FUNGAL COLONISATION OF EXTRACTION WOUNDS IN CONIFERS

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ABSTRACT

Four compartments of Norway spruce (Picea abies L. Karst) and Japanese larch (Larix kaempferi Lamb. & Carr.) were examined for the occurrence of extraction damage on the stems and superficial roots and the associated microflora. The study was carried out in two forests (Glentress and Yair Hill Forest) in the Tweed Valley in Scotland. Trees were dissected and the spread of discolouration and decay assosiated with extraction damage were recorded.

Stereum sanguinolentum (Alb. and Schw. ex Fr.) Fr. was the most common species isolated from infected Norway spruce , whereas Chaetomium cochliodes Palliser was the dominant species in Japanese larch. Four strains of S.sanguinolentum were isolated during the course of experiments and a detailed study on these strains was carried out. The expansion of decay by S.sanguinolentum was much greater in Norway spruce than in Japanese larch. Extraction wound and tree vigour were the most important factors that influenced the development of decay in Norway spruce. Fast growing trees showed the greatest expansion of decay as compared with slow growing trees. A number of correlations were demonstrated between wound , decay spread and tree vigour which might enable the forest manager to estimate the spread of decay by S.sanguinolentum with the knowledge of at least one variable. A total of 156 Norway spruce trees were artificially inoculated with S.sanguinolentum to study the

effect of inoculation site treatment (open or sealed), tree vigour and time of year at which the inoculations were made on the frequency and development of decay. Open inoculation sites were more likely to be infected compared with sealed inoculation sites. Decay was much greater in trees inoculated in July as compared with trees inoculated in February. Most of the correlations derived from artificial inoculation results confirmed the results obtained from naturally infected trees.

Trichoderma viride Fr. showed a greater tendency to replace and suppress S.sanguinolentum in living trees as well as in wood blocks in the laboratory. Wood samples from naturally and artificially inoculated trees as well as wood blocks artificially inoculated with S.sanguinolentum were analysed chemically to examine the effect of the fungus on cellulose and lignin. 8 years after natural infection with S.sanguinolentum an approximately 50% loss of cellulose was recorded in dominant Norway spruce trees. The destruction of cellulose and lignin was consistently much greater in fast growing trees as compared with slow growing trees.

DECLARATION

This thesis has been composed by myself and it has not been submitted for any previous degree. The work reported was executed by myself unless otherwise stated.

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CHAPTER 1

1.INTRODUCTION

Tree crops differ from almost other crops in that a significant part of the total production of any stand is harvested at intervals, before the crop as a whole is fully mature or before the periodic mean annual increment reaches its peak. With the recognition that the total production from any forest site is finite the forester is faced with a compromise in any management prescription. He may,for example, decide to concentrate his production upon a large number of small stems or else decide to carry out thinning operations so that the bulk of the production is concentrated upon a smaller number of larger trees (Baker,1934). Smith (1962) mentioned that the reduction in number of stems per unit area is not entirely a steady and progressive selection of the most vigorous trees because this process may be interrupted or reversed by natural accidents that eliminate trees at random. The most vigorous trees from the standpoint of natural selection are not necessarily the best ones from the forest manager's viewpoint. Therefore, thinning has two objectives: to stimulate the growth of trees which remain after removing the undesirable ones and to increase the total yield of utilisable material from the stand. Implicit in any decision to thin (unless,of course,thinning to waste is prescribed) is the fact that the felled material must , at some stage, be removed from within the residual stand. Shigo (1966a) has posed the whole problem as follows: "Growing high-quality

timber is one purpose of all forest managers. More and more attention has been paid to quality in recent years. And as emphasis on quality has increased,forest managers have felt a need for more information about any management operation that affects the quality of individual trees. What can happen to a tree that can affect its quality? Logging,for one thing. The trend in logging today is toward bigger machines and fewer men,and toward shorter intervals between the timber harvests. This leads to greater productivity,but it brings with it new problems. One is the increase in logging damage to the trees that are left". Therefore,thinning should be done properly and in such a way to avoid damaging the residual crop,especially where heavy machinery is used for extracting the felled stems;since wounding of tree stems can lead to serious infection by wood-decay fungi that are capable of entering through extraction wounds.

Although its activities had been previously noted by a number of workers ,not until 1960 did Gladman and Low draw attention to the fact that Stereum sanguinolentum (Alb. and Schw. ex Fr.) Fr. was a very common wound pathogen with a wide host range in the UK. They noted that it was also a very serious decay fungus associated with wounds,particularly those occurring on Norway spruce (Picea abies L. Karst.). They also observed fruit bodies occurring on the surfaces of wounds , on conifer stumps and on cut logs on the forest floor.

A later study in five Scottish forests by Pawsey and

Gladman (1965) confirmed that S.sanguinolentum was the most common decay fungus invading extraction wounds of Norway spruce ; Sitka spruce (P.sitchensis (Bong.) Carr.); Japanese larch (Larix kaempferi (Lamb. and Carr.) and European larch (L.decidua (Mill.) and was isolated from 27% of the samples examined.

Isolations carried out by Pawsey and Stankovicova (1974a) on injured crops of young Norway spruce in the Forest of Dean,Southern England, showed that S.sanguinolentum was the only decay fungus in association with evident decay in these stands.

Although information concerning some aspects of the distribution of S.sanguinolentum in parts of the UK is known , there appeared to be little critical information on the ability of this fungus to attack different conifers commonly planted in the UK.

The present study was therefore undertaken with the following objectives :

1. To investigate the occurrence of damage on Norway spruce and Japanese larch that resulted from extraction operations and to examine the associated decay occurring through extraction wounds.

2. To isolate different microorganisms from the infected trees and to identify the fungus or fungi responsible for decay

3. To examine the effect of different microorganisms on the development of decay

4. To determine the rate of development of decay

vertically,radially and by volume

5. To investigate factors that influence the development of decay

6. To inoculate some trees artificially and to examine the effect of inoculation site treatment,tree vigour and the time at which the inoculations were made on the frequency of successful infection and the development of decay

7. To determine the effect of the decay fungus or fungi in question on the major wood constituents e.g cellulose and lignin and to determine the rate at which they are degraded

8. To carry out physiological studies on the major decay fungus or fungi,isolated during the course of experiments, that may assist in developing possible control measures

The study was carried out in two forests in the Tweed Valley (Glentress forest in Peeblesshire and Yair Hill forest in Selkirkshire) in Scotland.

CHAPTER 2

2. *REVIEW OF THE PREVIOUS WORK*

2.1. Extraction damage in

A. Conifers

A.1. Britain

In Britain, damage of tree stems and superficial roots occurs frequently due to improper use of machinery or horses for the extraction of logs. This resulted in the initiation of decay in the residual crop. However, little has been done until recently to minimise such damage and its consequences. Generally,the incidence of decay through extraction wounds is relatively low probably due to the relative immaturity of conifers in Britain. The damage may increase as trees approach maturity (Gladman and Low,1960). The extent of damage is higher especially in North America and Canada where many conifer crops are mature or overmature. _Fomes annosus_ (Fr.)Cke. butt and root rot is the most important problem in British forests and Rishbeth (1950,1951,1957 & 1959) provided much valuable information on the biology of _F.annosus_ and its mode of infection in East Anglia pine plantations. The fungus was found to invade trees through freshly-cut stump surfaces,and rarely through extraction wounds, by means of air-borne spores. Infection then spreads downwards to the roots. The disease further spreads through root contacts. Accordingly,Rishbeth initiated chemical stump treatment to control infection by this fungus. This method proved to be efficient. When other fungi are concerned,however,the possibility of other alternative entry courts for decay

fungi should be considered. Therefore intensive surveys have been carried out recently on the problem of infection of conifers through extraction wounds on both stems and superficial roots. The early studies were made by Pawsey and Gladman(1965) in 5 Scottish forests (Monaughty forest (Moray), South Laggan (Inverness-shire),Inverliever (Argyll),Glentress (Peebles-shire) and Benmore (Argyll). The species studied were Norway spruce (*Picea abies*) Sitka spruce (*P.sitchensis*), Japanese larch (*Larix kaempferi*) and European larch (*L.decidua*) . A total of 2444 scars on these trees were examined. Dissection showed that 148 scars(6.1%)were infected with decay fungi. Norway spruce had the highest level of infection compared with the other species. The incidence of infection was three times greater in stem scars than in root scars. Cultural isolations showed that *Stereum sanguinolentum* (Alb.& Schw.exFr.)Fr. was the most common decay fungus,being isolated from 27% of the samples examined. It had a vertical expansion of 40.6 cm per year. In contrast,*F.annosus* was much less frequently isolated (0.25%). *Stereum chailletii* (Pers.)Fr. was isolated from only 3 samples (0.12%) and may be of a potential importance as it showed a fast rate of growth at Inverliever (Argyll,Scotland). Generally,wound surfaces were colonized by saprophytic fungi ,especially those on roots, as they are in contact with the soil and probably moisture content and microbial environment is suitable for the growth of soil saprophytes. Fructifications of other fungi were observed on the surfaces of some scars e.g

Polyporus stipticus (Pers.)Fr. which has also been noted as of potential importance as a wound pathogen on the continent.

Pawsey (1971),reported the occurrence of decay in association with extraction damage in crops of Norway spruce in Southern England. Damage was found to increase with crop age and was also potentially serious in compartments with steep extraction racks. Line thinning, where regular racks were cut through the crop, causes much less damage compared with selection thinning. Financial losses,due to decay,ranked next to those caused by _F.annosus_ rot e.g decay through extraction wounds by _S.sanguinolentum_ and _P.stipticus_ , amounted to 70% of the total decay loss in one compartment of Norway spruce in South West England. Generally,crops which were damaged repeatedly over a number of thinnings,were most heavily infected,but even in this case some crops showed only little decay. There is a considerable variation in the frequency and severity of infection. The worst occurrence of decay was reported in a 49-year-old Norway spruce crop in Hazelborough forest. A total of 225 injured trees were examined: 40% had scars;of these 26% had decay. A total of 27% of the scars with decay yielded _S.sanguinolentum_ in culture and 23% yielded _P.stipticus_ . The average and maximum annual development of decay were 15.5 and 26.7 cm ; and 7.9 and 18.3 cm for _S.sanguinolentum_ and _P.stipticus_ respectively. In the Forest of Dean in a 60 years old crop of Norway spruce ,approximately 20% of the decay following

extraction damage was caused by the above mentioned species. The corresponding rates of annual development of decay were: 6.4 and 8.9 cm for S.sanguinolentum and 5.1 and 9.7 cm for P.stipticus respectively. The incidence of infection by S.sanguinolentum was attributed to the discharge of the spores throughout the year whereas those of P.stipticus were available for a shorter period of time mainly in the autumn. The successful development of fungi below extraction scars depends on microclimatic and the biological conditions on the surfaces of wounds and tree response to wounding; availability of viable spores; resin content and the presence of competing microorganisms e.g Cryptopsporiopsis abietina (Rostr.)Petr. which has been reported to inhibit the invasion of extraction wounds by decay fungi. Other species isolated were: Odontia bicolor (A. and S. ex.Fr.)Pers. both in Southern England and in Scotland; Amylostereum chailletii (Pers. ex Fr.)Boidin; S.chailletii only in Scotland; Coniophora puteana (Fr.)Karst.; Calocera viscosa Fr. and Coryne sarcoides (Jacq.)Tul. (Pawsey, 1971).

A more recent study on the decay of spruce crops following extraction damage was carried out in Southern England by Pawsey and Stankovicova (1974a). It was confined to injured young spruce crops which were damaged within 6 years period. A variation in the extent and type of fungal invasion of wounds was reported. Where decay occurred, the only decay-fungus isolated was S.sanguinolentum . A variety of fungi were found colonizing the wood closer to the wounds. The most common species

isolated were: Graphium spp ; Cephalosporium spp and Sclerophoma pityophila (Corda.)Hohnel. There was no evidence that these fungi have actually influenced the establishment and development of S.sanguinolentum . The growth of Graphium spp was mainly associated with knots.

A.2. Europe

Introduction of heavy machinery in thinning operations has resulted in damage to stems and roots of residual crops in Sweden (Nillson and Hyppel,1968). Examining 1036 scars on 219 Norway spruce trees ranging from 6-8,10 to 33 years,they reported that S.sanguinolentum and F.annosus were the most successful decay fungi that caused well established rots. Karkkainen (1971)examined 78 damaged spruce crops in Northern Finland. Scars on these trees were 12 years old and the stand age was 110 years. Decay had become established both on stems and roots. More recently Norokorpi (1979) investigated wound decay in Northern Finland. A total of 6275 trees,150 years old,were examined for decay through extraction wounds. Decay entering through extraction wounds accounted for 5% of the total number of trees with decay and the associated mean volume of decay recorded was 2.6 m^3 per ha (4.9% of the under bark volume). Results of isolations showed that members of the Basidiomycotina were the predominant group. The species associated most frequently with wounds was S.sanguinolentum and was responsible for 35.9% of the total wound decay frequency. Next most frequent was Phellinus chrysoloma (Fr.)Donk. accounting for 13.8% and

Peniophora septentrionalis (M.)Laur.(12.3%). An interesting result was that F.annosus was not found entering through wounds,but it did occur as a butt rot fungus. Other species isolated were: Aspergillus spp (2%) and Penicillium spp (11%). The author referred to Bavarian spruce stands where Penicillium spp were isolated from 45% of the wound-decay columns. Bacteria were isolated from about half of the decay columns and they were found in both butt rot and wound-decay columns.

In Germany,a total of 2083 spruce trees, subjected to bark stripping by goats,were examined by Bazzigher (1973). A total of 30% showed open wounds; 46% healed wounds and 24% with no injuries. Injured trees were dissected and 441 discs were examined. Of these 73% showed decay; 10% discolouration and 17% with no decay or discolouration. Further, Schonhar (1975),examined 10 injured spruce trees in each of 24,50-80 years old crops. Scars were 1 and 3 years old. A total of 35% of the younger scars were decayed compared with 63% of the older ones. S.sanguinolentum was the most common decay fungus isolated from 37% of the samples examined,followed by S.areolatum (Fr.) (13%) and F.annosus (1%).

A.3. North America and Canada

A series of studies on the problem of fungal colonization of mechanical injuries and tree defects have been conducted in North America and Canada. For ease of clarity,these have been reported by host-species as follows:

A.3.1. Douglas fir (Pseudotsuga menziesii [Mirb.]Franco.

Investigating the decay of Douglas fir , Boyce (1923) gave a simple account of the mechanism of infection through wounded,exposed tissues. Wind blown-spores of wood-decay fungi that attack heartwood of living trees,must alight on exposed dead wood for infection to occur. The entry points can be mechanical injuries,fire scars,knots or branch stubs. Boyce found that knots,especially on Douglas fir,were the most important entry courts for decay-fungi, being responsible for 90% of the total decay volume,whereas fire scars accounted for only 4% and the remaining 6% were due to various injuries. In another survey in Western Oregon and Washington, Boyce (1932) examined the decay of Douglas fir through mechanical scars,fire scars,knots and branch stubs and confirmed that knots were most serious avenue for the entry of wood-decay fungi. Young stands of Douglas fir,18-20 years old,were examined by Hubert (1935). Mottled bark was the symptom of infection by S.sanguinolentum ,the resulting discolouration spreading as ray-like,irregular rays or patches. According to these symptoms and the occurrence of sporophores, S.sanguinolentum was identified as the causal organism and is locally termed the slash fungus,living as a saprophyte on dead and down material on the forest floor.

A.3.2. Western hemlock (Tsuga heterophylla [Raf.]Sarg.

The first investigations on the extraction wound-decay of this species were done by Englerth (1942)in Western

Oregon and Washington. Decay-fungi were found to have entered through knots,falling tree scars, broken tops,fire scars,lightning scars and frost cracks. F.annosus was the most common fungus attacking injured trees occurring frequently on moist sites. The rot column can reach up to 12.2 metres or more, resulting in a hollow butt for as much as 7.6 metres. In such instances the whole heartwood was decayed, leaving a thin layer of sapwood. Nevertheless, F.annosus is capable of invading both the heart and sapwood. Mechanical injuries were extremely serious in Western hemlock due to its thin bark,which is relatively susceptible to damage and therefore provides only little protection. Further the high moisture content of Western hemlock butts makes them very susceptible to severe decay by F.annosus . Of a total of 304 scars on 654 trees dissected,57% were found to be infected with decay fungi. Fire scars were less common,but 8 trees with fire scars were all infected and an extensive rot was established 87 years after fire injury. This study was followed by another investigation in the same area i.e Western Oregon and Washington,by Englerth and Isaac (1944). A total of 56 wounds on Western hemlock were examined of which 52 were infected with decay-fungi. Wounds were 12 years old and the maximum vertical expansion of decay recorded was 2.44 metres, the average being 0.73 metres. The radial penetration of decay ranged from zero to 30.5 cm,the average being 12.2 cm. The predominant fungus isolated in association with decay of logging scars , was F.annosus ,

followed by Poria colorea (Overh.& Engler.) and P.subacida (Peck)Sacc. Fomes pinicola (Fr.)Cke and Fomes applanatus (Wall.)Gill. were isolated mainly in association with bole scars and broken tops respectively. Rhoads and Wright (1946),reported a widespread trunk rot caused by F.annosus entering through extraction wounds on Western hemlock in Western Oregon and Washington. The decay volume exceeded that caused by all other fungi combined. It was not found entering through roots except when these are injured above the ground level. In Queen Charlotte Islands,British Columbia,Foster and Foster (1951) found that logging scars provided the most important and frequent entry courts for decay-fungi. Decay loss averaged 10.9% of the total wood volume. S.sanguinolentum was isolated frequently in association with extraction injuries. Kimmey (1964),continued the studies on the decay of young Western hemlock through mechanical injuries,fire scars,pruning wounds,and broken tops. White rots were the dominant rots found and were responsible for 87% of the decay volume inland and 75% in the coastal region.

A.3.3. Sitka spruce and True firs [P.sitchensis and Abies spp]

S.sanguinolentum was recorded as being responsible for 54% of the decay volume in balsam fir in New England (Spaulding and Hansbrough,1944). The entry courts were dead branches and branch stubs (79%), forks (13%) and through broken and dead tops ,mechanical wounds and frost cracks in the remaining 8%.

Wright,Rhoads and Isaac (1947),investigated wound-decay

in stands of Western hemlock and Sitka spruce. Out of 600 trunk scars on Western hemlock and 104 on Sitka spruce,33% were basal and 10% occurred on superficial roots. Scarring was very severe and individual scars ranged from 0.6 to 1.2 metres long and about 0.3 metres wide. Scars varied in age from 5 to 32 years. Results of dissections showed that 50% of the scars were infected and decayed. The loss due to decay amounted to 11.1% of the gross volume of Western hemlock and 12% of that of Sitka spruce. A loss of 10.6% and 11% in sawtimber was recorded for Western hemlock and Sitka spruce respectively. Many decay fungi were associated with one wound,but always one species predominated. Fomes annosus was the predominant species isolated. It attacks both young and old trees and produces white pockets and occasional black spots at the advanced stage of decay. F.pinicola was frequent in spruce wounds and responsible for 60% of the decay volume of Sitka spruce and 20% of that in Western hemlock. Wood infected by F.annosus can be utilized in the production of low grade paper,whereas that attacked by F.pinicola is quite useless. Working in the Franklin River area,Buckland, Foster and Nordin (1949) reported that scars had played an important role in the decay of Western hemlock and firs (mainly Abies amabilis (Loud.)Forb.). Infection through wounds accounted for 59% of the total infections. Fir seemed to be more resistant to decay than did Western hemlock. The fungal colonisation of extraction wounds on Western hemlock , Sitka spruce and true firs(grand fir (A.grandis (Dougl.)Lindl.

and silver fir (A.amabilis)) was studied by Wright and Isaac (1956) in Western Oregon and Washington. A total of 51% of the scars on Western hemlock were infected and decayed in the coastal area ,and 78% of the scars were rotted in inland stands,whereas 58% and 90% of the scars on Sitka spruce were decayed in the coastal area and inland respectively. A large number of trees were left standing after a light thinning, increasing the probability of damage to a large number of trees compared with a heavy thinning where a smaller number of trees are left. Nevertheless, direct exposure of Western hemlock to sunlight,following a heavy thinning, will result in extreme sunscald injury that may afford access to various decay-fungi. Hence,the authors suggested that heavy thinnings in Western hemlock stands should be avoided as much as possible. Infection was reported to be more frequent immediately after wounding and this was clearly shown by the fact that the rate of radial penetration of decay decreased with time but has not ceased completely. This study has provided valuable graphical data to estimate the amount of decay ,provided that scar size and age are already known. This is applicable to scars up to 0.93 m^2 in area and from 3 to 30 years of age. F.annosus and Stereum rots were the most common in Western hemlock. In Sitka spruce F.pinicola accounted for 34% of the rots and Lentinus kauffmanii Bier & Nobles was responsibe for 11% of the total infections. A total of 90% of the scars on fir were infected by at least one decay fungus. Further studies on the deterioration of

Western hemlock and Sitka spruce were carried out by Shea (1960). Trees examined were 90 years old and had 17-year-old scars. The frequency of infection by decay fungi was 63% of the scarred Sitka spruce and 55% of Western hemlock. The frequency of infection in trunk scars was 91% and 88% in Sitka spruce and Western hemlock respectively. The resulting loss was 1% of the merchantable volume of Western hemlock and 0.8% of Sitka spruce, and the average for both species amounting to 0.9%. F.annosus was the most common decay fungus isolated from infected Western hemlock,whereas F.pinicola accounted for 30% of the decay volume of Sitka spruce. A direct relationship was reported between the intensity of thinning and the amount of decay. Decay volume in individual infected trees was higher in heavily thinned stands and this was due to the creation of a better environment for decay fungi, as the stands contain fewer,more scattered trees as a result of heavy thinning. Maloy and Robinson (1968)reported the occurrence of decay in injured grand fir crops. The most important species capable of causing decay through extraction wounds on standing trees were Echinodontium tinctorium (Ellis & Everh.) and S.sanguinolentum .

A.3.4. Western hemlock and Douglas fir [T.heterophylla & P.menziesii]

Shea (1961)carried out a study on the decay through extraction wounds on these species. The loss due to decay during the 10-year interval since logging, was 86% of the volume increment of the butt logs of injured Douglas fir

and 142% of the butt logs of Western hemlock. Isolation from Douglas fir showed that F.pinicola was the most common decay-fungus in this species. Blue stain fungi were found to have preceded decay in all the cases studied. The occurrence of damage was mostly on trunk bases and roots, suggesting that skidding tractors were the major cause of damage. Decay following thinning injury was reported on young Western hemlock and Douglas fir by Hunt and Krueger (1962). Decay was detected in 61% of the scars on Western hemlock and the loss was 3.4% of the gross merchantable volume,whereas in Douglas fir 30% of the wounds were decayed and in the two sites studied the decay percent averaged 0.75 of the gross volume. F.pinicola accounted for 11% of the decayed volume of Douglas fir.

A.3.5. Engelmann spruce [Picea engelmanii Parry]

Aho (1971) reported that basal injuries, trunk wounds frost cracks,dead branches,broken tops and roots have all provided entry courts for decay fungi. Roots and basal injuries were responsible for 83.5% of decay by wound-invading fungi. The most important decay fungi isolated were S.sanguinolentum and S.chailletii .

A.4. Africa,New Zealand and Australia

The first report on the occurrence of S.sanguinolentum in Kenya,was made by Gibson (1964). The fungus was fruiting on Pinus radiata (D.Don) and P.patula (Sch. and Cham.). Almost all of the infections originated through wounds made by elephants and big game animals. Gibson (1965) reported that S.sanguinolentum was the cause of

heartrot of pines in the Southern Hemisphere. The darkening and reddish discolouration of woody tissues ,in a wedge-shape,were considered as typical symptoms of the infection by S.sanguinolentum through wounds.

Gilmour (1966) reported that S.sanguinolentum was responsible for the saprot of damaged Pinus nigra (Arn.),colonising dead wood and responsible for the saprot following pruning of P.radiata and as a wound pathogen that causes the saprot of Pinus taeda (L.). Generally, S.sanguinolentum causes the saprot of Pinus spp and occasional heartrot through wounds and branch stubs.

B. Hardwoods

B.1. Sugar maple [Acer saccharum March.], red maple [A.rubrum L.] ,yellow birch [Betula alleghaniensis Britt.], paper birch [B.papyrifera March.],American beech [Fagus grandifolia Ehrh.] and white ash [Fraxinus americana L.].

Decay fungi are capable of attacking hardwoods as well as conifers through extraction wounds (Shigo,1966a). Shigo examined the decay of Northern hardwoods in North America. Trees ranged from 15.2 to 66.0 cm in diameter and having 2 to 60-year-old wounds. Those with basal and root wounds were reported to die within a few years. The darker colour of logging wounds was considered as a symptom of infection by decay fungi. The author mentioned that insect holes have played a role in the spread of decay fungi. The amount and pattern of decay varied considerably in trees of the same species and in similar sized and aged wounds. However,decay and discolouration were severe in

those with previous defects e.g branch stubs. In such trees columns of discolouration and decay,due to logging wounds and other defects, were united,in which case it was difficult to differentiate between each type. Following logging wounds,discolouration becomes characteristically wedge-shaped with its point near the pith. The annual rings which are produced at the time of wounding become dark in colour and have been used to estimate time since wounding. Fruiting bodies of decay fungi were found frequently on the surfaces of wounds. Bacteria and non-Hymenomycetes were the first to invade. Decay fungi followed and grew longitudinally,but never beyond the discoloured tissues. As decay progresses,the surrounding tissues continue to support growth of Bacteria and non-Hymenomycetes. Thus, a bacterium -fungus complex often exists. The distribution of decay fungi was not uniform e.g an organism isolated from one point might not be present at a point only 2.5 cm away. The most common species isolated was Phialophora melinii (Nann.)Con. Other species isolated were Nectria spp, Fusarium spp, Phomopsis spp , Penicillium spp, Leptographium spp, Streptomyces spp and Trichoderma spp and also Bacteria. Ohman (1968) confirmed the occurrence of wound decay in injured sugar maple. Almost all decay fungi and other non-decay microorganisms have gained entry through pruning or logging scars. It was confirmed that discolouration precedes decay for some distance. Bacteria and non-decay microorganisms might have inhibited the radial penetration

of decay fungi. The author suggested that decay always starts at the discoloured tissues,but the presence of discolouration does not necessarily indicate decay.In sugar maple he found that Polyporus glomeratus (Peck.) was responsible for 25% of decay and the following species each accounted for 10% or less: Hypoxylon deustum (Hoff.ex Fr.)Grev.; Fomes connatus (Weinm.)Gill.; Steccherinum septentrionale (Fr.)Banker ; Pholiota adiposa (Fr.)Kummer; P.spectabilis (Fr.)Kummer and F.igniarius (L.ex Fr.)Kickx.

B.2. Oak [Quercus spp]

Berry and Beaton (1971) found that Northern red oaks (Q.rubra L.) were invaded by decay fungi entering through basal fire scars, dead branch stubs and open branch-stubs scars. About 25% of decay was caused by Poria oleraceae (D. and L.) and P.cocos (Sch.) Wolf. Further,Berry and Beaton (1972a) investigated the occurrence of decay and discolouration in black oak (Q.velutina Lam.); scarlet oak (Q.coccinea Muench.); chestnut oak (Q.prinus L.) and white oak (Q.alba L.). They found that decay fungi attacked the exposed heartwood as a result of injuries in the bark and/or the sapwood. Scarring of trees occurs as a result of fire or dead branch stubs. Fire scars were the most frequent entry courts for decay fungi,being responsible for almost 25% of all infections and more than 40% of the total decay volume. Decay in association with branch stubs accounted for about 33% of that associated with fire scars. Nine fungi were responsible for 80% of the infections the most important of which were: Poria andersonii (Ell. and

Ev.) Neuman; S.frustalatum (Fr.)Fr.; S.gausapatum (Fr.)Fr. and Polyporus compactus (Overh.). Over 80% of all infections were caused by white-rot fungi.

Berry (1973) examined decay entering through wounds and dead tissues of oak trees in the Central region of the U.S. Entry courts were: fire scars,dead branches,damaged or dead tops,dead roots and mechanical injuries. In this region fire scars were the most common entrance courts for decay fungi as they provide a large area of exposed wood and a long time is needed for the growth of a protective callus. The pattern of fungal colonisation was similar to that described by Shigo (1966a). Bacteria and non-Hymenomycetes were the first colonisers and responsible for discolouration that preceded decay. In many instances decay fungi attack the wood at a late stage and cause its ultimate decomposition. Among the species isolated were S.frustulatum and S.gausapatum .

B.3. Aspen [Populus tremuloides Michx]

Wounds are very common on this species because of its fragile bark. They afford access to canker-causing organisms (Hinds and Krebill,1975). The occurrence of trunk wounds was due to wild fires, falling trees,lightning,broken branches,snow, sunscald lesions,deer,big game animals,voles,sapsucker birds and human beings. Three types of cankers were found to invade wounded trees:

a. Highly parasitic fungi e.g Cenangium and Hypoxylon cankers

b. Mildly parasitic fungi e.g Ceratocystis cankers

c. Weakly parasitic and mostly saprophytic fungi e.g *Cytospora* canker Hinds (1976) reported that mechanical injuries provide entry courts for serious fungi and harmful insects. Mechanical wounds were found on at least 62.5% of aspen trees on the average in all plots examined. Most of the damage in camping grounds was caused by recreationists knives and axes. In 3 camping grounds,95% of the standing trees had mechanical injuries, 68% had cankers,32% being girdled by more than 50% by cankers. Canker causing organisms isolated were: *Ceratocystis* spp; present in 29% of the trees; *Cytospora* spp in 10%; *Cenangium* spp in 5%; *Cryptosphaeria* spp in 2% and *Hypoxylon* spp in 1% of the trees.

B.4. Yellow poplar [*Liriodendron tulipifera* L.],black gum [*Nyssa sylvatica* Marsh.] and ash [*Fraxinus* spp]

Berry (1977) reported that 57% of the above species become decayed following injury. The most frequent entry courts for decay fungi were fire scars. These were associated with almost 63% of the total decay volume. Mechanical wounds and felling and skidding wounds were the next most important followed by broken tops,unsound branch stubs and parent stumps. The most important fungi isolated were: *Spongipellis delectans* (Pk.)Murr. in black gum and *Pholiota adiposa* in yellow poplar.

B.5. Hickory [*Carya* spp]

A total of 600 trees of *Carya* spp were examined by Berry and Beaton (1972b). Decay fungi invaded living trees through wounds or dead tissues. Fire scars alone were

reponsible for more than 57% of the decay volume as entry points for decay-causing fungi. About 33% of decay volume was associated with open branch-stubs scars, unsound branch stubs and various mechanical injuries. Almost 75% of decay columns were inactive after wounds were healed. No decay fungi isolated from such wounds,but Bacteria and non-decay fungi were isolated. Poria spiculosa (Camb. and David.) was responsible for more than 50% of the total decay volume.

B.6. Southern and Appalachian hardwoods

Roth (1959) found that entry points for decay fungi were fire scars,logging scars,branch stubs and branches killed by suppression. The most common decay fungi were: S.frustulatum , S.gausapatum , Polyporus sulphureus (Fr.), Hydnum erinaceus (Fr.), P.hispidus (Fr.) and Poria spp. Further,Toole (1960) continued the examination of decay in Southern hardwoods. Almost 80% of the infections were in association with fire wounds and the rest had started in injuries during logging operations. Generally,decay was mainly caused by Polyporus spp, Fomes spp, Stereum spp, Poria spp and Pleurotus spp.

2.2.Relationships between different parameters in the process of decay

2.2.1. Wound parameters

Generally,almost all investigators found that the frequency and severity of decay depends on wound size,location,age and depth. Boyce (1923) mentioned that knots on Douglas fir were associated with the largest

decay volume as they occur low down on the bole where there are larger amounts of heartwood to be decayed. This was due to the fact that older heartwood is more susceptible to infection compared with younger heartwood higher up on the bole. Cartwright (1941) reported that recently formed heart-wood is more resistant to decay than older heartwood i.e resistance to decay increases up the tree. Horizontally,more fungitoxic material is present in the outer heartwood,adjoining the sapwood,than in the central heartwood. This can be explained by the fact that the amount of fungitoxic substance is higher in young and in the outermost heartwood and lower in older and in central heartwood. An ideal condition for decay fungi to grow is created when a larger amount of heartwood is exposed (Englerth,1942). Usually decay fungi attack basal wounds where there are greater amounts of exposed heartwood compared with a low level of infection and establishment of decay fungi in injuries close to the top of the tree where there is little or no heartwood to attack . The amount of decay,generally depends on the scar age and size and the causal organism. Further,Englerth and Isaac (1944) confirmed that scar position is an essential factor in influencing the severity of decay. Decay initiated from basal scars was found to be more advanced than that from scars higher up the bole. Decay fungi have also penetrated deeper in wide basal scars than in long ,narrow upper bole scars. Radial and vertical penetration of decay were directly related to the scar age and area (Wright,Rhoads

and Isaac,1947). Wright and Isaac (1956) reported that root scars and scars close to the ground were more frequently infected than upper bole scars. Further,the vertical and radial expansion of decay were directly related to scar age. Roth (1959) reported that the amount of decay is in direct relation with the wound width and age e.g an oak (Quercus spp) with a 30- year-old basal wound 20-33 cm in width showed a loss of 1.9 (board metre) of cull due to decay ,compared with another tree with a wound of the same age but 66-79 cm wide where the associated cull was 8.4 (board meter). Toole (1960) found that the extent of decay above the scars on hardwoods was much larger in older than in younger scars.

Parker and Johnson (1960) have carried out a comprehensive study on the factors associated with the infection of wounds on living trees by various decay fungi. The study confirmed that the infection level of wounded spruce (Picea glauca (Moench.)Voss.var. albertiana (S.Brown)Sarg. and balsam (alpine fir) (Abies lasiocarpa (Hook)Nutt.,varied with scar position,size,depth and age. There seemed to be an interaction between these factors. Scar size has influenced the infection frequency during the few years following wounding e.g scar size had little effect 15 years after wounding. Scars up to 365.8 cm;(2) showed a decreasing level of infection up the tree. Scars larger than that were nearly 100% infected. 15 years from scarring,all root and ground contact scars were infected, whereas the average infection level was 70% and 38% in

butt and upper bole scars respectively. The results suggest that scar size mainly influenced the frequency of infection of the butt and upper bole scars,but had little effect on root and ground contact scars. The interaction between the scar age and size was as follows:

15 years after scarring,the infection frequency in scars up to 91.4,94-365.8 and >365.8 cm^2 was 45,90 and 100% respectively. Scar depth seemed to influence infection level of some 5 and 15 years old scars on the butt and the upper bole,but not other scars. Scar age had also influenced the radial penetration of decay e.g in spruce decay had extended from 5-year-old scars on average by 3.8 cm. From 15 -year-old scars the average radial penetration was 7.6,12.7 and 17.8 cm in scars 91.4,94-365.8 and >365.8 cm^2 respectively. 31 years later,the average radial penetration was 10.2,15.2 and 22.9 cm for the same scar sizes respectively. Thus,scar size,age and location interact to affect the establishment and frequency of infection by decay fungi. (Shea,1961) found that the majority of loss due to decay occurred at the lower parts of Western hemlock and Sitka spruce trees. Hunt and Krueger (1962) reported that roots and wounds in contact with the ground level,on young Western hemlock and Douglas fir,were more frequently infected with decay fungi compared with other scars. The average surface area of wounds with decay was 5 times greater than those free of decay. A higher incidence of infection in stem scars compared with a lesser frequency of infection in root scars was reported in

wounds of spruce (Norway and Sitka spruce) and larch (Japanese and European larch) by Pawsey and Gladman (1965). This was explained by the fact that the average surface area of stem scars was much larger than that of root scars,probably due to the fact that a much larger area of stems is exposed to damage by tractors or horses,compared with a relatively smaller area of roots being exposed. Scars with broken tissue were more frequently infected compared with scars where only the bark is removed . An attempt to relate the scar age to the establishment and rate of decay failed to show a relation. Shigo (1966a), investigating decay of injured Northern hardwoods, mentioned that the decay process was generally influenced by wound age,severity and size. Time since wounding was the most important factor that influenced decay development. This can be attributed to the fact that a certain time is required for changes in air and moisture content of the exposed wood to become suitable for the successful establishment of decay fungi. This was confirmed by Nilsson and Hyppel (1968). Based on their results on the infection of wounded Norway spruce in Sweden,they reported that there should be exposed woody tissues with a suitable moisture content for the growth of decay fungi. Such exposed zones occur at certain positions on the tree. The scar position on the root is of considerable importance e.g the infection frequency in root scars occurring at 0-50 cm from the stem base was 50-100%;10-25% in scars 50-100 cm and 0-5% in scars> one metre away from the stem base.

