE NO., TRANSFORM CODE FOR X AND Y.
C.....ICODE NOT 0, 1 OR 2.
      DO 9010 IW = 1, ISEGNO
      IK = ISEG(IW)
      ICNT = 0
      IF(INUM.EQ.1)CALL ARDYFL( VCONM,IXVAL,NOXVAL,NBL,IK,ICNT,ARY)
      IF(INUM.EQ.2)CALL ARDYFL(VWLSEG,IXVAL,NOXVAL,NBL,IK,ICNT,ARY)
      IF(INUM.EQ.3)CALL ARDYFL( VRTCM,IXVAL,NOXVAL,NBL,IK,ICNT,ARY)
      IF(INUM.EQ.4)CALL ARDYFL(VWCPCV,IXVAL,NOXVAL,NBL,IK,ICNT,ARY)
      WRITE(6,9004) ( TITLE(J), J = 1, 80)
9004  FORMAT ( 1H1, 2X, 80A1 )
      DO 9005 J = 1, 80
      IF ( TITLE(J) .NE. 1H  ) TLINE(J) = 1H-
      WRITE(6,9006) (TLINE(J), J = 1, 80)
9005  CONTINUE
9006  FORMAT ( 3X, 80A1, /  )
      WRITE (6,9011) IK
9011  FORMAT ( 3X, 14HSEGMENT NUMBER, I2 )
C.....ARGUMENTS FOR LINREGA
C.....IA.......NUMBER OF X-VALUES = NOXVAL
C.....ICODE....INPUT CODE--NOT 0 1 2 (FOR INPUT FROM CARDS)
C.....ITRANX...TRANSFORMATION CODES FOR X-VALUES
C.....ITRANY...TRANSFORMATION CODES FOR Y-VALUES
C.....TALPH....T VALUE FROM TABLES
C.....ALPHA....CONFIDENCE VALUE 0.05
C.....IFREQ....ARRAY WITH NO. OF Y-VALUES AT EACH X = NBL
      IDF = ICNT - 2
      IF ( IDF .GT. 30 ) IDF = 30
      TALPH = TAT5(IDF)
      CALL LINREGA(NOXVAL,ICODE,ITRANX,ITRANY,TALPH,0.05,NBL,ARX,ARY,
     2 SUMX2, SUMXY, SSEXP, SSUMEX )
      ASUMX2(IW) = SUMX2
      ASUMXY(IW) = SUMXY
      ASSEXP(IW) = SSEXP
```

```
      ASSUMEX(IW) = SSUMEX
 9010 CONTINUE
      CALL REGCOMP(ISEGNO, NOXVAL, ASUMX2, ASUMXY, ASSEXP, ASSUMEX)
      GO TO 2001
C.....TO END--TITLE READS--END OF REGRESSIONS. NEXT CARD IS 01 000
 9112 CALL STGTST
      RETURN
      END


      SUBROUTINE ARRYFL(VALU,IXVAL,NOXVAL,NBL,IK,ICNT,ARY)
      DIMENSION VALU(14,6,12), IXVAL(14), NBL(14), ARY(100)
      DO 9100 IV = 1, NOXVAL
      IF = NBL(IV)
      IT = IXVAL(IV) / 14
      DO 9100 IL = 1, IF
      ICNT = ICNT + 1
      ARY(ICNT) = VALU(IT,IL,IK)
 9100 CONTINUE
      RETURN
      END


      SUBROUTINE LINREGA(IA, ICODE, ITRANX, ITRANY, TALPH, ALPHA,
     2  IFREQ, ARRAYX, ARRAYY, SUMX2, SUMXY, SSEXP, SSUMEX )
C.....LINEAR REGRESSION ANALYSIS.  USES SUBROUTINES INPUTA AND TRANSG
      DIMENSION IFREQ(20), YBAR(20), VAR(20), X(20), FREQ(20),
     A  ARRAYY(70), ARRAYX(20)
      I1 = 1
      WRITE(6,3) IA, ICODE, ITRANX, ITRANY, TALPH, ALPHA
    3 FORMAT ( 1H0 , I5, 9H X-VALUES, I10, 13H = INPUT CODE, 10X,
     2 22H TRANSFORMATION CODES ,I3, 1H , I3,  10X, 7HT =, F5.3,
     2  7H AT P =, F6.4/)
      DF = 0.0
      DO 1001 I = 1, IA
      X(I) = ARRAYX(I)
 1001 CONTINUE
      CALL INPUTA(IA, IFREQ, YBAR, VAR, WAVVAR, ITRANY, ARRAYY)
C.....COMPUT REGRESSION STATISTICS
      SUMX = SUMY = TOTN = 0.0
      DO 25 I = 1, IA
      X(I) = TRANSG( ITRANX, X(I))
      FREQ(I) = IFREQ(I)
      SUMX = SUMX + FREQ(I) * X(I)
      SUMY = SUMY + FREQ(I) * YBAR(I)
   25 TOTN = TOTN + FREQ(I)
      NTOTM1 = TOTN - 1
      IAM1 = IA - 1
      IAM2 = IA - 2
      FIAM1 = IAM1
      FIA = IA
      DF = TOTN - FIA
      NDF = DF
      XBAR = SUMX / TOTN
      YBARB = SUMY / TOTN
      SUMX2 = SUMY2 = SUMXY = 0.0
      DO 30 I = 1, IA
      XD = X(I) - XBAR
      YD = YBAR(I) - YBARB
      SUMX2 = SUMX2 + XD *XD * FREQ(I)
      SUMY2 = SUMY2 + YD * YD * FREQ(I)
   30 SUMXY = SUMXY + XD * YD * FREQ(I)
      VARX = SUMX2 / ( TOTN - 1.0)
      SSERR = DF * WAVVAR
      SSTOT = SUMY2 + SSERR
      VARY = SSTOT / (TOTN-1.0)
      B = SUMXY / SUMX2
      A = YBARB - B * XBAR
      WRITE(6,41) A, B, XBAR, YBARB, TOTN, VARX, VARY
   41 FORMAT ( /31H THE REGRESSION EQUATION IS Y =, F10.5, 2H +, F10.5,
     2 2H X // , 12H MEAN OF X =, F10.5, 3X, 12H MEAN OF Y =, F10.5, 3X,
     3 9HTOTAL N=, F5.0 // 16H VARIANCE OF X =, F15.5, 3X,
     4 15HVARIANCE OF Y =, F15.5 / )
      VARG = SUMY2 / FIAM1
      SSEXP = SUMXY * SUMXY / SUMX2
      SSUNEX = SUMY2 - SSEXP
      VUNEX = SSUNEX / FLOAT ( IA - 2 )
C.....CONFIDENCE LIMITS TO SLOPE
      SBXT = SQRT ( VUNEX / SUMX2 ) * TALPH
      B1 = B - SBXT
      B2 = B + SBXT
      PCENT = ( 1.0 - ALPHA ) * 100.0
      WRITE(6,42) PCENT, B1, B2
   42 FORMAT ( F6.1, 44H PERCENT CONFIDENCE LIMITS FOR THE SLOPE ARE,
     2 F10.4, 4H AND, F10.2)
      FSGP = -9999.9999
      FSDEV = -9999.9999
      IF ( NDF ) 45, 45, 43
   43 FSGP = VARG / WAVVAR
      FSDEV = VUNEX / WAVVAR
   45 FSEXP = SSEXP / VUNEX
```

```
      C.....PRINT ANOVA TABLE
            WRITE(6,50)   SUMY2,     IAM1,      VARG,      FSGP,
     58.   2              SSEXP,     I1,        SSEXP,     FSEXP,
           3              SSUNEX,    IAM2,      VUNEX,     FSDEV,
           4              SSEPR,     NDF,       WAVVAR,
           5              SSTOT,     NTOTM1
        50 FORMAT ( //25X, 21H ANALYSIS OF VARIANCE//
     59.   27H SOURCE, 7X, 2HSS, 20X, 2HDF, 14X, 2HMS, 10X, 2HFS //
           37H GROUPS,      F13.4,     I17,       2F20.4/2X,
           46HLINEAR,       F15.4,     I17,       2F20.4/2X,
           54HDEV,          F17.4,     I17,       2F20.4//
           66H ERROR,       F14.4,     I17,        F20.4//
           76H TOTAL,       F14.4,     I17)
      C.....PREPARE TABLE OF YHAT, CONFIDENCE LIMITS AND DEVIATIONS
            WRITE(6,60)
     60.
     61.    60 FORMAT ( //2X, 6HSAMPLE, 5X, 1HX, 11X, 1HY, 10X, 2HLI, 10X,
           2 4HYHAT, 8X, 2HL2, 9X, 9HDEVIATION )
            DO 70 I = 1, IA
     62.    YHAT = A + B * X(I)
     63.    DEV = YBAR(I) - YHAT
     64.    STDRXT = SQRT ( VUNEX * ( 1.0 / TOTN + ( X(I) - XBAR) *
     65.   2  ( X(I) - XBAR) / SUMX2) ) * TALPH
            Y1 = YHAT - STDRXT
     66.    Y2 = YHAT + STDRXT
     67.    WRITE(6,65) I, X(I), YBAR(I), Y1, YHAT, Y2, DEV
     68.
     69.    65 FORMAT ( I8, 6F12.5 )
     70.    70 CONTINUE
     71.       WRITE ( 6,1002)
     72.  1002 FORMAT(1H0, 9X, 8HSUMXSQRD, 4X, 6HSUMX*Y, 2X, 8HSSGROUPS, 3X,
           2 7HSMYHTSQ, 2X, 7HSMQSQYX, 3X, 8HSSWITHIN, 5X, 4HB YX, 3X,
           3 7HBARBARY, 5X, 5HBAR X,   3X, 7HA(YINT))
     73.    WRITE (6,1003) SUMX2, SUMXY, SUMY2, SSEXP, SSUNEX, SSEPR, A,
           2  YBARB, XBAR, A
     74.  1003 FORMAT ( 8X, 10F10.2)
     75.       RETURN
     76.       END


      1.       SUBROUTINE INPUTA ( IA, IFRE, YBAR, VAR, WAVVAR, ITRANS, ARRAYY)
      2.       DIMENSION IFRE(20), YBAR(20), VAR(20), DATA( 4), ARRAYY(70)
      3.       WRITE(6,30)
      4.    30 FORMAT (1H 3X, 6HSAMPLE, 2X, 1HN, 5X, 4HMEAN, 5X, 8HVARIANCE)
      5.       SUMDF = 0.0
      C.....CALCULATION OF MEANS, VARIANCES, SUM OF DF, AND WEIGHTED AVERAGE
      6.       WAVVAN = 0.0
      7.       JB = 0
      8.       DO 70 I = 1, IA
      9.       IFREQ = IFRE(I)
     10.       FREQ = IFREQ
     11.       DF = FREQ - 1
     12.       DO 1000 JA= 1, IFREQ
     13.       JB = JB + 1
     14.       DATA(JA) = ARRAYY(JB)
     15.  1000 CONTINUE
     16.       SUM = 0.0
     17.       DO 40 J = 1, IFREQ
     18.       IF (ITRANS) 35, 35, 31
     19.    31 DATA(J) = TRANSG(ITRANS,DATA(J))
     20.    35 SUM = SUM + DATA(J)
     21.    40 CONTINUE
     22.       YBART = SUM / FREQ
     23.       YBAR(I) = YBART
     24.       VARN = 0.0
     25.       IF(IFREQ-1) 45, 45, 49
     26.    45 VART = 0.0
     27.       GO TO 51
     28.    49 DO 50 J = 1, IFREQ
     29.       VARN = VARN + ( DATA(J) - YBAR(I) ) **2
     30.    50 CONTINUE
     31.       VART = VARN / DF
     32.    51 VAR(I) = VART
     33.       SUMDF = SUMDF + DF
     34.       WRITE(6,60) I, IFREQ, YBART, VART , (DATA(JK),JK=1,IFREQ)
     35.    60 FORMAT ( I7, I4, F10.4, F12.4, 6(F10.3,1X) )
     36.       WAVVAN = WAVVAN + VARN
     37.    70 CONTINUE
     38.       WAVVAR = WAVVAN / SUMDF
     39.       RETURN
     40.       END