These variations were related to changes in moisture content with distance from the tree base. Older scars were more frequently infected than younger ones. Vertical development of decay from younger scars was 2-3 metres and 5 metres or more from older scars,provided that the scar age ranged from 6-8,10 to 33 years. Results of isolations from wounded Norway spruce,in the Forest of Dean (Southern England),showed that fungal development increased considerably during the second year following wounding (Pawsey and Stankovicova ,1974a). Artificial wounding experiments in Finland showed that 9.5% of wounded Norway spruce crops,were infected by F.annosus one year after scarring. Three years later the infection level increased from 9.5 to 17%. The rate of development of discolouration was 50 and 30 cm per year,one year after wounding,in dominant and suppressed trees respectively. This rate increased from 50 to 61 cm per year in the first three years following wounding. There is evidence that S.sanguinolentum frequently invades larger wounds and that it rarely attacks smaller ones (Kallio,1976). Berry and Beaton (1972 a) found that usually fire scars are associated with the largest amount of decay as the area of wood exposed by this type of scar is relatively large. In addition,such scars require a long time to heal. Larger and deeper wounds are associated with the highest frequency of infection and growth rate of wood-decay fungi in wounded Norway spruce. In artificial wounding experiments, S.sanguinolentum infected 15% of trees 3-6 years after

wounding. However,9 years later the infection level had increased to 25% (Hansen and Hansen,1980,1981).

2.2.2. Tree parameters

Boyce (1932) noted that in virgin mature and over mature forest stands, decay is mainly balanced by growth. Up to a certain age,tree gross increment exceeds decay increment, but beyond that age the reverse is true. Boyce found that at ages between 300 and 350 years,gross increment of Douglas fir exceeded the decay increment. After that age the reverse happened i.e trees started to decay faster than they grew. In poor sites younger suppressed crops between 125 and 165 years of age were highly decayed compared with dominant trees. However,beyond these ages the opposite was true. This was confirmed by Englerth (1942). He found that Western hemlock crops are sound up to 200 years and beyond this age,trees were more susceptible to extreme damage by decay fungi. However,decay per cent was low in crops below 180 years old compared with that in ages between 181 and 300 years. In crops of balsam fir damaged by S.sanguinolentum Spaulding and Hansbrough (1944) found that decay frequency increased with tree age,being 20% and 50% in 90 and 150 year old crops respectively. Buckland,Foster and Nordin (1949) reported a direct relationship between tree diameter at breast height and per cent loss due to decay. The per cent loss in Western hemlock crops from 38 to 76 cm dbh varied from 10 to 13. For one tree of 139.7 cm dbh,42% of the gross volume was lost to decay. Generally,trees > 114

cm dbh showed a much greater decay increment than tree gross increment. Fir trees mainly (Abies amabilis). of 101.6 cm dbh lost 29% of their net volume to decay. Beyond this diameter,trees lost more volume than had been gained by an increase in diameter i.e tree volume increment is not enough to compensate for the volume lost to decay. The increase in decay amount with an increase in dbh was further confirmed by Foster and Foster (1951). Minor losses in wounded Western hemlock .< 101.6 cm dbh were reported. Within a range of 101.6to 177.8 cm dbh,decay had increased from 8 to 44% and from 10 to 46% in cubic and board metres respectively. According to Wright and Isaac (1956),the same amount of decay penetration that will destroy a smaller tree could be of a relatively minor importance in a larger tree. This means that larger diameter trees are capable of producing larger amounts of sound merchantable wood to compensate for the loss due to decay compared with slow growing or smaller trees. Studies on wound decay by Basham (1957),on Jack pine (Pinus banksiana Lamb.) , red pine (P.densiflora Sieb. & Zucc.) and white pines (P.strobus L.), showed that the width of the annual rings is directly related to the tree diameter. Larger or fast growing trees were found to have wider and fewer annual rings in the outer bole per unit distance compared with smaller or slow growing trees. The outer walls of the annual rings act as physical barriers against fungal penetration into the wood. Wider annual rings provide a wide layer of sapwood for decay fungi to attack.

Slow growing trees have a large number of annual rings with relatively thicker cell walls compared with a lesser number of rings with thinner cell walls in fast growing or large trees. Thus, the average radial penetration of decay was greater in larger trees and smaller in small trees. Frequency of infection and volume loss in fir (Abies spp) increased progressively with tree diameter between 38 and 127 cm. Within this range,the infection level increased from 21 to 100%. Volume loss also increased from 6 to 47%. Both infection frequency and volume loss increased with tree age e.g no infection was recorded in fir crops under 150 years. But beyond this age and up to 500 years, the infection frequency had increased from 4 to 100% and the average volume loss increased from 0.1 to 39% (Foster,Brown and Foster,1958). Radial penetration of decay is directly related to tree volume (Parker and Johnson,1960). This further supports previous investigations in concluding a direct relationship between frequency and amount of decay and tree volume or dbh. Furthermore,Aho (1971),reported that incidence and severity of decay usually increased with tree age e.g in a 100-year-old crops of Engelmann spruce (Picea engelmanii) decay was negligible,whereas it increased to 5-6% in trees aged 100-149 and decreased to 5% and 4.2% in the 150-199 and 200-249 age classes respectively. The same relation is applicable to tree diameter as it is a function of tree age. In contrast,Karkkanen (1971) reported a non-significant, negative correlation between tree growth rate and the amount of decay in crops of Norway

spruce in Southern Finland. He determined the following non-significant correlations were determined:

Variables	Correlation coefficient
Advance of decay upward/scar size	0.22
" " " " /scar depth	0.25
" " " " /No. of rings per cm	0.07
" " " downward/scar size	0.27
" " " " /scar depth	0.29
" " " " /No.of rings per cm	0.00

More recently,Norokorpi (1979) found a direct relationship between dbh and tree age; decay volume and dbh and decay volume and tree age derived from his work on decayed Norway spruce crops in Northern Finland. The frequency of infection was found to be dependant on tree age i.e as trees become older, the higher will be the incidence of decay. A comparable situation occurred in the case of hardwoods where Shigo (1966a) showed that radial penetration of decay in wounded hardwoods was directly related to tree diameter. Berry and Beaton (1971) reported that decay losses in Northern red oaks have slightly increased to 3.3% in trees over 90 years of age. Berry (1977)confirmed that severity of decay was found to increase progressively with tree age and diameter. Moreover,since tree diameter is a function of its age,the per cent decay volume increases with tree diameter.

2.3. Artificial wounding and inoculation experiments

A number of recent studies on the artificial inoculation of Norway spruce crops have been carried out. The effect of wound size,depth and position and the influence of minor and decay fungi on the development of the main

wound-decay fungi,inoculated as mycelial or spore suspensions,via artificial wounds,were all examined.

In Britain,Pawsey and Stankovicova (1974b) examined the development of S.sanguinolentum in inoculated wounds on Norway spruce. Square wounds were cut or else an increment borer was used and wounds either sealed or left open. The felling was done at three intervals: 3,6 and 9 months after inoculation. Mycelial and spore suspensions were used as inocula. A number of fungi were isolated from the superficial wood beneath 10 cm^2 scars. These were: Cephalosporium spp, Graphium spp, Sclerophoma pityiophila and Cryptosporiopsis abietina . All of these seemed to have no influence on the development of S.sanguinolentum . The establishment of S.sanguinolentum was consistently greater beneath 10 cm^2 scars where the tissues were broken than where the bark was only removed,provided that the inocula used were mycelial suspensions. However,the difference was insignificant when the inocula were spore suspensions. The greater devlopment of the fungus in splintered wood occurred irrespective of whether the wound was inoculated already or natural infection occurred. The development of the fungus was also more successful from borer holes inoculated with mycelial suspensions than from square wounds inoculated with spore suspensions. The rate of growth of S.sanguinolentum varied in different trees. Generally,the establishment was greater from inoculated borer holes that were left open than from sealed ones. No

consistent correlation was found between the time of year when inoculations were made and the establishment of S.sanguinolentum . No bacterial growth were observed on the wound surface. Kallio (1976) reported that inoculation of Norway spruce wounds with Peniophora gigantea (Fr.) Massee. has increased tree resistance against infection by F.annosus . However, S.sanguinolentum was found to attack even wounds which had been previously inoculated with P.gigantea ,and Kallio concluded that S.sanguinolentum was the most serious decay fungus attacking spruce wounds in South Finland. The fungus mostly infects sapwood injuries occurring at breast height and to a lesser extent root injuries occurring at soil level. S.sanguinolentum appeared to attack sapwood injuries more frequently than increment-borer holes,probably due to the larger wounded surface area exposed. Hansen and Hansen (1980) carried out the examination of 286 Norway spruce , 55-60 years old which were wounded artificially to investigate the fungal colonisation of wounds. S.sanguinolentum was the most common decay-fungus isolated from wounds. It occurred in 29% of all trees examined, followed by Cylindrobasidium evolvens (Fr. ex Fr.) Julich. (19%), Peniophora pithya (Pers.)J.E. (7%), Fomes annosus (6%) and unidentified species (3%). Larger and deeper wounds yielded the highest infection frequency and decay growth rate. Season of wounding seemed to have a considerable effect on growth rate of F.annosus . However,the variation in tree dbh and wound height above the stump had no significant effect.

The mean vertical spread of decay was found to decrease from one year to another i.e increasing but at a decreasing rate. F.annosus attacks younger wounds more frequently than older ones. Hansen and Hansen suggested that it is sometimes replaced by secondary organisms. Frequency of infection was higher in mid-summer,probably due to availability of larger amounts of spores compared with those of competing S.sanguinolentum . F.annosus was isolated mainly from one year old wounds and occasionally from 2 or 4 years old wounds,suggesting that it is a primary wound invader. Hansen and Hansen(1981) continued their experiments by making artificial wounds on roots of Norway spruce with a hammer to simulate those made by cattle in southern Norway. Felling and fungal isolations were done after 3,6,9 and 12 years. It seemed that time since wounding,wound distance from the stump,wound area and age had no significant effect on the fungal establishment due to limited data collected,but certainly the relationship between these parameters usually holds good for Norway spruce. S.sanguinolentum was again the most common fungus isolated,and had infected 15% of all the wounds,3 and 6 years after wounding,compared with 25% 9 years after wounding. The maximum growth rate recorded was 45 cm per year with an average of 24 cm per year. Isolates of the fungus were quite variable and it was possible to differentiate between these different isolates by their morphological characters in culture. In one of these localities studied F.annosus was isolated from 20% of

the wounded roots after the second wounding. The maximum vertical expansion of this species was 345 cm within 9 years i.e 38 cm per year. The global average was 20 cm per year. Nectria fuckeliana Booth has attacked 70% and 59% of the wounds in Hurdal and Ogndal respectively ,but was completely absent from Alvdal. Other species isolated were Ascocoryne spp and Neobulgaria premnophila (F. & H.). Trichoderma spp were present in one root and two wounded roots. Bacteria were isolated from wood close to the wounds from 15% of the samples examined. Although wound size and depth had no significant effect on the establishment of microorganisms in general,nevertheless,it seemed to have an effect on the development of the most important decay-fungi ; S.sanguinolentum and F.annosus . It was also reported that the chance of wound infection by both fungi increases with increasing wound size and depth(Hansen and Hansen,1981).

2.4. S.sanguinolentum as a saprophyte and a wound pathogen

In 1929,Spaulding reported that S.sanguinolentum had invaded insect weakened trees in Southern New England and drew attention to the possibility that the fungus might also attack healthy standing trees. Hubert (1935) reported that three years after natural infection by S.sanguinolentum Douglas fir (Pseudotsuga menziesii) crops suffered a loss of 50% of the total number of trees. In addition,the fungus was also found attacking dead and down material on the forest floor. Cartwright (1937) attributed a reddish stain in living Norway spruce trees to infection

by this fungus and noted that the entry court was broken branches. He considered it to be the principal fungus invading pruning wounds,noting that it was also common on dead stumps and branches of conifers in Britain and Europe. Spaulding and Hansbrough (1944) noted that S.sanguinolentum occurred abundantly on logs and conifer slash in North America. In 1946,Cartwright and Findlay observed that the fungus was isolated from freshly felled spruce and larch poles and even sometimes from living trees. In investigations in Western Oregon and Washington,Wright and Isaac (1956) found that rots caused by S.sanguinolentum and other Stereum spp were most common in association with wounded Western hemlock (Tsuga heterophylla) and ranked second,numerically, to those caused by Fomes annosus . Rots caused by Stereum spp (including S.sanguinolentum) accounted for 7% and 26% of all the infections along the coast and inland respectively. In British Columbia, investigations by Foster,Brown and Foster (1958) into the deterioration of Western hemlock (T.heterophylla) and amabilis fir (Abies amabilis) reported that S.sanguinolentum was responsible for 11% of the infections and 3% of the total decay volume of A.amabilis . Although S.sanguinolentum was of a minor importance on Abies spp in the Kitimat region it is a serious decay fungus of Abies spp in the interior of British Columbia and Eastern North America and accounted for 11% of the total infections of Abies lasiocarpa in the Southern Interior and 34% in the Upper Fraser region respectively. Meredith (1959) mentioned

that the fungus frequently colonises dead trees and fallen branches, considering it to be one of the earliest invaders of fallen tree stumps. Investigations by Parker and Johnson (1960) showed that,in spruce crops,the fungus was isolated from 81% of scars yielding decay. In Scotland,Gladman and Low (1960) considered S.sanguinolentum to be a very common wound pathogen with a wide host range. They noted that it was also a very serious decay fungus associated with wounds,particularly those occurring on Norway spruce. These workers noted fruit bodies occurring on the surfaces of wounds,on conifer stumps and on cut logs on the forest floor. In the Pacific North West,Shea (1961) reported a similar distribution of S.sanguinolentum in Western hemlock injured during logging operations. Here the fungus caused severe decay and was found fruiting abundantly on the surfaces of extraction wounds. In the same region,Hunt and Krueger (1962) estimated that the fungus accounted for 72% of decay volume in Douglas fir,and in the same year Loman (1962) reported that S.sanguinolentum was one of the most frequent decay fungi on the logging slash of lodgepole pine (Pinus contorta (Dougl.). Davidson and Etheridge (1963) examined the degree of infection of balsam fir (Abies balsamea) by S.sanguinolentum . The results showed that the fungus invades through stem wounds,broken tops and living branch injuries. Out of total of 370 branches examined,26% had the characteristic red rot of S.sanguinolentum . Etheridge and Morin (1963) described S.sanguinolentum as the most destructive and dangerous

agent causing heartrot of living conifers. It has been frequently isolated by Etheridge and Morin from artificially topped and stem wounded balsam fir. It prefers heartwood more than the sapwood of balsam fir. Roff and Whittaker (1963) reported S.sanguinolentum as one of the major red-stain fungi in lodgepole pine (P.contorta) logs,accounting for a 6% loss in weight of inoculated wood blocks within 15 weeks (approximately 4 months). Bjorkman (1964) reported that S.sanguinolentum was capable of invading Norway spruce crops through broken tops and was responsible for both firm light and dark rots at the advanced stage. S.sanguinolentum accounted for 4% of the identified infections in black spruce (Picea mariana (Mill.)B.S.P.; 4.5% in white spruce (P.glauca); 36.5% in balsam fir and 54.3% in Western hemlock (Basham and Morawski, 1964). Almost all of the trunk rot in balsam fir and Western hemlock was caused by S.sanguinolentum . They estimated the annual loss in revenue to be 24 million American dollars. In five Scottish forests,S.sanguinolentum was the most common decay fungus invading extraction wounds of spruce (Norway spruce and Sitka spruce) and larch (Japanese and European larch) and was isolated isolated from 27% of the samples examined (Pawsey and Gladman,1965). Investigations by Gibson (1964,1965) in Kenya and South Africa and by Gilmour (1966) in New Zealand have also confirmed that this fungus is a wound invader and also acts as a decay fungus on down and dead material on the forest floor. More recent Swedish studies by Nilsson

and Hyppel (1968) confirm that S.sanguinolentum and F.annosus are the most serious invaders of wounds on Norway spruce. Etheridge (1969) reported that S.sanguinolentum has a powerful competitive ability and a wide range of temperature at which it can penetrate wood,which makes it the most destructive decay fungus of balsam fir. Basham (1973) reported that S.sanguinolentum was one of a number of Basidiomycetes isolated from trunks of defective black spruce,mainly as an incipient red rot fungus. Isolations carried out by Pawsey and Stankovicova (1974a) on injured crops of young Norway in the Forest of Dean,Southern England,showed that it was the only decay fungus in association with evident decay in these stands. The occurrence of S.sanguinolentum as a wound invader and decay fungus was further confirmed by Schonhar's (1975) investigations in Germany and by those of Kallio (1976) in Finland respectively. On injured Western white pine (Pinus monticola Dougl.ex D.Don),it was one of the most common decay fungi recorded (Kulhavy,Chako and Partridge,1978). Investigations by Enerstvedt and Venn (1979) on living Norway spruce showed that S.sanguinolentum , together with F.annosus and Armillaria mellea (Fr.) Kummer. were the most important decay fungi, Stereum spp accounting for 4.6% of infections occurring as a result of wounding. Finally,recent studies by Norokorpi(1979) and by Hansen and Hansen(1980, 1981) in Fennoscandia have re-emphasised the role of S.sanguinolentum both as a wound invader and as a decay fungus.

2.5. Fomes annosus as a wound pathogen

It is evident that F.annosus is capable of causing serious infections through extraction wounds and other tree defects e.g pruning wounds,fire scars ,lightning scars,frost cracks and knots (Englerth,1942). Englerth showed that it was responsible for 21% of the volume loss in Western hemlock (Tsuga heterophylla). Entry courts were knots, falling tree scars,roots,broken tops,fire and lightning scars and frost cracks. F.annosus occurred most frequently in large overmature trees in moist sites in Western Oregon and Washington. These results were confirmed in the Wind River region by Englerth and Isaac (1944). It was described as the most frequent fungus in association with decay in extraction wounds on Western hemlock. Out of 56 wounds on Western hemlock examined,52 have afforded access to infection by F.annosus . Rhoads and Wright (1946) reported that the fungus was considered as a wound pathogen of Western hemlock rather than a root invader. This is because of the fact that it has never been found attacking uninjured roots. It was responsible for the largest amount of decay in Western hemlock. Furthermore,Wright and Isaac (1956) found that F.annosus was a serious wound invader of Western hemlock and responsible for 63% of the total infections in this species along the coast and 40% inland in Western Oregon and Washington. Rishbeth (1950,1951,1957 and 1959) provided valuable data on the biology of F.annosus as a butt and root rot pathogen attacking pine plantations in East Anglia.

It was reported invading freshly-cut stump surfaces by means of air-borne spores and then spreads through root contacts, but does not grow freely in the soil.

It is interesting to note that the American experience showed that F.annosus is a serious wound invader of some coniferous species e.g Western helock,whereas U.K. experience proved that it is almost never a wound invader.

2.6. Physiological studies on S.sanguinolentum

Cartwright and Findlay (1938) mentioned that initial growth in culture was generally sparse,arachnoid and patchy. Colour develops later over the inoculum and then in patches over the culture, but never uniformly. Usually the fungus stains the medium red. Colours in culture ranged from antimony yellow,yellow ochre,ochraceous orange to buckthorn brown. The mycelial development is more compact in the light than in the dark. Larger hyphae with reddish contents were observed,in addition to spherical swellings with dense contents. Hyphae tend to break down into short segments that act as oidia. Robak (1942) provided detailed information on the physiology of the fungus. Different isolates showed a considerable variation in culture. These variations were of a wider range than those mentioned in the previous study e.g the occurrence of large conducting hyphae with red contents was not considered as an important diagnostic feature as their occurrence is a chance phenomenon. Moreover,the spherical swellings with dense contents were not always present. Clamp connections were occasionally numerous and on other

occasions completely absent. Two strains were characterized by a distinct discolouration of the medium within six weeks. Growth of different strains has increased slowly with increasing temperature. It was inhibited at about 32C°. Temperature optimum for different strains ranged from 15-23,20-26 and 15-26 C°. For a number of strains temperature optimum was between 20-23C°. Nobles (1965) mentioned that the fungus has covered petri dishes within three or five weeks. A characteristic diagnostic feature of the fungus in culture is its sweet smell. Yee (1974) confirmed the variation of the fungus in culture. The variation between different isolates is mainly on the macroscopic features e.g colour,shape and size of the sporophores and the appearance of the aerial mycelium. There were no significant microscopic differences except for the absence of clamp connections in one isolate (No.4). Wide hyphae from 5 to 7 microns ,narrow ones from 2 to 3 microns and larger hyphae were observed in culture. Single and multiple clamp connections,sweet fruity smell and initially thin and arachnoid growth in culture confirmed previous findings. A week after the initial growth,the variation in growth and colour has started to appear in differnt isolates. Colour ranged from pale salmon,through orange to dark rusty brown. Minute liquid drops were produced on the aerial hyphae of the majority of the isolates,on liquid media,but never on solid media. The fungus produces extracellular oxidase in culture. Hyphae were characterised by resinous

masses sticking to the hyphal walls. Sporophores are thin,forming a bracket ,cream to greyish brown with or without margin and sometimes with concenteric zones. The lower surface bleeds red when scratched,hence the nomenclature sanguinolentum (Robak,1942,and Nobles,1965).

2.7. Effect of insect boring

In (1929),Spaulding reported that S.sanguinolentum can easily attack trees weakened by insect activity. Insect activity on wounded Western hemlock might have assisted in the fungal spread via wounds (Englerth and Isaac,1944). S.sanguinolentum has been repeatedly isolated from woody tissues surrounding the larval and ovipositor tunnels of Ureceros gigas L. (Cartwright and Findlay,1946). This suggests a possible relationship between the fungal spread and the insect tunnels. Moreover,oidia of the fungus were found in the glands at the base of the ovipositor,which would assist in the fungal spread from one tree to another. More recently, Basham (1959) focussed attention on the possible importance of insects in the infection of woody tissues as vectors or by exposure of woody tissues to attack by various decay fungi. Molnar (1965) reported that mortality in alpine fir (Abies lasiocarpa) was caused by a beetle-fungus complex. The Western balsam bark beetle (Dryocoetes confusus (Sw.) was found to attack first and this results in lesions that act as entry courts for some harmful fungi, the most destructive of which is Ceratocystis dryocoetidis (K. & M.).

The association between insect attack and fungal

spread is more clear in hardwoods than in conifers (Shigo,1966b). Attack by wood boring insects usually follows the occurrence of logging wounds. Insect holes provide a chance for decay fungi to enter and advance at least to the depth of these holes. The adult Ambrosia beetle (*Xyloterinus politus* Say.) was found to bore through lenticels,mechanical injuries,living agents and other avenues. The beetle eventually departs leaving galleries. Bacteria and fungi often enter through these galleries and start a process of discolouration which might lead to ultimate decay under favourable conditions. The extent of damage depends on the number of holes and extent and location of internal defects already present in the tree at the time of attack. Discolouration usually takes three patterns:

1. Spindle-shaped
2. Irregular patches
3. Circular shape in cross-section

This pattern sometimes includes proper decay. The scale insect (*Xylococculus betulae* (Perg.)Morris. expels sugary substance through long wax tubes that protrude from their bodies out through the holes. Hence,wound healing is retarded. The problem is compounded when woodpeckers, searching for scale insects as their food source,tear the bark. Shigo and Larson (1969) examined the effect of insect injury on decay of American beech (*Fagus grandifolia*); Paper bich (*Betula papyrifera*); Yellow birch (*B.alleghaniensis*); Sugar maple (*Acer saccharum*); Red maple (*A.rubrum* L.) and ash (*Fraxinus americana*). The following insects were found to injure the above mentioned species:

Glucobius speciosus (Say.) bores into the wood of sugar maple and makes galleries. Eventually the bark falls off exposing the inner woody tissues. It has been reported that Fomes connatus is capable of invading these exposed tissues. Sphyrapicus varius drills into the tree to suck the sap feeding on it and makes three types of wounds:

- Scattered rows of wounds in sugar maple. These result in streaks of discolouration. The flowing sap is often covered with dark coloured fungi indicating the presence of sap suckers.
- One spot wounds formed during several seasons.
- One spot wounds formed during one season resulting in dark bands. Phytobia spp was found to attack Northern hardwoods. Larvae mine down the stem through the cambium resulting in limited discolouration known as pith flecks. Ambrosia beetles (Xyloterinus politus) usually invade birch trees already injured by other agents. The damage is disscused above. The stem borer (Xylotrechus aceris Fish.): invades young red maple crops and creates small wounds which are associated with dark wet discolouration and decay. The bronze birch borer (Agrilus anxius Gory.) and pigeon tremex (Tremex columba L.) both attack beech and expose the wood to further attacks by microorganisms that might cause decay and discolouration. A strong association between Armillaria mellea and beetles attacking Douglas fir, ponderosa pine (Pinus ponderosa Dougl. ex Laws.) and grand fir,has been reported by Partridge and Miller (1972). In New Zealand,Faulds (1973) found that

beetles (Platypus spp) bore into wood of Nothofagus fusca (Hook.)Oerst. Two species of fungi were associated with the beetle tunnels. These were Ceratocystis spp and Endomycopsis platypodis Baker et Kreger-Van Ry. A study of decay on Western aspen (Populus tremuloides) by Hinds and Krebill (1975) reported that mortality in alpine fir (Abies lasiocarpa) has been ,showed that insect injuries have provided entry courts for cankers caused by Cenangium spp; Hypoxylon spp; Ceratocystis spp and Cytospora spp. Hertert,Miller and Partridge (1975) confirmed the previous results of Partrige and Miller (1972) in finding evident association between insect damage and fungal attack on conifers.

More recently,Berry (1978) reported that injuries caused by the red oak (Quercus rubra) borer (Enaphalodes rufulus Hald.); the white oak (Q.alba) borer (Goes tigrinus DeG.) and the carpenter worm (Prionoxystus robiniae Peck.), have all provided entry courts for various decay fungi. In one experimental area, 16% of the infections by decay fungi have developed from borer wounds and accounting for 9% of the total decay volume. In another region approximately 10% of the infections have occurred through borer wounds. The most common decay fungi associated with insect injuries were: Polyporus compactus ,responsible for about 33% of the total decay volume developing from insect injuries; Merulius stremello Schrad.(ex Fr.) and Stereum frustulatum accounting for 0.26 and 0.13% of decay volume respectively. Brown rot fungi e.g Laetiporus sulphureus

(Bull.ex Fr.) Bond.& Sing. and Poria cocos ranked next. All the species mentioned were responsible for 83% of the total decay volume in association with borer galleries in oak trees (Quercus spp).Decay volume amounted to 2.2% in scarlet oak (Q.coccinea), 1.3% in black oak (Q.velutina) and less than 1% of the gross volume in both Northern red and white oaks.

2.8. Succession and interaction of microorganisms

Almost all workers agree that succession and interaction take place in the process of wound infection by Hymenomycetes, non-Hymenomycetes and Bacteria. The disagreement has originated from whether non-Hymenomycetes are necessary or capable of causing discolouration and decay and which precedes which. Generally,it seems that the pattern of succession is highly dependant on the tree species and the type of microorganisms in question e.g different tree species are susceptible to invasion by different kinds of microorganisms which vary in the qualitative and quantitative production of anti-fungal and anti-bacterial products. These substances actually determine which organisms are inhibited and which ones are favoured. Nevertheless,there are two distinct concepts in the succession and interaction process that dominated all the others.

A. First concept

The first concept concludes that the process of tree decay by microorganisms involves a series of events that finally lead to the deterioration of the tree species in

question. Shigo(1965),suggested the following phases in tree decay:

Phase one: When a tree is wounded a series of chemical changes take place in the exposed tissues. These involve the excretion of certain chemicals by the injured,exposed parenchyma cells. The exposure of these substances to the air, leads to their oxidation and hence to a change in the original chemical composition of the injured tissues. This process may result in chemical discolouration resulting from exposure that leads to changes in moisture content and pH of the exposed tissues.

Phase two: Pioneer organisms e.g Bacteria and non-Hymenomycetes,are the first invaders of the chemically altered wood and they cause further changes in the nature of these tissues.

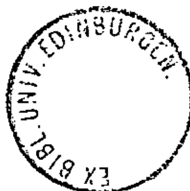
Phase three: Finally,Hymenomycetes attack and eventually decay takes place. From these stages Shigo concluded that for phase three to occur phase one and two must take place first. Although phases one and two precede phase three,the occurrence of phases one and two does not necessarily lead to phase three taking place. This is because the process may cease at phase one or two e.g as a result of wound healing or unfavourable environmental conditions. This concept,therefore, clearly suggests that some changes in the chemical structure of the injured tissues,should take place as a precondition for further invasion and ultimate decay by Hymenomycetes. This concept has been supported by some workers and criticised

by others.

The fact that host response to injury can start the discolouration process without any microbial involvement was observed by Sucoff,Ratsch and Hook in 1967. Their experiments showed that microbial antibiotics failed to reduce or prevent discolouration. The only factor that controls this process is the exposure of the injured tissues to the atmosphere . Exposed,wounded samples (aspen) were discoloured,whereas those kept unexposed failed to show any discolouration. It has been concluded,therefore, that atmospheric exposure is required as it stimulates factors that lead to discolouration e.g oxidation of leucophenols,changes in moisture content and oxygen and carbon dioxide exchange. While in some cases decay fungi benefit from the presence of other microorganisms,it is also true that in other cases antagonism occurs. Henningsson (1967a) mentioned that the succession of microorganisms is mainly controlled by nutritional factors. Fungi which have minor wood-decay capabilities invade first. These are dependant on simple,soluble carbohydrates which are readily available during the early stages of colonisation. These soluble carbohydrates will be exhausted at a certain stage. When this happens,early colonisers give room to other species capable of breaking down polymeric carbohydrates e.g lignin and cellulose. Inoculation experiments of American beech,yellow birch, paper birch and red oak,with _Fomes igniarius_ and _Fomes_ spp, showed the previously mentioned phases (Shigo, 1965) that lead to

ultimate decay (Shigo and Sharon,1968). Some workers have even suggested a climax species that invades late but then dominates all the others (Maloy and Robinson,1968). They reported that a clear succession of microorganisms attacking grand fir (Abies grandis) is evident. Finally,the wood is colonised by Echinodontium tinctorium which is considered as the climax species. S.sanguinolentum is the second most frequent fungus isolated. The early colonisers of wounded balsam fir,non-Hymenomycetes, may be replaced at a later stage by aggressive wood-destroying fungi e.g S.sanguinolentum (Etheridge,1969). In an investigation of the discolouration and decay of sugar maple,following inoculation with Fomes fomentarius (L.ex Fr.) and F.connatus , Bacteria and non-Hymenomycetes were frequently isolated from the distal parts of the infected columns in advance of Hymenomycetes which were never isolated from these parts. This indicates that Hymenomycetes have invaded already altered tissues only as a result of the host response to injury and invasion by pioneer microorganisms (Shigo and Sharon,1970). Examination of the amount of phenolic compounds present in the reaction zone of Norway spruce crops by Shain and Hillis (1971) showed that it contains 4 to 28 times more phenolic compouds compared with the sap or heartwood. Moreover,the pH of the reaction zone is too high for fungal growth to occur. Thus,resistance is achieved in this zone in two ways:

1. The presence of fugitoxic substances.
2. The high alkalinity that creates unfavourable



conditions for fungal growth. This mobilization of fungitoxic substances occurs in the cambial region and parenchyma cells close to the injured zone. Howland (1971) reported that the inoculation of sugar maple with Bacillus spp and Pseudomonas spp may have a role in predisposing the wood to further colonisation by organisms other than Bacteria. Dissection of wounded red and white oaks showed a consistent presence of Bacteria and other non-Hymenomycetes e.g Trichoderma spp, Mortierella spp and Mucor spp. These were isolated only from discoloured wood and the distal parts of the decayed columns. Hymenomycetes were more frequent at the border of discoloured and decayed wood. This pattern of distribution suggests that Hymenomycetes follow other pioneer microorganisms (Shigo,1972). Further,the same pattern of isolation and succession was reviewed by Shigo and Hillis in 1973. Shigo(1974) conducted an experiment to show the effect of non-decay fungi on the inhibition or otherwise promotion of decay fungi inoculated artificially on to sugar maple trees. Isolation results proved that Phialophora melinii (Nann.)Con.; Cytospora spp; Graphium spp and Bacteria are aggressive pioneer invaders of the recently exposed wood. Hymenomycetes attack later after the wood has been altered in some way. This was further supported by the fact that decay fungi i.e F.igniarius and F.connatus , failed to grow on heat-killed wood blocks,sterile and clear wood,while they grew and further decayed blocks of wood which are discoloured and slightly decayed. This proves

that the alteration of wood tissues by pioneer organisms has promoted decay. The same results and pattern of isolation previously reported by Howland (1971); Shigo (1972) and Shigo and Hillis (1973), have been recently confirmed by Manion and Zabel (1978). Interaction between Hymenomycetes on the one hand and Bacteria and yeasts on the other have been further studied by Blanchette and Shaw (1978). They inoculated wood samples with Hymenomycetes alone and others with composite inocula of Hymenomycetes,Bacteria and yeasts. Results showed that an additional 10% weight loss has been obtained from samples treated with the composite inocula. This could be attributed to the possibility that Bacteria may provide essential vitamins and other growth substances that promoted the growth of decay-fungi. In return,Bacteria use some cell-wall components which are broken down by enzymatic activity of decay fungi.

B. Second concept

Earlier studies by Henningsson (1967b) suggested that some microorganisms are capable of breaking down toxic phenolic compounds and thus detoxifying them. By doing this they pave the way for decay-fungi to attack the detoxified wood substances. The process of discolouration and decay usually results in a reduction in the total extractable phenols compared with healthy wood (Tattar ,1971). He found that total phenols in sugar maple was highest(10mg/gm) near the cambium,where injury has occurred,decreasing progressively towards the pith.

Eventually,the presence of phenols is restricted to clear wood. Further,Shain (1978) suggested that when a tree is injured or attacked by microorganisms,it forms a reaction zone that seperates the infected tissues from the healthy ones as a host-defense mechanism. Certain fungitoxic substances which are mainly phenolic compounds accumulate at this zone in large quantities compared with clear wood. This second concept was summarised recently by Shortle and Cowling (1978). They noted that the discolouration process is actually initiated by the activity of decay-fungi rather than by Bacteria and non-Hymenomycetes. The authors concluded that as a result of activities of decay-fungi,wood is discoloured . As a host response,dying and injured parenchyma cells excrete products which are toxic to decay fungi,but not to phenol tolerant microorganisms. The non-Hymenomycetes may be present in the sapwood before or after wounding and discolouration. These organisms can tolerate the toxic effect of the phenolic compounds produced by the host. At this stage they are more frequently isolated than the suppressed, phenol-intolerant decay-fungi. After detoxification of these substances by the activity of non-Hymenomycetes, decay fungi regain activity and readily replace other microorganisms. This concept differs from the previous hypothesis in that phase three can actually start first i.e for phase three to take place,it is not a precondition that phase one and two should occur. However,Shigo (1965) have considered it impossible for phase three to take place

before phase one and two.

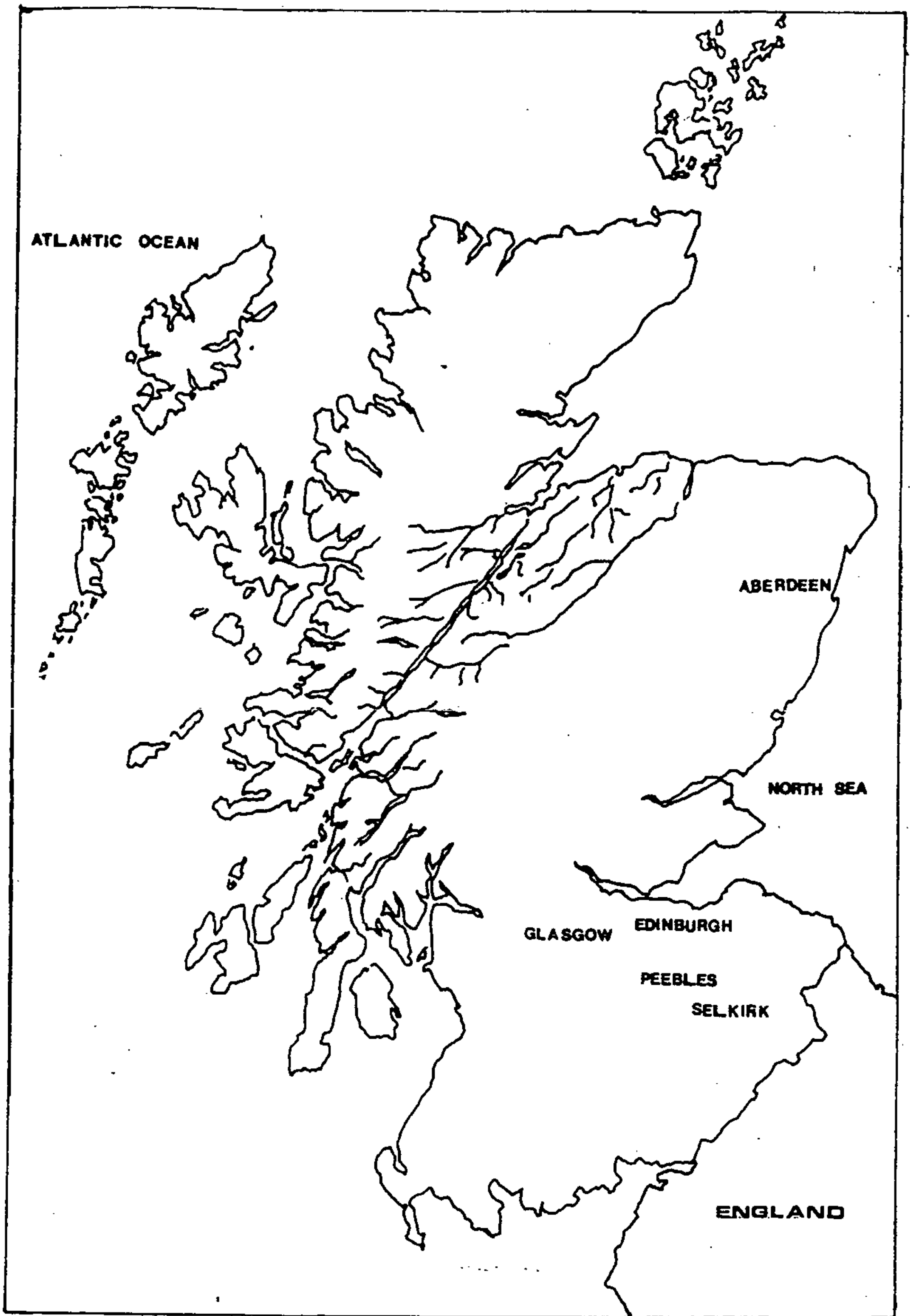
CHAPTER 3

3. MATERIALS AND METHODS

Norway spruce and Japanese larch were examined in this study because of their frequent occurrence in British forests and their susceptibility to decay (Pawsey and Gladman,1965). A total of four compartments were chosen where extraction damage had occurred in 1975 and 1979. Trees from younger Norway spruce crops (P 52) were taken from compartment 11 (16 acres) of Yair Hill forest (Map 1 and 2). in Selkirkshire (National grid reference : NT 442322) from an even-aged mixed stand. Older Norway spruce trees (P 29) were taken from compartment 1016 (7 acres) of Glentress forest (Map 1 and 3) in Peeblesshire (National grid reference : NT 276415) from a pure even-aged stand. [illegible] Japanese larch trees (P 53) were taken from compartment 3 (10 acres) of Yair Hill forest (National grid reference : NT 455322) from an even-aged mixed stand. [illegible] Japanese larch (P 54) were taken from compartment 21 (22 acres) of Yair Hill forest (National grid reference : NT 442328) from an even-aged mixed stand.