      1.       FUNCTION TRANSG( ICODE, DATA )
      C.....THIS SUBROUTINE PERFORMS VARIOUS COMMON TRANSFORMATIONS
      C.....TH FOLLOWING IS AN ARITHMETIC STATEMENT FUNCTION
      2.       ASIN(X) = ATAN ( X / SQRT ( 1.0 - X * X ) )
      3.       IF ( ICODE) 99, 99, 100
      4.    99 TRANSG = DATA
      5.       RETURN
      6.   100 GO TO (1,2,3,4,5,6,7,8,9), ICODE
      7.     1 TRANSG = ABS(DATA)
      8.       RETURN
      9.     2 TRANSG = SQRT(DATA)
     10.       RETURN
```

```
      3 TRANSG = SQRT( DATA + 0.5 )
        RETURN
      4 TRANSG = DATA * DATA
        RETURN
      5 TRANSG = ALOG10(DATA)
        RETURN
      6 TRANSG = ALOG10(DATA + 1.0 )
        RETURN
      7 IF ( DATA - 1.0 ) 75, 71, 71
     71 TRANSG = 90.0
        RETURN
     75 TRANSG = ASIN( SQRT (DATA) ) * 57.29578
        RETURN
      8 IF ( DATA - 100.0 ) 85, 71, 71
     85 TRANSG= ASIN ( SQRT ( DATA / 100.0 ) ) * 57.29578
        RETURN
      9 TRANSG = 1.0 / DATA
        RETURN
        END


      SUBROUTINE REGCOMP ( ISEGNO, NOXVAL, ASUMX2, ASUMXY, ASSEXP,
     2 ASSUNEX )
      DIMENSION ASSEXP(12), ASUMXY(12), ASUMX2(12), ASSUNEX(12)
      Q1 = Q2 = Q3 = SMSSUNX = 0.0
      DO 7000 I0 = 1, ISEGNO
C.....QUANTITY 1 IS SUM OF ( SUM YHAT SQUARED )
      Q1 = Q1 + ASSEXP(IU)
C.....QUANTITY 2 IS SUM OF ( SUM PRODUCTS X&Y )
      Q2=Q2 + ASUMXY(IU)
C.....QUANTITY 3 IS SUM OF ( SUM OF X SQUARED )
      Q3=Q3 + ASUMX2(IU)
C.....SMSSUNX IS SUM OF ( UNEXPLAINED SUM OF SQUARES)
      SMSSUNX = SMSSUNX + ASSUNEX(IU)
 7000 CONTINUE
      Q4 = ( Q2 * Q2 ) / Q3
      Q5 = Q2 / Q3
      Q6 = Q1 - Q4
      DFF = ISEGNO - 1
      Q7 = Q6 / DFF
      SMNGPS = NOXVAL * ISEGNO
      DFMS = SMNGPS - ( 2 * ISEGNO)
      Q8 = SMSSUNX / DFMS
      FS = Q7 / Q8
      WRITE(6,7001)
 7001 FORMAT( 1H1, 45HTEST FOR EQUALITY OF SLOPE OF REGRESSION , INFS)
      WRITE(6,7002)
 7002 FORMAT ( 1H0, 25X, 20HANALYSIS OF VARIANCE)
      WRITE(6,7003)
 7003 FORMAT ( 1H0, 5X, 19HSOURCE OF VARIATION, 4X, 2HDF, 8X, 2HSS,
     2  8X, 2HMS, 8X, 1HF)
      WRITE(6,7004) DFF, Q4, Q7, FS
 7004 FORMAT( 1H0, 11X, 7HAMONG B, 3X, 4F10.2)
      WRITE(6,7005)
 7005 FORMAT(7X, 17HWARN AMONG REGRNS)
      WRITE(6,7006) DFMS, SMSSUNX, Q8
 7006 FORMAT(1H0, 7X, 11HWT AV DEVNS, 3X, 3F10.2)
      WRITE(6,7007)
 7007 FORMAT ( 7X, 21HAV VARN WITHIN REGRNS)
      RETURN
      END

      SUBROUTINE SIGTST
      WRITE(6,8000)
 8000 FORMAT ( 1H0,       107HIF FSGROUPS IS GREATER THAN F FROM TABLES
     2(FOR DFGROUPS VERSUS DF ERROR) THEN SAMPLES DIFFER SIGNIFICANTLY )
      WRITE(6,8001)
 8001 FORMAT ( 1H0,       107HIF FSDEV IS GREATER THAN F FROM TABLES
     2(FOR DFDEV VERSUS DFERROR) THEN LINE IS LINEAR                   )
      WRITE(6,8002)
 8002 FORMAT ( 1H0,       107HIF FS LINEAR IS GREATER THAN F FROM TABLES
     2(FOR DFLINEAR VERSUS DFDEV) THEN REGRESSION IS SIGNIFICANT       )
      WRITE(6,8003)
 8003 FORMAT ( 1H0,       107HIF FAMONGB IS GREATER THAN F FROM TABLES
     2(FORDFAMONGB VERSUS DFWTAV) THEN SLOPES ARE SIGNIF DIFFERENT     )
      WRITE(6,8004)
 8004 FORMAT ( 1H0,       107HA POSTERIORI TESTS FOR DIFFERENCES AMONG A
     2SET OF REGRESSION COEFFS, BY THE SIMULTANEOUS TEST PROCEDURE     )
      WRITE(6,8005)
 8005 FORMAT ( 1H0,       107HSSCRIT = DFAMONGB  X  MSWTAVDFVN  X  F FRO
     2M TABLES FOR DFAMONGB VERSUS DFWTAV                              )
      WRITE(6,8006)
 8006 FORMAT ( 1H0,       107HTO COMPARE A SET OF COEFFS WITH SSCRIT
     2                                                                 )
      WRITE(6,8007)
 8007 FORMAT ( 1H0,        41HQUANTITY 1 = SUM OF ( YHATSQRD ) FOR SET )
      WRITE(6,8008)
 8008 FORMAT(1H0,          41HQUANTITY 2 = SUM OF ( SUMX*Y ) FOR SET   )
      WRITE(6,8009)
 8009 FORMAT(1H0,          41HQUANTITY 3 = SUM OF ( SUMX2 ) FOR SET    )
      WRITE(6,8010)
 8010 FORMAT(1H0,          41H QUANT1 - ((QUANT2*QUANT2) / QUANT3)     )
      WRITE(6,8011)
 8011 FORMAT(1H0,       107HIF SSCALCULATED IS GREATER THAN SSCRIT THE
     2N IT IS SIGNIFICANT                                              )
      RETURN
      END
```

LINREG1 Input

Cards 1 - n : As in TIGROT and HISTGRF

Card n + 1 : Number of sample times, number of blocks sampled at each time.

e.g. 06_04_03_03_03_03_03

Card n + 2 : Title card

e.g. WATER CONTENT SEGS 1 TO 12 SAMPLE TIMES 70 TO 154 BIN 12

Card n + 3 : Code number ; 1 = MC, 2 = WL, 3 = AR, 4 = WC, 5 = Stop. Number of X-values (Sample times), actual X-values (days)

e.g. 04_06_070_084_112_126_140_154

Card n + 4 : Number of segments to be analysed, Code number of segments

e.g. 12_01_02_03_04_05_06_07_08_09_10_11_12

Card n + 5 : Code number for input (Not 0,1 or 2 which are for input of Means from punched cards), Transform codes for X and Y (0 = no transform)

e.g. 03_00_00

Cards n + 6 to n + 9 : Same as n + 2 to n + 5 to continue data processing

To end processing :

Card n + 6 : Title card to end program

e.g. END OF REGRESSIONS

Card n + 7 : Code number card which selects option 5 (Stop)

e,g. 05_01_000

LINREG1 Output

WATER CONTENT SEGS 1 TO 12 SAMPLE TIMES 70 TO 154 BIN 12

SEGMENT NUMBER 1

A X-VALUES 4 = INPUT CODE TRANSFORMATION CODES 0 0 T = 2.110 AT P = .0500

SAMPLE	N	MEAN	VARIANCE				
1	4	62.5934	34.8986	6.821	59.429	56.381	69.744
2	3	64.3704	2.1493	63.111	66.000	64.000	
3	3	80.7450	32.8184	6.667	81.524	84.044	
4	3	73.9785	101.2470	75.378	63.289	83.269	
5	3	71.7434	74.1383	77.977	75.378	67.935	
6	3	72.7704	7.2481	69.689	74.667	73.956	

THE REGRESSION EQUATION IS Y = 55.47189 + .13500 X

MEAN OF X = 112.00000 MEAN OF Y = 73.59245 TOTAL N= 18.

VARIANCE OF X = 980.00000 VARIANCE OF Y = 70.78034

95.0 PERCENT CONFIDENCE LIMITS FOR THE SLOPE ARE -.0263 AND .2963

ANALYSIS OF VARIANCE

SOURCE	SS	DF	MS	FS	PROB
GROUPS	734.0429	5	146.8085	3.5343	.030906
LINEAR	321.5127	1	321.5127	3.1175	.141255
DEV.	412.5294	4	103.1324	2.4828	.094909
ERROR	540.0000	13	41.5388		
TOTAL	1274.0441	18			

SAMPLE	X	Y	L1	YHAT	L2	DEVIATION
1	70.00000	62.59341	56.55076	64.92224	73.29372	-2.32883
2	84.00000	64.37037	60.13601	66.81231	73.48861	-2.44196
3	112.00000	80.74497	65.47655	70.59245	75.50835	10.15252
4	126.00000	73.97849	67.07254	72.48252	77.89249	1.49598
5	140.00000	71.76342	67.69629	74.37259	81.04889	-2.60916
6	154.00000	72.77037	67.89118	76.26266	84.63414	-3.49229

SUMXSQR	SUMXSY	SSGROUPS	SMYHTSQ	SMDSQYX	SSWITHIN	R YX	RADBARY	RAR X	A(YINT)
17640.00	2381.49	734.04	321.51	412.53	540.00	.16	70.59	112.00	55.47

GRFPLT2

Program GRFPLT2 was used to analyse water content data collected from the segments of wood blocks exposed to soil and described in Sections 2 to 9. The program uses the average water content of a segment at each sample time and calculates a moving average and the slope of the curve of water content against time at each sample time. The technique is described by Gregg, Hossell and Richardson (1964), and in Section 11.2.2. If the slope, logarithm$_{10}$ of slope, slope ÷ moving average, $\log_{10}$ (slope ÷ moving average), and $\log_{10}$ (slope ÷ moving average squared) are plotted against sample time, then the characteristics of the resulting curve indicate the type of curve which will fit the original data.

The main program reads in the title, the number of curves to be analysed and the number of sample times for each curve, and each sample time in days. It then reads in the values of water content for a segment. Subroutine MASLP is called which calculates the slopes and moving averages for the curve at each sample time. This is repeated for each curve. The characteristic values for each curve are calculated and the main program then calls GRAFIT nine times to plot out the data. Further data can be analysed or the program terminated.

```
      PROGRAM GRFPLT2 (INPUT,OUTPUT,TAPE5=INPUT,TAPE6=OUTPUT)
      DIMENSION T(170),B(170), F(170), G(170), H(170),P(170), Q(170),
     2 Y(170), Z(170), SLP(170), MOVAV( 25), XTIME(25), YPTS(20), W(170)
     3 , ATITLE(80)
      REAL K, MOVAV
      IY=0
      READ(5,3007) (ATITLE(NC),NC=1,80)
 3007 FORMAT(80A1)
 3008 READ(5,3000) NCURV, NV, NPTS
 3000 FORMAT ( 3I3 )
C.....NCURV IS NO. OF CURVES TO BE ANALYSED ( 12 OR LESS )  000 FOR STOP
C.....NV IS NO. OF SAMPLE TIMES ( 14 OR LESS )
C.....  NCURV * NV NOT GREATER THAN 170
      IF ( NCURV .EQ. 0 ) STOP
      READ ( 5,3001) (XTIME(I1), I1 = 1, NV )
 3001 FORMAT ( 20F4.0)
      DO 3002 I8 = 1, NCURV
      READ ( 5,3003) ( YPTS (I3), I3 = 1, NV )
 3003 FORMAT ( 8F10.3 )
      CALL MASLP ( YPTS, NV, 5, SLP, MOVAV )
      DO 3004 IK = 1, NV
      IY= IY + 1
      W(IY) = YPTS(IK)
      T(IY) = XTIME(I )
      Y(IY) = SLP(IK)
      Z(IY) = MOVAV(IK)
 3004 CONTINUE
 3002 CONTINUE
      I4 = NCURV * NV
      DO 3005 IX = 1, I4
      B(IX) = 0.0
      F(IX) = 0.0
      G(IX) = 0.0
      H(IX) = 0.0
      P(IX) = 0.0
      Q(IX) = 0.0
 3005 CONTINUE
      WRITE (6,3009) ( ATITLE(NC), NC= 1,80)
 3009 FORMAT (1H1, 80A1 )
      WRITE ( 6,2006 )
 2006 FORMAT ( 1HO, 6X, 5HINDEX, 2X, 4HTIME, 8X, 4HDATA, 6X,
     2 9HMOVING AV, 3X, 5HSLOPE, 5X, 9HLOG SLOPE, 2X, 8HLOG DATA, 3X,
     3 9HSLOP/MOVAV, 2X, 9HLOGSL/MA, 2X, 10HRATIO DATA, 2X,
     4 13HLOG RATIO, / )
      DO 3006 IX = 1, I4
      B(IX) = ALOG10(W(IX))
      F(IX) = 1. / W(IX)
      IF ( Y(IX) .LT. 1.0E-10 ) G(IX) = 0.0
      IF ( Y(IX) .LT. 1.0E-10 ) GO TO 4000
      G(IX) = ALOG10 (Y(IX))
 4000 IF ( Z(IX) .LT. 1.0E-10 ) E(IX) = 0.0
      IF ( Z(IX) .LT. 1.0E-10 ) GO TO 4001
      IF ( Y(IX) .LT. 1.0E-10 ) GO TO 4001
      E(IX) = Y(IX) / Z(IX)
 4001 IF ( E(IX) .LT. 1.0E-10 )  F(IX) = 0.0
      IF ( E(IX) .LT. 1.0E-10 ) GO TO 4002
      F(IX) = ALOG10 (E(IX))
 4002 IF(E(IX) .LT. 1.0E-10 .OR. Y(IX) .LT. 1.0E-10) Q(IX) = 0.0
      IF(Z(IX) .LT. 1.0E-10 .OR. Y(IX) .LT. 1.0E-10) GO TO 2007
      R = Y(IX) / ( Z(IX) * Z(IX) )
      IF ( R .LT. 1.0E-10 ) Q(IX) = 0.0
      IF ( R .LT. 1.0E-10 ) GO TO 2007
      Q(IX) = ALOG10 (R)
 2007 WRITE(6,2005) IX, T(IX), W(IX), Z(IX), Y(IX), G(IX), B(IX), E(IX),
     2  F(IX), P(IX), Q(IX)
 2005 FORMAT ( 1X, I10, 10F11.6 )
 3006 CONTINUE
      CALL GRAFIT ( T, W, IX, 50, NV )
      CALL GRAFIT (T, Z, IX, 50, NV )
      CALL GRAFIT ( T, Y, IX, 50, NV )
      CALL GRAFIT ( T, G, IX, 50, NV )
      CALL GRAFIT ( T, B, IX, 50, NV )
      CALL GRAFIT ( T, E, IX, 50, NV )
      CALL GRAFIT ( T, F, IX, 50, NV )
      CALL GRAFIT ( T, P, IX, 50, NV )
      CALL GRAFIT ( T, Q, IX, 50, NV )
      GO TO 3008
      END
                              SUBROUTINE MASLP ( A, NUM, L, SLP, MOVAV )
                              DIMENSION A(20), SLP(20), MOVAV(20)
                              REAL MOVAV
                              DO 4003 IE = 1, NUM
                              SLP(IE) = 0.0
                              MOVAV(IE) = 0.0
                         4003 CONTINUE
                              IB = ( L / 2 ) + 1
                              IC = NUM - ( L / 2 )
                              DO 4002 IT = IB, IC
                              IF ( L .GT. 5 ) GO TO 4002
                              SLP(IT)=-A(IT+1) + (2. * A(IT+2))- (2. * A(IT-2)) - A(IT-1)
                              MOVAV(IT) = (A(IT-2) + A(IT-1) + A(IT) + A(IT+1) + A(IT+2))/5.
                         4002 CONTINUE
                              RETURN
                              END
```

GRFPLT2 Input

Card 1 : Title card

e.g. BIN 16 BIRCH 45 PC SMC WATER CONTENT TIMES 0 - 84 DAYS SEG 7-12

Card 2 : Number of curves to be analysed, number of points in each curve.

e.g. 006_15

Card 3 : List of 15 sample times in days

e.g. 000•014•028•042•056•070•084•098•112•126•140•154•168•182•196•

Card 4 & 5 : List of water contents at each sample time for each segment.

e.g. 3•3_____40•296___52•530___72•533___74•108___80•654___78•222___78•0
78•0_____78•0_____78•0_____78•0_____78•0_____78•0_____78•0

Card 6 & 7, 8 & 9, 10 & 11, 12 & 13, 14 & 15 : Same format

Card 16 : Title card

e.g. BIN 16 BIRCH 45 PC SMC WATER CONTENT TIMES 0-84 DAYS SEG 7-12

Card 17 - 24 : Title cards for graphs

e.g. MOVING AVERAGES
SLOPE....IF LINE IS HORIZONTL = STRT LINE....IF AT ANGLE = PARABOLA
LOGARITHM OF SLOPE....IF LINE SLOPES DOWN TO RIGHT = SIMPLE MOD EXPNTL
LOGARITHM OF DATA
SLOPE / MOVING AVERAGE....IF HORIZONTL = SIMP EXP....IF AT ANGLE = LOG PAR
LOG (SLOPE/MOVING AVERAGE)....IF DOWN TO RIGHT = GOMPERTZ
RECIPROCAL DATA
LOG(SLOPE/MOVING AVERAGE SQUARED)....IF DOWN TO RIGHT = LOGISTIC

Card 25 : Card to finish analysis

e.g. 000_00000

FIT3PT

Program FIT3PT was used to analyse water content data collected from the segments of wood blocks which had been exposed to soil and described in Sections 2 to 9. The program calculates the equation of the curve for a parabolic, log parabolic, simple modified exponential, Gompertz and logistic curve, using the 3 - point method described by Gregg, Hossell and Richardson (1964).

The main program reads in the average water content of each sample time for each segment and then calls subroutine PT3. This routine calls ABCFAC to calculate a, b, and c in the equations :

$$y = a + bt \quad \text{(Straight line)}$$

$$\log y = a + bt \quad \text{(Simple exponential)}$$

$$y = a + bt + ct^2 \quad \text{(Parabola)}$$

$$\log y = a + bt + ct^2 \quad \text{(Log parabola)}$$

It then calls subroutine RST which calculates a, b and r in the equations

$$y = a - br^t \quad \text{(Simple modified exponential)}$$

$$\log y = a - br^t \quad \text{(Gompertz)}$$

$$\frac{1}{y} = a + br^t \quad \text{(Logistic)}$$

Subroutine PT3 then writes out the results.

```
      PROGRAM FIT3PT  (INPUT,OUTPUT,TAPE5=INPUT,TAPE6=OUTPUT)
      DIMENSION AFAC(20), BFAC(20), CFAC(20), YPTS(20), XTIME(25),
     2  ATITLE(80)
      COMMON AFAC, BFAC, CFAC, RM
      READ(5,3007) ( ATITLE(NC),NC=1,80)
 3007 FORMAT(80A1)
      READ(5,4010) NPFT, RM
 4010 FORMAT ( I3, 7X, F10.0 )
      READ(5,4001) ( AFAC(L), BFAC(L), CFAC(L), L=1, NPFT )
 4001 FORMAT ( 3F10.0 )
 3008 READ(5,3000) NCURV, NV, NPTS
 3000 FORMAT ( 3I3 )
C.....NCURV IS NO. OF CURVES TO BE ANALYSED (12 OR LESS )    000 FOR STOP
C.....NV IS NO. OF SAMPLE TIMES ( 14 OR LESS )
C.....   NCURV * NV NOT GREATER THAN 170
      IF ( NCURV .EQ. 0 ) STOP
      READ ( 5,3001) (XTIME(I1), I1 = 1, NV )
 3001 FORMAT ( 20F4.0 )
      DO 3002 I2 = 1, NCURV
      READ ( 5,3003 ) ( YPTS(I3), I3 = 1, NV )
 3003 FORMAT ( 8F10.3 )
      WRITE(6,3009) ( ATITLE(NC),NC=1,80)
 3009 FORMAT ( 1H1, 80A1)
      CALL PT3 ( YPTS, NPFT )
 3002 CONTINUE
      GO TO 3008
      END
```

```
      SUBROUTINE ABCFAC ( V, IV, A, B, C )
      DIMENSION AFAC(20), BFAC(20), CFAC(20), V(20)
      COMMON AFAC, BFAC, CFAC, RM
      SM1 = SM2 = SM3 = 0.0
      DO 1000 L = 1, NV
      SM1 = SM1 + ( V(L) * AFAC(L) )
      SM2 = SM2 + ( V(L) * BFAC(L) )
      SM3 = SM3 + ( V(L) * CFAC(L) )
 1000 CONTINUE
      A = SM1 / RM
      B = SM2 / RM
      C = SM3 / RM
      RETURN
      END
```

```
      SUBROUTINE RST ( W, NPT, A, B , RR )
      DIMENSION W(20)
      IF ( NPT .NE. 12 ) RETURN
      RSUM = SSUM = TSUM = 0.0
      DO 1000 I = 1, 5
      RSUM = RSUM + W(I)
      K = I + 7
      TSUM = TSUM + W(K)
 1000 CONTINUE
      DO 1001 J = 4, 9
      SSUM = SSUM + W(J)
 1001 CONTINUE
      R = RSUM / 5.0
      T = TSUM / 5.0
      S = SSUM / 6.0
      TSSR = ( T-S ) / ( S-R)
      RNPT = NPT
      RRLOG = ( 2.0 / ( RNPT - 5.0 ) ) * ALOG10(TSSR)
      RR = 10.0 ** RRLOG
      STR = ( ( 2.0 * S ) -T ) -R
      A = ((S*S) - (T*R)) / STR
      R2 = RR * RR
      R4 = R2 * R2
      RPR = RR + R2 + (R2*RR) + R4 + (R4*RR)
      B = (5.0 / RPR ) * (((S-R) * (S-R) ) / STR )
      RETURN
      END
```