1. Investigation of the occurrence of extraction damage and decay

Every extraction rack in compartments 11 (Norway spruce) ;3 (Japanese larch) and 21 (Japanese larch) all in Yair Hill forest and in compartment 1016 (Norway spruce) in Glentress forest were checked for the occurrence of extraction damage on the stems and superficial roots. The extraction racks were marked with coloured tapes so that they could be recognized when later revisited. The occurrence of extraction wounds was examined by working



Map 1: Map of Scotland

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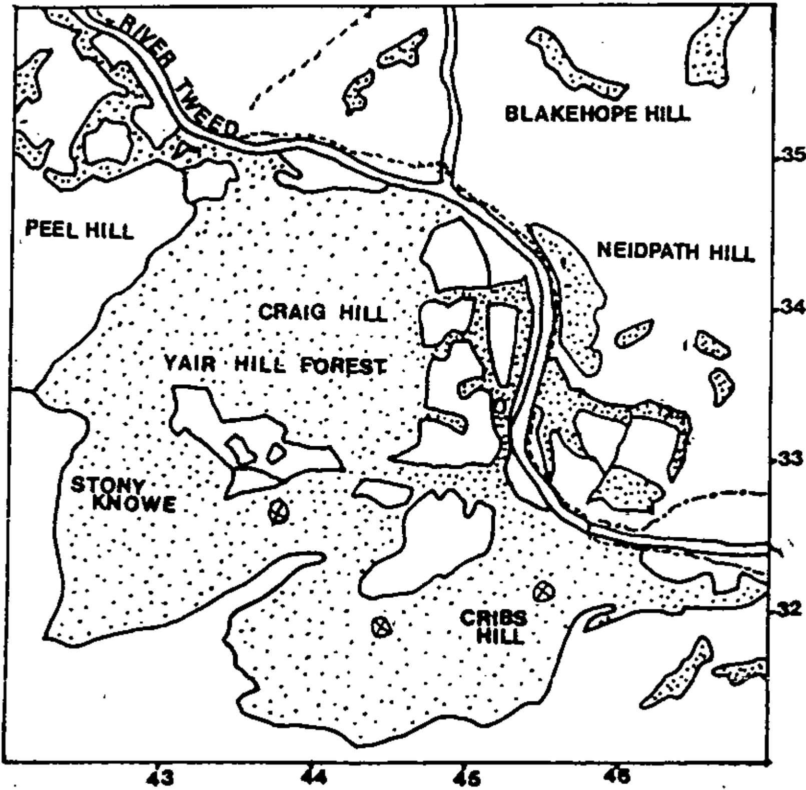

Map 2 : Location of the experimental sites in Yair Hill Forest

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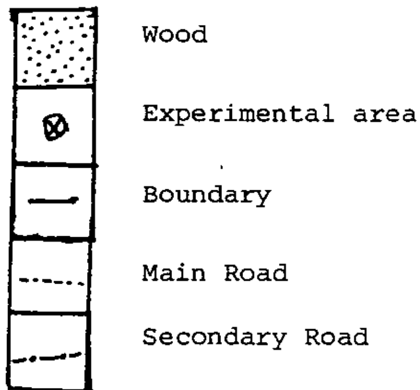

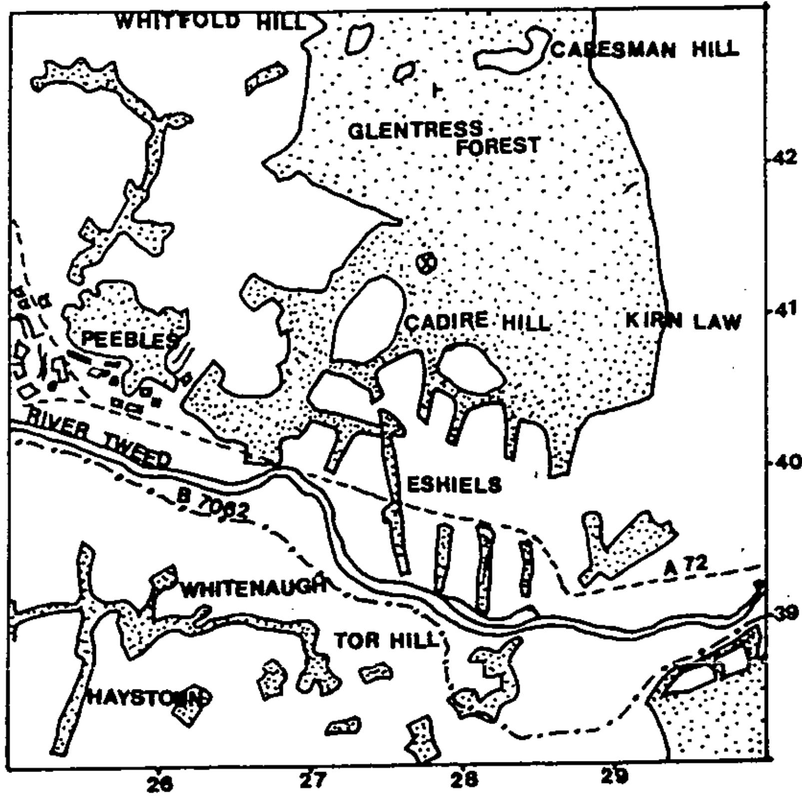

Map 3 : Location of the experimental site in Glentress Forest

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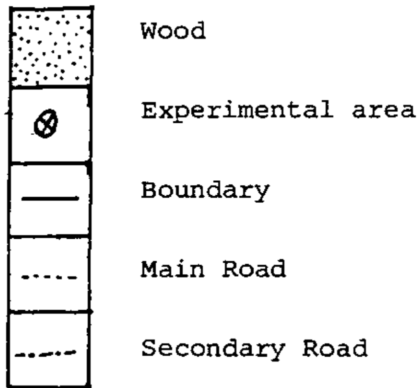

from one tree to the next in a systematic way. Every wounded tree was carefully labelled and numbered. The diameter at breast height and the position of the wounds were recorded. A core was taken at the middle of the wound inwards by means of an increment borer to check the extent of discolouration or decay. The use of the borer was not always reliable and sometimes an infected tree might be overlooked because of the improper positioning of the borer such that it took cores from healthy wood only. This was found to be particularly so if the infection column was very narrow and discontinuous as in most Japanese larch trees. Therefore,every wounded tree was felled and divided into approximately 50 cm lengths as far as any discolouration or decay could be traced. A total of 103 Norway spruce and 93 Japanese larch trees were felled and dissected. Each of the 50 cm lengths were carefully labelled and given a number showing the tree it was taken from and its position in that tree. All wood samples were moved from forest to laboratory as quickly as possible and kept in a cool room at about 5 C^{o} until further analysis was carried out.

In the laboratory,each wound on a tree was drawn on tracing paper. Using a dot grid the surface area of the wound was determined in square centimeters. A small disc was sawn from the middle of the wound and the number of growth rings per 5 cm from the outer bark towards the pith was counted. This ring count enabled the time at which the damage occurred and hence the wound age to be

determined. Using the same disc the radial penetration of decay was recorded. The vertical expansion of decay was also recorded. The decay and tree volume were determined according to Smalian's formula which gives the volume of a log in terms of its length and its two ends areas:

$$V = (A + a)/2 \times L$$

where V = volume; A = the area at the large end of the log; a = the area at the small end of the log and L = the length of the log. For the determination of the log volume the length was determined from the basal part of the log up to 7 cm diameter.

Sampling of the infected wood was carried out after the method of Hansen and Hansen (1980). 3 cm thick discs were taken from each log for culturing in the middle of the wound,5 cm above and below the edges of the wound and 10,20,40 cm and then every 20 cm from the site of the wound as far as any discolouration or decay could be traced (Fig.1). The position of each sample was carefully marked on neighbouring thin discs. Samples taken from the disc through the middle of the wound were taken at 1 cm intervals along a radius from the centre of the wound inwards and on both sides of the wound,inside and outside the decay column. 1 cm cubes of wood were taken from each disc and the number taken depends on the expansion of decay. These cubes were surface sterilised by dipping into 0.1% silver nitrate solution ; 0.1% sodium chloride solution and sterile distilled water for about one minute respectively (Ward,1952) . Cubes were then plated

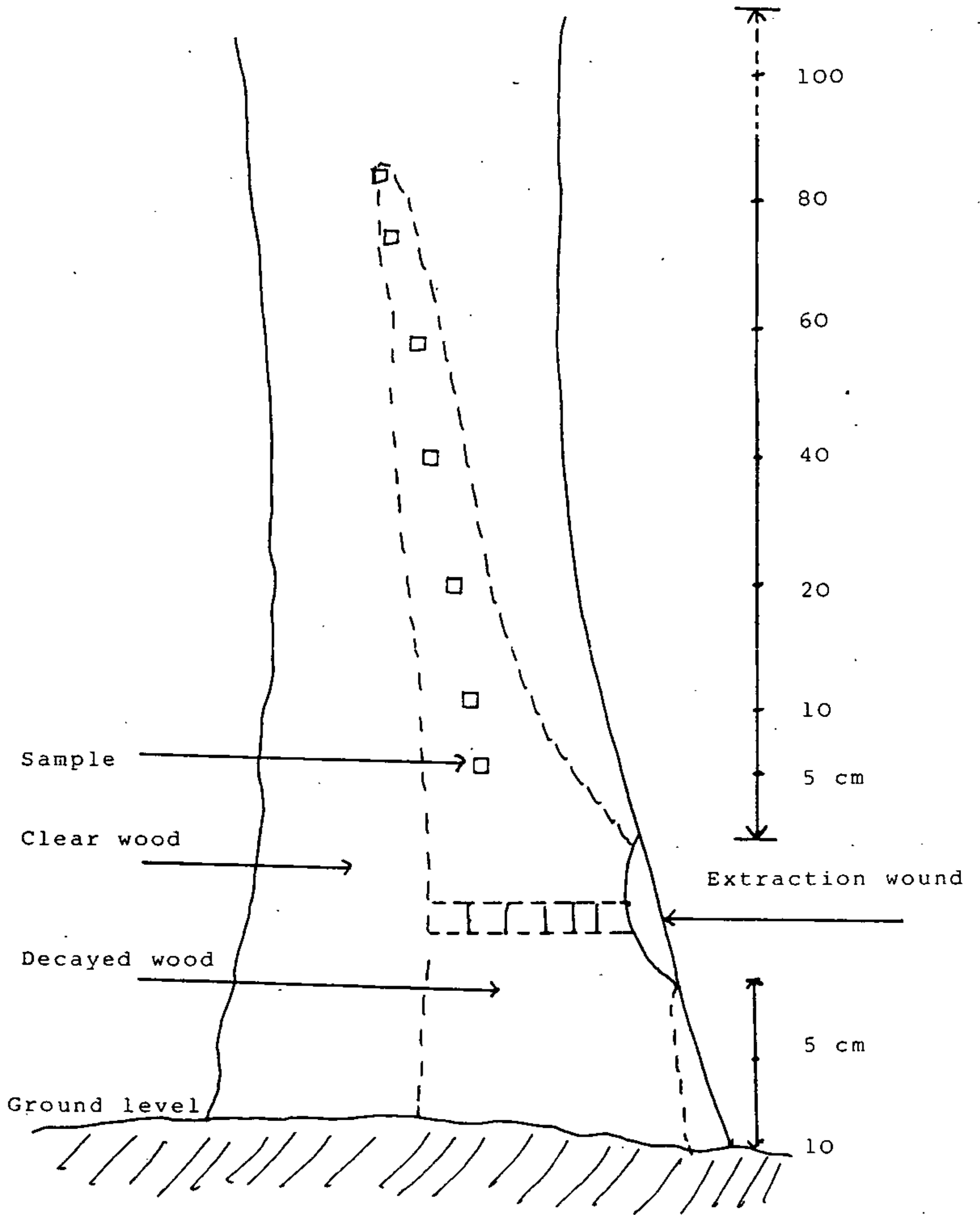

Fig.1: Position of samples taken from trees damaged during extraction operations

aseptically onto petri dishes containing 20 ml of 3% malt extract agar and incubated at 25 C° . Cultures were checked continuously for the occurrence of microbial growth. The identification of cultures was assisted by Dr A.J.Hayes and by the Commonwealth Mycological Institute (Kew).

2. Artificial inoculation experiments

The objective of this experiment was to study the development of S.sanguinolentum and other microorganisms that might influence its expansion. A second ojective was to investigate the effect of tree vigour (i.e dominant,codominant and subdominant),inoculation site treatment (i.e sealed or left open) and the time of year at which the inoculations were made.

2.1. Preparation of inocula

Wood plugs measuring 0.5 x 0.5 x 1 cm were cut from a healthy Norway spruce tree (P 52) taken from compartment 11 in Yair Hill forest. Plugs were autoclaved at 115 C° for 15 minutes and further sterilised by dipping into 0.1% silver nitrate solution ,0.1% sodium chloride solution and sterile distilled water for about one minute respectively (Ward,1952). Plugs were then transfered aseptically to flat inoculation flasks (Plate 1) that contained 2 week-old S.sanguinolentum cultures growing actively on 3% malt extract agar. The plugs were in direct contact with S.sanguinolentum colonies.Cultures were kept humid throughout experiments by adding sterile distilled water in the chamber provided whenever required. Each flask was

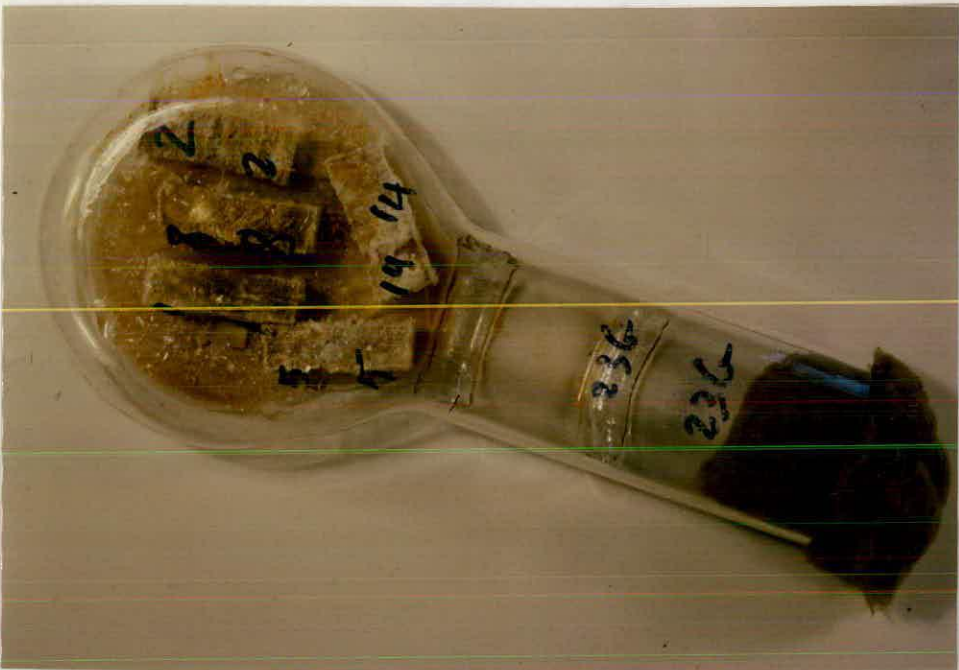

Plate 1: Wood plugs inoculated with *S.sanguinolentum* growing on 3% malt extract agar at 25 C° for artificial inoculation experiments

plugged with a piece of non-absorbent sterilised cotton-wool that allowed the passage of sterile air. Cultures were incubated at 25 ^{o}C. There was a low level of contamination and every contaminated culture was immediately replaced. After 2 months S.sanguinolentum successfuly grew in all the plugs and by that time they were ready for inoculation.

2.2. Selection of trees for inoculation

Norway spruce trees in compartment 11 of Yair Hill forest were chosen for this experiments because they were free of infection by F.annosus . Trees were classified into dominant,codominant and subdominant on the basis of their crowns in relation to the average canopy according to Spurr and Barnes (1980). A total of 26 healthy trees in each class were labelled and the dbh was recorded. In each tree a hole was made on the stem 20 cm from ground level facing the extraction rack and another hole was made opposite to the first one. These were left as controls. Holes were made by an increment borer after the removal of the bark and the borer was resterilised by dipping for about 30 seconds into 95% alcohol between the making of successive holes. The holes facing the extraction rack were inoculated by inserting one wood plug previously inoculated with S.sanguinolentum in each hole (Fig.2). Half the number of the inoculated holes in each tree vigour class (13) were left open,whereas the other half (13) were sealed with paraffin wax immediately after inoculation. In this way a total of 78 trees (26 in each tree vigour class) were

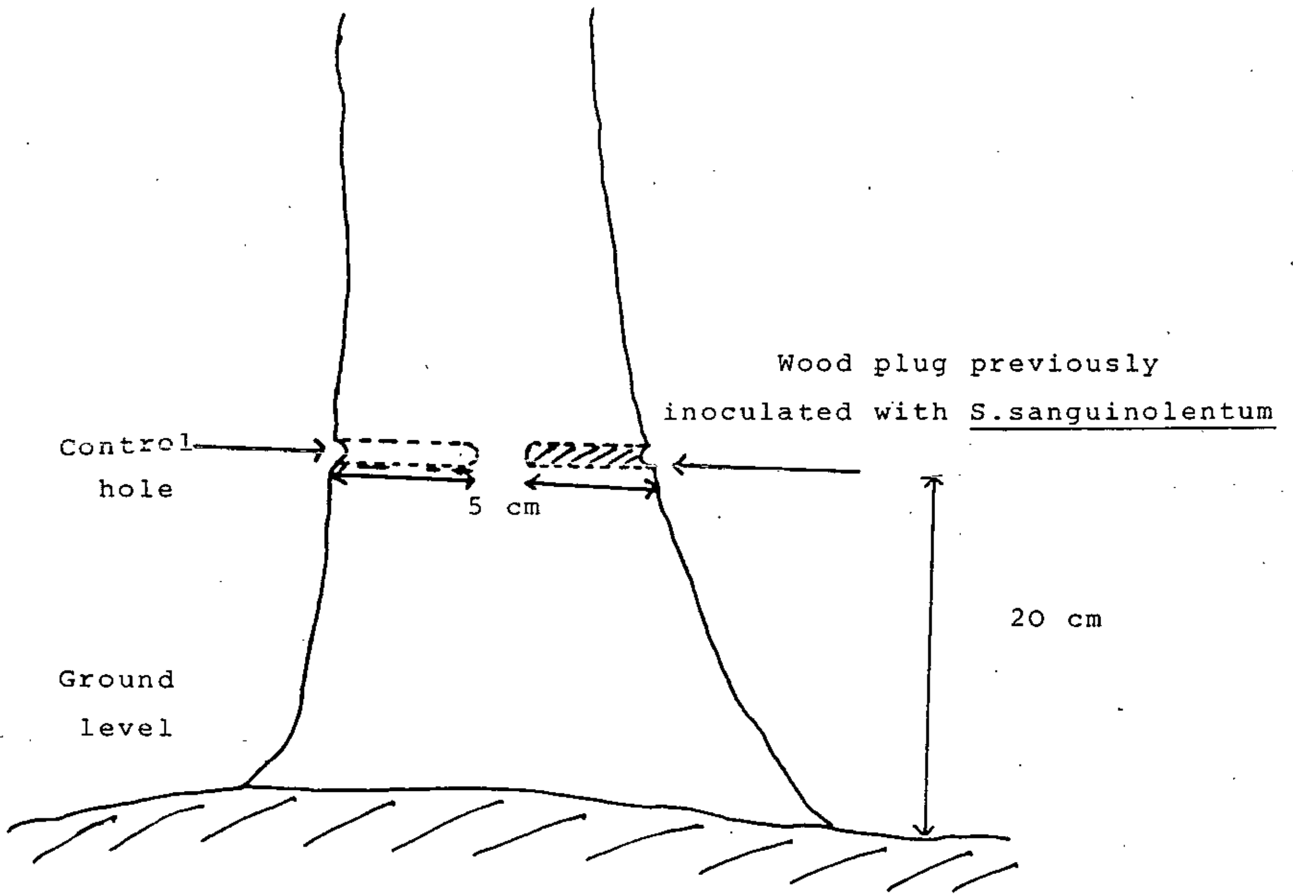

Fig.2 : Method of inoculating standing trees with wood plugs previously inoculated with S.sanguinolentum

inoculated in July 1982 and another series of 78 trees were inoculated in the same manner in February 1983. Eventually in each series there were 39 inoculated trees with open inoculation sites and another 39 in which their inoculation sites had been sealed with paraffin wax. Trees were felled six months after inoculation and transported to the Forestry department (Edinburgh University) and kept in a cool room at about 5 ^{o}C until further analysis was carried out. The method of sampling was made as discussed on page61(after Hansen and Hansen,1980). Culturing was also carried out in a manner similar to that described under 1.

3. Growth studies

3.1. Effect of media and temperature

Each S.sanguinolentum strain was first isolated separately into pure culture. The objective of this experiment was to study possible differences between strains and to determine the effect of various media ,temperature and pH on the growth of all strains.

Media used were 3% malt extract agar (Oxoid), 3% corn meal agar (Oxoid), Czapek dox agar (Oxoid) and spruce-sawdust agar. In Czapek dox agar the basal medium was kept constant:

Magnesium glycerophosphate	0.5 gm
Potassium chloride	0.5 gm
Ferrous sulphate	0.01 gm
Potassium sulphate	0.35 gm
Agar No. 3 (Oxoid)	12 gm
Water	1 litre

Different carbon and nitrogen sources were used and

were added to the basal medium as 2 gm/ℓ of nitrogen source and 30 gm/ℓ of carbon source as indicated in Czapek dox agar. The following carbon sources were used:

Sucrose
Fructose
Lactose
Glucose
Maltose
Starch

The choice of the carbon sources was made in such a way to include monosaccharides e.g glucose and fructose; disaccharides e.g maltose,sucrose and lactose and polysaccharides e.g starch and cellulose as part of the spruce-sawdust agar formulation. The nitrogen sources added separately at each time were:

A. Inorganic nitrogen sources

A.1. Sodium nitrate
A.2. Ammonium sulphate

B. Organic nitrogen sources

B.1. Asparagine
B.2. Leucine
B.3. Glutamic acid

The basal Czapek dox agar medium was therefore kept constant and a carbon and a nitrogen source were added to it for each experiment,the exception being organic nitrogen sources which were combined with sucrose only. Spruce-sawdust agar was prepared by the addition of 30 gm of spruce-sawdust to 12 gm of agar No. 3 (Oxoid). Sawdust was obtained from a healthy Norway spruce tree and was added to one litre of distilled water containing agar in a flask which was autoclaved at 120 C° for 15

minutes. 20 ml of each sterile medium were poured in sterile petri dishes. The inocula used were 10 mm in diameter (Simpson and Hayes,1978) and were taken from the perimeter of 4 week-old stock cultures. A 10 mm core borer was used to take 10 disks of S.sanguinolentum . Each inoculum was placed at the centre of the petri dish. All cultures were incubated at 5,10,15,20,25,30 and 35 C° . For each treatment 5 replicates were used at each temperature. The total number of replicates was: 4 (strains) x 18 (media) x 7 (temperatures) x 5 (replicates) = 2520 cultures.

Each petri dish was divided by means of two lines at right angles on the reverse surface of the petri dish. Colony diameter was measured weekly for four weeks. Two assessments of the colony diameter,set at right angles were averaged (Hayes and Manap,1975). Optimum temperature for growth was recorded for all strains.

3.2. Effect of pH

All S.sanguinolentum strains were grown on 3% malt extract agar at 25 C° . The pH levels used were 4,5,6,6.5,7,7.5 and 8 (after Simpson and Hayes,1978). The adjustment of pH was carried out,after sterilisation,by using 0.1N sodium hydroxide or 0.1N hydrochloric acid. Measurement of the colony diameters was carried out as disscused above.

4. Cellulose and lignin decomposition

1. Norway spruce naturally infected with S.sanguinolentum

Trees in compartment 1016 were chosen for this

experiment because they exhibited advanced decay. 3 trees from each tree vigour class (i.e dominant, codominant and subdominant) showing well developed advanced decay were randomly selected and labelled. Extraction damage in this compartment had occurred in 1975. Each tree was dissected longitudinally through the decay column as far as any decay could be traced.

A total of 10 samples were taken from each tree in the following manner (Fig.3):

Samples 1,3,4,6,7 and 9 were control samples,being taken from healthy wood 10 cm from the boundary of the decay columns on either side. Sample 2 was taken from the top of the decay column; sample 5 from the middle part of the decay column and sample 8 from the bottom part of the decay column. Sample 10 had one half outside the decay column and the other half inside the decay column.Samples 1 and 3 were the control samples for sample 2; 4 and 6 were controls for 5 and 7 and 9 were controls for both sample 8 and 10. Each sample measured 1.5 x 2 x 5 cm. A pilot experiment showed that these dimensions would produce an oven-dry weight slightly more than 5 gm which was necessary for chemical analysis. All samples were pooled and oven-dried until constant weights were obtained. The samples were then ground in a laboratory mill through a 2 mm diameter grate,and kept in a desiccator for further chemical analysis.

Chemical analysis was carried out using a technique described by Wise et al (1946).

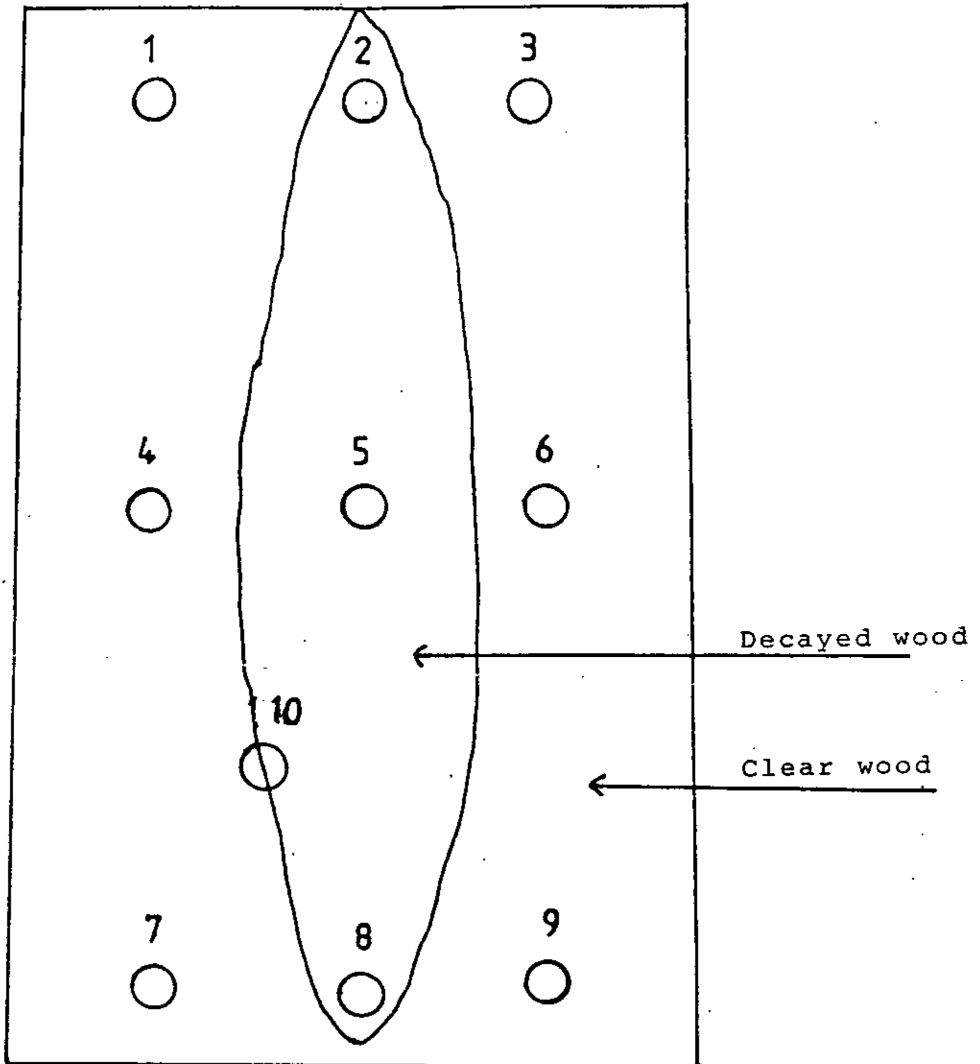

Fig. 3: Position of wood samples taken from infected trees for chemical analysis experiments

(Longitudinal section)

(i) An extraction thimble was filled with 5 gm oven-dry weight. Each sample was washed with distilled water at 21 C^{o}, air-dried and extracted with petroleum ether (boiling point 40-60 C^{o}) in a soxhlet apparatus for six hours to remove lipids. The defatted material was oven-dried and weighed. This weight was subtracted from the original oven-dry weight to give the weight of lipids present in the sample.

(ii) The determination of lignin content was carried out on each defatted sample,the weight of which was known. Each sample was suspended in about 160 ml of distilled water in a 250 ml flask. A 25 ml conical flask was inverted in the top of the large flask,as a gas trap,and the whole apparatus was immersed to its neck in a water bath at 75 C^{o} in a fume chamber. At hourly intervals,for four hours,ten drops of glacial acetic acid and 1.5 gm of sodium chlorite were added. After four hours the contents of each flask were filtered through a tared sintered glass funnel. Then the flask was washed repeatedly with ice water to remove adhering matter and the contents of the funnel were washed repeatedly with ice water and then with acetone and ether until the residue was white in colour. The residue was oven-dried and weighed. By a subtraction of weights before and after the treatment,lignin content was determined.

(iii) The oven-dry weight of the sample after the extraction of lipids and lignin

gives the weight of crude cellulose,including hemicellulose,in the sample. A total of 90 samples were analysed from all trees.

4.2. Norway spruce artificially inoculated with S.sanguinolentum

A total of 3 successfuly infected Norway spruce trees from each tree vigour class (i.e dominant,codominant and subdominant) were randomly selected and labelled. Method of sampling and analysis was similar to that described above (Wise et al ,1946).

4.3. Analysis of wood blocks artificially inoculated with S.sanguinolentum

The objective of this experiment was to determine the effect of S.sanguinolentum on the wood substance 1,2 and 3 months after artificial inoculations. 3 healthy Norway spruce trees in compartment 11 (Yair Hill forest) were randomly selected from each tree vigour class. Trees were felled and moved to the laboratory as quickly as possible and kept in a cool room at 5 C° for further analysis. A slab of wood measuring 10 x 9 x 5 cm was taken from the basal part of the stem 20 cm from the ground level from each tree. Each slab was further cut into 1.5 x 2 x 5 cm blocks. Each slab produced 30 wood blocks 1.5 x 2 x 5 cm each. Of the 30 wood blocks,5 were left as controls and the neighbouring 5 were inoculated with S.sanguinolentum for one month. Another series of 5 wood blocks were inoculated with S.sanguinolentum for 2 months and the neighbouring 5 blocks were left as controls. Of the remaining samples 5 were artificially inoculated with

S.sanguinolentum for 3 months and the remaining 5 were left as controls. Therefore, of the total number of samples in each tree (i.e 30), 5 were blocks inoculated with S.sanguinolentum for one month; 5 for two months and 5 for three months and the remaining 15 samples were left as controls. The sampling method was summarised in Fig.2. The inoculation method used was the same as described under 2.1. Control as well as inoculated samples were chemically analysed (after Wise et al 1946) for the effect of S.sanguinolentum on wood substance after 1,2 and 3 months of artificial laboratory inoculations.

5. Determination of wood density

Samples for this experiment were taken from healthy parts of Norway spruce trees used for artificial inoculation with S.sanguinolentum for six months. To avoid any infected wood,one sample of 1.5 x 1.5 x 5 cm (Campbell,1932a) was taken 10 cm above the ground level from the basal part of the stem opposite the extraction rack. Samples were taken from 26 trees in each tree vigour class (i.e dominant,codominant and subdominant). The 5 cm length was chosen so that a reasonable ring count was possible. Trees in each vigour class were chosen in such a way that they were closely comparable in terms of ring frequency and dbh (Southam and Ehrlich,1943). Samples were then oven-dried at 105°C to constant weight. The weight of wood was then determined in gm/cm^3 for each sample.

6. Statistical Analysis:

Tests of significance (i.e t-test ; analysis of variance and regression analysis) were carried out using methods

described by Snedecor and Cochran (1974) and Parker (1979). Statistical analysis was carried out by using three different computer packages: SPSS (statistical package for social sciences,SPSS INC., Chicago); BMDP (Bio-Mathematics Department Package,University of California,Los Angeles) and Presto (Department of Forestry and Natural Resources,University of Edinburgh). Data derived from the artificial inoculation of Norway spruce was analysed partly using the test of significance of a binomial proportion (Chambers,1955 and Snedecor and Cochran,1974) or where appropriate,a chi-square test (Snedecor and Cochran, 1974) was carried out using 2 x n contingency tables. Chi-square and binomial tests were carried out for the analysis of frequencies and percentages.

T-tests of significance were carried out using the SPSS package for paired or independent populations. In the latter a seperate variance computation was done for each population and on basis of whether the variances are equal or not a certain formula for the t-value and the associated degrees of freedom was demonstrated. Presto package was used for the analysis of variance to test differences between more than two populations and another series of t-tests was also carried out to determine the source of variation. In addition Presto was also used for the drawing of most of the graphs. The BMDP package was used for the computation of the regression analysis. The output also included analysis of variance as a result of regression and the regression equation. Tests of binomial distribution were carried out according to the advice of Dr. Renshaw,Department of Statistics, University of Edinburgh.

RESULTS

CHAPTER 4

4. INVESTIGATION OF THE FREQUENCY OF EXTRACTION DAMAGE AND DECAY

4.1. Frequency of injured and infected trees

4.1.1. Younger Norway spruce [Picea abies]

Trees from younger Norway spruce crops, (P52) from compartment 11 of YAIR HILL FOREST,were examined for extraction damage. Line thinning had been carried out in 1979 where one row in each 4 was removed. In this compartment a total of 49 trees were injured during extraction operations; damaged trees being scattered over 4 extraction racks. Of these 40 (82%) were stem wounds and the remaining 9 (18%) were wounds occurring on superficial roots. The majority of stem wounds occurred on the basal part of the stem with their lower edges in contact with the ground level. The frequency of infection by S.sanguinolentum entering through extraction wounds was 20 (50%) in stem wounds and 3 (33%) in root injuries. The overall frequency of infection (irrespective of the wound location) was 23 (47%). The fact that most of the extraction damage occurred on the basal part of stems suggested that it was caused by skidding tractors. Most of the infected trees had incipient decay where tissues were discoloured but still firm. A ring count showed that the extraction damage occurred in 1979 (i.e 4 years old at the time of examination). S.sanguinolentum sporophores were found on the wound surfaces of 5 stems and on one injured root. In addition,the sporophores were also abundant on the cut surfaces of logs (Plate 2) and dead branches on the forest floor,being most evident during the

Plate 2: S.sanguinolentum sporophores on the cut-surface of a Norway spruce

wet part of the season.