```
      SUBROUTINE PT3 ( Y, NP )
      DIMENSION Y(20), AFAC(20), BFAC(20), CFAC(20), YLOG(20), RY(20)
      COMMON AFAC, BFAC, CFAC, RM
      DO 1000 L = 1, NP
      YLOG(L) = ALOG10 ( Y(L) )
      RY (L) = 1.0 / Y(L)
 1000 CONTINUE
      CALL ABCFAC(Y, NP, APAR, BPAR, CPAR )
      WRITE(6 ,1001)
 1001 FORMAT ( 1H0, 2X, 5HCURVE, 27X, 1HA, 19X, 1HB, 19X, 1HC, 19X,1HR)
      WRITE(6,1002) APAR, BPAR, CPAR
 1002 FORMAT ( 1H0, 2X, 3HPARABOLA, 10X, 3F20.5)
      CALL ABCFAC ( YLOG, NP, ALP, BLP, CLP )
      WRITE(6,1003) ALP, BLP, CLP
 1003 FORMAT ( 1H0, 2X, 12HLOG PARABOLA, 6X, 3F20.5)
      CALL RST ( Y, NP, ASME, BSME , RRSME )
      WRITE(6,1004) ASME, BSME, RRSME
 1004 FORMAT ( 1H0, 2X, 12HSIMP MOD EXP, 6X, 2F20.5, 20X, F20.5)
      CALL RST ( YLOG, NP, AGOM, BGOM , RRGOM )
      WRITE(6,1005) AGOM, BGOM, RRGOM
 1005 FORMAT ( 1H0, 2X, 8HGOMPERTZ, 10X, 2F20.5, 20X, F20.5)
      CALL RST ( RY, NP, ALOG, BLOG , RRLOG )
      WRITE(6,1006) ALOG, BLOG, RRLOG
 1006 FORMAT ( 1H0, 2X, 8HLOGISTIC, 10X, 2F20.5, 20X, F20.5)
      RETURN
      END
```

FIT3PT Input

Card 1 : Title of analysis

e.g. BIN 16 BIRCH 45 PC SMC WATER CONTENT SEGMENT 7

Card 2 : Number of points in curve, curve coefficient (which depends on the number of sample times)

e.g. 012_____4004•

Cards 3 - 14: List of factors for the calculation of a, b and c. One card per sample time.

e.g. ____3003•0____-869•0_____55•0

Card 15 : Number of curves to be analysed (12 or less), number of sample times (14 or less), number of points per curve (000 to end)

e.g. 012008012

Card 16 : Sample times

e.g. 014•028•042•056•070•084•098•112•126•140•154•168•182•196•

Card 17 : Average water content values at each sample time

e.g. 3•3_____40•296____52•530____72•533____74•108____80•654____78•222____78•0
78•0_____78•0_____78•0_____78•0_____78•0_____78•0_____78•0

Card 18 : Repeat cards 15 - 17 for next data set (000 to end)

e.g. 000000000

NLIN

Program NLIN was used to fit a Gompertz curve to the values of water content in wood blocks against time of soil exposure and described in sections 2 to 10. The technique is described in Section 11.2.3. The program is a modification of the NLIN2 program in the IBM SHARE program library, originally programmed by Baumeister, Sheldon and Stanley. It uses the Marquardt algorithm for least squares estimation of the parameters of a non-linear curve. The program was modified by P.W. Mueller of Imperial College to allow implementation on the CDC installation at Imperial College, and by the author to allow the analysis of the water content data in the format used in the GRFPLT2 and FIT3PT programs.

The main program NLIN calls the subroutine NLIN3. This reads in the data and calls SUBZ to calculate the means and deviations for use by FCODE. This calculates the values of the function given in FCODE for each data point. NLIN3 then compares the calculated and observed results and changes the values of the curve's parameters systematically until the calculated and observed results are the same, or there is no further convergence, or a specified number of iterations is reached. The values of the parameters, and their confidence limits is printed out with a graph of the calculated and observed results.

The program was tested using the data supplied with the listing, and with the data for 'Commodity A' given by Gregg, Hossell and Richardson. The program listing is given here, but no information on input or output, which is available from the documentation supplied with the program.

```
      PROGRAM NLIN(INPUT,OUTPUT,TAPE3,TAPE5=INPUT,TAPE6=OUTPUT)
      COMMON X(100,5),Y(100)
      CALL NLIN3(X,Y,100,5)
      STOP
      END


      SUBROUTINE NLIN3(X,Y,NPWM1,NPWM2)

      NONLINEAR LEAST SQUARES
      BY      D. W. MARQUARDT
      PROGRAMMED BY      T. BAUMEISTER III,
                         J. ANN SHELDON AND RUBY M. STANLEY
                  IBKT=1 MEANS USE UPPER A MATRIX
                  IBKT=2 MEANS USE TAPE 3

      DIMENSION FMT(12),PRNT(5),SPRNT(5)
      DIMENSION BS(50),DB(50),BA(50),G(50),W(51),IB(49),SA(50),P(50),A(5
     *0,51),B(50),TITLE(10)
      DIMENSION X(NPWM1,NPWM2),Y(NPWM1)
      DIMENSION CONS(25)

      COMMENT     THIS IS A MODIFICATION OF THE ORIGINAL MARQUARDT SHAREPROGRAM
      IT IS ARRANGED TO BE A SUBROUTINE WITH THE MAIN X AND Y ARRAYS AS
       CALLING ARGUMENTS
       IT CANNOT WRITE ONTO THE ON-LINE PRINTER (TAPE12)
       IT DOES NOT USE SENSE SWITCHES (CAN READ EQUIVALENT COMMANDS)
       THE OUTPUT IS MODIFIED SLIGHTLY TO MAKE IT MORE COMPATIBLE WITH
       INTERACTIVE TELETYPE USAGE.

      ------------------------------------------------------------------
      MAX NO. OF PARAMETERS IS K=50
      MAX NO. OF IND VARS IS M=10
      MAX NO. OF OBSERVATIONS IS N=500
                  IWHER =-1 MEANS DO ANY SPECIAL INITIALIZING FOR CASE
                  IWHER = 0 MEANS START NEW CASE OR END RUN
                  IWHER = 1 MEANS GET P S AND F
                  IWHER GREATER THAN 1 MEANS GET F ONLY
      NPRNT=0
  650 IWHER = 0
  652 GO TO 4
  653 IWHER = IWHER
C     IS IWHER = 0 OR 1 OR GREATER THAN 1 ********************************
      IF (IWHER.GT.0)GO TO 654
      IF (IWHER.EQ.0)GO TO 660
  651 CONTINUE
                  CODING FOR CASE INITIALIZING GOES HERE
      ------------------------------------------------------------------
      CALL SUBZ(Y,X,B,PRNT,NPRNT,N)
      ------------------------------------------------------------------
      IF (IBOUT.EQ.0) GO TO 652
C     IS IBOUT = 0 OR NOT ************************************************
      GO TO 650
  654 CONTINUE
                  CODING TO MAKE F GOES HERE
                  F IS Y HAT (1)
C     NPRNT IS THE NO. OF OTHER WORDS TO BE PRINTED
C     THE WORDS TO BE PRINTED ARE IN PRNT(1)...PRNT(5)
      CALL FCODE (Y,X,B,PRNT,F,I,RES)
      IF (IWHER.NE.1)GO TO 652
  656 IF (IFSS2.NE.0) GO TO 652
  658 CONTINUE
C                 CODING TO MAKE DF/DB GOES HERE
C                 MAKE K OF THEM. CALL THEM P(J)
C                 THEY ARE MADE FROM X(I,L) AND B(J)
C     ------------------------------------------------------------------
C     IS IWHER = 0 OR 1 OR GREATER THAN 1 ********************************
      CALL PCODE(P,X,B,PRNT,F,I)
C     ------------------------------------------------------------------
      GO TO 652
  660 STOP
C                 THIS IS THE END OF THE MAIN ROUTINE
C     ------------------------------------------------------------------
    4 IWHER = IWHER
C                 = 0 ?
C     IS IWHER = -1 OR 0 OR 1 ********************************************
      IF (IWHER.LT.0)GO TO 59
      IF (IWHER.EQ.0)GO TO 10
C                 1  2  3  4
    8 GO TO (75,304,606,620), IWHER
C                 READ FIRST CARD OF NEXT CASE
   10 ITCT=0
      IBOUT=0
      READ (5,940) N,K,IP,M,IPP,NCONS,(TITLE(I),I=1,10)
      WRITE(6,941) (TITLE(I),I=1,10)
      NTILDA=N*NCONS
      XNT=NTILDA
      IF (N.LE.0)GO TO 20
      READ (5,900)IWS1,IWS2,IWS3,IWS4,IWS5,IWS6
      IFSS1=2
      IF (IWS5.EQ.0)GO TO 210
C     PAUSES INACTIVATED AND SENSE SWITCHES REPLACED BY READS
C     PAUSE 5
C     CALL SSWTCH(1,IFSS1)
      PRINT 1000
 1000 FORMAT(50H TYPE 5 SENSE SWITCHES (1 DIGIT EACH) 0=OFF 1=ON   )
      READ 1001,IFSS1,IFSS2,IFSS3,IFSS4,IFSS5
 1001 FORMAT(5I1)
      IF(IFSS1.NE.1)IFSS1=2
      IF(IFSS2.NE.1)IFSS2=2
      IF(IFSS3.NE.1)IFSS3=2
      IF(IFSS4.NE.1)IFSS4=2
      IF(IFSS5.NE.1)IFSS5=2
  210 CONTINUE
  211 GO TO 21
C                 END OF LAST PROBLEM
   20 GO TO (  19,  17),IBKT
      REWIND 3
C     IS IBKT = 1 OR NOT (IBKT = 2) **************************************
   19 IWHER=0
      GO TO 653
   21 IF (IPP.LE.0)GO TO 22
   23 CONTINUE
      CALL ATHRUZ(IBCH,1H )
      CALL ATHRUZ(IOCH,1HO)
      CALL ATHRUZ(IPCH,1HP)
      CALL ATHRUZ(IYCH,1HY)
      CALL ATHRUZ(IXCH,1HX)
      READ (5,930)YMW,SPRD
   12 IF(IP.LT.0) GOTO 30
   24 READ(5,900)(IB(I), I = 1,IP)
      DO 26 I=1,IP
      IF (IB(I).GT.0)GO TO 26
   25 WRITE (6,926)
      IBOUT=1
   26 CONTINUE
```

```
      10 READ (5,931, FF,T,E,TAU,XL,GAMCR,DEL,ZETA
C                     DUB IN INPUT CONSTANTS IF NOT SUPPLIED
C                     ( XL IS CHECKED IN FIRST ITERATION )
      IF(FF.GT.0.. GOTO 34
   32 FF=4.
   34 IF(E.GT.0. GOTO 37
   36 E=.00005
   37 IF(TAU.GT.0.) GOTO 39
   38 TAU=.001
   39 IF(T.GT.0.  GOTO 42
   40 T=2.
   42 IF (K .GT.25)GO TO 46
   44 IBKT=1
      GO TO 50
   46 IBKT=2
      REWIND 3
   50 IF(GAMCR.GT. 0.) GOTO 52
   51 GAMCR = 45.
   52 IF (DEL.GT. 0.) GO TO 55
      DEL=.00001
   55 IF (ZETA.GT. 0.) GO TO 53
      ZETA=.1E-30
   53 XDG = 1.
   54 CONTINUE
C                     READ IN INITIAL B GUESSES 7 TO THE CARD
      READ (5,901)(B(I),I=1,K)
      READ(5,3001, (X(I,1),I = 1, N)
 3001 FORMAT (16F5.1)
      READ(5,3002) (Y(I), I = 1, N)
 3002 FORMAT(F10.3.
      IWHER=1
      GO TO 663
   59 IBKA=1
C
C     ..................................................................
C                     START THE CALCULATION OF THE PTP MATRIX
   58 WRITE (6,907)N,K,IP,M,IFP,GAMCR,DEL,FF,T,E,TAU,XL,ZETA
  213 GO TO 61
   60 CONTINUE
      IF (IWS5.NE.0) GO TO 61
      IWS3=IW53-1
      IWS3=MAXO(IWS3,0)
   61 DO 62 I=1,K
      G (I) =0.
      DO 62 J=1,K
   62 A (I,J)=0.
      GO TO (63,69,69),IBKA
   63 IF (IWS6.EQ.0)GO TO 630
C     CALL SSWTCH (3,IFS63)
C     CALL SSWTCH (2,IFS52)
C     CALL SSWTCH (1,IFS51)
      GO TO (66,64),IFSS3
  630 IFSS3=IWS3
      IFSS2=IWS2
      GO TO 70
   64 IFSS3=0
   66 GO TO (70,65), IFSS2
   65 IFSS2=0
      GO TO 70
   69 CONTINUE
   70 WRITE (6,908) ITCT,(B(J),J=1,K)
      IF (IFSS3.EQ.0 GO TO 73
   71 IF (IFP.LE.0.60 TO 68
   67 WS = YMN-SPRD
      WRITE( 6,906)YMN,WS
      GO TO 73
   68 WRITE( 6,910)
   73 I=1
      PHI=0.
      PHIN=0.
      ICONS=1
      IF (IFSS2.EQ.0) GO TO 57
      GO TO 600
   72 IF (IFSS2.EQ.1)GO TO 602
C                     THIS IS THE ANALYTICAL P S ROUTINE
   57 IWHER=1
C                     GET P S AND F
      GO TO 663
   75 IF (IP.LE.0)GO TO 80
   76 DO 77 II=1,IP
      IWS=IB(II)
   77 P(IWS)=0.
      GO TO 80
C     ..................................................................
C                     THIS IS THE ESTIMATED P S ROUTINE
  600 CONTINUE
  602 IWHER=3
      GO TO 653
  606 RWS=RES
      FSAVE=F
      DO 607 II=1,NPRNT
  607 SPRNT(II)=PRNT(II)
      J=1
  608 IF (IP.LE.0)GO TO 618
  610 DO 612 II=1,IP
      IF ((J-IB(II)).EQ.0)GO TO 621
  612 CONTINUE
  618 DBW=B(J)*DEL
      TWS=B(J)
      B(J)=B(J)+DBW
      IWHER=4
      GO TO 653
  620 B(J)=TWS
      P(J)=-(RES-RWS)/DBW
      GO TO 622
  621 P(J)=0.
  622 J=J+1
      IF ((J-K).LE.0)GO TO 608
  624 RES=RWS
      F=FSAVE
      DO 625 II=1,NPRNT
  625 PRNT(II)=SPRNT(II)
C                     END OF ESTIMATED P S ROUTINE
C     ..................................................................
C                     NOW, USE THE P S TO MAKE PARTIALS MATRIX
   80 DO 82 JJ=1,K
      G(JJ)=G(JJ)+RES*P(JJ)
      DO 82 II=JJ,K
      A(II,JJ)=A(II,JJ)+P(II)*P(JJ)
   82 A(JJ,II)=A(II,JJ)
      IF (IFP.LE.0)GO TO 318
  800 IF (IFSS3.EQ.0.OR.I.GT.W) GO TO 314
                     PLOTTING Y(I),F
  802 IO = (Y(I)-YMN)*100./SPRD
      IPP = (F-YMN)*100./SPRD
      IF (IO.EQ.IPP)GO TO 808
      IF (IO.GT. IPP)GO TO 812
C                     Y(I) OUT FIRST
  804 IF1=IOCH
      IF2=IPCH
      II=IO
      IE=IPP
```