4.1.2. Older Norway spruce

Older Norway spruce trees (P 29) were examined in compartment 1016 in GLENTRESS FOREST. In this compartment a total of 54 trees had been damaged during extraction operations. Out of the total number of injured trees 39 (72%) had stem damage mostly occurring on the basal part and in the remaining 15 (28%) damage occurred on superficial roots. Of these 29 (54%) were infected by S.sanguinolentum entering through extraction wounds irrespective of the wound location. The infection frequency was 24 (62%) in stem wounds and 5 (33%) in root injuries. A ring count showed that the extraction damage occurred in 1975 (i.e 8 years old at the time of examination) and was probably caused by skidding tractors. S.sanguinolentum sporophores were noted on wound surfaces occurring on the stems of 6 trees and in two instances on wounded roots. The sporophores were also found on logs and dead branches on the forest floor. S.sanguinolentum sporophores usually occur in clusters and are rarely found solitary (Plate 3). This compartment was heavily dominated by F.annosus sporophores occurring on the stumps of felled trees. Most of the infected trees had advanced decay caused by S.sanguinolentum .

4.1.3. Japanese larch [Larix kaempferi] (P 53)

Japanese larch trees (P 53) were examined in compartment 3 of YAIR HILL FOREST. A 1 row in 4 line thinning had been carried out. A total of 50 trees had

Plate 3: Clusters of S.sanguinolentum sporophores on the wound surface of a Norway spruce tree

been injured during extraction operations in this compartment. Most of the injury occurred on stem bases and less frequently on superficial roots. Most of the damage occurred on the basal parts of the stems resulting in wounds with their lower edges in contact with the ground level and a few occurring at about 20 cm from the ground level. Of the total number of damaged trees 12 (24%) were infected with S.sanguinolentum entering through extraction wounds. Most of the infection occurred through basal stem wounds,the exception being one tree where S.sanguinolentum entered through a broken top and produced numerous sporophores on the broken part and on the bark. The ring count showed that the age of the extraction damage was 4 years i.e damage occurred in 1979. All the infected trees had only incipient decay. S.sanguinolentum sporophores were found on some wound surfaces and were most evident on the broken top of one tree.

4.1.4. Japanese larch (P54)

Japanese larch trees (P 54) were examined in compartment 21 of YAIR HILL FOREST. Skyline thinning had previously been carried out in this compartment. A total of 43 trees had been injured during extraction operations. Of these 16 (37%) were infected with S.sanguinolentum entering through extraction wounds. The extraction racks in this compartment were very steep and it was quite common to find up to 3 wounds on the same tree. Most of the lower edges of extraction wounds occurred between 10 and 160 cm from the ground level. All the infections

occurred through stem injuries,whereas all root injuries (2) were free of infection. The ring count showed that the extraction damage had occurred in 1979 i.e age was 4 years. The sporophores of S.sanguinolentum were found on injured surfaces of 4 trees and occurred in clusters and rarely found individually. Infected trees had decay at its very early stages.

4.2. Description of the infection column

4.2.1. Norway spruce

Following infection, S.sanguinolentum expanded radially,downwards and upwards from the wound site. Incipiently decayed tissues were discoloured but still firm and ranged in colour from pale red through pale brown to reddish brown. Tissues at the advanced stage of decay were softer and usually reddish brown or dark brown compared with healthy tissues (Plate 4). The maximum radial expansion of decay occurred consistently at the wounded site of the tree,decreasing up the infection column forming a spindle-shaped zone of decay. Eventually the infection column occupied the whole of the central portion of the tree (Plate 5). The infection column was consistently surrounded by a zone of dry wood. Enclosing both the infection column and the dry zone was a grey zone afew millimeters wide (Plate 6). Resin was produced at the site of the wound and drained from the wound surface onto the bark. If the infection occurred in two separate extraction wounds,,two infection columns were produced (Plate 7 & 8). The occurrence of knots disturbed

Plate 4: A cross section in a Norway spruce (P 29) infected with S.sanguinolentum through an extraction wound (8-year-old)

Plate 5: A cross section in a Norway spruce (P 29) showing part of the decay column (central) caused by S.sanguinolentum through an 8-year-old extraction wound

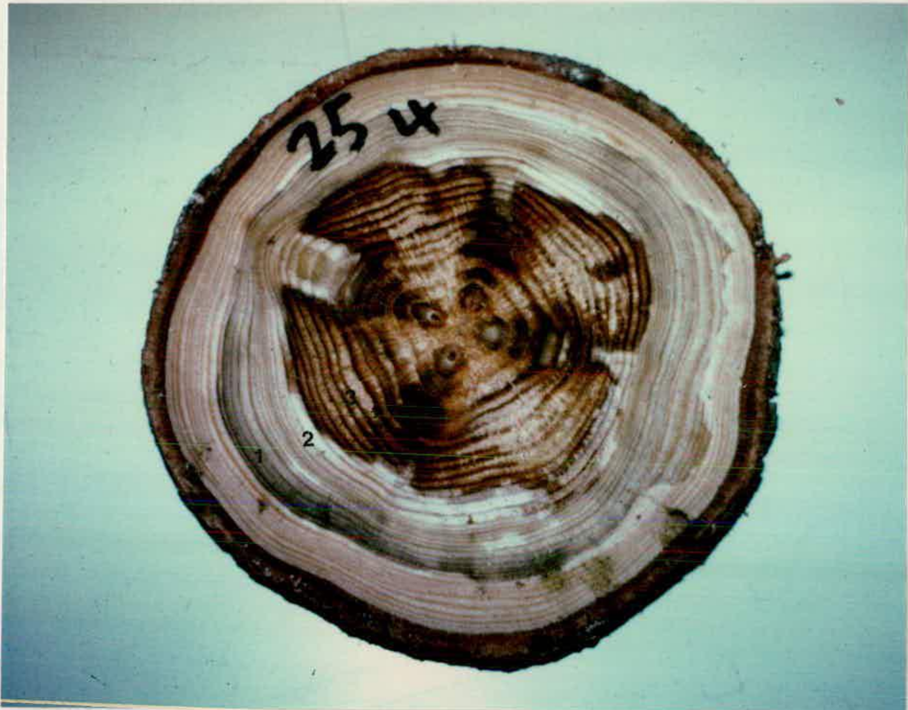

Plate 6: A cross section in a Norway spruce (P 29) infected with S.sanguinolentum through an extraction wound :

1: The grey zone which was produced as a response against infection.

2: Clear wood

3: Decayed wood

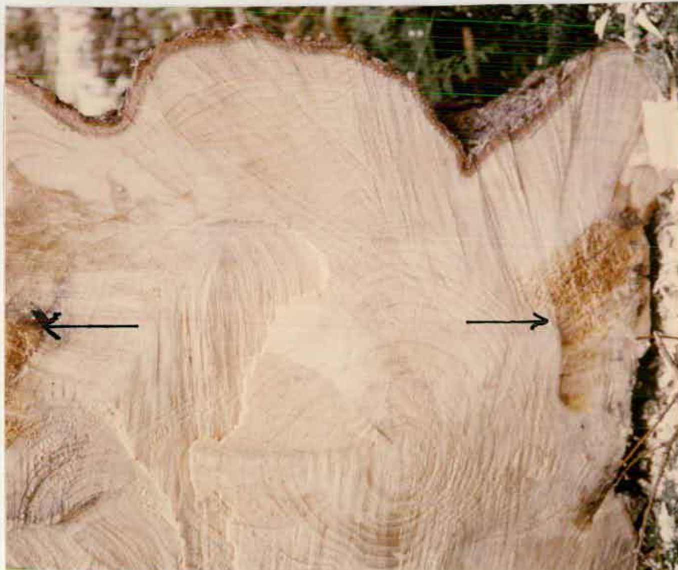

Plate 7: Two basal extraction wounds in a Norway spruce which were infected with S.sanguinolentum

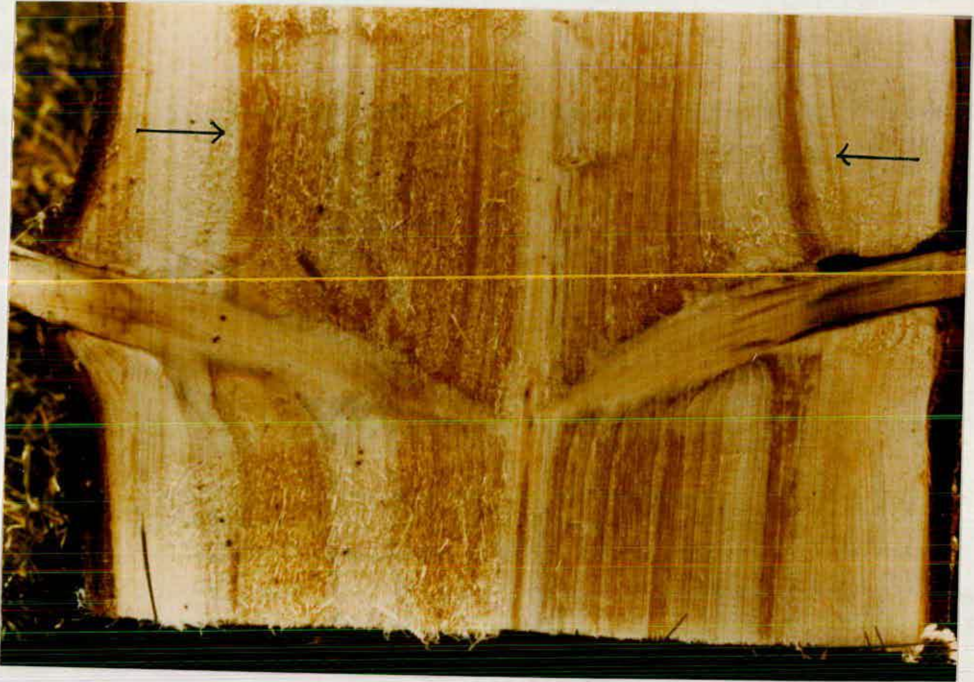

Plate 8: Two seperate decay columns in a Norway spruce tree that resulted from the infection of two basal extraction wounds with S.sanguinolentum

the continuity of the infection column (Plate 9).

4.2.2. Japanese larch

Following infection with S.sanguinolentum , decay spread radially in form of extremely irregular brown or red patches expanding up and down the wounded site. The spread of the infection was restricted to a few centimeters both radially and vertically. Usually the infection column was narrow,short and discontinuous. The tracing of the infection column presented some difficulties because it resembles the heartwood in colour (Plate 10). The maximum radial penetration of decay occurred consistently at the wounded site. The dry wood and grey zone that surrounded the infection columns in Norway spruce were completely absent in infected Japanese larch. Injured Japanese larch showed relatively better capabilities of wound healing yet most of the tissues at the injured site were exposed.

4.3. Rate of progress of decay

Tables 1 and 2 summarise the rate of decay caused by S.sanguinolentum entering through extraction wounds in Norway spruce and Japanese larch crops respectively.

The mean radial penentration of decay was significantly ($P<0.001$) different in Norway spruce (Compartment 11) and Japanese larch crops in compartment 3 and 21 (Table 3). Mean radial penetration of decay was minimum (1.8 cm) in Japanese larch (Compartment 21) and maximum (9.3 cm) in Norway spruce (Compartment 11) with Japanese larch crops in compartment 3 occupying an intermediate position (2.3

Plate 9: A cross section in a Norway spruce which was infected with S.sanguinolentum entering through an extraction wound. The occurrence of knots disturbed the continuity of the decay column

Plate 10: A cross section in a Japanese larch which was infected with S.sanguinolentum entering through extraction wounds

A. Clear heartwood

B. Infected wood

Table 1:RATE OF PROGRESS OF DECAY CAUSED BY *S.sanguinolentum* IN NORWAY SPRUCE CROPS (COMPARTMENTS 11 and 1016)

COMPARTMENT	PLANTED	WOUND AGE (YEARS)	MINIMUM	MAXIMUM	MEAN
RATE OF RADIAL PENETRATION OF DECAY				(CM/YEAR)	
11	1952	4	1.2	4.0	2.3
1016	1929	8	0.6	7.4	3.3
RATE OF VERTICAL EXPANSION OF DECAY				(CM/YEAR)	
11			12.5	72.5	35.0
1016			07.5	80.0	37.5
RATE OF PROGRESS OF DECAY				(M^3 /YEAR)	
11			0.0010	0.01	0.003
1016			0.0003	0.02	0.003

Table 2:RATE OF PROGRESS OF DECAY CAUSED BY *S.sanguinolentum* IN JAPANESE LARCH CROPS (COMPARTMENTS 3 and 21)

COMPARTMENT	PLANTED	WOUND AGE (YEARS)	MINIMUM	MAXIMUM	MEAN
RATE OF RADIAL PENETRATION OF DECAY				(CM/YEAR)	
3	1953	4	0.4	0.8	0.6
21	1954	4	0.2	0.9	0.5
RATE OF VERTICAL EXPANSION OF DECAY				(CM/YEAR)	
3			12.5	25	17.5
21			7.5	25	15
RATE OF PROGRESS OF DECAY				(M^3) /YEAR)	
3			0.0001	0.001	0.0003
21			0.0001	0.001	0.0002

Table 3: COMPARISON OF DECAY DEVELOPMENT CAUSED BY *S.sanguinolentum*
IN NORWAY SPRUCE AND JAPANESE LARCH CROPS
(ANALYSIS OF VARIANCE)

SOURCE OF VARIATION	DF	SS	MSS	F ratio	PROBABILITY
1.RADIAL PENETRATION OF DECAY:					
TREATMENT	2	685.89136	342.94556	59.35722	< 0.001
ERROR	48	277.32739	5.77765		
TOTAL	50	963.21875			
2.VERTICAL EXPANSION OF DECAY:					
TREATMENT	2	6.48213	3.24107	18.74763	< 0.001
ERROR	48	8.29817	0.17288		
TOTAL	50	14.78030			
3.DECAY VOLUME:					
TREATMENT	2	0.00157	0.00079	9.19261	< 0.001
ERROR	48	0.00411	0.00009		
TOTAL	50	0.00569			

cm). The difference in the mean radial penetration of decay between Japanese larch crops in compartment 3 and 21 was not significant (Table 4).

Similarly mean vertical expansion of decay was significantly ($P<0.001$) different comparing Norway spruce (Compartment 11) and Japanese larch in compartments 3 and 21 (Table 3). Mean vertical expansion of decay was maximum (1.4 metres) in Norway spruce (Compartment 11); minimum (0.6 metres) in Japanese larch (Compartment 21) and was intermediate (0.7 metres) in Japanese larch in compartment 3. However,differences in the mean vertical expansion of decay in Japanese larch crops in compartments 3 and 21 were not significant (Table 4).

The same trend was observed in the mean decay volume which was significantly ($P<0.001$) different comparing Norway spruce and Japanese larch (Table 3). Mean volume of decay was maximum (0.0122 m^3) in Norway spruce (Compartment 11); minimum (0.0009 m^3) in Japanese larch (Compartment 21) and intermediate (0.0012 m^3) in Japanese larch in compartment 3. The difference in mean volume of decay was not significant comparing Japanese larch in compartments 3 and 21 (Table 4).

In another series of studies, mean radial and vertical expansion of decay and mean volume of decay were compared in younger (P 52,compartment 11,YAIR HILL FOREST) and in older (P 29,compartment 1016,GLENTRESS FOREST) Norway spruce crops having extraction damage 4 years and 8 years old (1979 and 1975) extraction damage respectively.

Table 4: COMPARISON OF DECAY DEVELOPMENT CAUSED BY S.sanguinolentum AFTER FOUR YEARS IN NORWAY SPRUCE AND JAPANESE LARCH CROPS (T-TEST)

COMPARTMENT	SPECIES	MEAN	T-VALUE	PROBABILITY
1.RADIAL PENETRATION OF DECAY		(CM)		
11	NORWAY SPRUCE	9.3478	8.2241	< 0.001
3	JAPANESE LARCH	2.3083		
11	NORWAY SPRUCE	9.3478	9.7097	< 0.001
21	JAPANESE LARCH	1.7500		
3	JAPANESE LARCH	2.3083	0.6083	> 0.05
21	JAPANESE LARCH	1.7500		
2.VERTICAL EXPANSION OF DECAY:		(M)		
11	NORWAY SPRUCE	1.3656	4.6533	< 0.001
3	JAPANESE LARCH	0.6767		
11	NORWAY SPRUCE	1.3656	5.4349	< 0.001
21	JAPANESE LARCH	0.6300		
3	JAPANESE LARCH	0.6767	0.2939	> 0.05
21	JAPANESE LARCH	0.6300		
3.DECAY VOLUME:		(M^3)		
11	NORWAY SPRUCE	0.0122	3.3250	< 0.01
3	JAPANESE LARCH	0.0012		
11	NORWAY SPRUCE	0.0122	3.7568	< 0.001
21	JAPANESE LARCH	0.0010		
3	JAPANESE LARCH	0.0012	0.1020	> 0.05
21	JAPANESE LARCH	0.0010		

Mean radial penetration of decay was significantly ($P<0.01$) greater in older Norway spruce crops (13.1 cm) compared with younger Norway spruce (9.4 cm) (Table 5). On the other hand, there were no significant differences in mean vertical expansion of decay and mean volume of decay comparing younger and older Norway spruce crops. Mean vertical expansion of decay was 1.5 and 1.4 metres and mean volume of decay was 0.0119 and 0.0122 m^3 in older and in younger Norway spruce crops respectively (Table 6).

4.4. Factors affecting the development of decay

Data on tree and decay parameters were summarised in appendices 1,2,3 and 4.

4.4.1. Norway spruce

[i] Younger Norway spruce (Compartment 11)

The following correlations were demonstrated :

(i) A significant positive correlation ($r=0.559, P<0.01$) between radial penetration of decay and tree dbh. Radial penetration of decay is dependent on the dbh. $Y=0.51029\ X + 0.55003$) (Fig.4).

(ii) A significant negative correlation ($r=-0.761, P<0.001$) between the number of annual rings per 5 cm from the outer bark inwards and tree dbh. The number of annual rings/5 cm is dependent on the dbh. $Y=40.808-1.27477\ X$ (Fig. 5).

(iii) A significant negative correlation ($r=-0.431, P<0.05$) between radial penetration of decay and the number of annual rings/5 cm. Radial penetration of decay is dependent on the number of annual rings/5 cm.

Table 5: COMPARISON OF DECAY DEVELOPMENT CAUSED BY S.sanguinolentum
IN YOUNGER AND IN OLDER NORWAY SPRUCE CROPS
(RADIAL PENETRATION OF DECAY)

COMPARTMENT	PLANTED	WOUND AGE (YEARS)	MEAN (CM)	T-VALUE	PROBABILITY
11	1952	4	9.3910		
				2.64	> 0.01
1016	1929	8	13.1414		

Table 6: COMPARISON OF DECAY DEVELOPMENT CAUSED BY S.sanguinolentum
IN YOUNGER AND IN OLDER NORWAY SPRUCE CROPS

COMPARTMENT	PLANTED	WOUND AGE (YEARS)	MEAN	T-VALUE	PROBABILITY
VERTICAL EXPANSION OF DECAY:			(M)		
11	1952	4	1.3657		
				0.66	> 0.05
1016	1929	8	1.5048		
DECAY VOLUME:			(M^3)		
11			0.0122		
				0.08	> 0.05
1016			0.0119		

—— Compartment 1016 (Y = 0.63 X - 2.39)

– – – Compartment 11 (Y = 0.51 X + 0.55)

Radial penetration of decay (cm)

dbh (cm)

Fig. 4 Relationship between radial penetration of decay and dbh

Norway spruce :

+ (P29)

× (P52)

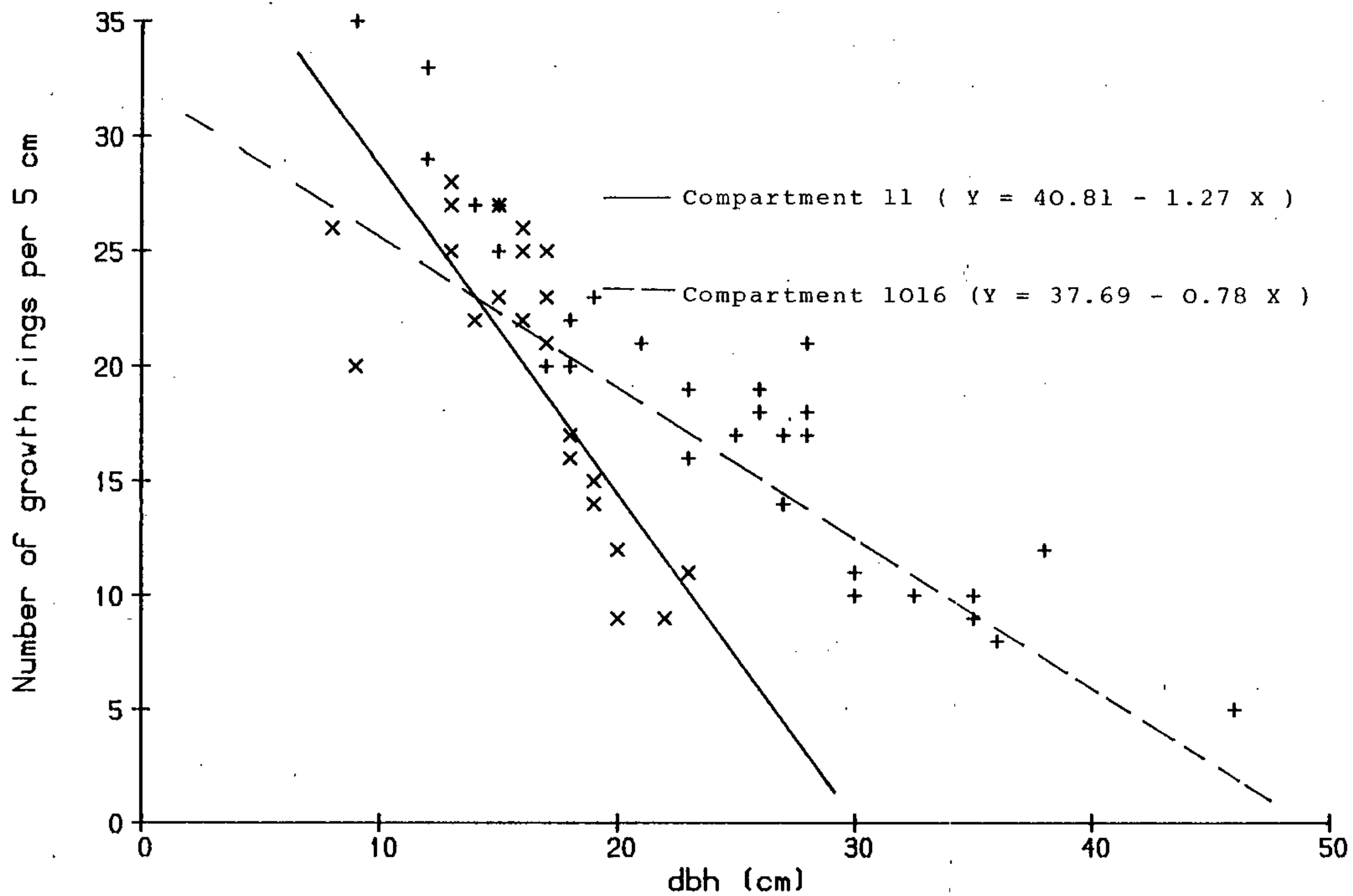

Fig.5 Relationship between the number of rings per 5 cm and dbh

Norway spruce:

+ (P29)

X (P52)

Table 7: RESULTS OF ANALYSIS OF VARIANCE (YOUNGER NORWAY SPRUCE) COMPARING RADIAL DECAY PENETRATION WITH NUMBER OF ANNUAL RINGS AND DBH.

	SUM OF SQUARES	DF	MEAN SQUARE	F ratio	P
REGRESSION	77.4786	2	38.7393	4.553	> 0.01
RESIDUAL	170.1653	20	8.5083		

Table 8: RESULTS OF REGRESSION ANALYSIS (YOUNGER NORWAY SPRUCE) COMPARING DECAY VOLUME WITH DBH AND WOUND SURFACE AREA

ANALYSIS OF VARIANCE:

	SUM OF SQUARES	DF	MEAN SQUARE	F ratio	P
REGRESSION	0.0011	2	0.0006	3.791	> 0.01
RESIDUAL	0.0030	20	0.0001		

T-TEST:

VARIABLE	STANDARD ERROR	REG.COEFFICIENT	T-VALUE	PROBABILITY
X1	0.00002	0.084	0.436	> 0.05
X2	0.001	0.529	2.754	< 0.01

X1= WOUND SURFACE AREA

X2= TREE DBH

Y=13.59228-0.23492 X (Fig .6).

(iv) A significant positive correlation ($r=0.894, P<0.001$) between vertical expansion of decay and wound surface area. Vertical expansion of decay is dependent on the area of exposed tissues. $Y=0.00354 X + 0.56543$ (Fig. 7).

(v) A significant positive correlation ($r=0.531, P<0.01$) between decay volume and tree volume. Decay volume is dependent on tree volume. $Y=0.06813 X - 0.00083$ (Fig.8).

(vi) A significant positive correlation ($r=0.518, P=0.01$) between decay volume and tree dbh. The larger the dbh the greater is the decay volume. $Y=0.00192 X -0.01931$ (Fig.9).

(vii) Multiple regression analysis (Table 7) showed that the radial penetration of decay was dependent on the number of annual rings and the dbh. $Y=0.50167 X1 -0.00677 X2 + 0.82613$, where Y= the radial penetration of decay; X1=number of annual rings per 5 cm and X2=dbh.

(viii) Multiple regression analysis (Table 8) also showed that decay volume was more dependent on the dbh than on the wound surface area. $Y=0.00001 X1 + 0.00196 X2 - 0.02177$, where Y=decay volume; X1=wound surface area and X2=dbh.

The following correlations were not significant

(i) Decay volume/wound surface area

(ii) %Decay volume/tree volume

(iii) Radial penetration of decay/wound surface area

(vi) Radial/vertical expansion of decay

(v) Vertical expansion of decay/tree volume

[ii] Older Norway spruce (Compartment 1016)

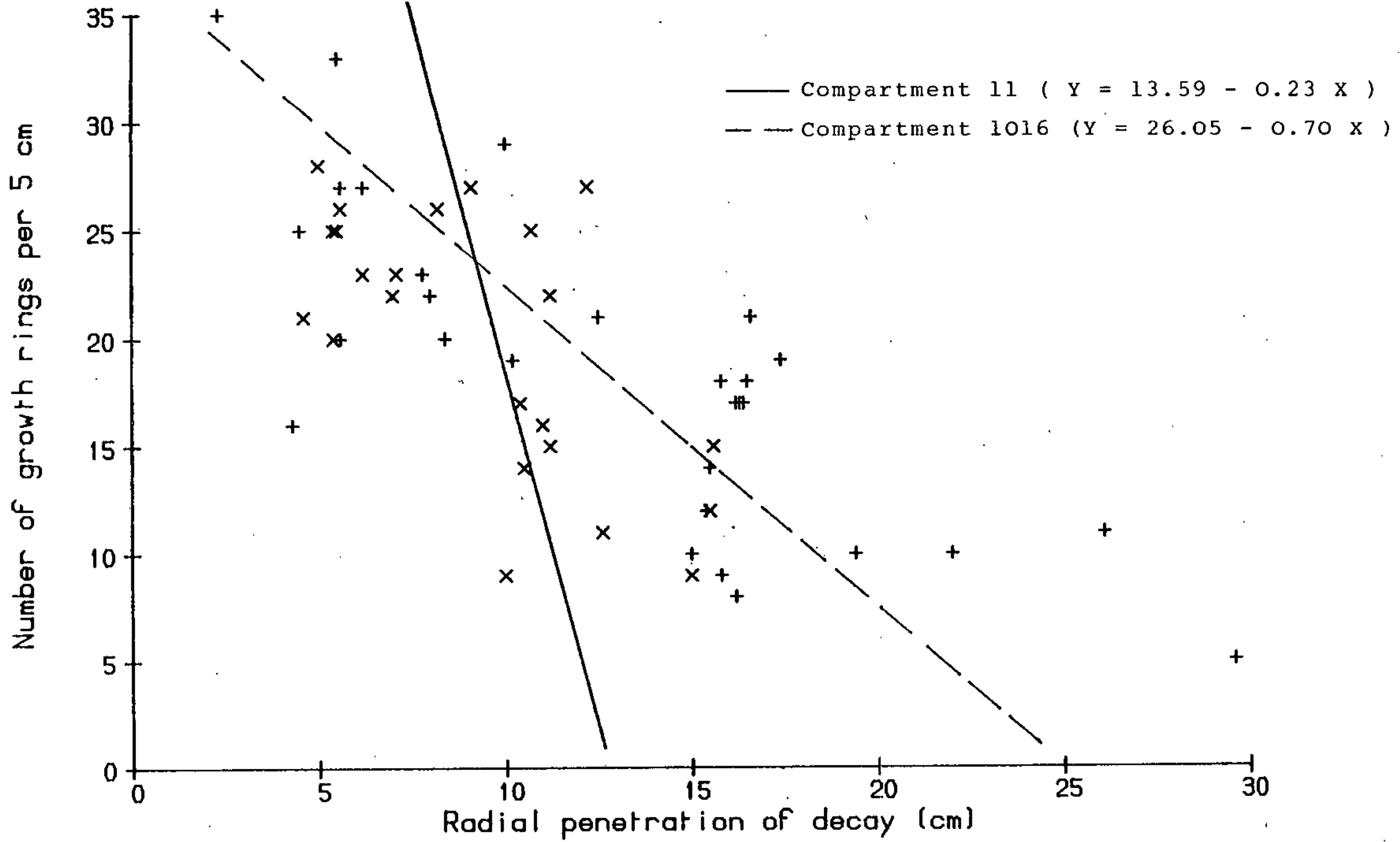

Fig.6 Radial penetration of decay VS number of rings per 5 cm

Norway spruce;

+ (P19)

x (P52)

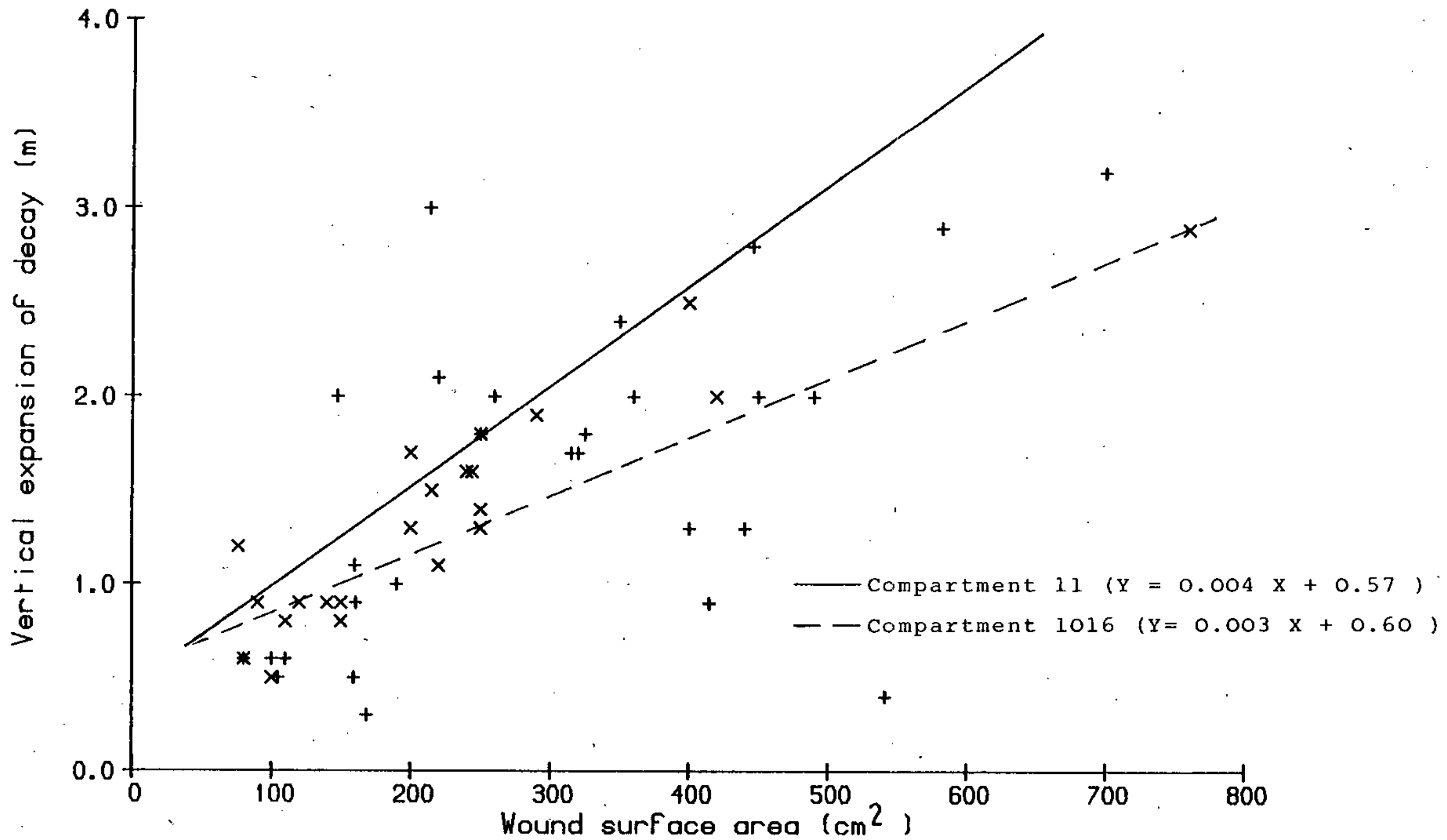

Fig.7 Wound surface area VS vertical expansion of decay

Norway spruce:

+ (P19)

X P52

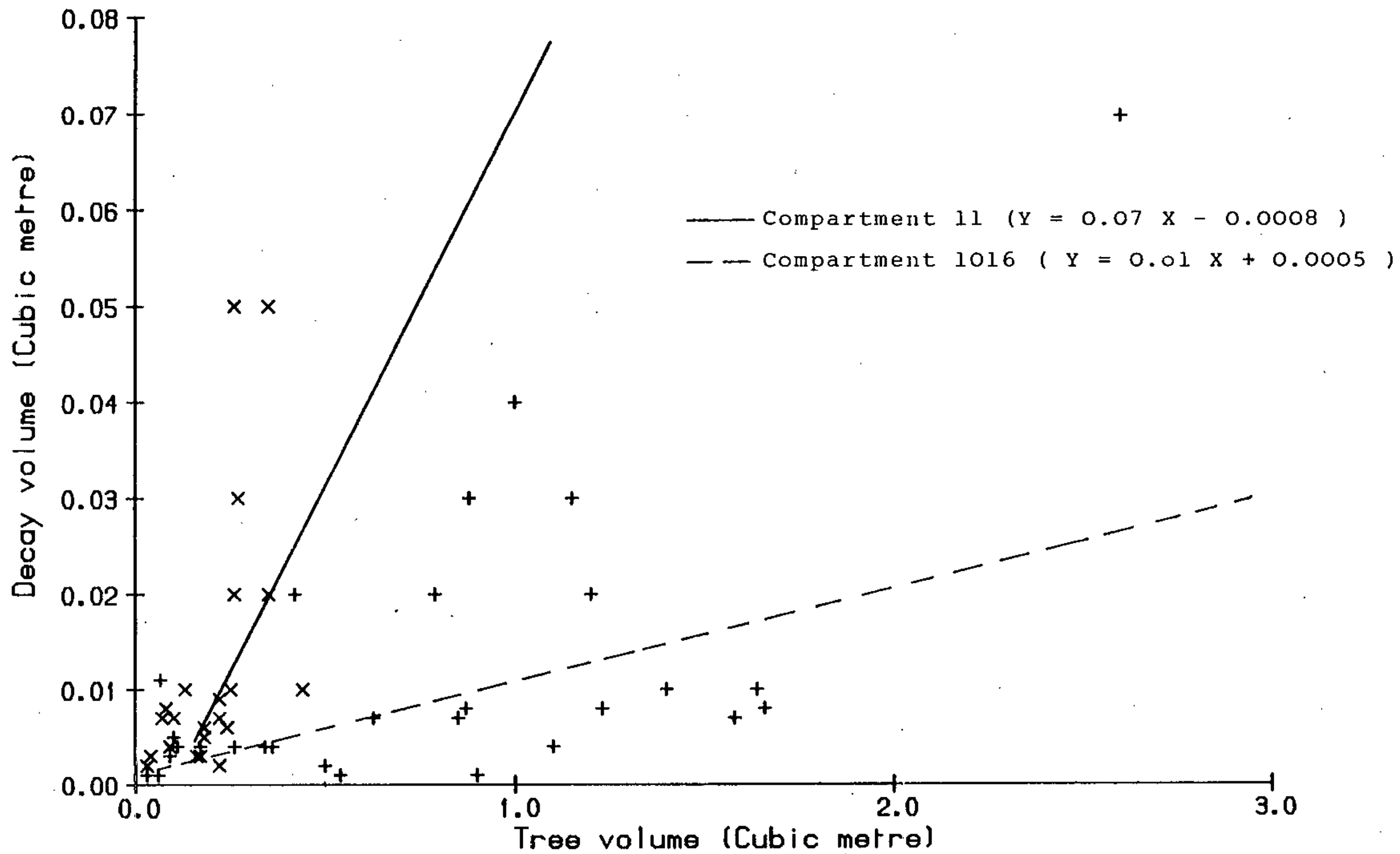

Fig.8 Relationship between tree volume and decay volume

Norway spruce

+ (p29)

x (p52)

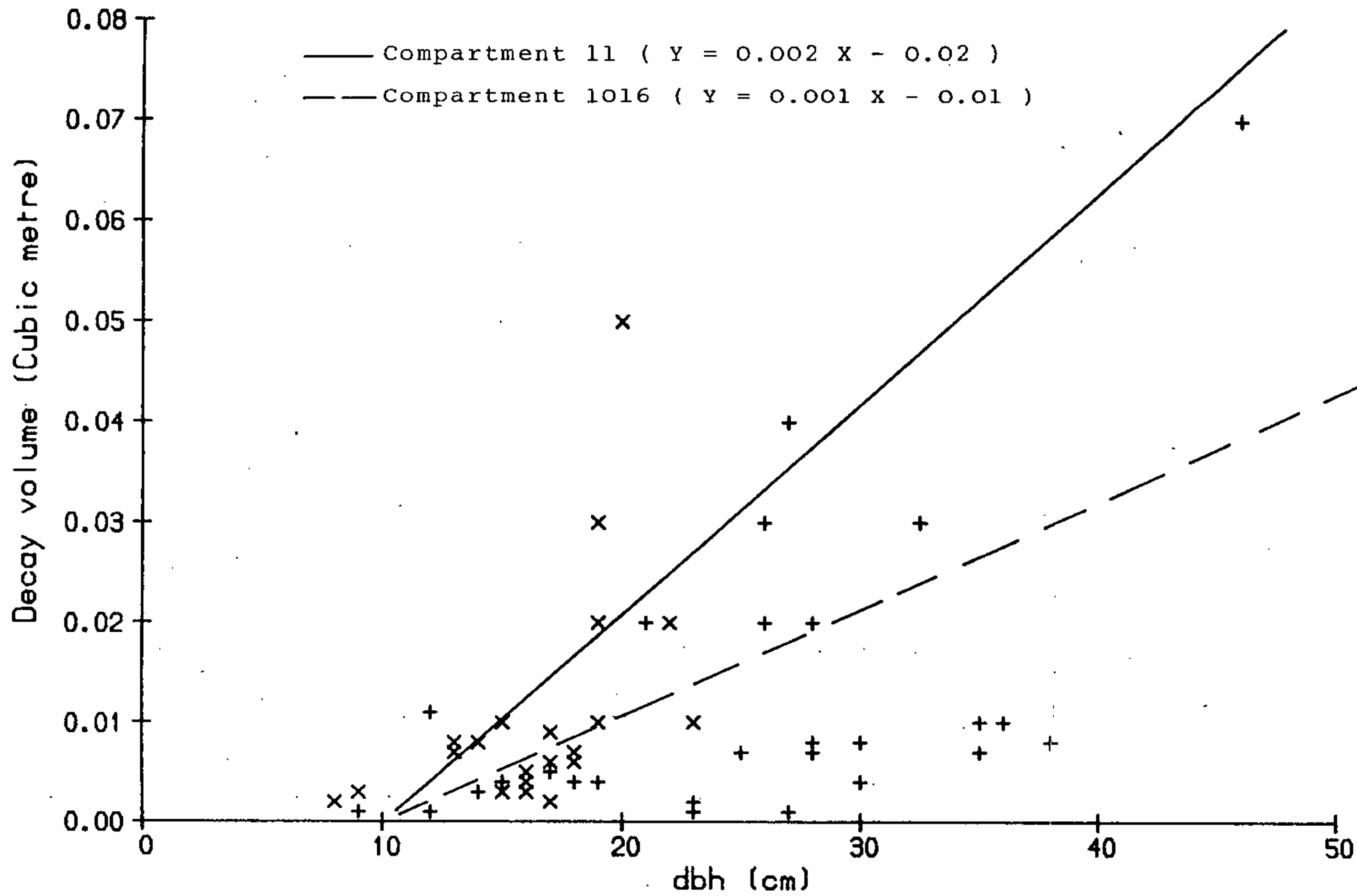

Fig.9: Relationship between decay volume and dbh

Norway spruce:

+ (p29)

X (p52)

The following correlations were demonstrated :

(i) A significant positive correlation (r=0.838,$P<0.001$) between radial penetration of decay and tree dbh.The former is dependent on the latter. Y=0.63132 X - 2.39118 (Fig.4).