```
182.        GO TO 816
            C                       ONLY ONE CHARACTER
  808 IP1=IYCH
      IP2=IBCH
      I1=IO
      I2=IPP
      GO TO 816
            C                       F OUT FIRST
  818 IP1=IPCH
      IP2=IOCH
      I1=IPP
      I2=IO
            C                       ZERO PLOTS IN THE LEFT HAND COLUMN, SO I1 IS ITS
            C                       OWN BLANK COUNTER
            C                       OVERFLOWS PLOT X IN COLUMN 102
            C                       UNDERFLOWS ALSO PLOT X IN COLUMN ZERO
  816 IF (I2.LE.101)GO TO 819
  817 I2=101
      IP2=IXCH
      IF (I1.LT.101)GO TO 819
  818 I1=101
      IP1=IXCH
      IP2=IBCH
      GO TO 825
  819 IF (I1.GE.0)GO TO 825
  822 I1=0
      IP1=IXCH
      IF (I2.GT.0)GO TO 825
  823 I2=1
      IP2=IBCH
  825 I1M1=I1
      I1M2=I2-I1-1
      IF (I1M1.GT.0)GO TO 832
  820 IF (I1M2.GT.0)GO TO 828
  824 WRITE (6,928)IP1,IP2
      GO TO 844
  828 WRITE (6,928)IP1,(IBCH,II=1,I1M2),IP2
      GO TO 844
  832 IF (I1M2.GT.0)GO TO 840
  836 WRITE (6,928)(IBCH,II=1,I1M1),IP1,IP2
      GO TO 844
  840 WRITE (6,928)(IBCH,II=1,I1M1),IP1,(IBCH,II=1,I1M2),IP2
  844 GO TO 314
  318 WS=RES
      IF (IFSS3.EQ.0.OR.I.GT.N) GO TO 314
  308 IF (NPRNT.GT.0)GO TO 312
  310 WRITE (6,925)Y(I),F,WS
      GO TO 314
  312 WRITE (6,925)Y(I),F,WS,(PRNT(JJ),JJ=1,NPRNT)
  314 WS=RES
      PHI=PHI*WS*WS
      IF (I.GT.N) GO TO 313
      PHIN=PHIN*WS*WS
      GO TO 315
  313 CONS(ICONS)=RES
      ICONS=ICONS+1
  315 I=I+1
      IF (I.LE.NTILDA) GO TO 72
   84 IF (IP.LE.0)GO TO 88
   85 DO 87 JJ=1,IP
      IWS=IB(JJ)
      DO 86 II=1,K
      A(IWS,II)=0.
   86 A(II,IWS)=0.
   87 A(IWS,IWS)=1.
   88 GO TO (90,704,703),IBKA
                        SAVE SQUARE ROOTS OF DIAGONAL ELEMENTS
   90 DO 92 I=1,K
   92 SA(I)=SQRT (A(I,I))
      DO 106 I=1,K
      DO 100 J=1,K
      WS = SA(I)*SA(J)
      IF(WS.GT.0.) GOTO 98
   96 A(I,J) =0.
      GO TO 100
   98 A(I,J)=A(I,J)/WS
  100 CONTINUE
      IF(SA(I).GT.0.) GOTO 104
  102 G(I)=0.
      GO TO 106
  104 G(I)=G(I)/SA(I)
  106 CONTINUE
      DO 110 I=1,K
  110 A(I,I)=1.
  120 PHIZ=PHI
            C                       WE NOW HAVE PHI ZERO
      GO TO (1132,1130),IBKT
 1130 WRITE (3)A
      REWIND 3
      GO TO 1134
 1132 DO 1133 II=1,K
      III=II+25
      DO 1133 JJ=1,K
 1133 A(III,JJ)=A(II,JJ)
            C ..........................................................................
 1134 CONTINUE
      IF (ITCT.NE.0) GO TO 163
            C                       FIRST ITERATION
  150 IF(XL.GT.0.) GOTO 154
  152 XL=0.01
  154 DO 161 J=1,K
  161 BS(J)=B(J)
            C                       BS(J) CORRESPONDS TO PHIZ
  163 IBK1=1
      WS=N-K*IP
      ITCT=ITCT+1
      SE=SQRT(PHIN/WS)
      IF (IFSS3.GT.0)GO TO 165
  162 IF (IFSS2.EQ.0) GO TO 168
  167 WRITE (6,911)PHIZ,SE,XLL,GAMMA,XL
      IF(IFSS1.NE.1)GO TO 221
      ITCT=ITCT+1
  221 CONTINUE
      GO TO 169
            C     NEXT CARD INACTIVATED BY PWM FOR TELEX USE**********
      C 168  WRITE (6,912)PHIZ,SE,XLL,GAMMA,XL
  168  CONTINUE
      GO TO 169
  165 IF (NCONS.EQ.0) GO TO 166
      WRITE (6,938) (JJ,CONS(JJ),JJ=1,NCONS)
  166 WRITE (6,939)
  111 DO 114 I=1,K
      WRITE (6,937) I, (A(I,J),J=1,K)
  114 CONTINUE
      IF (IFSS2.EQ.0) GO TO 1661
      WRITE (6,903) PHIZ,SE,XL
      GO TO 169
 1661 WRITE (6,909)PHIZ,SE,XL
  169 GO TO 200
  164 PHIL=PHI
```

```
C                    WE NOW HAVE PHI LAMBDA
      DO 170 J=1,K
      IF (ABS(DB(J)/(ABS(B(J)) + TAU)).GE.E) GOTO 172
  170 CONTINUE
      WRITE (6,923) (TITLE(I),I=1,10)
      GO TO 700
  172 IF (IWS5.EQ.0) GO TO 1720
C     CALL SSWTCH(4,IFSS4)
      GO TO (171,173),IFSS4
 1720 IF (IWS4.EQ.0)GO TO 173
      IF (IWS4.EQ.1)GO TO 171
      IWS4=IWS4-1
      GO TO 173
  171 WRITE (6,924) (TITLE(I),I=1,10)
      GO TO 700
  173 XKDB = 1.
      IF (PHIL.GT.PHIZ)GO TO 190
  174 XLS=XL
      DO 176 J=1,K
      BA(J)=B(J)
  176 BS(J)=B(J)
      IF (XL.GT..00000001)GO TO 175
 1175 DO 1176 J=1,K
      B(J)=BA(J)
 1176 BS(J)=B(J)
      GO TO 60
  175 XL=XL/10.
      IBKI=2
      GO TO 200
  177 PHL4=PHI
C                    WE NOW HAVE PHI(LAMBDA/10)
      IF(PHL4.GT.PHIZ) GOTO 184
  182 DO 183 J=1,K
  183 BS(J)=B(J)
      GO TO 60
  184 XL=XLS
      DO 186 J=1,K
      BS(J)=BA(J)
  186 B(J)=BA(J)
      GO TO 60
  190 IBKI=4
      XLS=XL
      XL=XL/10.
      DO 185 J=1,K
  185 B(J)=BS(J)
      GO TO 200
  187 IF (PHI.LE.PHIZ)GO TO 196
  191 XL=XLS
      IBKI=3
  192 XL=XL*10.
  195 DO 193 J=1,K
  193 B(J)=BS(J)
      GO TO 200
  194 PHIT4=PHI
C                    WE NOW HAVE PHI(10*LAMBDA)
  180 IF (PHIT4.GT.PHIZ.GO TO 198
  196 DO 197 J=1,K
  197 BS(J)=B(J)
      GO TO 60
  198 IF (GAMMA.GE.GAMCR.GO TO 192
  199 XKDB = XKDB/2.
      DO 1199 J=1,K
      IF (ABS(DB(J)/(ABS(B(J))+TAU)).GE.E)GO TO 195
 1199 CONTINUE
      DO 1200 J=1,K
 1200 B(J)=BS(J)
      WRITE (6,934) (TITLE(I),I=1,10)
      GO TO 700
C
C     ------------------------------------------------------------------
C                    SET UP FOR MATRIX INVERSION
  200 GO TO (1102,1100),IBKT
 1100 READ (3)A
      REWIND 3
      GO TO 1104
 1102 DO 1103 II=1,K
      III=II+25
      DO 1103 JJ=1,K
 1103 A(II,JJ)=A(III,JJ)
 1104 DO 202 I=1,K
  202 A(I,I)=A(I,I)+XL
C                    GET INVERSE OF A AND SOLVE FOR DB (J)S
      IBKM=1
C     ------------------------------------------------------------------
C                    THIS IS THE MATRIX INVERSION ROUTINE
C                    K IS THE SIZE OF THE MATRIX
  404 CALL GJR(A,K,ZETA,MSING)
      GO TO (415,650), MSING
  415 GO TO (416,710), IBKM
C                    END OF MATRIX INVERSION, SOLVE FOR DB(J)
  416 DO 420 I=1,K
      DB(I)=0.
      DO 421 J=1,K
  421 DB(I)=A(I,J)*G(J)+DB(I)
  420 DB(I)=XKDB*DB(I)
      XLL=0.
      DTG = 0.
      GTG = 0.
      DO 250 J=1,K
      XLL=XLL+DB(J)*DB(J)
      DTG = DTG + DB(J)*G(J)
      GTG = GTG + G(J)**2
      DB(J)=DB(J)/BA(J)
  250 B(J)=B(J)-DB(J)
      KIP=K-IP
      IF (KIP.EQ.1) GO TO 1257
      CGAM=DTG/SQRT(XLL*GTG)
      JGAM = 1
      IF(CGAM.GT..0) GOTO 253
  251 CGAM = ABS(CGAM)
      JGAM = 2
  253 GAMMA = 57.2957795*(1.5707288-CGAM*(-0.2121144+CGAM*(0.074261
     *-CGAM*.0187293)))*SQRT(1.-CGAM)
      GO TO (257,255), JGAM
  255 GAMMA = 180.-GAMMA
      IF (XL.LT.1.0)GO TO 257
 1255 WRITE(6,922) (TITLE(I),I=1,10),XL,GAMMA
      GO TO 700
 1257 GAMMA=0.
  257 XLL=SQRT(XLL)
      IBKZ=1
      GO TO 300
  252 IF (IFSS3.EQ.0)GO TO 256
  254 WRITE (6,904)(DB(J),J=1,K)
      WRITE (6,905)PHI,XL,GAMMA,XLL
  256 GO TO (144,177,194,187),IBKI
C     ------------------------------------------------------------------
C                    CALCULATE PHI
```