(ii) A significant negative correlation (r=-0.933,$P<0.001$) between the number of annual rings per 5 cm and tree dbh. The number of annual rings/5 cm is dependent on the dbh. Y=37.686-0.78472 X (Fig.5).

(iii) A significant negative correlation (r=-0.784,$P<0.001$) between radial penetration of decay (dependent) and and the number of annual rings/5 cm. Y=26.04892-0.70229 X (Fig.6).

(iv) A significant positive correlation (r=0.586,$P<0.001$) between vertical expansion of decay and wound surface area. Y=0.00305 X + 0.60318 (Fig.7).

(v) A significant positive correlation (r=0.613,$P<0.001$) between decay volume and tree volume. The former is dependent on the latter. Y=0.01469 X + 0.00045 (Fig.8)

(vi) A significant positive correlation (r=0.546,$P>0.001$) between decay volume and tree dbh. Decay volume is dependent on the dbh. Y=0.001 X - 0.01068 (Fig.9).

Multiple regression analysis (Table 9) showed that decay volume was more dependent on the dbh than on the wound surface area. Y=0.00085 X1 + 0.00001 X2 - 0.01329,where Y=decay volume;X1=dbh and X2=wound surface area.

Multiple regression (Table 10) also suggested

Table 9: RESULTS OF REGRESSION ANALYSIS (OLDER NORWAY SPRUCE) COMPARING DECAY VOLUME WITH DBH AND WOUND SURFACE AREA

ANALYSIS OF VARIANCE:

	SUM OF SQUARES	DF	MEAN SQUARE	F ratio	P
REGRESSION	0.0020	2	0.0010	6.184	< 0.01
RESIDUAL	0.0042	26	0.0002		

T-TEST:

VARIABLE	STANDARD ERROR	REG.COEFFICIENT	T-VALUE	P
X1	0.00002	0.162	0.970	> 0.05
X2	0.00030	0.504	3.017	< 0.01

X1= WOUND SURFACE AREA

X2= TREE DBH

Table 10:RESULTS OF REGRESSION ANALYSIS (OLDER NORWAY SPRUCE) COMPARING RADIAL PENETRATION OF DECAY,TREE DBH AND NUMBER OF ANNUAL RINGS

ANALYSIS OF VARIANCE:

	SUM OF SQUARES	DF	MEAN SQUARE	F ratio	P
REGRESSION	879.5459	2	439.7729	30.661	< 0.001
RESIDUAL	372.9211	26	14.3431		

T-TEST:

VARIABLE	STANDARD ERROR	REG.COEFFICIENT	T-VALUE	P
X1	0.22412	0.823	2.766	< 0.01
X2	0.26649	-0.016	-0.055	> 0.05

X1= TREE DBH

X2= NUMBER OF ANNUAL RINGS PER 5 CM FROM OUTER BARK INWARDS

that the radial penetration of decay was more dependent on the dbh than on the number of annual rings/5 cm.Y=0.6199 X1 - 0.01455 X2 - 1.84279,where Y=the radial penetration of decay; X1=dbh and X2=the number of annual rings/5 cm.

The following correlations were not significant :

(i) Decay volume/wound size

(ii) %Decay volume/tree volume

(iii) Radial penetration of decay/wound size

(iv) Radial/vertical expansion of decay

(v) Vertical expansion of decay/tree volume

4.4.2. Japanese larch

[i] Japanese larch (Compartment 3)

The following correlations were demonstrated :

(i) A significant negative correlation ($r=-0.798, P>0.001$) between the percentage of decay volume (dependent) and tree volume. $Y=1.41326-3.08071\ X$ (Fig.10).

(ii) A significant negative correlation ($r=-0.686, P=0.01$) between the percentage of decay (dependent) and tree dbh. $Y=1.86296-0.06087\ X$ (Fig.11).

The following correlations were not significant :

(i) Radial penetration of decay/dbh

(ii) Radial penetration of decay/number of annual rings per 5 cm

(iii) Number of annual rings per 5 cm/dbh

(iv) Vertical expansion of decay/wound surface area

(v) Decay volume/dbh

(vi) Decay volume/tree volume

[ii] Japanese larch (Compartment 21)

The following correlations were demonstrated :

(i) A significant negative correlation ($r=-0.594, P>0.01$) between the percentage of decay volume (dependent) and dbh. $Y=6.10947-0.31444\ X$ (Fig.11).

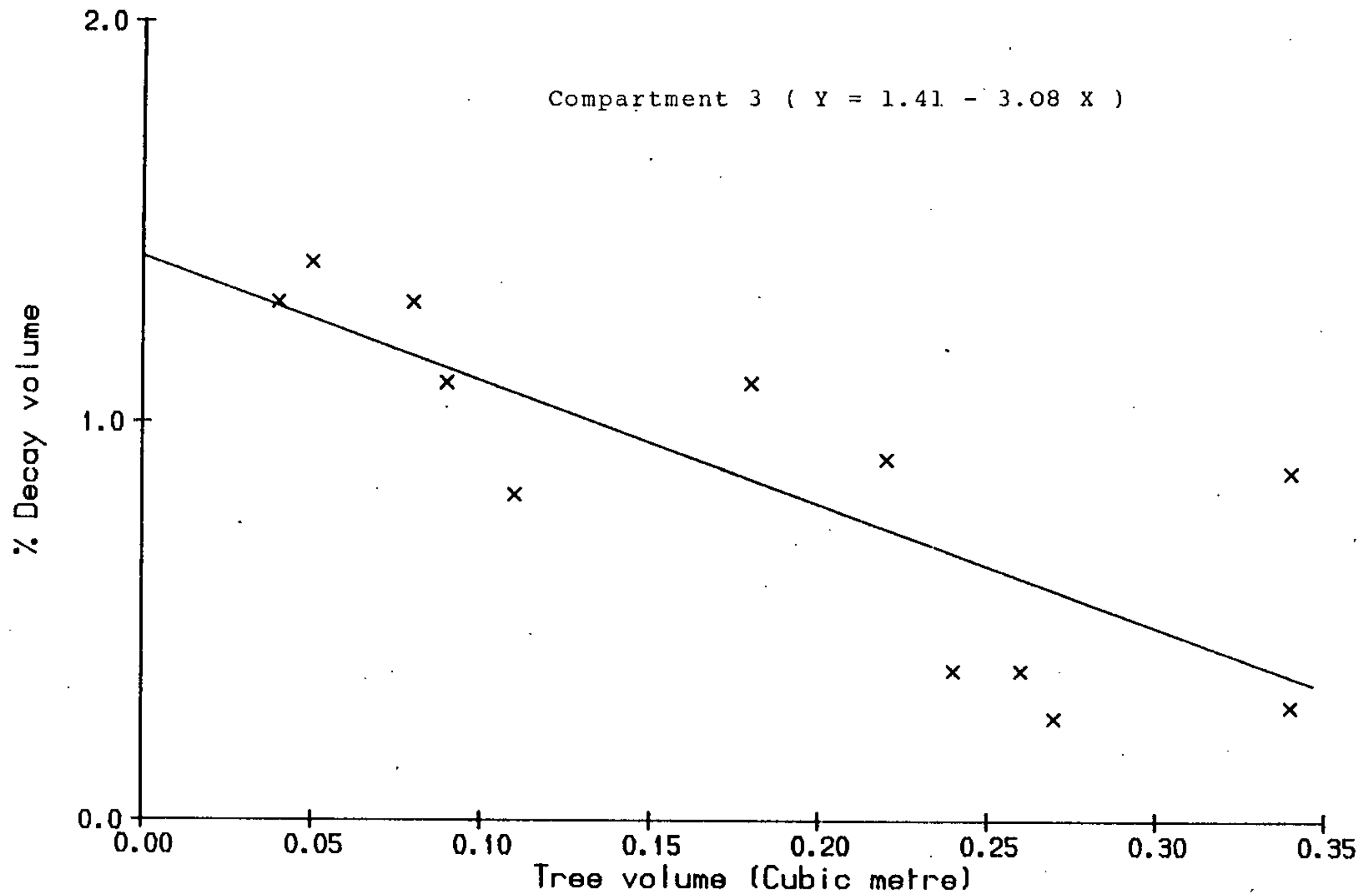

Fig.10 Relationship between tree volume and percent decay volume

Japanese larch (P53)

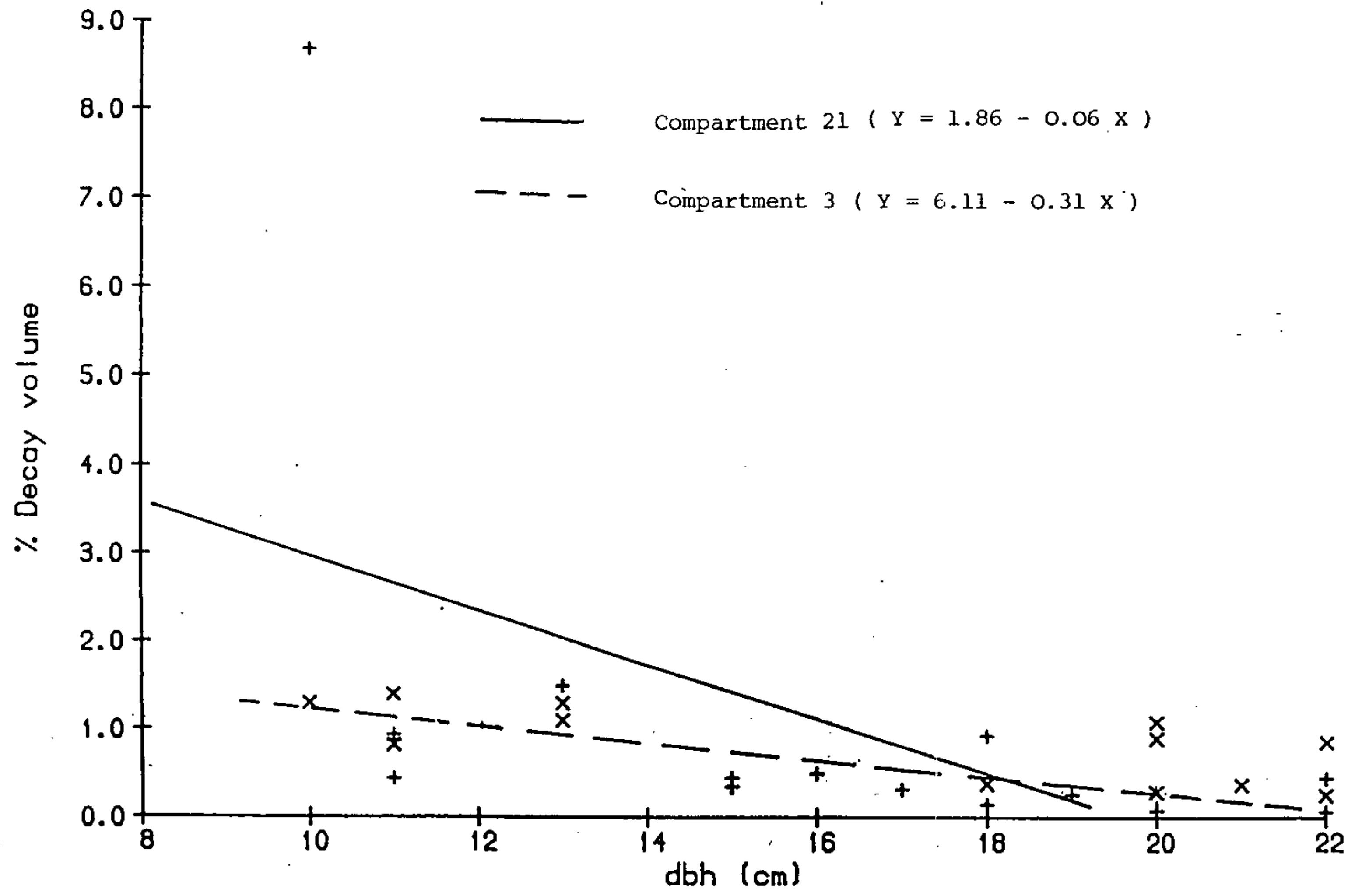

Fig. 11 Relation between dbh and percent decay volume

Japanese larch :

× (P53)

+ (P54)

(ii) A significant positive correlation ($r=0.510, P>0.01$) between the wound surface area and the vertical expansion of decay (dependent). $Y=0.00061\ X + 0.47772$ (Fig.12).

The following correlations were not significant :

(i) %Decay volume/tree volume

(ii) Radial penetration of decay/dbh

(iii) Radial penetration of decay/number of annual rings per 5 cm

(iv) Number of annual rings per 5 cm/dbh

(v) Decay volume/dbh

(vi) Decay volume/tree volume

4.5. Frequency of microorganisms isolated

[A] Younger Norway spruce (Compartment 11)

A.1. Isolations from the surface inwards at the wound site

Table 11 shows the frequency and distribution of microorganisms isolated at the site of infection inwards at 1 cm intervals for >10 cm towards the pith. S.sanguinolentum was the dominant species,being isolated from 70.8% of all samples. T.viride ranked next(8.6%). Other species isolated were: Penicillium spp (6.6%); Bacteria (3.1%); Fusarium sambucinum (Fuckel.) (1%); Chaetomium cochliodes (Palliser) (0.8%); Mucor hiemalis (Wehm.) (0.4%); F.annosus (0.1%) and sterile unidentified mycelia (1.3%). 7% of the samples remained sterile.

The frequency of S.sanguinolentum increased progressively towards the pith and reached 100% in the samples taken between 7 and >10 cm from the outer side. Within this sample range no other microorganisms were isolated. On the other hand,the frequency of all other microorganisms decreased considerably with distance from the outer surface of the stem inwards. At the 1st cm

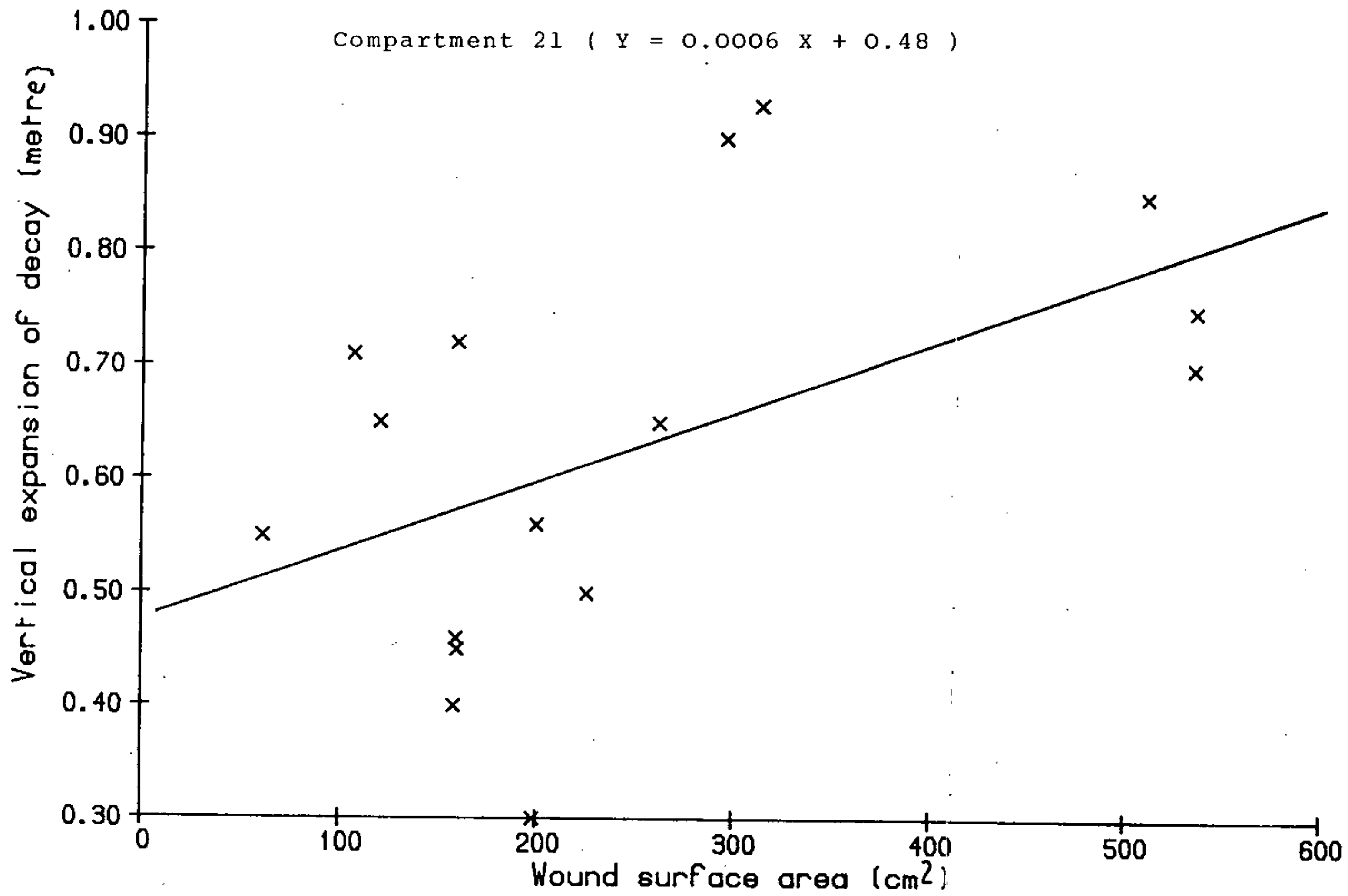

Fig.12: Wound surface area VS vertical expansion of decay

Japanese larch (p54)

Table 11:%FREQUENCY OF MICROBES ISOLATED FROM THE SURFACE INWARDS AT WOUND SITES IN YOUNG NORWAY SPRUCE CROPS (COMPARTMENT 11,YAIR HILL FOREST)

CM INTO WOOD	1	2	3	4	5	6	7	8	9	10	>10	MEAN
SPECIES					%FREQUENCY							
S.sanguinolentum	22	24	30.4	45	73.4	84	100	100	100	100	100	70.8
T.viride	27.6	22.4	21.5	19	4.1	0	0	0	0	0	0	8.6
F.annosus	0	0	1	0	0	0	0	0	0	0	0	0.1
C.cochliodes	4	4.2	1	0	0	0	0	0	0	0	0	0.8
F.sambucinum	7.4	3.4	0	0	0	0	0	0	0	0	0	1.0
Penicillium spp	20	19	16.5	16.9	0	0	0	0	0	0	0	6.6
M.hiemalis	2	2	0	0	0	0	0	0	0	0	0	0.4
Sterile mucelia	7	4	3	0	0	0	0	0	0	0	0	1.3
Bacteria	10	12.4	8	4.1	0	0	0	0	0	0	0	3.1
No growth	0	8.6	14.6	15	22.5	16	0	0	0	0	0	7.0

T.viride dominated all other microorganisms;being isolated from 27.6% of all samples. Between 2 and 5 cm T.viride was consistently the next most common species. None of the microorganisms isolated,with the exception of S.sanguinolentum , were ever found beyond 6 cm from the outer surface of the infection site,a distance beyond which S.sanguinolentum was the only microorganism isolated. T.viride showed the next greatest radial expansion as it was the only microorganism isolated together with S.sanguinolentum at the 5th cm from the bark,a depth at which other microorganisms were not isolated. At the 1st cm all samples examined yielded at least one microorganism,but between 2 and 6 cm some samples (8.6;14.6;15;22.5 and 16% respectively) failed to show microbial growth.

A.2. Isolations above and below the wound site

Table 12 summarised the results of isolations made above and below the wounded site. S.sanguinolentum was the dominant species isolated (88.2%). T.viride ranked second ; being isolated from 3.7% of all samples. Third in frequency were sterile unidentified mycelia(2.3%). Other microorganisms isolated were: Penicillium spp (2%); C.cochliodes (1.2%); M.hiemalis and Bacteria (1% each); Fusarium sambucinum (0.4%); and F.annosus (0.2%). With the exception of S.sanguinolentum and T.viride all other microorganisms were isolated from samples taken between 1 and 12 cm above and below the infection site. Both S.sanguinolentum and T.viride expanded as far as the distal

Table 12:%FREQUENCY OF MICROBES ISOLATED ABOVE AND BELOW THE WOUND SITE IN YOUNG NORWAY SPRUCE CROPS (COMPARTMENT 11,YAIR HILL FOREST)

SPECIES	%FREQUENCY
S.sanguinolentum	88.2
F.annosus	0.2
T.viride	3.7
C.cochliodes	1.2
F.sambucinum	0.4
Penicillium spp	2.0
M.hiemalis	1.0
Sterile mycelia	2.3
Bacteria	1.0

parts of the decay columns. In one tree T.viride dominated all other microorganisms including S.sanguinolentum . F.annosus was isolated from only 2 of the infected trees.

B. Older Norway spruce (Compartment 1016)

B.1. Isolations from the surface inwards at the wound site

The results were summarised in table 13. S.sanguinolentum was the dominant species; being isolated from 67.7% of all samples. T.viride was the next most common species isolated (7%). A variety of other microorganisms occurred between 0.1 and 4.3%. These were: Penicillium spp (4.3%); C.cochliodes (4%); Fusarium sambucinum (2.6%); M.hiemalis (2.3%); Acremonium spp (1.7%); F.annosus (0.1%); sterile unidentified mycelia (1.2%) and Bacteria (4.3%). 5.5% of all samples remianed sterile.

Again the frequency of S.sanguinolentum increased progressively from the outer side towards the pith and became 100% in the samples taken between 8 and >10 cm from the outer surface. Within this sample range no other microorganisms were isolated. On the other hand,the frequency of all other species dropped dramatically with distance from the outer surface of the stem inwards. T.viride showed the next greatest radial expansion as it was the only species isolated together with S.sanguinolentum at the 6th cm from the bark,a depth at which other microorganisms disappeared. Between 1 and 3 cm F.sambucinum ; Bacteria and C.cochliodes were the next most common species respectively. Between 4 and 6 cm T.viride was the next most common microorganism isolated.

Table 13:%FREQUENCY OF MICROBES ISOLATED FROM THE SURFACE INWARDS

CM INTO WOOD	1	2	3	4	5	6	7	8	9	10	>10	MEAN
SPECIES	%FREQUENCY											
S.sanguinolentum	18	20	26.4	34	62	89	95	100	100	100	100	67.7
F.annosus	0	0	1	0	0	0	0	0	0	0	0	00.1
T.viride	9	12	18	16	20	2	0	0	0	0	0	07.0
C.cochliodes	12	10	20	2	0	0	0	0	0	0	0	04.0
F.sambucinum	14	10	5	0	0	0	0	0	0	0	0	02.6
Penicillium spp	10	13	12	4	8	0	0	0	0	0	0	04.3
M.hiemalis	8	10	7.6	0	0	0	0	0	0	0	0	02.3
Acremonium spp	9	6	1	3	0	0	0	0	0	0	0	01.7
Sterile mycelia	8	4	1	0	0	0	0	0	0	0	0	01.2
Bacteria	12	15	8	12	0	0	0	0	0	0	0	04.3
No growth	0	0	0	29	18	9	5	0	0	0	0	05.5

Within the first 3 cm all samples yielded at least one microorganism,but between 4 and 7 cm some samples (29;18;9 and 5%) failed to show microbial growth.

B.2. Isolations above and below the wound site

Table 14 shows the frequency of microorganisms isolated above and below the infection site. S.sanguinolentum was the most common species;being isolated from 76.4% of all samples. Bacteria ranked next (8%). Other microorganisms isolated were: T.viride (5.2%); C.cochliodes (4%); Fusarium sambucinum (2.2%); M.hiemalis (1.2%); Penicillium spp (0.5%); Acremonium spp (0.3%) and 0.2% of the samples yielded sterile unidentified mycelia. 2% of the samples yielded no microorganism. With the exception of S.sanguinolentum ; T.viride and Bacteria all other microorganisms were isolated from samples taken between 1 and 8 cm above and below the infection site. S.sanguinolentum ; T.viride and Bacteria all expanded as far as the distal parts of the decay columns. Most of the extremely decayed samples yielded only Bacteria and these were very wet samples.

C. Isolations above and below the wound site

C.1. Japanese larch (Compartment 3)

The %frequency of microbes isolated are summarised in table 15. C.cochliodes was the dominant microorganism;being isolated from 62% of all samples. Penicillium spp ranked next (22.3%). S.sanguinolentum was the third most common species; being isolated from only 7.7% of all samples. Other species isolated were: T.viride (4%);Mortierella ramanniana

Table 14: %FREQUENCY OF MICROBES ISOLATED ABOVE AND BELOW THE WOUND SITES IN OLDER NORWAY SPRUCE CROPS (COMPARTMENT 1016, GLENTRESS FOREST)

SPECIES	%FREQUENCY
S.sanguinolentum	76.4
F.annosus	0
T.viride	5.2
C.cochliodes	4.0
F.sambucinum	2.2
Penicillium spp	0.5
M.hiemalis	1.2
Acremonium spp	0.3
Sterile mycelia	0.2
Bacteria	8.0
No growth	2.0

(Moller)Linn. (3%);sterile unidentified mycelia (0.5%) and 0.7% of the samples yielded Bacteria.

C.2. Japanese larch (Compartment 21)

Again <u>C.cochliodes</u> was the most common microorganism;being isolated from 60.1% of all samples (Table 16). <u>S.sanquinolentum</u> ranked next (26.5%). Other microorganisms occurred between 0.3 and 9.5%.These were: <u>T.viride</u> (9.5%); <u>Penicillium</u> spp (2.4%); <u>M.ramanniana</u> (0.3%); sterile unidentified mycelia (0.9%) and 0.3% of the samples yielded Bacteria.

In both Japanese larch compartments all microorganisms,with the exception of <u>C.cochliodes</u> ; <u>T.viride</u> and <u>S.sanquinolentum</u> , were isolated between 1 and 5 cm above and below the infection site. <u>S.sanquinolentum</u> was isolated only as traces as it was dominated by <u>C.cochliodes</u> in particular and by <u>T.viride</u> which both expanded as far as the distal parts of the decay columns.

It is important to note that 4 strains of <u>S.sanquinolentum</u> were isolated during the course of experiments and a detailed description of these strains was included in chapter 6.

Table 15:%FREQUENCY OF MICROBES ISOLATED ABOVE AND BELOW THE WOUND SITE ON JAPANESE LARCH CROPS (COMPARTMENT 3)

SPECIES	%FREQUENCY
S.sanguinolentum	07.7
C.cochliodes	61.8
Penicillium spp	22.3
T.viride	4.0
M.ramanniana	3.0
Sterile mycelia	0.5
Bacteria	0.7

Table 16:%FREQUENCY OF MICROBES ISOLATED ABOVE AND BELOW THE WOUND SITE ON JAPANESE LARCH CROPS (COMPARTMENT 21)

SPECIES	%FREQUENCY
S.sanguinolentum	26.5
C.cochliodes	60.1
Penicillium spp	2.4
T.viride	9.5
M.ramannniana	0.3
Sterile mycelia	0.9
Bacteria	0.3

CHAPTER 5

5. ARTIFICIAL INOCULATION EXPERIMENTS

5.1. Description of the infection column

The infection columns were similar to those resulting from natural infection. Following infection, S.sanguinolentum expanded radially, downwards and upwards from the point of inoculation,causing incipient decay in all trees infected. The infection column varied from pale brown to reddish brown. It ended abruptly and was consistently surrounded by a grey zone a few millimetres wide. Usually the upward expansion of decay was much larger than the downward expansion (Plate 11). The maximum radial expansion of decay occurred consistently at the point of inoculation, decreasing gradually up and down the infection column, forming a spindle-shaped zone of decay. Enclosing both the infection column and the grey zone was a dry zone of clear wood usually wider than either of them. Resin was produced at the inoculation site and drained down the holes onto the bark. Initially this resin was white but the colour changed to yellow and eventually became dark in colour particularly on the bark.

5.2. Frequency of successful infection

5.2.1. Trees inoculated in July 1982

Table 17 summarises the frequency of infection in Norway spruce inoculated in July 1982. In some cases frequency of infection varied with tree vigour (dominant, codominant or subdominant) ;inoculation site treatment (whether the inoculating hole was sealed or left open after inoculation) and time of year at which the

Plate 11: A longitudinal section in a Norway spruce 6 months after artificial inoculation with S.sanguinolentum (Middle arrow indicates the inoculation site)

Table 17: %FREQUENCY OF INFECTION OF TREES INOCULATED IN JULY(1982)

TREE CLASS		TREATMENT OF INOCULATION SITE SEALED	OPEN	TOTAL
DOMINANT	INFECTED	9	11	20
	NOT INFECTED	4	2	
CODOMINANT	INFECTED	9	10	19
	NOT INFECTED	4	3	
SUBDOMINANT	INFECTED	4	7	11
	NOT INFECTED	9	6	
TOTAL(INFECTED)		22	28	50

inoculations were made.

Within individual tree vigour classes and inoculation treatments, infection frequency was significantly (P = 0.01) greater in dominant and codominant trees (P = 0.05) and the difference was not significant in subdominants in open inoculation sites respectively (Table 18). There was no significant difference in the infection frequency comparing all tree vigour classes in case of sealed inoculation sites (Table 19).

Irrespective of inoculation site treatment mean infection frequency was significantly (P < 0.01) much greater in dominant and in codominant trees (P = 0.05) but the difference was not significant in subdominant trees (Table 20) (i.e the frequency of infected and not infected trees was similar). Mean infection frequency for sealed and open inoculation sites (pooling all values for tree vigour) was 22 (56.4%) and 28 (71.8%) respectively. Open inoculation sites were significantly (P < 0.01) more frequently infected compared with sealed inoculation sites (Table 21).

A low incidence of natural infections occurred and it was significantly (P < 0.001) less than from artificially inoculated sites (Table 21A) in all trees examined. The incidence of these natural infections was not related significantly with tree vigour and the mean infection frequencies were: 3(11.5%),5 (19.2%) and 4 (15.4%) in dominant,codominant and subdominant trees respectively.

5.2.2. Trees inoculated in February 1983

The frequency of infection from inoculation

Table 18: ARTIFICIAL INOCULATIONS OF TREES WITH S.sanguinolentum FOR SIX MONTHS (SERIES INOCULATED IN JULY 1982)

TREE CLASS	INFECTED	NOT INFECTED	S.D*	O-E/S.D	PROBABILITY
BINOMIAL TEST ON OPEN INOCULATION SITES					
DOMINANT	11	2	1.87	2.49	0.01
CODOMINANT	10	3	1.87	1.93	0.05
SUBDOMINANT	7	6	1.81	0.28	> 0.05

* $S.D = \sqrt{(npq)}$ IN ALL THE FOLLOWING BINOMIAL TESTS IN THIS CHAPTER.

Table 19: ARTIFICIAL INOCULATION RESULTS (AFTER SIX MONTHS, SERIES JULY 1982) BINOMIAL TEST ON SEALED INOCULATED SITES

TREE CLASS		FREQUENCY	S.D	O-E/S.D	PROBABILITY
DOMINANT	INFECTED	9	1.87	1.38	> 0.05
	NOT INFECTED	4			
CODOMINANT	INFECTED	9	1.87	1.38	> 0.05
	NOT INFECTED	4			
SUBDOMINANT	INFECTED	4	1.87	1.38	> 0.05
	NOT INFECTED	9			

Table 20:BINOMIAL TEST ON POOLED DATA SIX MONTHS AFTER INOCULATION (SERIES JULY 1982)

TREE CLASS		FREQUENCY	S.D	O-E/S.D	PROBABILITY
DOMINANT	INFECTED	20	2.56	2.73	< 0.01
	NOT INFECTED	6			
CODOMINANT	INFECTED	19	2.56	2.34	< 0.05
	NOT INFECTED	7			
SUBDOMINANT	INFECTED	11	2.56	0.78	> 0.05
	NOT INFECTED	15			

Table 21:BINOMIAL TEST ON POOLED DATA (SIX MONTHS AFTER INOCULATION) (SERIES JULY 1982)

INOCULATION SITE		FREQUENCY	S.D	O-E/S.D	PROBABILITY
SEALED	INFECTED	22	3.13	0.8	> 0.05
	NOT INFECTED	17			
OPEN	INFECTED	28	3.13	2.72	< 0.01
	NOT INFECTED	11			

Table 21A: CHI-SQUARE TEST ON CONTROLS AND ARTIFICIALLY INOCULATED TREES EXAMINED AFTER SIX MONTHS

SAMPLE	OBSERVED	EXPECTED	O-E	Chi-square	DF	P
CONTROLS	12	31	-19	23.290	1	< 0.001
INOCULATED	50	31	+19			

experiments carried out in February is summarised in table 22A. The results are similar to July inoculations in that in some cases there was an evident influence of tree vigour and inoculation site treatment upon the inoculation success rate.

Within the individual tree vigour classes and inoculation treatments ,infections were not significantly different (Table 22). Mean infection frequencies were 7 (53.8%) and 8 (61.5%) in dominant trees; in codominants 5 (38.5%) and 6 (46.2%) and in subdominants 5 (38.5%) and 8 (61.5%) in sealed and in open inoculation sites respectively.

The global frequency of infection combining all tree vigour classes and the two inoculation treatments was 39 (50%) . In dominant trees mean infection frequency was 15 (57.7%) ;in codominants 11 (42.4%) and 13 (50%) in subdominants respectively.

There was no significant difference in infection frequency between open and sealed inoculation sites (Table 23). Pooling all values for tree vigour no significant difference in infection frequency between dominant, codominant and subdominant was observed (Table 24) and mean infection frequencies were 15 (57.7%) ;11 (42.4%) and 13 (50%) respectively.

5.3. Comparison between July and February inoculations

5.3.1. Frequency of infection

Pooling all values for inoculation treatments, there was no significant difference in the infection frequency in sealed sites inoculated in July compared with February

Table 22A: %FREQUENCY OF TREES INOCULATED IN FEBRUARY(1983)

TREE CLASS		TREATMENT OF INOCULATION SITE		
		SEALED	OPEN	TOTAL
DOMINANT	INFECTED	7	8	15
	NOT INFECTED	6	5	
CODOMINANT	INFECTED	5	6	11
	NOT INFECTED	8	7	
SUBDOMINANT	INFECTED	5	8	13
	NOT INFECTED	8	5	
TOTAL(INFECTED)		17	22	39

Table 22: BINOMIAL TESTS ON DATA OBTAINED SIX MONTHS AFTER INOCULATION (FEBRUARY 1983 SERIES)

TREE CLASS		FREQUENCY	S.D	O-E/S.D	PROBABILITY
BINOMIAL TEST ON SEALED SITES					
DOMINANT	INFECTED	7	1.81	0.28	> 0.05
	NOT INFECTED	6			
CODOMINANT	INFECTED	5	1.81	0.83	> 0.05
	NOT INFECTED	8			
SUBDOMINANT	INFECTED	5	1.81	0.83	> 0.05
	NOT INFECTED	8			
BINOMIAL TEST ON OPEN SITES					
DOMINANT	INFECTED	8	1.81	0.28	> 0.05
	NOT INFECTED	5			
CODOMINANT	INFECTED	6	1.81	0.83	> 0.05
	NOT INFECTED	7			
SUBDOMINANT	INFECTED	8	1.81	0.28	> 0.05
	NOT INFECTED	5			

Table 23:BINOMIAL TEST ON POOLED DATA SIX MONTHS AFTER INOCULATION (SERIES (FEBRUARY 1983)

INOCULATION SITE		FREQUENCY	S.D	O-E/S.D	PROBABILITY
SEALED	INFECTED	17	3.13	0.8	> 0.05
	NOT INFECTED	22			
OPEN	INFECTED	22	3.13	0.8	> 0.05
	NOT INFECTED	17			

inoculations (Table 25). However,the infection frequency was significantly ($P < 0.01$) greater in open sites inoculated in July compared with open sites inoculated in February (Table 26). Within the individual tree vigour classes and inoculation treatments it was evident that the frequency of infection was much higher in dominant and codominant trees compared with subdominant trees all inoculated in July. In sites inoculated in February there was no significant difference in the infection frequency which was similar in all tree vigour classes.

In the July series,open inoculation sites had a much higher frequency of successful infections compared with sealed sites. In contrast,the difference was not significant comparing sealed and open sites inoculated in February.

The global mean infection frequency was much greater in trees inoculated in July (50) compared with February inoculations (39 trees).

Pooling all July and February values,no significant difference in the infection frequency comparing tree vigour classes was observed in sealed inoculation sites (Table 27). However,infection frequency was significantly ($P = 0.05$) greater in dominant trees compared with codominant and subdominant trees (Table 28) in open inoculation sites.

5.3.2. Development of incipient decay

Using 't' tests very highly significant differences were shown to exist between radial and vertical expansions of decay in trees inoculated in July compared with those inoculated in February (Table 29). The global mean radial

Table 24: BINOMIAL TEST ON FEBRUARY 1983 SERIES SIX MONTHS AFTER INOCULATION

TREE CLASS		FREQUENCY	S.D	O-E/S.D	PROBABILITY
DOMINANT	INFECTED	15	2.56	0.78	> 0.05
	NOT INFECTED	11			
CODOMINANT	INFECTED	11	2.56	0.78	> 0.05
	NOT INFECTED	15			
SUBDOMINANT	INFECTED	13	2.56	0.00	> 0.05
	NOT INFECTED	13			

Table 25: BINOMIAL TEST ON SEALED INOCULATION SITES (TREE CLASSES POOLED) EXAMINED AFTER SIX MONTHS

INOCULATION TIME		FREQUENCY	S.D	O-E/S.D	PROBABILITY
FEBRUARY	INFECTED	17	3.13	0.8	> 0.05
	NOT INFECTED	22			
JULY	INFECTED	22	3.13	0.8	> 0.05
	NOT INFECTED	17			

Table 26: BINOMIAL TEST ON OPEN INOCULATION SITES (TREE CLASSES POOLED) EXAMINED AFTER SIX MONTHS

INOCULATION TIME		FREQUENCY	S.D	O-E/S.D	PROBABILITY
FEBRUARY	INFECTED	22	3.13	0.8	> 0.05
	NOT INFECTED	17			
JULY	INFECTED	28	3.13	2.72	< 0.01
	NOT INFECTED	11			

Table 27: BINOMIAL TEST ON SEALED INOCULATION SITES (POOLED DATA) EXAMINED AFTER SIX MONTHS

TREE CLASS		FREQUENCY	S.D	O-E/S.D	PROBABILITY
DOMINANT	INFECTED	16	2.56	1.17	> 0.05
	NOT INFECTED	10			
CODOMINANT	INFECTED	14	2.56	0.39	> 0.05
	NOT INFECTED	12			
SUBDOMINANT	INFECTED	9	2.56	1.56	> 0.05
	NOT INFECTED	17			

Table 28: BINOMIAL TEST ON OPEN INOCULATION SITES (POOLED DATA) EXAMINED AFTER SIX MONTHS

TREE CLASS		FREQUENCY	S.D	O-E/S.D	PROBABILITY
DOMINANT	INFECTED	19	2.56	2.34	0.05
	NOT INFECTED	7			
CODOMINANT	INFECTED	16	2.56	1.17	> 0.05
	NOT INFECTED	10			
SUBDOMINANT	INFECTED	15	2.56	0.78	> 0.05
	NOT INFECTED	11			

Table 29: VARIATION IN THE RADIAL AND VERTICAL EXPANSION OF DECAY WITH TIME OF INOCULATION AND INOCULATION SITE TREATMENT

VARIABLE	NO. OF CASES	MEAN	STANDARD DEVIATION	STANDARD ERROR	T VALUE	DF	2-TAIL PROBABILITY
RADIAL JULY	50	6.8560	1.979	0.280	5.56	87	< 0.001
RADIAL FEBRUARY	39	4.5872	1.817	0.291			
VERTICAL JULY	50	46.1260	11.434	1.617	11.28	87	< 0.001
VERTICAL FEBRUARY	39	20.7821	09.200	1.473			
RADIAL JULY #	28	06.5714	01.959	0.370	-1.15	48	> 0.05
RADIAL JULY *	22	07.2182	01.988	0.424			
VERTICAL JULY #	28	47.8071	10.980	2.075	1.18	48	> 0.05
VERTICAL JULY *	22	43.9773	11.905	2.538			
RADIAL FEBRUARY #	22	04.7500	02.188	0.467	0.68	34	> 0.05
RADIAL FEBRUARY *	17	04.3765	01.213	0.294			
VERTICAL FEBRUARY #	22	20.5136	10.245	2.184	-0.20	37	> 0.05
VERTICAL FEBRUARY *	17	21.1294	07.939	1.925			

RADIAL JULY = RADIAL PENETRATION OF DECAY IN TREES INOCULATED IN JULY(1982) IRRESPECTIVE OF INOCULATION SITE TREATMENT.

RADIAL FEBRUARY = RADIAL PENETRATION OF DECAY IN TREES INOCULATED IN FEBRUARY(1983) IRRESPECTIVE OF INOCULATION SITE TREATMENT

RADIAL JULY # = RADIAL PENETRATION OF DECAY IN OPEN SITES INOCULATED IN JULY(1982).

RADIAL JULY * = RADIAL PENETRATION OF DECAY IN SEALED SITES INOCULATED IN JULY (1982).

VERTICAL JULY # = VERTICAL EXPANSION OF DECAY IN OPEN SITES INOCULATED IN JULY (1982).

VERTICAL JULY * = VERTICAL EXPANSION OF DECAY IN SEALED SITES INOCULATED IN JULY (1982).

RADIAL FEBRUARY # = RADIAL PENETRATION OF DECAY IN OPEN SITES INOCULATED IN FEBRUARY (1983).

RADIAL FEBRUARY * = RADIAL PENENTRATION OF DECAY IN SEALED SITES INOCULATED IN FEBRUARY(1983).

VERTICAL FEBRUARY # = VERTICAL EXPANSION OF DECAY IN OPEN SITES INOCULATED IN FEBRUARY (1983).

VERTICAL FEBRUARY * = VERTICAL EXPANSION OF DECAY IN SEALED SITES INOCULATED IN FEBRUARY(1983).

VERTICAL JULY = VERTICAL EXPANSION OF DECAY IN TREES INOCULATED IN JULY (1982) IRRESPECTIVE OF THE INOCULATION SITE TREATMENT

VERTICAL FEBRUARY = VERTICAL EXPANSION OF DECAY IN TREES INOCULATED IN FEBRUARY (1983) IRRESPECTIVE OF THE INOCULATION SITE TREATMENT.

penetration of decay from sealed and open sites was significantly (P< 0.001) higher in trees inoculated in July (6.9 cm) than in trees inoculated in February (4.6 cm). It is also evident that the global mean vertical expansion of decay was significantly (P< 0.001) greater in trees inoculated in July (46.1 cm) than in those inoculated in February (20.8 cm). Statistical analysis failed to show any significant difference in the global mean radial and vertical expansion of decay from inoculation sites which had been left open compared with sealed sites regardless of inoculation time (Table 29).

5.4. Factors affecting the development of decay

Data on decay and tree parameters were summarised in tables 30;30A and 31. The frequency of infection and the development of decay are influenced by tree vigour (dominant;codominant or subdominant); inoculation site treatment (sealed or left open) and the number of annual rings per 5 cm measured from the outer bark towards the pith. The influence of tree vigour and treatment of inoculation site were discussed in section 3.

The following correlations were demonstrated for trees inoculated in July 1982:

A. A significant positive correlation ($r = 0.505, P < 0.001$) between radial penetration of decay and tree dbh. Radial penentration of decay is dependent on tree growth rate expressed as its dbh ($Y = 0.21333 X + 3.42739$) (Fig.13).

B. A significant negative correlation ($r = -0.764, P < 0.001$) between tree dbh and the number of annual rings per

Table 30 : DECAY SPREAD IN NORWAY SPRUCE (COMPARTMENT 11, YAIR HILL) EXAMINED SIX MONTHS AFTER INOCULATION (SERIES JULY 1982)

	DBH (CM)	MAXIMUM RADIAL PENETRATION OF DECAY (CM)	NO.OF RINGS PER 5 CM	DOWNWARD SPREAD OF DECAY (CM)	UPWARD SPREAD OF DECAY (CM)	TOTAL VERTICAL EXPANSION OF DECAY (CM)
			DOMINANT			
*	17.0	06.5	15.00	15.00	34.70	49.70
*	20.0	09.9	10.00	17.20	41.40	58.60
*	24.0	10.2	05.00	25.40	44.20	69.60
*	19.0	08.7	11.00	20.00	29.70	49.70
*	23.0	05.0	16.00	13.00	16.20	29.20
*	24.5	07.4	13.00	19.30	32.20	51.50
*	25.0	10.0	07.00	15.70	30.40	46.10
*	22.5	08.2	14.00	20.00	25.60	45.60
*	20.0	09.4	12.00	18.60	31.00	49.60
#	18.0	07.2	11.00	20.00	29.30	49.30
#	22.0	08.8	12.00	20.00	35.40	55.40
#	19.0	08.2	12.00	07.60	31.00	38.60
#	22.0	09.0	18.00	20.00	39.70	59.70
#	24.0	08.5	13.00	18.60	37.80	56.40
#	22.5	07.6	14.00	16.70	29.80	46.50
#	23.0	06.2	16.00	19.70	40.60	60.30
#	18.5	07.8	14.00	18.40	30.00	48.40
#	20.0	09.2	11.00	17.00	39.00	56.00
#	23.6	06.1	15.00	20.00	31.40	51.40
#	17.0	05.7	17.00	19.40	30.30	49.70

* INOCULATION SITE SEALED

INOCULATION SITE OPEN

Table 30A: DECAY SPREAD IN NORWAY SPRUCE (COMPARTMENT 11, YAIR HILL) EXAMINED SIX MONTHS AFTER INOCULATION (SERIES JULY 1982)

	DBH (CM)	MAXIMUM RADIAL PENETRATION OF DECAY (CM)	NO. OF RINGS PER 5 CM	DOWNWARD SPREAD OF DECAY (CM)	UPWARD SPREAD OF DECAY (CM)	TOTAL VERTICAL EXPANSION OF DECAY (CM)
			CODOMINANT			
*	14.0	07.0	19.00	20.00	20.70	40.70
*	13.0	05.1	20.00	20.00	27.40	47.40
*	13.0	07.0	18.00	08.50	22.60	31.10
*	14.0	06.0	19.00	20.00	35.10	55.10
*	12.0	10.0	17.00	12.20	50.00	62.20
*	14.0	06.0	19.00	03.00	29.30	32.30
*	15.0	09.2	12.00	09.40	20.70	30.10
*	12.0	06.7	19.00	19.70	26.50	46.20
*	14.0	05.7	19.00	10.00	37.60	47.60
#	12.0	07.6	15.00	19.30	41.40	60.70
#	12.0	08.5	18.00	20.00	40.50	60.50
#	15.0	05.2	22.00	13.10	34.10	47.20
#	14.0	03.5	21.00	11.10	21.20	32.30
#	12.0	09.0	19.00	12.60	27.00	39.60
#	14.0	10.2	20.00	20.00	29.70	49.70
#	14.0	06.7	18.00	20.00	38.70	58.70
#	12.0	04.2	22.00	19.00	37.20	56.20
#	16.0	06.2	19.00	07.90	25.60	33.50
#	13.0	06.2	17.00	20.00	25.70	45.70
			SUBDOMINANT			
*	11.0	03.1	24.00	07.10	20.20	27.30
*	12.0	07.0	16.00	09.10	25.70	34.70
*	11.0	05.2	21.00	10.50	15.40	25.90
*	10.5	05.5	21.00	15.10	22.20	37.30
#	11.0	04.2	25.00	15.40	30.00	45.40
#	10.0	05.2	19.00	19.10	44.00	63.10
#	12.0	06.5	23.00	09.70	23.00	32.70
#	12.0	03.3	22.00	03.50	14.00	17.50
#	11.0	04.4	22.00	12.60	28.60	41.20
#	12.0	05.4	21.00	14.20	20.00	34.20
#	11.5	03.4	24.00	15.10	33.60	48.70
			ALL TREE VIGOUR CLASSES			
MIN.	10.0	03.1	05.00	03.00	14.00	17.50
MAX.	25.0	10.2	25.00	25.40	50.00	69.60
MEAN	16.7	06.9	16.90	15.60	29.70	46.10

* INOCULATION SITE SEALED

INOCULATION SITE OPEN

Table 31: DECAY SPREAD IN NORWAY SPRUCE TREES (COMPARTMENT 11,YAIR HILL) EXAMINED SIX MONTHS AFTER INOCULATION (SERIES FEBRUARY 1983)

TREE CLASS		DBH (CM)	MAXIMUM RADIAL PENETRATION OF DECAY (CM)	NO. OF RINGS PER 5 CM	DOWNWARD SPREAD OF DECAY (CM)	UPWARD SPREAD OF DECAY (CM)	TOTAL VERTICAL EXPANSION OF DECAY (CM)
D	*	20.0	06.4	13.00	11.60	18.30	29.90
O	*	25.0	05.0	18.00	11.30	15.60	26.90
M	*	19.0	05.7	17.00	15.70	17.20	32.90
I	*	20.0	03.2	13.00	10.30	19.30	29.60
N	*	18.0	05.0	16.00	14.70	16.70	31.40
A	*	22.0	04.2	17.00	09.30	15.80	25.10
N	*	17.0	05.4	15.00	12.40	16.20	28.60
T	#	20.0	07.7	11.00	10.00	14.30	24.30
	#	17.0	05.1	09.00	07.70	16.00	23.70
	#	20.0	05.2	13.00	10.70	12.00	22.70
	#	19.0	06.4	15.00	12.40	15.70	28.10
	#	22.0	06.3	06.00	09.20	15.10	24.30
	#	22.0	09.0	05.00	12.30	26.10	38.40
	#	19.0	07.2	12.00	12.60	25.20	37.80
	#	18.5	09.4	10.00	18.60	25.40	44.00
C	*	16.0	06.2	17.00	05.10	11.60	16.70
O	*	12.5	04.0	21.00	07.10	09.10	16.20
D	*	12.2	03.1	20.00	07.20	11.30	18.50
O	*	12.4	04.5	23.00	10.40	12.50	22.90
M	*	14.0	04.7	20.00	07.20	10.40	17.60
I	#	13.0	02.7	14.00	09.40	12.20	21.60
N	#	15.0	03.2	17.00	05.10	07.00	12.10
A	#	15.0	02.6	18.00	02.00	04.30	06.30
N	#	13.0	02.6	23.00	02.90	04.80	07.70
T	#	14.0	03.2	19.00	08.00	11.50	19.50
	#	16.0	06.1	14.00	07.60	12.70	20.30
S	*	11.4	03.1	24.00	02.80	04.10	06.90
U	*	10.2	03.2	18.00	07.90	09.50	17.40
B	*	10.7	02.4	21.00	03.70	06.50	10.20
D	*	10.2	03.1	24.00	04.50	07.20	11.70
O	*	12.5	05.2	21.00	06.30	10.40	16.70
M	#	10.0	02.0	21.00	03.20	04.00	07.20
I	#	11.0	02.7	22.00	05.10	05.40	10.50
N	#	11.0	04.9	19.00	08.30	13.20	21.50
A	#	12.0	03.6	19.00	03.70	11.00	14.70
N	#	11.0	03.5	22.00	09.00	16.10	25.10
T	#	11.0	04.6	19.00	05.60	11.20	16.80
	#	11.0	02.4	21.00	03.50	06.30	09.80
	#	11.0	04.1	20.00	06.40	08.50	14.90
MIN.		10.0	02.0	05.00	02.00	04.00	06.30
MAX.		25.0	09.4	24.00	18.60	26.10	44.00
MEAN		15.2	04.6	17.00	08.20	12.60	20.80

* INOCULATION SITE SEALED
# INOCULATION SITE OPEN

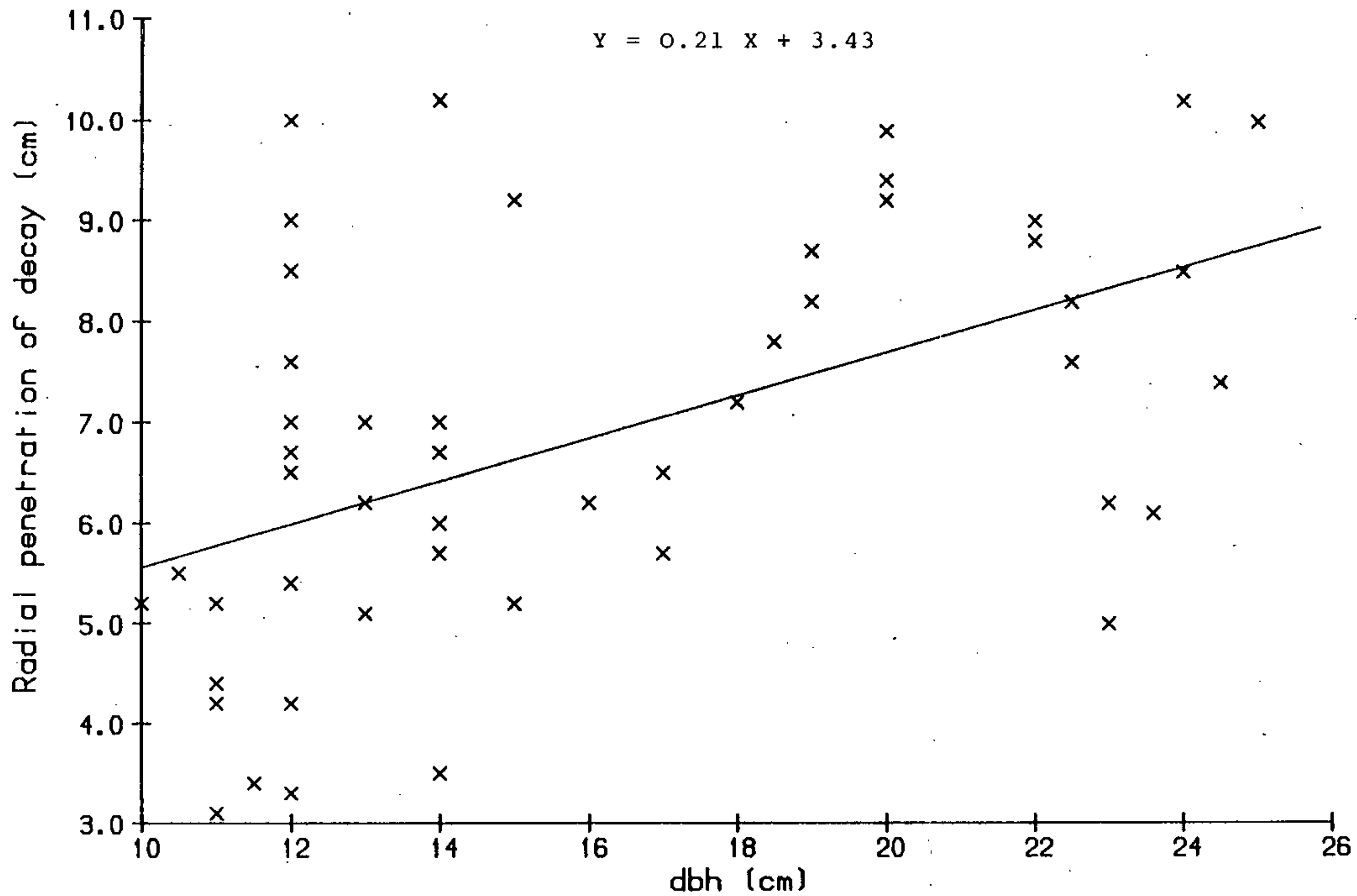

Fig.13 Relationship between radial penetration of decay & dbh

(Artificial inoculation, series July 1982)

5 cm measured from the outer bark towards the pith. The number of annual rings per 5 cm is inversely dependent on the dbh i.e the larger the tree the lesser is the number of rings per unit distance and vice versa ($Y = 28.72087 - 0.73302 X$) (Fig.14).

C. A significant negative correlation ($r = -0.757$, $P < 0.001$) between radial penetration of decay and the number of annual rings. The larger the number of annual rings the lesser is the radial penetration of decay ($Y = 12.49849 - 0.33309 X$) (Fig.15).

D. A significant positive correlation ($r = 0.335$, $P > 0.01$) between vertical expansion of decay and tree dbh. As the diameter increases the vertical expansion of decay increases ($Y = 0.81776 X + 32.97897$) (Fig.16).

E. A significant positive correlation ($r = 0.460$, $P < 0.001$)between vertical and radial expansion of decay i.e the greater the radial penentration of decay the greater is its vertical expansion ($Y = 2.65807 X + 27.89813$) (Fig.17).

F. A multiple correlation between the radial penentration of decay as a dependent variable on both tree dbh and the number of annual rings per distance and the figures showed that the radial penentration of decay is more dependent on the number of rings ($r = 0.765$, $P < 0.001$) ($Y = 14.68243 - 0.07392 X1 - 0.39188 X2$), where $X1$ = dbh and $X2$ = number of growth rings per 5 cm.

Similarly the following correlations were demonstrated for trees inoculated in February 1983:

A. A significant positive correlation ($r = 0.657$, $P <$

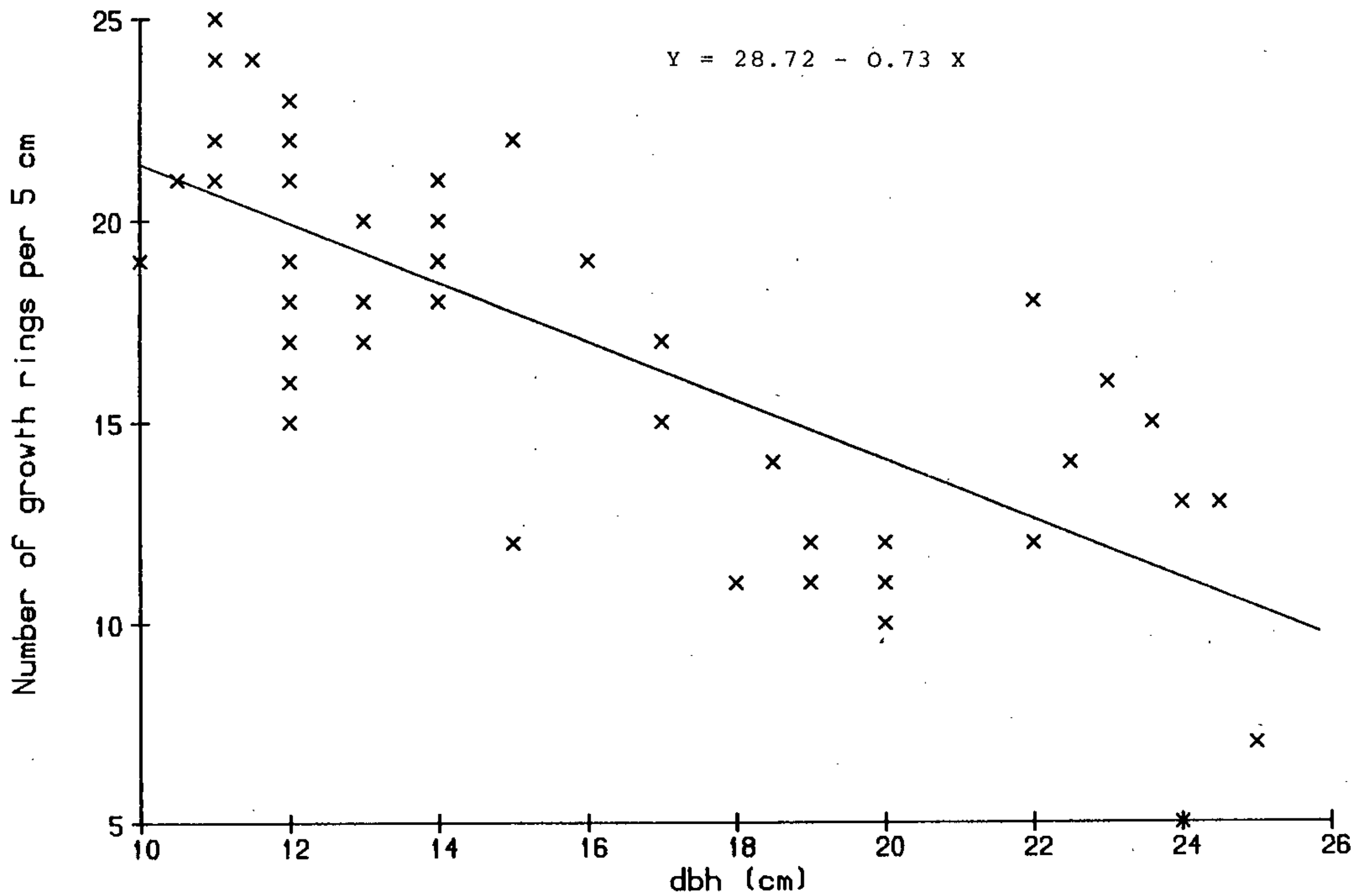

FIG.14 Relationship between number of rings per 5 cm and dbh

(Artificial inoculation, series July 1982)

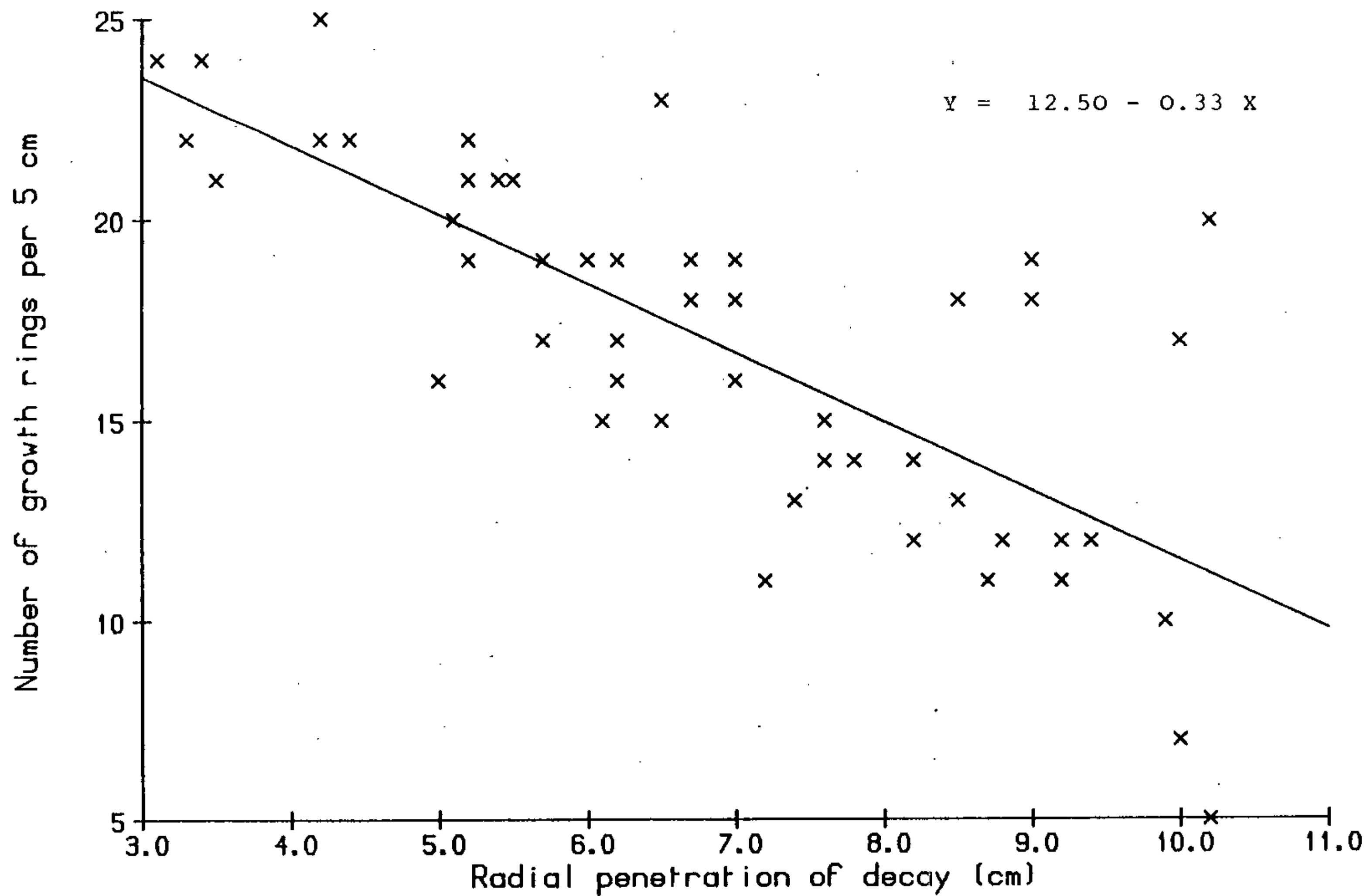

Fig.15 Radial penetration of decay VS number of rings per 5 cm

(Artificial inoculation, series July 1982)

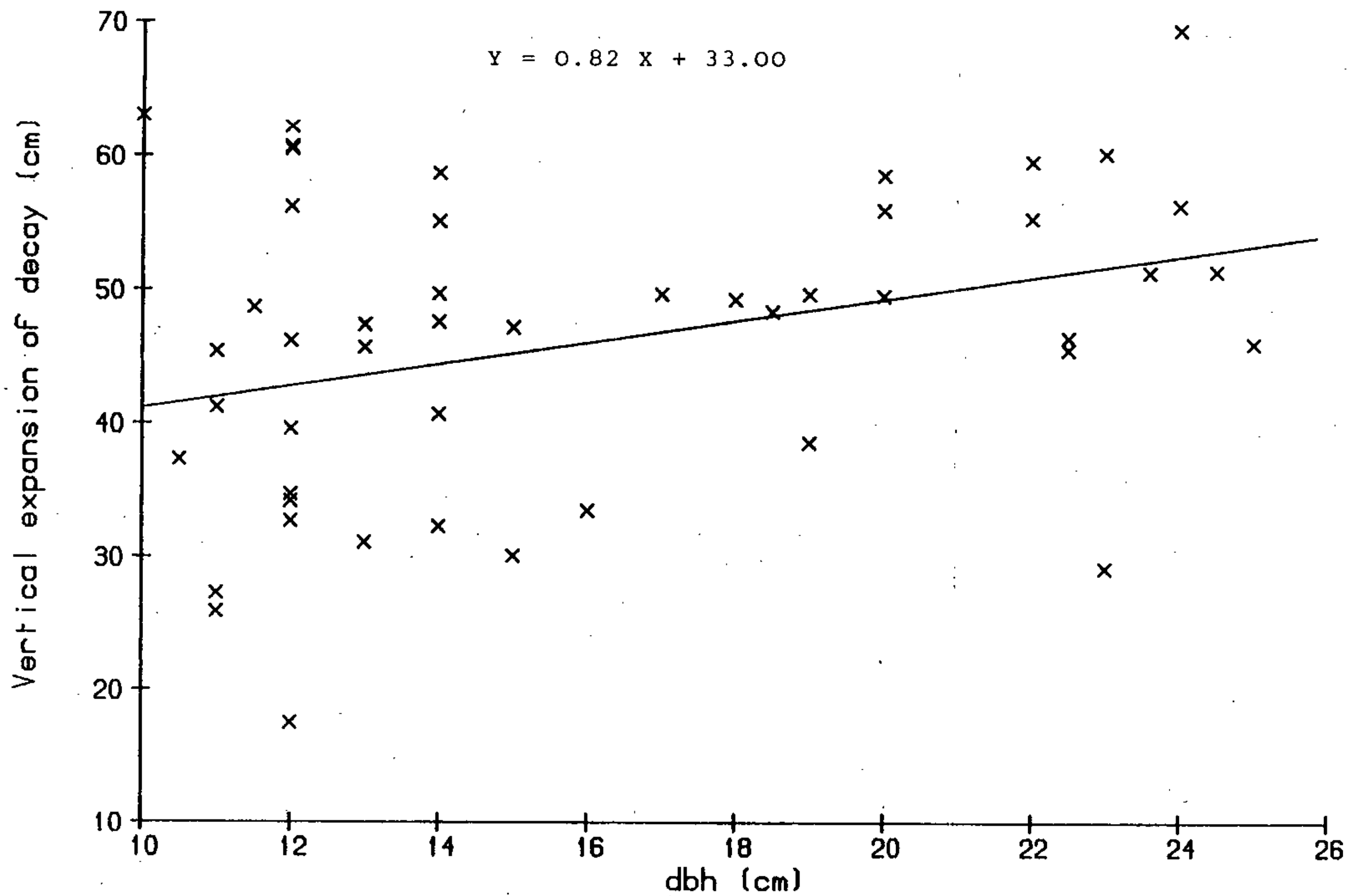

Fig.16 Relationship between vertical expansion of decay and dbh

(Artificial inoculation, series July 1982)

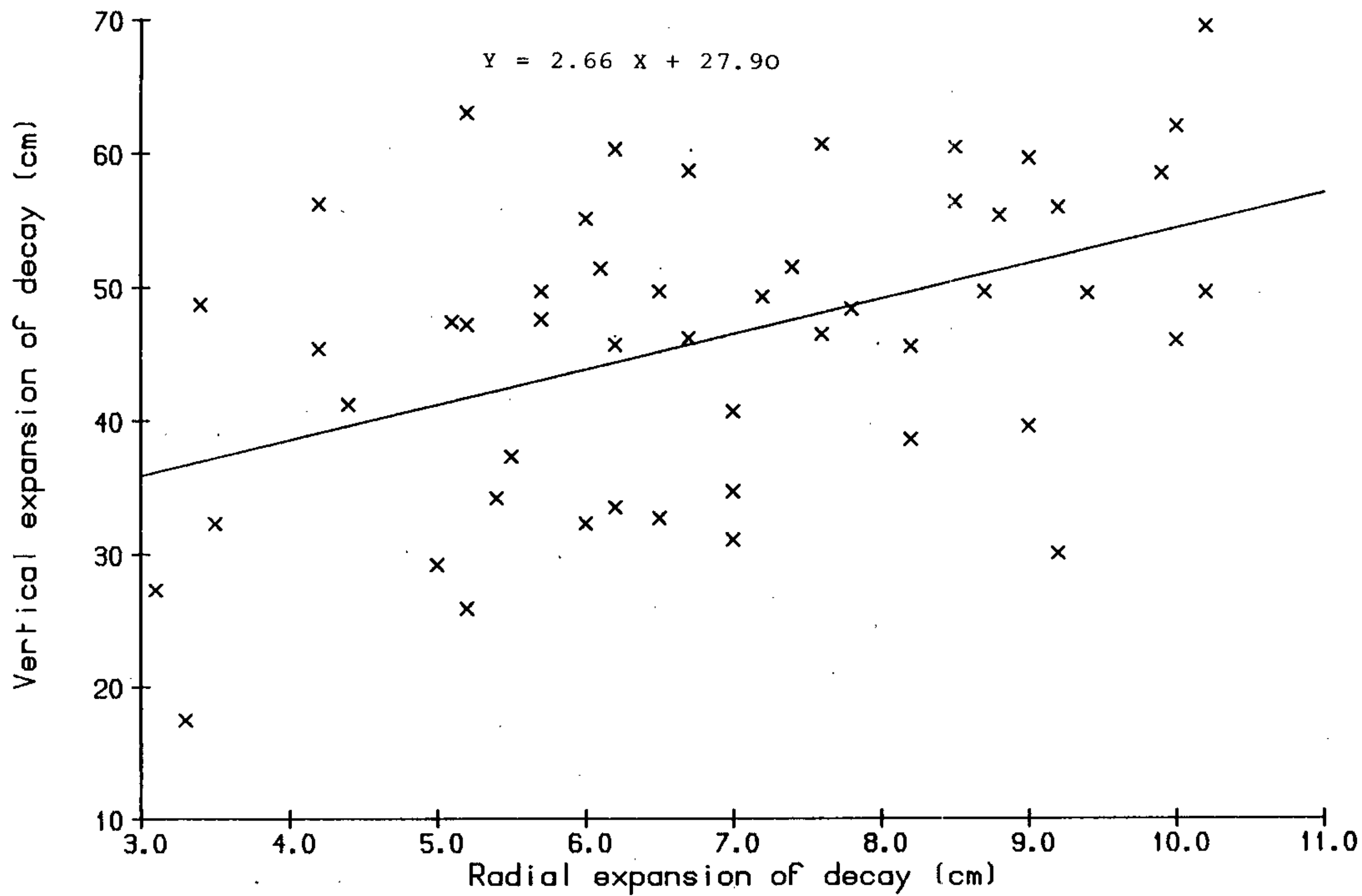

Fig.17 Radial VS vertical expansion of decay

(Artificial inoculation, series July 1982)

0.001) between radial penetration of decay (dependent) and dbh (Y = 0.28446 X + 0.25027) , where Y= radial penetration of decay and X = dbh (Fig.13A).