```
      300 I=1
          PHI=0.
          PHIN=0.
          IWHER=2
          IF (IWSS.EQ.0) GO TO 653
      C   CALL SSWTCH(5,NONSK)
          NONSK=IF655
          IF(NONSK.EQ.1) GOTO 650
      302 GO TO 653
      304 PHI=PHI+(RES**2)
          IF (I.GT.N) GO TO 305
          PHIN=PHIN+RES*RES
      305 I=I+1
          IF (I.LE.NTILDA) GO TO 302
      916 GO TO (252,780,704,762,766,772),IBK2
      C
      C
      C     ..................................................................
      C                    THIS IS THE CONFIDENCE LIMIT CALCULATION
      700 DO 702 J=1,K
      702 B(J)=BS(J)
          WRITE (6,933)N,K,IP,M,FF,T,E,TAU
          IBKA=2
          NTILDA=N
      C                    THIS WILL PRINT THE Y,YHAT,DELTA Y
          ITCT=ITCT-1
          IFSS3=1
          GO TO 62
      704 IF (IPP.LE.0) GO TO 703
      705 IBKA=3
          IFP=0
          GO TO 62
      703 IF (NCONS.EQ.0) GO TO 706
          WRITE (6,938) (JJ,CONS(JJ),JJ=1,NCONS)
      706 WS=N-K+IP
          SE=SQRT(PHI/WS)
          PHIZ=PHI
          IF (IFSS2.EQ.0)GO TO 709
      707 WRITE (6,903)PHIZ,SE,XL
          GO TO 708
      709 WRITE(6,909) PHIZ,SE,XL
      C                    NOW WE HAVE MATRIX A
      708 GO TO (1122,1120),IBKT
     1120 WRITE (3)A
          REWIND 3
          GO TO 1124
     1122 DO 1123 II=1,K
          III=II+25
          DO 1123 JJ=1,K
     1123 A(III,JJ)=A(II,JJ)
     1124 IBKN=2
          GO TO 404
      C
      C                    NOW WE HAVE C * A INVERSE
      710 DO 711 J=1,K
          IF(A(J,J).LT..0) GO TO 713
      711 SA(J)=SQRT(A(J,J))
          GO TO 715
      713 IBOUT=1
      715 KST=-4
          WRITE (6,916)
      234 KST=KST+5
          KEND=KST+4
          IF (KEND.LT.K) GO TO 719
          KEND=K
      719 DO 712 I=1,K
      712 WRITE (6,918)I,(A(I,J),J=KST,KEND)
          IF (KEND.LT.K) GO TO 234
          IF (IBOUT.EQ.0) GO TO 717
          WRITE (6,936)
          GO TO 650
      717 DO 718 I=1,K
          DO 718 J=1,K
          WS=SA(I)*SA(J)
          IF(WS.GT. 0.) GOTO 716
      714 A(I,J)=0.
          GO TO 718
      716 A(I,J)=A(I,J)/WS
      718 CONTINUE
          DO 720 J=1,K
      720 A(J,J)=1.
          WRITE (6,917)
          KST=-9
      721 KST=KST+10
          KEND=KST+9
          IF (KEND.LT.K) GO TO 722
          KEND=K
      722 DO 724 I=1,K
      724 WRITE (6,935)I,(A(I,J),J=KST,KEND)
          IF (KEND.LT.K) GO TO 721
      C                    GET T=SE*SQRT(C(I,I))
          DO 726 J=1,K
      726 SA(J)= SE*SA(J)
          GO TO (1112,1110),IBKT
     1110 READ (3)A
          REWIND 3
          GO TO 1114
     1112 DO 1113 II=1,K
          III=II+25
          DO 1113 JJ=1,K
     1113 A(II,JJ)=A(III,JJ)
     1114 CONTINUE
      740 WRITE (6,919)
          WS=K-IP
          DO 750 J=1,K
          IF (IP.LE.0)GO TO 743
      741 DO 742 I=1,IP
          IF (J.EQ.IB(I))GO TO 746
      742 CONTINUE
      743 MJTD=SQRT(WS*FF)*SA(J)
          STE=SA(J)
          OPL=BS(J)-SA(J)*T
          OPU=BS(J)+SA(J)*T
          SPL=BS(J)-MJTD
          SPU=BS(J)+MJTD
          WRITE ( 6,927)J,STE,OPL,OPU,SPL,SPU
          GO TO 750
      746 WRITE (6,913)J
      750 CONTINUE
      C                    NONLINEAR CONFIDENCE LIMIT
          IF (IWS6.EQ.1) GO TO 650
          WS=K-IP
          WS1=N-K+IP
          PKN=WS/WS1
          PC=PHIZ*(1.+FF*PKN)
          WRITE (6,920)PC
          WRITE (6,921)
          IFSS3=1
          DO 790 J=1,K
          IBKP=1
          DO 752 JJ=1,K
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      152 B(JJ)=BS(JJ)
          IF (IP.LE.0)GO TO 758
      754 DO 756 JJ=1,IP
          IF (J.EQ.IB(JJ))GO TO 787
      756 CONTINUE
      758 DD=-1.
          IBKN=1
      760 D=DD
          B(J)=BS(J)+D*SA(J)
          IBK2=4
          GO TO 300
      762 PHI1=PHI
          IF (PHI1.GE.PC)GO TO 770
      764 D=D*DD
          IF (D/DD.GE.5.)GO TO 768
      765 B(J)=BS(J)+D*SA(J)
          IBK2=5
          GO TO 300
      766 PHID=PHI
          IF (PHID.LT.PC)GO TO 764
          IF (PHID.GE.PC) GO TO 778
      770 D=D/2.
          IF (D/DD.LE..001)GO TO 788
      771 B(J)=BS(J)+D*SA(J)
          IBK2=6
          GO TO 300
      772 PHID=PHI
          IF (PHID.GT.PC)GO TO 770
      778 XK1=PHI2/D-PHI1/(1.-D)-PHID/(D*(D-1.))
          XK2=-(PHI2*(1.-D)/D-D/(1.-D)*PHI1-PHID/(D*(D-1.)))
          XK3=PHI2-PC
          BC = (SQRT(XK2*XK2-4.*XK1*XK3)-XK2)/(2.*XK1)
          XK3=PHI2-PC
          BC = (SQRT(XK2*XK2-4.*XK1*XK3)-XK2)/(2.*XK1)
          GO TO (779,784),IBKN
      779 B(J)=BS(J)-SA(J)*BC
          GO TO 781
      784 B(J)=BS(J)+SA(J)*BC
      781 IBK2=2
          GO TO 300
      780 GO TO (782,786),IBKN
      782 IBKN=2
          DD=1.
          BL=B(J)
          PL=PHI
          GO TO 760
      786 BU=B(J)
          PU=PHI
          GO TO (783,795,785,789),IBKP
      783 WRITE (6,918) J, BL, PL, BU, PU
          GO TO 790
      795 WRITE (6,915) J, BU, PU
          GO TO 790
      785 WRITE (6,918)J,BL, PL
          GO TO 790
      787 WRITE (6,913)J
          GO TO 790
      789 WRITE (6,914)J
          GO TO 790
      788 GO TO (791,792),IBKN
C                     DELETE LOWER PRINT
      791 IBKP=2
          GO TO 780
      792 GO TO (793,794),IBKP
C                     DELETE UPPER PRINT
      793 IBKP=3
          GO TO 780
C                     LOWER IS ALREADY DELETED. SO DELETE BOTH
      794 IBKP=4
          GO TO 780
      790 CONTINUE
          GO TO 10
C     ------------------------------------------------------------------
      900 FORMAT (25I3)
      901 FORMAT (7F10.0)
      902 FORMAT(12A6 )
      903 FORMAT (/13X, 4H PHI, 14X, 4H S E , 9X, 7H LAMBDA, 6X,
     .     24H ESTIMATED PARTIALS USED, /, 5X, 2E18.8, E13.3)
      904 FORMAT(/12H INCREMENTS 5E18.8/(12X,5E18.8) )
      905 FORMAT ( 13X, 4H PHI, 10X, 7H LAMBDA, 6X, 7H GAMMA , 6X, 7H LENGTH
     .     , /, 5X, E18.8, 3E13.3)
      906 FORMAT(1X,1E9.2,86X,1E9.2 /1X,1H, 99X,1H' )
      907 FORMAT ( 5HON = , I3, 5X, 5H K = , I3, 5X, 5H P = , I3, 5X,
     .     5H M = , I3, 5X, 7H IPP = , I3, 5X, 13HGAMMA CRIT = , E10.3,
     .5X,          6HDEL = , E10.3, /, 6H FF = , E10.3, 5X, 5H T = , E10
     ..3, 5X,                5H E = , E10.3, 5X, 7H TAU = , E10.3, 5X, 6H
     .XL = , E10.3, 4X,                7HZETA = , E10.3, /)
      908 FORMAT(1X,I3,12H PARAMETERS ,6G16.8/(16X,5G16.8))
      909 FORMAT (/13X, 4H PHI, 14X, 4H S E, 9X, 7H LAMBDA, 6X,
     .     25H ANALYTIC PARTIALS USED  , /, 5X, 2E18.8, E13.3)
      910 FORMAT(1H /5X,9X,4H OBS 13X,5H PRED 13X,5H DIFF  )
      911 FORMAT (/13X, 4H PHI, 14X, 4H S E, 11X, 7H LENGTH, 6X, 7H GAMMA ,
     .     6X, 7H LAMBDA, 6X, 25HESTIMATED PARTIALS USED  , /, 5X, 2E18.
     .8,          3E13.3)
      912 FORMAT (/13X, 4H PHI, 14X, 4H S E, 11X, 7H LENGTH, 5X, 7H GAMMA ,
     .     6X, 7H LAMBDA, 6X, 24HANALYTIC PARTIALS USED  , /, 5X, 2E18.8
     .,           3E13.3)
      913 FORMAT(2X,I3,20H PARAMETER NOT USED  )
      914 FORMAT(2X,I3,12H NONE FOUND  )
      915 FORMAT(2X,I3,36X,2E18.8 )
      916 FORMAT(1H /13H PTP INVERSE )
      917 FORMAT(1H /34H PARAMETER CORRELATION MATRIX  )
      918 FORMAT( 2X,I3,5E18.8)
      919 FORMAT ( ///, 13X, 4H STD, 17X, 16H ONE - PARAMETER, 21X,
     .     14H SUPPORT PLANE, /, 3X, 2H B, 7X, 6H ERROR, 12X, 6H LOWER,
     .12X,          6H UPPER, 12X, 6H LOWER, 12X, 6H UPPER)
      920 FORMAT ( ///, 30H  NONLINEAR CONFIDENCE LIMITS , //,
     .     16H PHI CRITICAL = , E15.8)
      921 FORMAT ( //, 7H PARAM , 5X, 8H LOWER B, 8X, 10H LOWER PHI, 10X,
     .     8H UPPER B, 8X, 10H UPPER PHI)
      922 FORMAT(2H1 ,10A6,18H GAMMA LAMBDA TEST,6X,2E13.3)
      923 FORMAT(2H1 ,10A6,18H       EPSILON TEST)
      924 FORMAT(2H1 ,10A6,18H          FORCE OFF)
      925 FORMAT(4X,3G18.8,5G11.4)
      926 FORMAT ( 40H BAD DATA. SUBSCRIPTS FOR UNUSED BS = 0   / / / )
      927 FORMAT(2X,I3,5E18.8 )
      928 FORMAT(1H , 110A1 )
      929 FORMAT(10A1)
      930 FORMAT (7F10.0)
      931 FORMAT (8F10.0)
      933 FORMAT (5HON = I3,5X,5H K = ,I3,5X,5H P = ,I3,5X,5H M = ,I3,5X,
     .     /6H FF = ,E10.3,5X,5H T = ,E10.3,
     .     5X,5H E = ,E10.3,5X,7H TAU = ,E10.3/)
      934 FORMAT(2H1 ,10A6,18H GAMMA EPSILON TEST)
      935 FORMAT (3X,15,2X,10F10.4)
      936 FORMAT (27HO NEGATIVE DIAGONAL ELEMENT  )
      937 FORMAT (3X,15,2X,10F10.4/(10X,10F10.4))
      938 FORMAT (1H /25H CONSTRAINT RESIDUALS .../(3X,15,33X,E18.8))
      939 FORMAT (1H /23H PTP CORRELATION MATRIX )
      940 FORMAT(6I3,2X,10A6)
      941 FORMAT 1H1,9X,10A6/)
          END
```