B. A significant negative correlation ($r = -0.747, P < 0.001$) between the number of growth rings per 5 cm from the outer bark inwards (dependent) and dbh (Y = 30.01735 - 0.84710 X) , where Y = the number of growth rings per 5 cm and X = dbh (Fig.14A).

C. A significant negative correlation ($r = -0.727, P < 0.001$) between radial penetration of decay (dependent) and the number of growth rings per 5 cm from the outer bark inwards (Y = 9.33335 - 0.27751 X) , where Y = radial penetration of decay and X = number of growth rings per 5 cm (Fig.15A).

D. A significant positive correlation ($r = 0.702, P < 0.001$) between vertical expansion of decay (dependent) and dbh (Y = 1.53987 X - 2.69501) , where Y = vertical expansion of decay and X = dbh (Fig.16A).

E. A significant positive correlation ($r = 0.793, P < 0.001$) between vertical (dependent) and radial expansion of decay (Y = 4.01772 X + 2.35196) , where Y = vertical expansion of decay and X = radial expansion of decay (Fig.17A.)

F. Multiple regression analysis showed that radial penetration of decay was more dependent ($r = 0.747, P < 0.001$) on the number of annual rings per 5 cm from the outer bark towards the pith than on dbh ($Y = 0.11173\ X1 - 0.20391\ X2 + 6.37111$) , where Y = radial penetration of

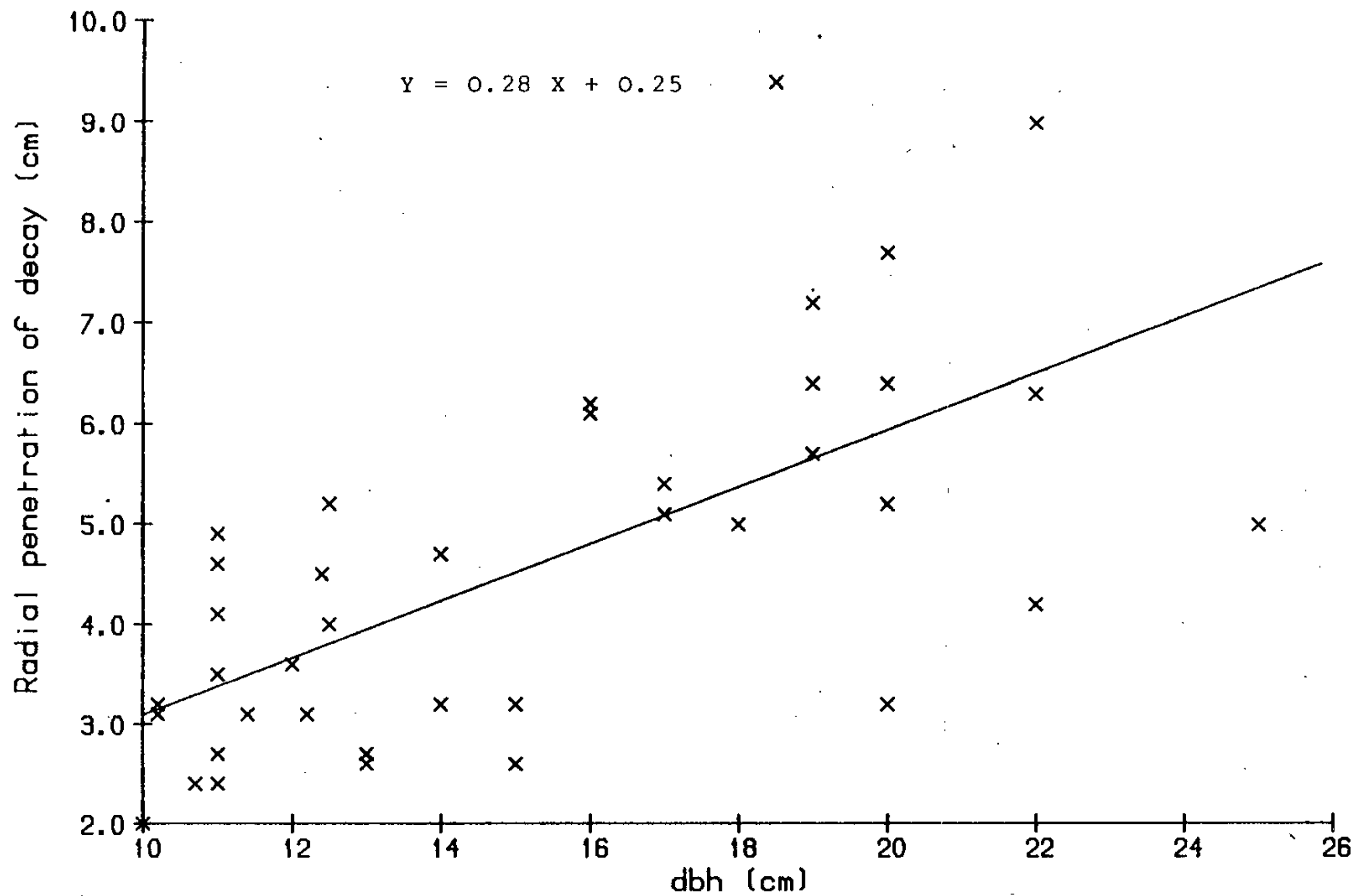

Fig.13A Relationship between radial penetration of decay and dbh

(Artificial inoculation, series February 1983)

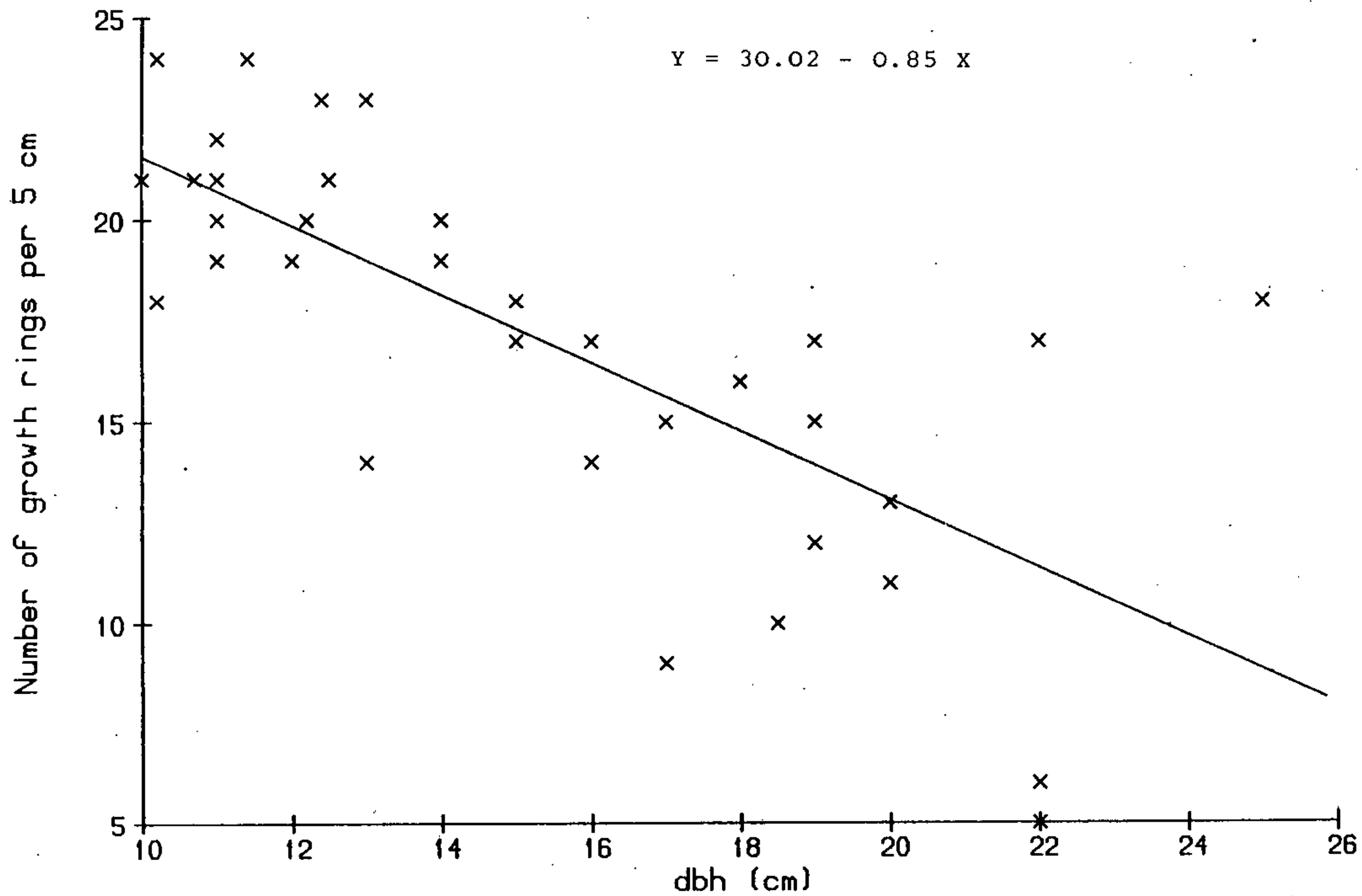

Fig.14A Number of growth rings per 5 cm VS dbh

(Artificial inoculation, series February 1983)

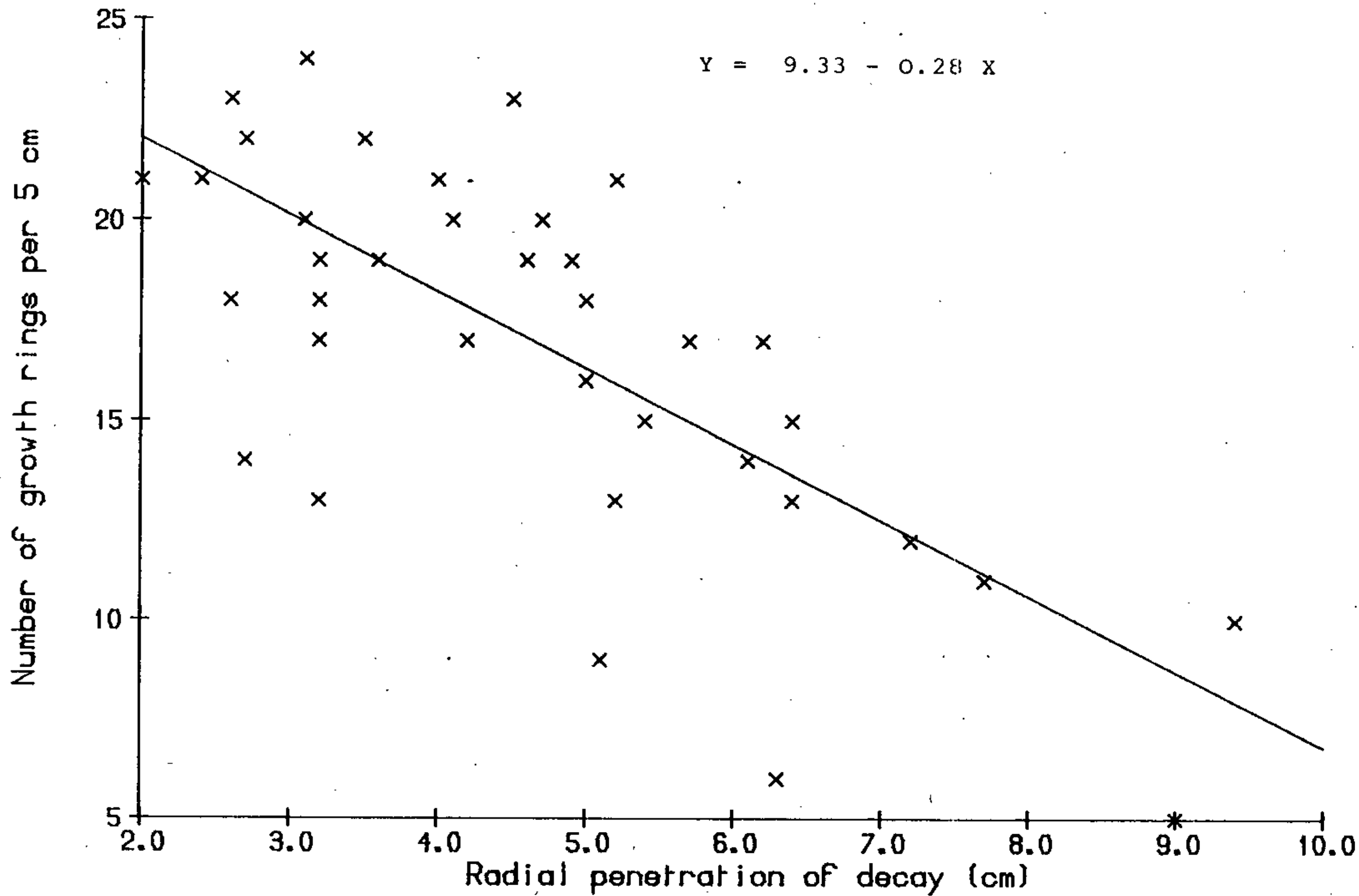

Fig.15A Radial penetration of decay VS number of rings per 5 cm

(Artificial inoculation, series February 1983)

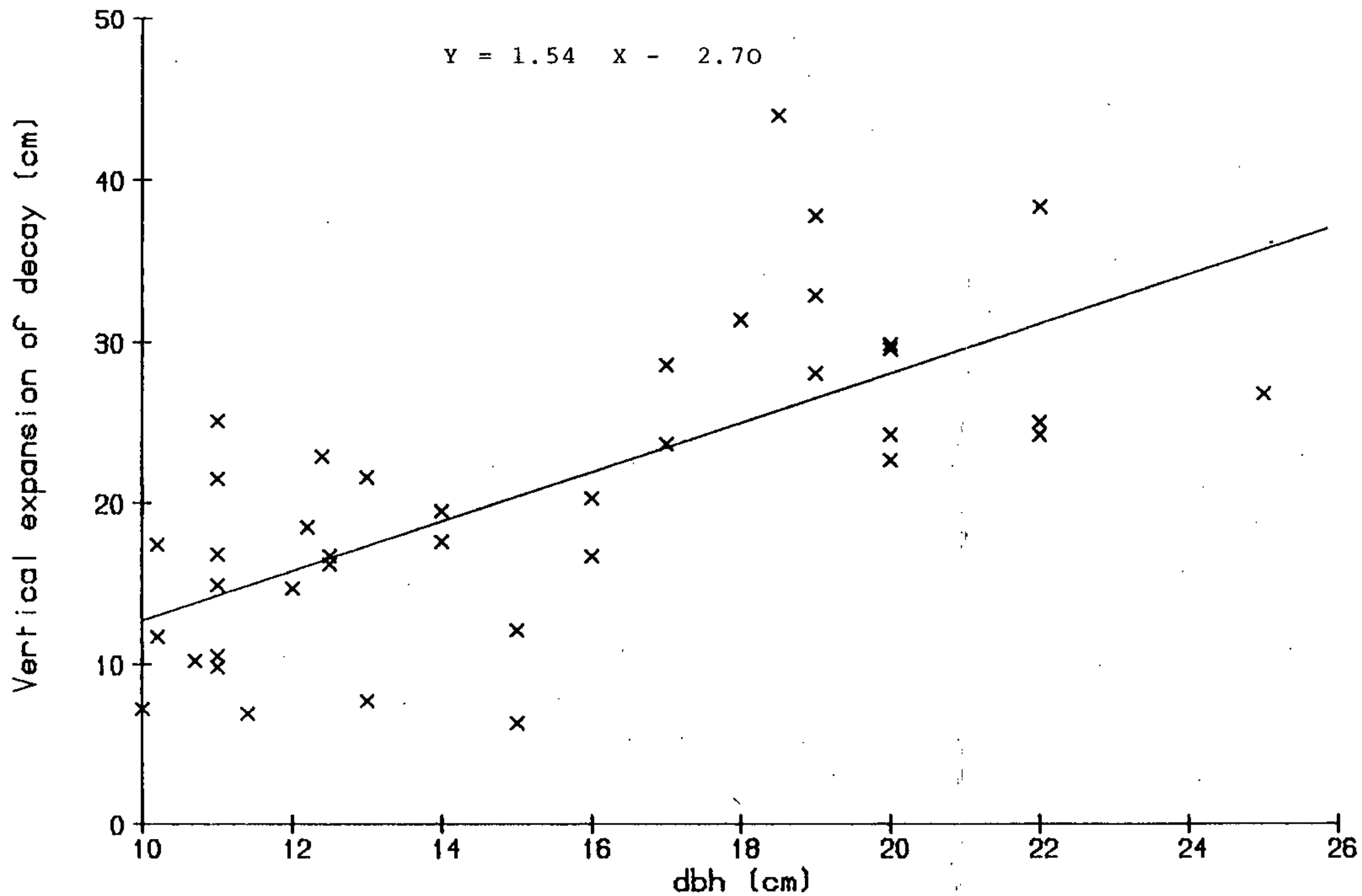

Fig.16A Relationship between dbh and vertical expansion of decay

(Artificial inoculation, series February 1983)

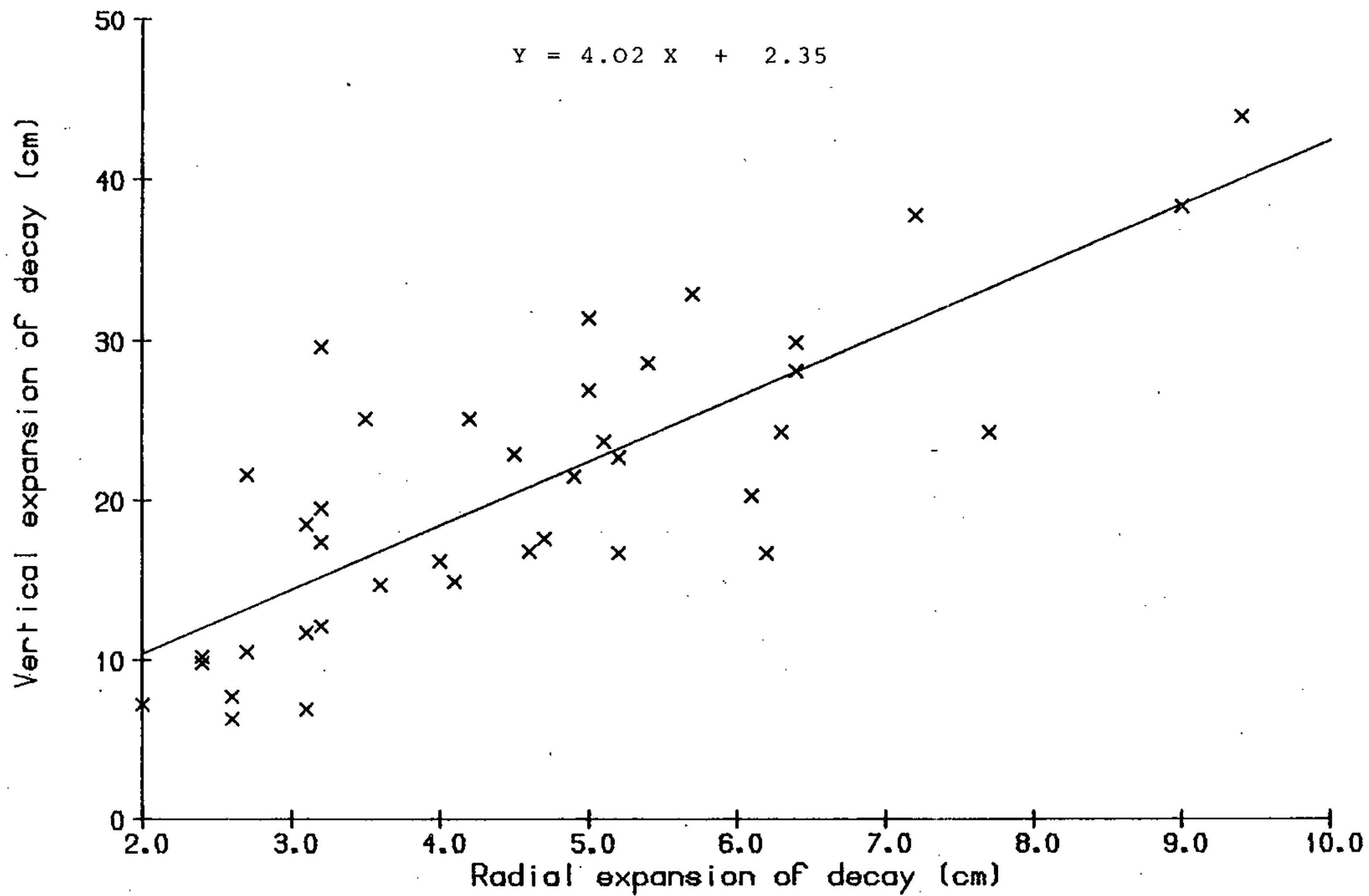

Fig.17A Radial VS vertical expansion of decay
(Artificial inoculation, series February 1983)

decay, X1 = dbh and X2 = number of growth rings per 5 cm

5.5. Frequency of microorganisms isolated

A. Sealed inoculation sites

A.1. Isolations at the site of inoculation inwards

Table 32 shows the frequency and distribution of microorganisms isolated at the site of inoculation inwards at 1 cm intervals for > 10 cm towards the pith (Fig. 1). S.sanguinolentum was the dominant species,being isolated from 72.4% of all samples . T.viride ranked next (6.7%) . Other species isolated occurred between 0.4 and 4.3%. These were: C.cochliodes (4.2%) ; Bacteria (4.3%) ; Penicillium spp (3.4%); F.sambucinum (2.5%) ;members of Dematiaceae (1.5%) ; M.hiemalis (1.4%) and sterile unidentified mycelia (0.4%) . 3.2% of the samples failed to yield any microbial growth.

The frequency of S.sanguinolentum increased progressively from the outside of the tree towards the pith and reached 100% in the samples taken between 7 and >10 cm from the outer surface. Within this sample range no other microorganisms were isolated. On the other hand the frequency of all other microorganisms dropped dramatically with distance from the outer surface of the stem inwards. Between 1 and 5 cm T.viride was consistently the next most common species; at the 6th cm C.cochliodes ranked next as T.viride failed to grow from that depth onwards. Of all microorganisms isolated ,with the exception of S.sanguinolentum ,8 were never found beyond 7 cm from the outer surface of the inoculation site,a distance beyond which S.sanguinolentum was the only

Table 32: ISOLATIONS MADE FROM SEALED INOCULATED SITES FROM THE OUTER SURFACE INWARDS AFTER SIX MONTHS (JULY 1982)

CM INTO WOOD	1	2	3	4	5	6	7	8	9	10	>10	MEAN
SPECIES	%FREQUENCY											
S.sanguinolentum	37.0	42.0	43.2	47.1	57.8	75.0	93.8	100	100	100	100	72.4
M.hiemalis	04.5	03.2	03.2	00.0	00.0	04.2	00.0	000	000	000	000	01.4
C.cochliodes	12.0	11.9	07.7	03.3	02.8	08.3	00.0	000	000	000	000	04.2
F.sambucinum	05.8	05.7	07.8	05.8	02.8	00.0	00.0	000	000	000	000	02.5
T.viride	15.8	14.4	17.4	14.5	11.3	00.0	00.0	000	000	000	000	06.7
Penicillium spp	05.8	05.7	04.7	05.0	05.6	04.2	06.2	000	000	000	000	03.4
Acremonium spp	02.0	02.0	00.0	00.0	00.0	00.0	00.0	000	000	000	000	00.4
Dematiaceae	07.0	06.9	02.3	00.0	00.0	00.0	00.0	000	000	000	000	01.5
Sterile mycelia	03.3	01.2	00.0	00.0	00.0	00.0	00.0	000	000	000	000	00.4
Bacteria	07.0	06.9	11.3	13.0	07.0	00.0	00.0	000	000	000	000	04.3
No growth	00.0	00.0	02.4	11.3	12.7	08.3	00.0	000	000	000	000	03.2

microorganism isolated. Penicillium spp showed the next greatest radial expansion as they were the only microorganisms isolated together with S.sanguinolentum at the 7th cm from the bark,a depth at which other species were not isolated. Within the first 2 cm all samples examined yielded at least one microorganism ,but between 3 and 6 cm some samples (2.4;11.3;12.7 and 8.3% respectively) failed to show microbial growth.

A.2. Isolations above the inoculation site

Results were summarised in table 33. S.sanguinolentum was the most common microorganism isolated (81.6%) . Next in frequency was C.cochliodes (4.9%) . Other species isolated were: Penicillium spp (4.1%) ; T.viride (3.8%) ; M.hiemalis (1.5%) Bacteria (1.4%); F.sambucinum (1.3%) ; sterile mycelia (0.5%) and members of Dematiaceae (0.9%). All the samples examined yielded one or more microorganism(s). With the exception of S.sanguinolentum , T.viride and C.cochliodes all other microorganisms were isolated from samples taken between 1 and 5 cm above the site of inoculation. All attempts to isolate microorganisms beyond that zone failed. The distribution of S.sanguinolentum was uniform, whereas other species were characterized by an irregular pattern of distribution e.g one organism might be isolated from one position ,but uncommon or even absent just a few cm from that position. S.sanguinolentum ; T.viride and C.cochliodes all expanded as far as the distal parts of the decay columns. All isolations from clear wood adjoining the decay columns yielded no microorganisms.

Table 33: ISOLATIONS ABOVE SEALED INOCULATED SITES ASSOCIATED WITH DECAY SIX MONTHS AFTER INOCULATION (JULY 1982)

SPECIES	%FREQUENCY
S.sanguinolentum	81.6
Mucor hiemalis	1.5
Chaetomium cochliodes	4.9
Fusarium sambucinum	1.3
Trichoderma viride	3.8
Penicillium spp	4.1
Dematiaceae	0.9
Sterile mycelia	0.5
Bacteria	1.4
TOTAL	100

A.3. Isolations below the inoculation site

S.sanguinolentum was again dominant ,being isolated from 74.1% of the samples taken below the inoculation site (Table 34). Penicillium spp ranked next (10.1%). F.sambucinum and T.viride were isolated by almost the same frequency (5 and 4.9% respectively); M.hiemalis (2.7%); C.cochliodes (1.6 %) and Bacteria (1.7%). In contrast to the situation at the site of inoculation Dematiaceae and sterile mycelia were not isolated. All samples yielded microorganism(s). With the exception of S.sanguinolentum ,all microorganisms occurred in scattered samples with no definite pattern of distribution.

A.4. Isolations from the distal parts of the decay columns

S.sanguinolentum was the most common fungus and was isolated from 92.5% of the samples yielding microorganisms (Table 35). Other species isolated were : C.cochliodes (2.5%) and T.viride (1.7%). 3.3% of the samples were sterile. Some microorganisms which were isolated from other positions with different frequencies were never isolated from the distal parts of the decay columns e.g M.hiemalis ; Dematiaceae and Bacteria. The occurrence of these microorganisms was restricted to a 5 cm zone above the site of inoculation and were also distributed irregularly up to, but not beyond , 20 cm below the site of inoculation.On the other hand, T.viride and C.cochliodes were capable of expanding as far as the distal parts of the decay columns, but with much less frequency than was S.sanguinolentum .

B. Open inoculation sites

Table 34: ISOLATIONS BELOW SEALED INOCULATED SITES ASSOCIATED WITH DECAY SIX MONTHS AFTER INOCULATION (JULY 1982)

SPECIES	%FREQUENCY
S.sanguinolentum	74.1
M.hiemalis	02.7
C.cochliodes	01.6
F.sambucinum	05.0
T.viride	04.9
Penicillium spp	10.1
Acremonium spp	00.0
Dematiaceae	00.0
Sterile mycelia	00.0
Bacteria	01.7
TOTAL	100

Table 35: ISOLATIONS FROM THE DISTAL PARTS OF DECAY COLUMNS ASSOCIATED WITH SEALED INOCULATED SITES (JULY1982) SIX MONTHS AFTER INOCULATION

SPECIES	%FREQUENCY
S.sanguinolentum	92.5
T.viride	01.7
C.cochliodes	02.5
No growth	03.3
TOTAL	100

B.1. Isolations at the site of inoculation inwards

S.sanguinolentum was the dominant species isolated at all wood depths examined (Table 36). The global frequency of occurrence was 83%. The frequency of S.sanguinolentum increased progressively with increasing depth of sample,being lowest at the 1st cm (41.6%) but still dominant,reaching 100% in the samples taken between 7 and > 10 cm . The next most common species isolated was T.viride (6.9%). The remaining microorganisms isolated were: Penicillium spp (2.4%) ; M.hiemalis (2%) ; C.cochliodes (1.7%); F.sambucinum (0.8%) ; sterile mycelia (0.4%) and Acremonium spp (0.4%). Bacteria were not isolated at all. 2.4% of the samples were sterile. The frequency of microorganisms ,with the exception of S.sanguinolentum , declined progressively with increasing depth of sample. Beyond 2 cm F.sambucinum was never isolated. Also all attempts to isolate C.cochliodes beyond 3 cm failed. Penicillium spp and M.hiemalis were not isolated beyond 4 and 3 cm respectively. T.viride showed the next greatest radial expansion (5-6 cm) but was considerably less frequent than S.sanguinolentum . Thus, T.viride could grow in a zone where only S.sanguinolentum grew,whereas other microorganisms failed. T.viride remained as the next most frequent species at depths between 1 and 6 cm. In samples deeper than 6 cm only S.sanguinolentum was isolated.

B.2. Isolations above the inoculation site

Table 37 shows that the most common species isolated was S.sanguinolentum (75.8%) ,followed by T.viride (9.4%) .

Table 36: ISOLATIONS MADE FROM OPEN INOCULATED SITES FROM THE OUTER SURFACE INWARDS AFTER SIX MONTHS (JULY 1982)

CM INTO WOOD	1	2	3	4	5	6	7	8	9	10	>10	MEAN
SPECIES	%FREQUENCY											
S.sanguinolentum	41.6	54.8	70.7	76.2	77.2	92.5	100	100	100	100	100	83.0
M.hiemalis	13.6	06.9	01.5	00.0	00.0	00.0	000	000	000	000	000	02.0
C.cochliodes	07.8	07.6	03.5	00.0	00.0	00.0	000	000	000	000	000	01.7
F.sambucinum	05.0	03.7	00.0	00.0	00.0	00.0	000	000	000	000	000	00.8
T.viride	16.5	15.9	12.4	14.3	08.8	07.5	000	000	000	000	000	06.9
Penicillium spp	10.7	06.9	06.0	03.2	00.0	00.0	000	000	000	000	000	02.4
Acremonium spp	03.4	01.4	00.0	00.0	00.0	00.0	000	000	000	000	000	00.4
Sterile mycelia	01.4	02.8	00.0	00.0	00.0	00.0	000	000	000	000	000	00.4
No growth	00.0	00.0	06.0	06.3	14.0	00.0	000	000	000	000	000	02.4

Table 37: ISOLATIONS ABOVE OPEN INOCULATED SITES ASSOCIATED WITH DECAY SIX MONTHS AFTER INOCULATION (JULY 1982)

SPECIES	%FREQUENCY
S.sanguinolentum	75.8
M.hiemalis	00.0
C.cochliodes	03.6
F.sambucinum	03.2
T.viride	09.4
Penicillium spp	02.8
Acremonium spp	02.5
Dematiaceae	00.0
Sterile mycelia	00.4
Bacteria	02.3
TOTAL	100

Other species isolated were: C.cochliodes (3.6%) ; F.sambucinum (3.2 %) ; Penicillium spp (2.8%); Acremonium spp (2.5%) ; sterile mycelia (0.4%) and Bacteria (2.3%). M.hiemalis and members of Dematiaceae were never isolated from this zone. All samples yielded at least one microorganism. All microorganisms,except S.sanguinolentum , T.viride and C.cochliodes were isolated from a zone 1 to 7 cm above the inoculation site,but never beyond that and their occurrence has no definite pattern of distribution.

B.3. Isolations below the inoculation site

Isolations from this zone further confirmed the dominance of S.sanguinolentum (69.5%) (Table 38). Tviride ranked next (13.7%) followed by Penicillium spp (4.4%) ; Bacteria (3.8%) ; Acremonium spp (2.5%) ; C.cochliodes and F.sambucinum (2.2% each) and M.hiemalis (1.8%). No sterile mycelia or Dematiaceae were isolated and all samples yielded microorganism(s). All microorganisms were isolated from a 20 cm zone below the site of inoculation with no consistent or uniform distribution of a particular microorganism.

B.4. Isolations from the distal parts of the decay columns

Only 3 microorganisms were isolated from this zone ,the most common of which was S.sanguinolentum (91.5%) (Table 39). T.viride was the next most common (6.4%) and the least frequent species was C.cochliodes (2.1%). All samples examined yielded at least one microorganism.

C. Naturally infected controls

Not all the controls remained sterile,and some natural

Table 38: ISOLATIONS BELOW OPEN INOCULATED SITES ASSOCIATED WITH DECAY SIX MONTHS AFTER INOCULATION (JULY 1982)

SPECIES	%FREQUENCY
S.sanguinolentum	69.5
M.hiemalis	01.8
C.cochliodes	02.2
F.sambucinum	02.2
T.viride	13.7
Penicillium spp	04.4
Acremonium spp	02.5
Dematiaceae	00.0
Sterile mycelia	00.0
Bacteria	03.8
TOTAL	100

Table 39: ISOLATIONS FROM THE DISTAL PARTS OF DECAY COLUMNS ASSOCIATED WITH INOCULATED OPEN SITES (JULY 1982) SIX MONTHS AFTER INOCULATION

SPECIES	%FREQUENCY
S.sanguinolentum	91.5
T.viride	06.4
C.cochliodes	02.1
TOTAL	100

infection took place. Within the first 2 cm the most frequent species isolated were Penicillium spp (17.2 and 16.5% respectively) (Table 40). Beyond that depth and up to > 10 cm zone S.sanguinolentum was the most common species isolated (22.5;17.7;23.6;69.7;100;100;100 and 100% respectively). Between 4 and 6 cm Bacteria ranked second to S.sanguinolentum . At depths between 7 and > 10 cm S.sanguinolentum was the only species isolated. The global mean frequencies of individual species were: S.sanguinolentum (56.3%) ; Bacteria (7%) ; T.viride (5.3%) and sterile mycelia (4.4%); 7.8% of the samples were sterile. As in the case of the inoculated sites, frequency of S.sanguinolentum increased progressively with depth and was isolated from 100% of the samples from the 7th cm onwards. On the other hand, the frequency of other microorganisms dropped dramatically with depth. T.viride ; sterile mycelia and Bacteria all reached the next greatest radial penetration(6 cm),whereas other species were restricted to more superficial samples.