```
      SUBROUTINE ATHRUZ(I,J)
C     ASSIGNS HOLLERITH CONSTANT TO INTEGER
      I=J
      RETURN
      END

      SUBROUTINE GJR(A,N,EPS,MSING)
C     GAUSS-JORDAN-RUTISHAUSER MATRIX INVERSION WITH DOUBLE PIVOTING.
      DIMENSION A(50,50),B(50),C(50),P(50),Q(50)
      INTEGER P,Q
      MSING=1
      DO 10 K=1,N
C     DETERMINATION OF THE PIVOT ELEMENT
      PIVOT=0.
      DO 20 I=K,N
      DO 20 J=K,N
      IF(ABS(A(I,J))-ABS(PIVOT))20,20,30
   30 PIVOT=A(I,J)
      P(K)=I
      Q(K)=J
   20 CONTINUE
      IF(ABS(PIVOT)-EPS)60,40,50
C     EXCHANGE OF THE PIVOTAL ROW WITH THE KTH ROW
   50 IF(P(K)-K)60,60,60
   60 DO 70 J=1,N
      L=P(K)
      Z=A(L,J)
      A(L,J)=A(K,J)
   70 A(K,J)=Z
C     EXCHANGE OF THE PIVOTAL COLUMN WITH THE KTH COLUMN
   80 IF(Q(K)-K)85,90,85
   85 DO 100 I=1,N
      L=Q(K)
      Z=A(I,L)
      A(I,L)=A(I,K)
  100 A(I,K)=Z
   90 CONTINUE
C     JORDAN STEP
      DO 110 J=1,N
      IF(J-K)130,120,130
  120 B(J)=1./PIVOT
      C(J)=1.
      GO TO 140
  130 B(J)=-A(K,J)/PIVOT
      C(J)=A(J,K)
  140 A(K,J)=0.
  110 A(J,K)=0.
      DO 10 I=1,N
      DO 10 J=1,N
   10 A(I,J)=A(I,J)+C(I)*B(J)
C     REORDERING THE MATRIX
      DO 155 M=1,N
      K=N-M+1
      IF(P(K)-K)160,170,160
  160 DO 180 I=1,N
      L=P(K)
      Z=A(I,L)
      A(I,L)=A(I,K)
  180 A(I,K)=Z
  170 IF(Q(K)-K)190,155,190
  190 DO 150 J=1,N
      L=Q(K)
      Z=A(L,J)
      A(L,J)=A(K,J)
  150 A(K,J)=Z
  155 CONTINUE
  151 RETURN
   40 PRINT 45,P(K),Q(K),PIVOT
   45 FORMAT(16HOSINGULAR MATRIX3H I=I3,3H J=I3,7H PIVOT=E16.8/)
      MSING=2
      GO TO 151
      END

      SUBROUTINE SUBZ(Y,X,B,PRNT,NPRNT,N)
      DIMENSION REC(12)
      DIMENSION Y(100),X(100,5)
      DIMENSION B(50),PRNT(5)
      READ(5,901) OPT
      READ(5,902) REC
      WRITE(6,902) REC
      IF(OPT.EQ.0.)GO TO 20
      SUMX=0.
      DO 10 I=1,N
   10 SUMX=SUMX+X(I,1)
      XN=N
      XBAR=SUMX/XN
      DO 15 I=1,N
   15 X(I,1)=X(I,1)-XBAR
   20 NPRNT = 4
      RETURN
  901 FORMAT(7F10.0)
  902 FORMAT(12A6)
      END
C*******************************************************************
C*******************************************************************
C*******************************************************************

      SUBROUTINE FCODE(P,X,B,PRNT,F,I)
      DIMENSION P(50),X(100,5),B(50),PRNT(5)
      RETURN
      END
C*******************************************************************
C*******************************************************************
C*******************************************************************
C*******************************************************************
C*******************************************************************

      SUBROUTINE FCODE(Y,X,B,PRNT,F,I,RES)
      DIMENSION Y(100),X(100,5)
      DIMENSION B(50),PRNT(5)
      PRNT(1) =X(I,1)
      PRNT(2) = EXP ( -B(3) * X(I,1))
      PRNT(3) = EXP ( -B(2) *PRNT(2) )
      PRNT(4) = B(1) * PRNT(3)
      F = PRNT(4)
      RES = Y(I) - F
      RETURN
      END
```

TIGFLD

This program calculates the moisture content, weight loss and water content of the slices cut from the field stakes and described in Section 12.

The main program calls the subroutine INDFL which reads in the data for each stake and the wet weight, dry weight and volume of each slice. STKOT is called which calculates the moisture content, weight loss and water content of each slice and writes out the initial weight, weight on removal, overall moisture content etc. for the stake. For treated stakes, WDTRT is called which calculates the retention of each stake and writes it out. For treated and leached stakes, the retention is calculated together with the moisture content during leaching and dry weight after leaching. The results are written out. The main program then calculates the moisture content, weight loss and water content of each slice, and writes the results out. A modification of the program using the subroutine HISTR from TIGROT allowed histograms of the results to be printed out. A further modification allowed the data to be displayed as the three-dimensional representations shown in Figures 89 to 94 in Section 12.

```
UNIVERSITY OF MINNESOTA 6600 FORTRAN COMPILER SCOPE  3.4.1 VER4.5     27/01/77  21.11.51.
MWF(D,T)
      1.        PROGRAM TIGFLD (INPUT,OUTPUT,TAPE5=INPUT,TAPE6=OUTPUT)
      2.        DIMENSION     SPEC (3),  IBLKP (3),  WETW (12,6),  WLSLC (12,6),
                2             NBIN (3),  ISAMT (3),  DRYW (12,6),  WCPCV (12,6),
                3             BLKN (3),  IDATE (3),  SLGT (12,6),
                4IVAL (12),   WINI(3),   WASAW (3),  CONM (12,6),
                5HISTG(100),  WREM (3),  ICODE (3),  WLSL (12,6)
      3.        COMMON   SPEC,  WETW,          NBIN,  DRYW,  IBLKP,  WLSLC,  ISAMT,
                2        BLKN,  SLGT,  IVAL,   WINI,  CONM,  WCPCV,  IDATE,  WASAW,
                3  WREM, ICODE,  WLSL,  NB,    NS,  SYMBOL,  HISTG,  SLCFAC
      4.   1011 CALL INDFL
      5.        DO 3402 L = 1, NB
      6.        IF ( ICODE(L) .EQ. 0 ) GO TO 1010
      7.        CALL STKOT(L)
      8.        IF ( ICODE(L) .EQ. 2 .OR. ICODE(L) .EQ. 5 ) CALL WDTRT(L)
      9.        IF ( ICODE(L) .EQ. 3 .OR. ICODE(L) .EQ. 6 ) CALL WDLCH(L)
     10.        WRITE (6,3409)
     11.   3409 FORMAT ( 1X,///,2X, 13HSLICE RESULTS, // )
     12.        WRITE (6,3410)
     13.   3410 FORMAT (2X, 10HSLICE NUMB, 2X, 10H WET SLICE, 2X, 10HDRY SLICE ,
                2 2X, 10HSLICE LGTH, 2X, 10HMOIST CONT, 2X, 10HWATER CONT, 2X,
                3 11HWEIGHT LOSS, 2X, 21HWEIGHT LOSS CORRECTED,//)
     14.        DO 3411 K = 1, NS
     15.        WRITE(6,3412) K,WETW(K,L), DRYW(K,L),SLGT(K,L), CONM(K,L),
                2  WCPCV(K,L), WLSL(K,L), WLSLC(K,L)
     16.   3412 FORMAT ( 7X, I3, 7X, 7 ( F7.2,5X))
     17.   3411 CONTINUE
     18.   3402 CONTINUE
     19.        GO TO 1011
     20.   1010 STOP
     21.        END


      1.        SUBROUTINE INDFL
      2.        DIMENSION     SPEC (3),  IBLKP (3),  WETW (12,6),  WLSLC (12,6),
                2             NBIN (3),  ISAMT (3),  DRYW (12,6),  WCPCV (12,6),
                3             BLKN (3),  IDATE (3),  SLGT (12,6),
                4IVAL (12),   WINI(3),   WASAW (3),  CONM (12,6),
                5HISTG(100),  WREM (3),  ICODE (3),  WLSL (12,6)
      3.        COMMON   SPEC,  WETW,          NBIN,  DRYW,  IBLKP,  WLSLC,  ISAMT,
                2        BLKN,  SLGT,  IVAL,   WINI,  CONM,  WCPCV,  IDATE,  WASAW,
                3  WREM, ICODE,  WLSL,  NB,    NS,  SYMBOL,  HISTG,  SLCFAC
      4.        READ(5,3010) NB, NS
      5.   3010 FORMAT ( I2, 1X, I2 )
      6.        DO 3014 L = 1, NB
      7.        READ (5,3011) SPEC(L),ICODE(L), BLKN(L), IBLKP(L),ISAMT(L),
                2 IDATE(L), WINI(L), WREM(L), WASAW(L)
      8.   3011 FORMAT( A5, 2X, I2, 2X, A4,2X,I2, 2X, I3, 2X, I4,2X,
                2   3( F4.2, 2X))
      9.        IF ( ICODE(L) .EQ. 0 ) RETURN
     10.        DO 3013 K = 1,4
     11.        READ(5,3012) NSEGA, WETW(NSEGA,L), DRYW(NSEGA,L), SLGT(NSEGA,L),
                2                NSEGB, WETW(NSEGB,L), DRYW(NSEGB,L), SLGT(NSEGB,L),
                3                NSEGC, WETW(NSEGC,L), DRYW(NSEGC,L), SLGT(NSEGC,L)
     12.   3012 FORMAT ( 3 ( 1X, I2, 1X, 2 ( F5.2, 1X), F4.1, 3X ))
     13.   3013 CONTINUE
     14.   3014 CONTINUE
     15.        RETURN
     16.        END


      1.        SUBROUTINE STKOT(L)
      2.        DIMENSION     SPEC (3),  IBLKP (3),  WETW (12,6),  WLSLC (12,6),
                2             NBIN (3),  ISAMT (3),  DRYW (12,6),  WCPCV (12,6),
                3             BLKN (3),  IDATE (3),  SLGT (12,6),
                4IVAL (12),   WINI(3),   WASAW (3),  CONM (12,6),
                5HISTG(100),  WREM (3),  ICODE (3),  WLSL (12,6)
      3.        COMMON   SPEC,  WETW,          NBIN,  DRYW,  IBLKP,  WLSLC,  ISAMT,
                2        BLKN,  SLGT,  IVAL,   WINI,  CONM,  WCPCV,  IDATE,  WASAW,
                3  WREM, ICODE,  WLSL,  NB,    NS,  SYMBOL,  HISTG,  SLCFAC
      4.        WETWSM = DRYWSM = SLGTSM = 0.0
      5.        DO 3502 K = 1, NS
      6.        WATC = SLCFAC = STKMC= STKWL=  STKWC= STKMCOR = STKWCOR = 0.0
      7.        WETWSM = WETWSM + WETW(K,L)
      8.        DRYWSM = DRYWSM + DRYW(K,L)
      9.        SLGTSM = SLGTSM + SLGT(K,L)
     10.        WATC = WETW(K,L) - DRYW(K,L)
     11.        CONM(K,L) = WATC * 100.0 / DRYW(K,L)
     12.        SLCFAC = SLGT(K,L) / 200.0
     13.        WLSL(K,L) = ((( WINI(L) * SLCFAC ) - DRYW(K,L))* 100.0)/(WINI(L)
                2  * SLCFAC )
     14.        WLSLC(K,L) = 0.0
     15.        WCPCV(K,L) = ( WATC * 100.0) / (SLGT(K,L) * 1.5)
     16.   3502 CONTINUE
     17.        IF ( WREM(L) .LT. 1.0 ) GO TO 3406
     18.        STKMCOR = ( WREM(L) - WINI(L) ) * 100.0 / WINI(L)
     19.        STKWCOR = ( WREM(L) - WINI(L) ) * 100.0 / 300.0
     20.   3406 STKMC = ( WETWSM - DRYWSM) * 100.0 / DRYWSM
     21.        STKWC = ( WETWSM - DRYWSM) * 100.0 / 300.0
     22.        STKWL=(((WINI(L)*SLGTSM/200.0)-DRYWSM)*100.0) /WINI(L)
     23.        WRITE (6,3403) SPEC(L),ICODE(L), BLKN(L), IBLKP(L), ISAMT(L),
                2  IDATE(L)
     24.   3403 FORMAT (1H1, 4HWOOD, 1X, A5, 3X, 4HCODE, 1X, I2, 3X,9HSTAKE NO.,
                2  1X, A4, 3X, 10HSTAKE POSN, 1X, I2, 3X, 11HSAMPLE TIME, 1X, I3,
                3  1X, 6HMONTHS, 3X, 4HDATE, 1X, I6, ///)
```

```
      WRITE (6,2420)
 2420 FORMAT (1HO)
      WRITE(6,3404)
 3404 FORMAT (2X, 10HINITIAL WT, 2X, 10HWT REMOVAL, 2X, 10HWT APT SAW,
     2  2X, 10HSUM WET WT, 2X, 10HSTAKE MCON, 2X, 11HSTAKE WCPCV, 1X,
     3  11HSTAKE WT LS, 2X, 10HSTK MC REM, 2X, 10HSTK WC REM//)
      WRITE(6,3405) WINI(L), WREM(L), WASAW(L), WETWSM, STKMC, STKWC,
     2  STKWL, STKMCOR, STKWCOR
 3405 FORMAT ( 5X, 9 ( F7.2, 5X) )
      RETURN
      END
      SUBROUTINE WOTRT(L)
      DIMENSION    SPEC (3),  IBLKP (3),  WETW (12,6),  WLSLC (12,6),
     2             NBIN (3),  ISAMT (3),  DRYW (12,6),  WCPCV (12,6),
     3             BLKN (3),  IDATE (3),  SLGT (12,6),
     4IVAL (12),  WINI(3),  WASAW (3),  CONM (12,6),
     5HISTG(100),  WREM (3), ICODE (3),  WLSL (12,6)
      COMMON   SPEC,  WETW,          NBIN,  DRYW,  IBLKP,  WLSLC,  ISAMT,
     2         BLKN,  SLGT,  IVAL,  WINI,  CONM,  WCPCV,  IDATE,  WASAW,
     3  WREM, ICODE,  WLSL,  NB,    NS,  SYMBOL,  HISTG, SLCFAC
      READ(5,3200) VOL, WTINIT, FINWT, WTAFT
 3200 FORMAT ( 1X, 3 (F5.1, 1X), F6.2)
C  PRESERVATIVE CONCENTRATION
      CONCN = 3.32
      GNINWT = FINWT - WTINIT
C  PRESERVATIVE IN STAKE IN GMS.
      PRSTK = GNINWT * ( CONCN / 100.0 )
C  RETENTION IN KGS/M3
      RETNTN = ( PRSTK / VOL) * 1000.0
      DO 3401 K = 1, NS
      SLCFAC = SLGT(K,L) / 200.0
C  WEIGHT OF PRESERVATIVE PER SLICE
      WTPSL = PRSTK * SLCFAC
C  ORIGINAL SLICE WEIGHT BEFORE BURIAL
      OSLW = WTAFT * SLCFAC
C  THEORETICAL WEIGHT OF SLICE WITHOUT PRESERVATIVE
      WSLNPC = OSLW - WTPSL
C  WEIGHT OF SLICE CORRECTED FOR PRESERVATIVE CONTENT
      WLSLC(K,L) = ((WSLNPC-(DRYW(K,L)-WTPSL))/WSLNPC)*100.0
C  WEIGHT LOSS DIRECT NOT CORRECTED FOR PRESERVATIVE CONTENT
      WLSL(K,L) = ( ( OSLW - DRYW(K,L) ) * 100.0 ) / OSLW
 3401 CONTINUE
      WRITE(6,3406)
 3406 FORMAT( 1X,//,   1X,10HVOLUME CM3, 2X, 10HINITIAL WT, 2X,
     2  10HWT WET TRT, 2X, 10HGAIN IN WT, 2X, 10HWT DRY TRT,
     3  2X, 16HRETENTION KG/M3, // )
      WRITE(6,3407) VOL, WTINIT, FINWT, GNINWT, WTAFT, RETNTN
 3407 FORMAT ( 5X, 6(F7.2,5X))
      RETURN
      END
      SUBROUTINE WDLCH(L)
      DIMENSION    SPEC (3),  IBLKP (3),  WETW (12,6),  WLSLC (12,6),
     2             NBIN (3),  ISAMT (3),  DRYW (12,6),  WCPCV (12,6),
     3             BLKN (3),  IDATE (3),  SLGT (12,6),
     4IVAL (12),  WINI(3),  WASAW (3),  CONM (12,6),
     5HISTG(100),  WREM (3), ICODE (3),  WLSL (12,6)
      COMMON   SPEC,  WETW,          NBIN,  DRYW,  IBLKP,  WLSLC,  ISAMT,
     2         BLKN,  SLGT,  IVAL,  WINI,  CONM,  WCPCV,  IDATE,  WASAW,
     3  WREM, ICODE,  WLSL,  NB,    NS,  SYMBOL,  HISTG, SLCFAC
      READ(5,3200) VOL,WTINIT,FINWT,WTAFT,WTWTLC,WTDYLC
 3200 FORMAT(1X,3( F5.1,1X), 3 ( F6.2,1X))
C  PRESERVATIVE CONCENTRATION
      CONCN = 3.32
      GNINWT = FINWT - WTINIT
C  PRESERVATIVE IN STAKE IN GMS.
      PRSTK = GNINWT * ( CONCN / 100.0 )
C  RETENTION IN KGS/M3
      RETNTN = ( PRSTK / VOL) * 1000.0
      IF ( WTWTLC .LT. 1.0 ) GO TO 3408
C   MOISTURE CONTENT DURING LEACHING
      CMDLC = ( ( WTWTLC - WTAFT) * 100.0) / WTAFT
      WCPCLC = ( ( WTWTLC - WTDYLC) * 100.0) / VOL
 3408 DO 3300 K = 1, NS
      SLCFAC = SLGT(K,L) / 200.0
C  WEIGHT OF PRESERVATIVE PER SLICE
      WTPSL = PRSTK * SLCFAC
C  ORIGINAL SLICE WEIGHT BEFORE BURIAL
      OSLW = WTDYLC * SLCFAC
C  THEORETICAL WEIGHT OF SLICE WITHOUT PRESERVATIVE
      WSLNPC = OSLW - WTPSL
C  WEIGHT OF SLICE CORRECTED FOR PRESERVATIVE CONTENT
      WLSLC(K,L) = ((WSLNPC-(DRYW(K,L)-WTPSL))/WSLNPC)*100.0
C  WEIGHT LOSS DIRECT NOT CORRECTED FOR PRESERVATIVE CONTENT
      WLSL(K,L) = ( ( OSLW - DRYW(K,L) ) * 100.0 ) / OSLW
 3300 CONTINUE
      WRITE(6,3406)
 3406 FORMAT( 1X,//,   2X,10HVOLUME CM3, 2X, 10HINITIAL WT, 2X,
     2  10HWT WET TRT, 2X, 10HGAIN IN WT, 2X, 10HWT DRY TRT,
     3  2X, 16HRETENTION KG/M3, // )
      WRITE(6,3407) VOL, WTINIT, FINWT, GNINWT, WTAFT, RETNTN
 3407 FORMAT ( 5X, 6(F7.2,5X))
      WRITE(6,3302)
 3302 FORMAT ( 1X,//, 14X,10HWT WET LCH, 2X, 10HWATCPC LCH, 2X,
     2 10HMCON LEACH,2X, 10HWT DRY LCH,//)
      WRITE(6,3303) WTWTLC, WCPCLC, CMDLC, WTDYLC
 3303 FORMAT ( 17X, 4 ( F7.2, 5X) )
      RETURN
      END
```

TIGFLD Input :

Card 1 : Number of stakes, number of segments per stake.

e.g. 02 12

Card 2 : Species code, Code for treatment (01=Untreated Scots pine, 02= Treated Scots pine, 03=Treated and Leached Scots pine, 04=Untreated Birch , 05=Treated Birch, 06=Treated and Leached Birch), Stake number, Stake position, Sample time, Date of Sample, Initial Wt., Wt. on removal, Wt. after sawing into segments.

e.g. SPNLC__03__EA06__22__001__060176__139•63__000•00__000•00

Card 3 - 6 : Segment number, Wet Wt., Dry wt., Segment length ; Segment number, Wet wt., Dry wt., Segment length ; Segment number, Wet wt., Dry wt., Segment length.

e.g. _01_25•34_16•20_15•5____02_15•37_10•24_15•5____03_15•83_10•26_15•5

Card 7 : Omitted if stake is untreated. If stake is treated and leached : Stake volume, Initial wt. before treatment, Wt. after treatment, Wet wt. during leaching, Dry wt. after leaching. If stake is treated, but not leached : Wet wt. during leaching and Dry wt after leaching are omitted.

e.g. _229•0_140•3_362•8_175•56_366•01_183•85

Cards 8 - 13 : Similar to cards 2 - 7.

Card 14 : End of run card

e.g. 00_00

TIGFLD Output :

WOOD SPNLC CODE 3 STAKE NO. EA06 STAKE POSN 22 SAMPLE TIME 1 MONTHS DATE 60176

INITIAL WT	WT REMOVAL	WT AFT SAW	SUM WET WT	STAKE MCON	STAKE WCPCV	STAKE WT LS	STK MC REM	STK WC REM
139.63	0.00	0.00	185.84	56.69	22.41	4.81	0.00	0.00

VOLUME CM3	INITIAL WT	WT WET TRT	GAIN IN WT	WT DRY TRT	RETENTION KG/M3
299.00	140.30	362.80	222.50	175.56	24.71

WT WET LCH	WATCPC LCH	MCON LEACH	WT DRY LCH
366.01	60.92	108.48	183.85

SLICE RESULTS

SLICE NUMB	WET SLICE	DRY SLICE	SLICE LGTH	MOIST CONT	WATER CONT	WEIGHT LOSS	WEIGHT LOSS CORRECTED
1	25.34	16.20	24.50	56.42	24.87	28.07	29.24
2	15.37	10.24	15.50	50.10	22.04	28.13	29.31
3	15.83	10.26	15.50	54.29	23.94	27.49	29.16
4	16.57	10.40	15.50	59.33	26.54	27.01	28.14
5	16.27	10.50	15.50	54.95	24.87	25.31	27.41
6	15.49	10.42	15.50	50.58	22.67	25.87	27.99
7	15.30	10.07	15.50	51.94	22.49	29.33	30.55
8	15.63	10.53	15.50	48.43	21.94	25.10	27.19
9	15.24	10.21	15.50	49.46	21.72	25.34	29.51
10	15.31	9.83	15.50	55.75	23.57	31.01	32.51
11	19.27	9.94	15.50	93.86	40.18	30.24	31.50

TIGFSG

This program calculates the moisture content, weight loss, acetylene reduction rate and water content in segments cut from slices of the stakes exposed in the field and described in Section 12. It is similar to TIGROT and calls BOTIN, which reads in the weights and volumes of the bottles used in the AR assay, and then INDAT reads in the data for each segment of each slice. The input data was recorded on forms similar to that shown in Figure 19, except that the segment volume replaces the segment length. The density map produced by MAPER represents the transverse face of the slices cut from the stakes.

A modification of the program, combined with the MATMAP package produced the three-dimensional representations shown in Figures 93 and 94.

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