5.6. Effect of inoculation site treatment on the frequency of S.sanguinolentum

Table 41 shows the frequency of S.sanguinolentum above and below the site of inoculation and at the distal parts of the decay columns. Statistical analysis (Table 42) of the data showed that the observed differences in the frequency of S.sanguinolentum in the three positions in sealed and open sites of inoculation are not significant. Table 43 shows the variation in the frequency of S.sanguinolentum at

Table 40: ISOLATIONS MADE FROM NATURALLY INFECTED CONTROL TREES (NORWAY SPRUCE) FROM THE BARK INWARDS AFTER SIX MONTHS

CM INTO WOOD / SPECIES	1	2	3	4	5	6	7	8	9	10	MEAN
	%FREQUENCY										
S.sanguinolentum	14.0	15.8	22.5	17.7	23.6	69.7	100	100	100	100	56.3
M.hiemalis	08.4	05.1	00.0	00.0	00.0	00.0	000	000	000	000	01.4
C.cochliodes	09.7	05.8	11.0	05.9	05.0	00.0	000	000	000	000	03.7
F.sambucinum	11.0	07.3	07.9	07.5	05.1	00.0	000	000	000	000	03.9
T.viride	12.4	10.8	12.5	07.7	05.5	03.0	000	000	000	000	05.2
Penicillium spp	17.2	16.5	14.9	09.6	07.1	00.0	000	000	000	000	06.5
Acremonium spp	02.7	05.8	03.1	05.9	02.1	00.0	000	000	000	000	02.0
Dematiaceae	07.7	05.1	05.6	00.0	00.0	00.0	000	000	000	000	01.8
Sterile mycelia	07.4	10.8	05.4	08.9	05.2	06.1	000	000	000	000	04.4
Bacteria	09.5	12.2	09.3	11.8	09.3	18.2	000	000	000	000	07.0
No growth	00.0	05.0	07.8	25.0	37.1	03.0	000	000	000	000	07.8

Table 41:

%FREQUENCY OF S.sanguinolentum AT DIFFERENT POSITIONS OF INOCULATED SITES SIX MONTHS AFTER INOCULATION (SERIES JULY 1982)

POSITION FROM INOCULATED SITE	INOCULATION SITE OPEN	INOCULATION SITE SEALED	GLOBAL MEAN
ABOVE	75.8	81.6	78.7
BELOW	69.5	74.1	71.8
DISTAL	91.5	92.5	92.0
MEAN	78.9	82.7	80.8

Table 42: VARIATION IN THE %FREQUENCY OF S.sanguinolentum ABOVE AND BELOW THE INOCULATION SITE AND AT THE DISTAL PART OF THE DECAY COLUMN IN OPEN AND SEALED SITES OF INOCULATION

VARIABLE	NO. OF CASES	MEAN	STANDARD DEVIATION	STANDARD ERROR	T VALUE	DF	2-TAIL PROBABILITY
ABOVE #	28	75.8000	27.181	5.137	-0.94	44.7	> 0.05
ABOVE *	22	81.6000	15.887	3.387			
BELOW #	28	69.5000	30.200	5.707	-0.65	46.7	> 0.05
BELOW *	22	74.1000	19.878	4.238			
DISTAL #	28	91.5000	12.079	2.283	0.21	-0.24	> 0.05
DISTAL *	22	92.5000	17.440	3.718			

ABOVE # = %FREQUENCY OF S.sanguinolentum ABOVE OPEN INOCULATION SITES

ABOVE * = %FREQUENCY OF S.sanguinolentum ABOVE SEALED INOCULATION SITES

BELOW # = %FREQUENCY OF THE FUNGUS BELOW OPEN INOCULATION SITES

BELOW * = %FREQUENCY OF THE FUNGUS BELOW SEALED INOCULATION SITES

DISTAL # = %FREQUENCY OF S.sanguinolentum AT THE DISTAL PARTS OF THE DECAY COLUMNS ASSOCIATED WITH OPEN INOCULATION SITES

DISTAL * = %FREQUENCY OF S.sanguinolentum AT THE DISTAL PARTS OF THE DECAY COLUMNS ASSOCIATED WITH SEALED INOCULATION SITES

Table 43: %FREQUENCY OF _S.sanguinolentum_ COMPARING OPEN AND SEALED INOCULATED SITES (JULY 1982 SERIES) EXAMINED AFTER SIX MONTHS

CM INTO WOOD												MEAN
OPEN SITE	41.6	54.8	70.7	76.2	77.2	92.5	100	100	100	100	100	83.0
SEALED SITE	37.0	42.0	43.2	47.1	57.8	75.0	93.8	100	100	100	100	72.4

different sample depths measured form the outer surface at the site of inoculation inwards. Differences in the mean frequency of the fungus was not significant at all depths comparing open with sealed inoculation sites (Table 44).

In three trees antagonism of T.viride on S.sanguinolentum was evident. In these trees discolouration had already started but did not develop further and was soon suppressed by the activity of T.viride. S.sanguinolentum was replaced by T.viride as the former was isolated only as traces ,whereas T.viride dominated throughout the discoloured samples examined. In another instance C.cochliodes showed the same effect on S.sanguinolentum which was replaced and isolated only sparsely. T.viride tended to be more frequent above open (9.4%) than above sealed inoculated sites (3.8%). A comparable situation was also found in the case of isolations below the site of inoculation. Here, T.viride was more frequent below open (13.7%) as compared with sealed sites of inoculation (4.9%). The same trend was also true in the case of the distal parts of the decay columns where the frequency of the fungus was 6.4 and 1.7% from open and sealed inoculation sites respectively. No consistent relationship was observed in the case of other microorganisms.

The antagonistic effect of T.viride on other decay fungi was also reprted by some other workers (Page 283).

Table 44: VARIATION IN THE %FREQUENCY OF S.sanguinolentum WITH TREATMENT OF INOCULATION SITE

VARIABLE	NO. OF CASES	MEAN	STANDARD DEVIATION	STANDARD ERROR	T VALUE	DF	2-TAIL PROBABILITY
%FREQUENCY #	11	83.0	20.678	6.235	1.03	20	> 0.05
%FREQUENCY *	11	72.4	27.194	8.199			

%FREQUENCY # = %FREQUENCY OF S.sanguinolentum WITHIN >10 cm FROM THE OUTER SURFACE OF INOCULATED OPEN SITES INWARDS.

%FREQUENCY * = %FREQUENCY OF S.sanguinolentum WITHIN >10 cm FROM THE OUTER SURFACE OF INOCULATED SEALED SITES INWARDS.

CHAPTER 6

6. GROWTH STUDIES

Generally,all fungi need the following materials for growth in addition to a supply of moisture and oxygen:

1. Carbon supply
2. Nitrogen supply
3. Certain inorganic ions e.g phosphorus,sulphur, potassium and magnesium.
4. Micronutrient elements including iron,copper,manganese and possibly calcium.
5. Other organic substances in very low concentrations,which include accessory growth factors e.g vitamins (Hawker,1950; Lilly and Barnett,1951; Cochrane,1958; Ainsworth and Sussman,1966; Ingold,1967 and Griffin,1972) and some amino acids.

Methods

All the methods and techniques were detailed in pages 67-69.Four seperate strains of S.sanquinolentum were tested for possible differences in nutritional and physical requirements for optimum growth.

Results

Strain 1

6.1. 3% MEA [malt extract agar]

This was slowest growing strain when grown on different solid media. In 3% MEA at 25 C° , two growth zones were formed. First,a narrow zone occurred immediately around the inoculum,consisting of dense aerial mycelium ranging in colour from white to dirty white. The second zone was white,wider and had relatively denser aerial mycelium. The colony margin was rarely uniform and

sometimes spider-shaped (Plate 12). Honey-like liquid drops were produced on 3%MEA after 4 weeks. Colonies attained 74 mm diameter after 2 weeks at 15 C° and at 20 C° (Fig. 18).

6.2. 3% CMA [corn meal agar]

Growth was extremly sparse and the mycelium was mainly submerged with only a few scattered aerial hyphae. Colonies remained white throughout the course of experiments. Colonies attained 74 mm diameter after 3 and 4 weeks at 15 C° and at 20 C° respectively (Fig. 18).

6.3. Spruce sawdust/agar

The mycelium was moderately dense. A characteristic staining of the medium occurred after 2 weeks. The colour was pale red,dark red or brown commencing in the area immediately surrounding the inoculum and later spread over the whole colony. The staining did not occur in cultures incubated at 5 C° where colonies remained white. The intensity of staining was directly related to the amount of growth,being well pronounced at optimum temperatures and pale at temperatures where growth was relatively slower (e.g it was well pronounced at 15 C°). Colonies attained 74 mm diameter after 4 weeks at 15 C° and at 20 C° (Fig. 18).

CDA [Czapek dox agar]

Generally,growth in CDA with different substitutions of carbon and nitrogen sources consisted of mainly thin submerged mycelium. Colonies produced very few scattered aerial hyphae. Usually growth in this medium resulted in coloured colonies ranging from red through reddish brown

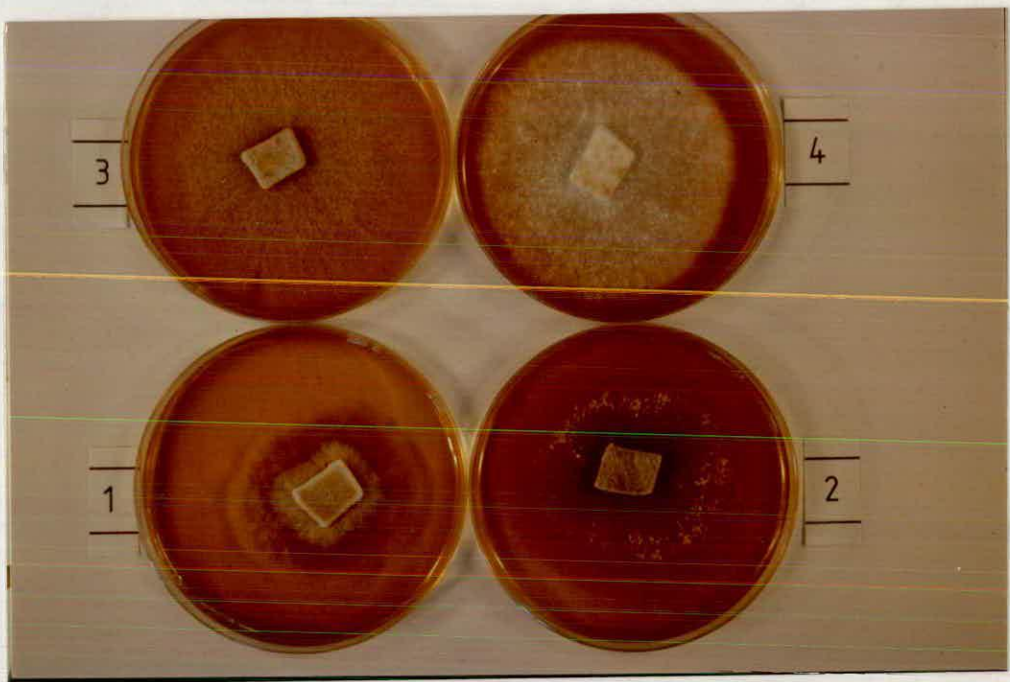

Plate 12: Four strains of S.sanguinolentum isolated from Norway spruce and Japanese larch following extraction damage and decay

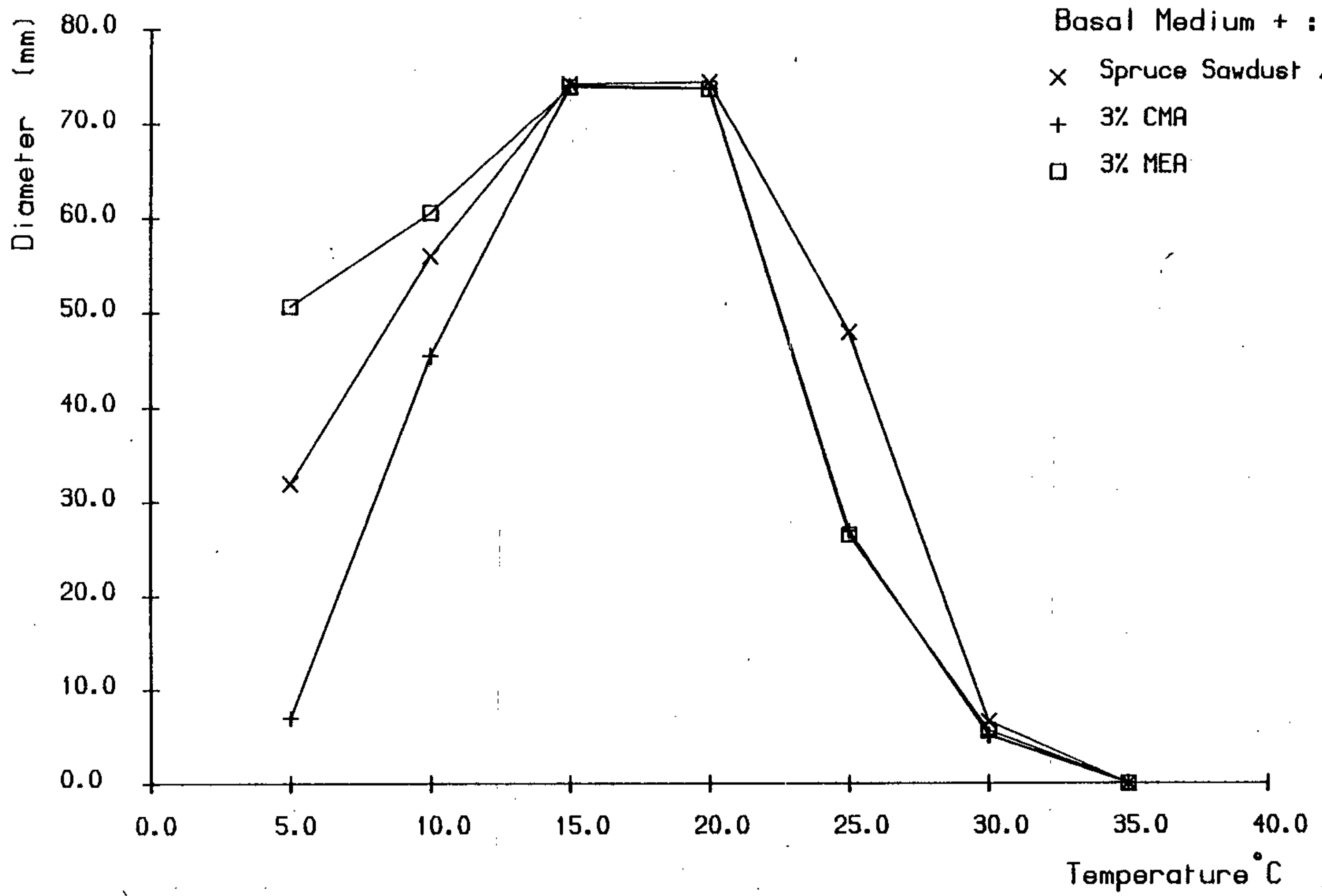

Fig:18 GROWTH STRAIN1

to dark brown.

6.4. Utilization of different carbon sources in combination with

6.4.1. Inorganic nitrogen sources

[i] Sodium nitrate

The total amount of growth was considered as indicative of the availability of the carbon source used since the nitrogen source and the basal medium were constants (chapter 3). Using sodium nitrate as sole nitrogen source,glucose,fructose,lactose and sucrose produced colonies of 38,64,53 and 49 mm diameter after 4 weeks at 15 C° respectively,whereas starch and maltose produced colonies of 64 and 28 mm diameter after 4 weeks at 20 and 25 C° respectively (Fig. 19 and 21). These results suggest that fructose and starch were more readily utilised as carbon sources,but the observed differences were not significant (Table 45).

[ii] Ammonium sulphate

If ammonium sulphate was used as sole nitrogen source,glucose,fructose, starch,maltose and sucrose produced colonies of 50,39,47,31 and 50 mm diameter after 4 weeks at 15 C° respectively, whereas lactose produced colonies of 65 mm diameter after 4 weeks at 20 C° (Fig.20 and 21). Significant differences were summarised in table 45.

[iii] Comparison between sodium nitrate and ammonium sulphate

Table 45 summarises paired-t tests between various carbon and nitrogen sources. It was evident that most carbon sources supported much better growth when combined with ammonium sulphate as compared with sodium

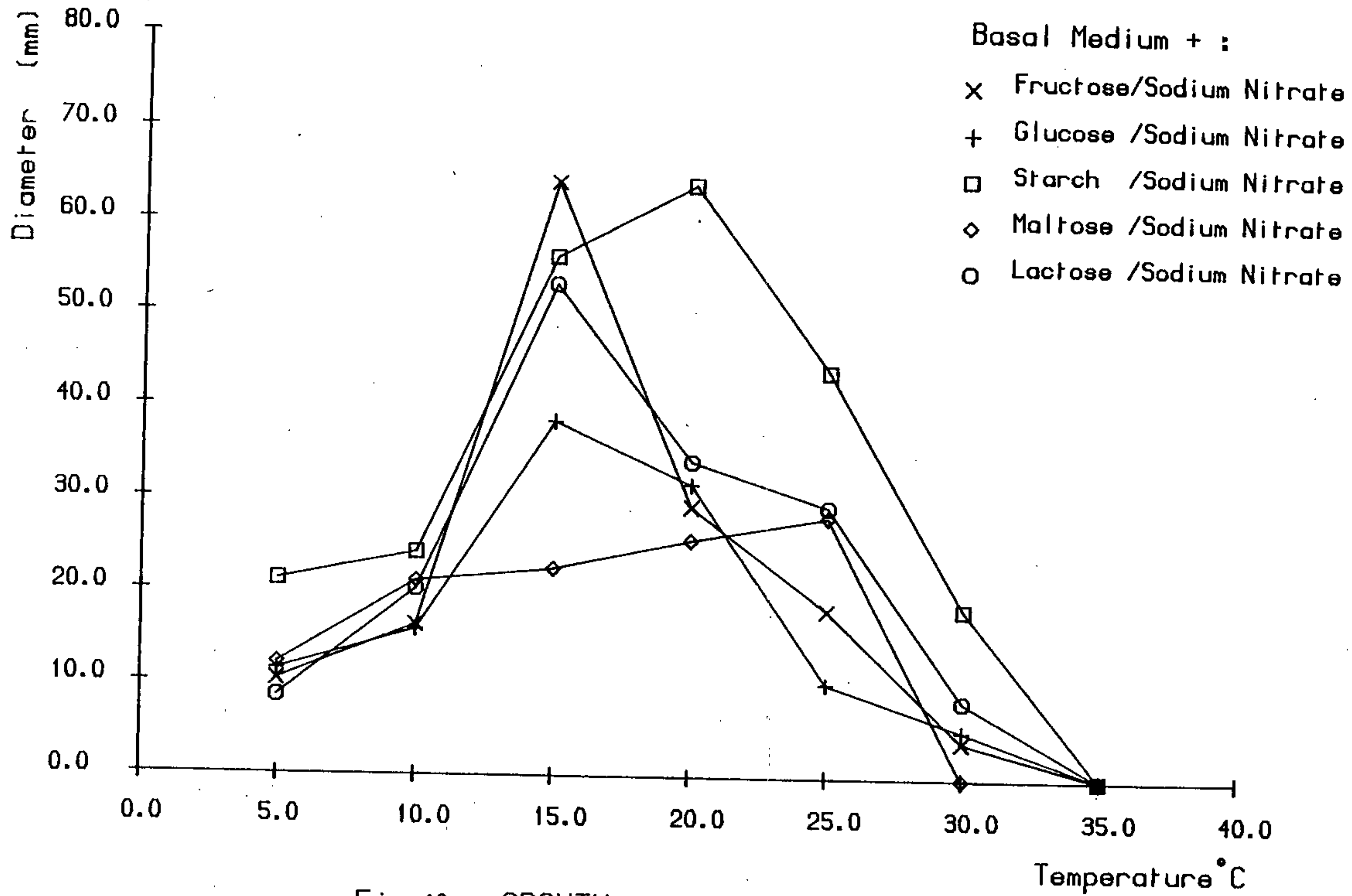

Fig:19 GROWTH — STRAIN1

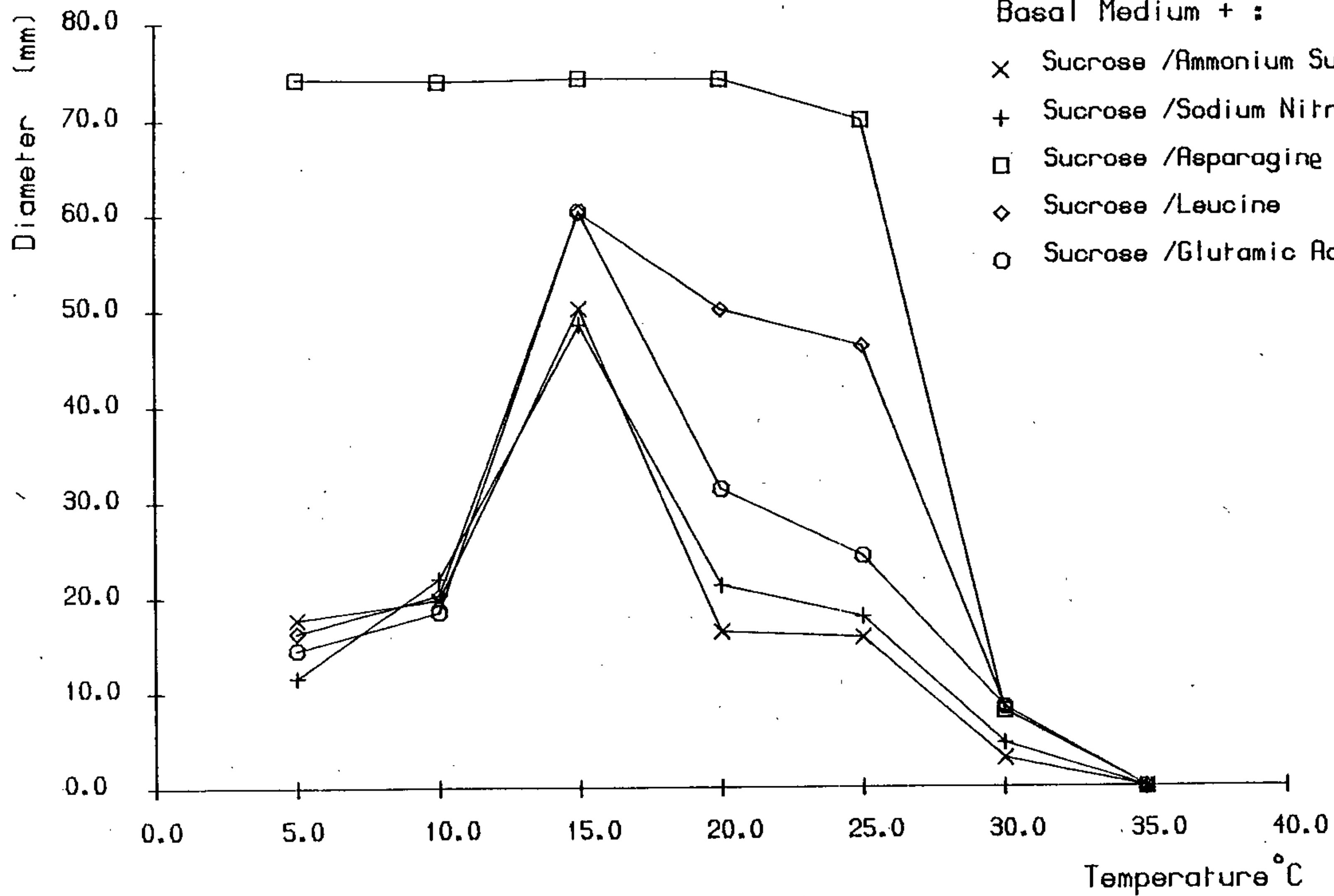

Fig: 21 GROWTH / STRAIN1

Table 45: RESULTS OF T-TESTS COMPARING GROWTH OS S.sanguinolentum STRAIN 1 AFTER 28 DAYS ON DIFFERENT COMBINATIONS OF CARBON AND NITROGEN SOURCES
BASAL MEDIUM +

CARBON/NITROGEN SOURCE	MEAN MAX.DIAMETER	T VALUE	DF	PROBABILITY
SUCROSE/SODIUM NITRATE	48.5	-12.33	4	< 0.001
SUCROSE/AMMONIUM SULPHATE	50.2			
GLUCOSE/SODIUM NITRATE	38.4	-58.84	4	< 0.001
GLUCOSE/AMMONIUM SULPHATE	50.1			
FRUCTOSE/SODIUM NITRATE	64.2	49.17	4	< 0.001
FRUCTOSE/AMMONIUM SULPHATE	38.7			
MALTOSE/SODIUM NITRATE	28.3	-4.77	4	< 0.01
MALTOSE/AMMONIUM SULPHATE	30.5			
LACTOSE/SODIUM NITRATE	53.1	-14.29	4	< 0.001
LACTOSE/AMMONIUM SULPHATE	65.0			
STARCH/SODIUM NITRATE	63.9	30.97	4	< 0.001
STARCH/AMMONIUM SULPHATE	47.0			

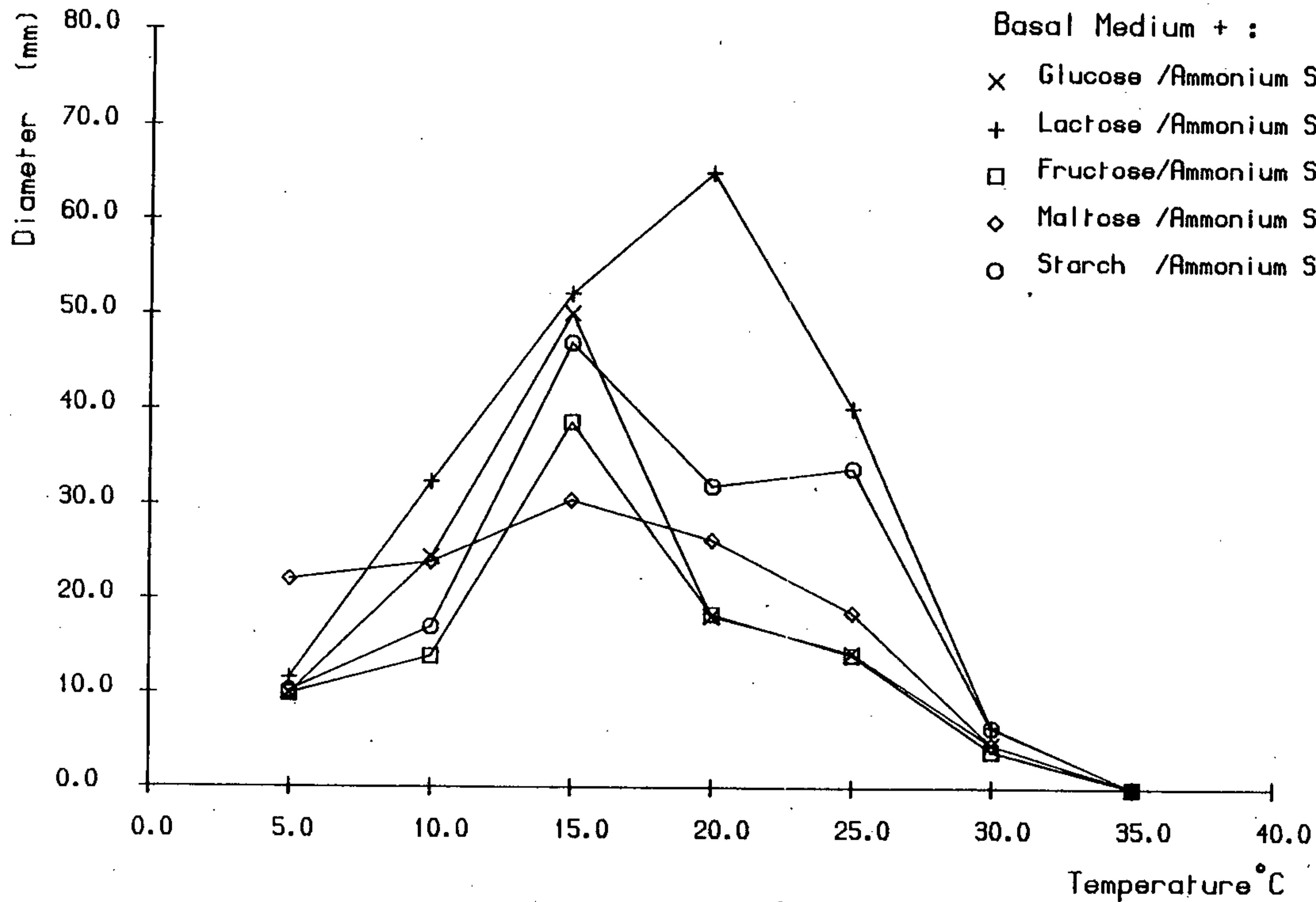

Fig:20 GROWTH — STRAIN1

nitrate ,the exceptions being fructose and starch. Both fructose and starch proved to produce faster growth when the sole nitrogen source was sodium nitrate compared with ammonium sulphate. Table 46 shows that mean colony diameter was 65 mm after 4 weeks where lactose and ammonium sulphate were used as carbon and nitrogen sources respectively,followed by fructose/sodium nitrate and starch/sodium nitrate which produced mean colony diameters of 64 mm, but the differences were not significant. Much smaller amounts of growth occurred where maltose/ammonium sulphate were used (31 mm diameter ,after 4 weeks).

[iv] Effect of carbon and nitrogen sources on optimum growth

The optimum temperature for growth was 15 C° where sucrose,glucose or fructose were used in combination with sodium nitrate or ammonium sulphate,whereas different temperature optima were recorded if other combinations of carbon and nitrogen sources.

6.4.2. Organic nitrogen sources

Sucrose was used as the sole carbon source in individual combination with asparagine, leucine or glutamic acid (chapter 3 ,page 68). Colonies grown on sucrose/asparagine (plus the basal medium) attained 74 mm diameter after 4,3,2 & 4 weeks at 5;10;15 and 20 C° respectively (Fig.21). Both the quantity of aerial mycelium and the vigour of growth were much greater as the temperature increased(i.e both were poor at 5 C° and were well pronounced at 15 and 20 C°). Sucrose/glutamic acid

Table 46: RESULTS OF T-TESTS COMPARING GROWTH OF *S.sanguinolentum* STRAIN 1 AFTER 28 DAYS ON DIFFERENT CARBON AND NITROGEN SOURCES

BASAL MEDIUM +

CARBON/NITROGEN SOURCE	MEAN MAX.DIAMETER	T VALUE	DF	PROBABILITY
LACTOSE/AMMONIUM SULPHATE	65.0	3.23	4	< 0.05
FRUCTOSE/SODIUM NITRATE	64.2			
LACTOSE/AMMONIUM SULPHATE	65.0	2.78	4	< 0.05
STARCH/SODIUM NITRATE	63.9			
STARCH/SODIUM NITRATE	63.9	0.58	4	> 0.05
FRUCTOSE/SODIUM NITRATE	64.2			

and sucrose/leucine produced similar amounts of growth (60 mm diameter after 4 weeks respectively). Table 47 shows that asparagine was the best overall nitrogen source. In addition organic nitrogen sources supported a much greater amount of growth compared with inorganic nitrogen sources.

6.4.3. Optimum temperature*

Out of a total of 18 different media examined 14 (78%) supported best growth at 15 C°. In the remaining combinations of substrates optimum growth occurred at temperatures between 5 and 25 C°.

Strain 2

6.1. 3% MEA

Aerial mycelium was moderately dense. The colony margin was uniform and developed white and vigorous mycelium (Plate 12). Honey-like droplets of liquid were observed on 3% MEA after 3 weeks of incubation at 25 C°. The aerial mycelium was relatively denser at the middle of the colony. Growth on 3% MEA resulted in the most intensive aerial mycelium compared with other media. Actively growing mycelium attained 74 mm in diameter within 2-3 weeks between 10 and 25 C° (Fig.22). Even at 5 C° growth was relatively good in contrast to the performance of this strain on other media at this temperature. Colonies attained 74 mm in diameter within 2 weeks at 15,20 and 25 C°,whereas it took 3 weeks at 10 C° to attain similar amounts of growth.

6.2. 3% CMA

*: It is important to note that because the size of the petri dish is limited it was not possible to determine precisely the limits of the optimum temperature and pH for growth.

Table 47: RESULTS OF T-TESTS COMPARING GROWTH OF S.sanguinolentum STRAIN 1 AFTER 28 DAYS ON DIFFERENT NITROGEN SOURCES SUCROSE + BASAL MEDIUM +

NITROGEN SOURCE	MEAN MAX.DIAMETER	T VALUE	DF	PROBABILITY
ASPARAGINE	74.3	-123.27	4	< 0.001
SODIUM NITRATE	48.5			
ASPARAGINE	74.3	-11.76	4	< 0.001
AMMONIUM SULPHATE	50.2			
ASPARAGINE	74.3	62.00	4	< 0.001
GLUTAMIC ACID	60.4			
ASPARAGINE	74.3	57.59	4	< 0.001
LEUCINE	60.3			
SODIUM NITRATE	48.5	-57.40	4	< 0.001
GLUTAMIC ACID	60.4			
SODIUM NITRATE	48.5	-43.98	4	< 0.001
LEUCINE	60.3			
SODIUM NITRATE	48.5	-1.94	4	> 0.05
AMMONIUM SULPHATE	50.2			
LEUCINE	60.3	-4.41	4	< 0.01
AMMONIUM SULPHATE	50.2			
GLUTAMIC ACID	60.4	-4.67	4	< 0.01
AMMONIUM SULPHATE	50.2			
GLUTAMIC ACID	60.4	0.18	4	> 0.05
LEUCINE	60.3			

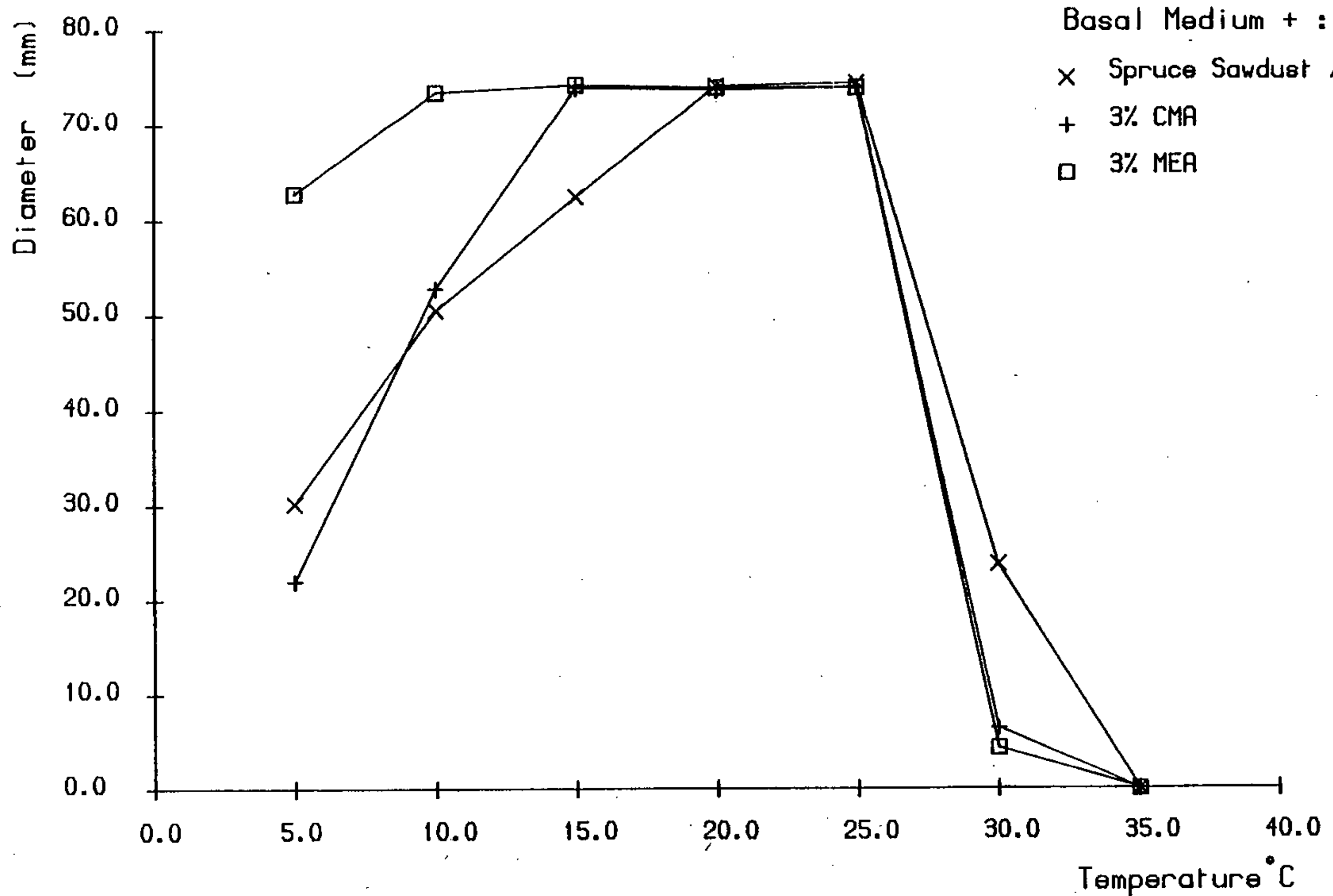

Fig:22 GROWTH STRAIN2

Colonies attained 74 mm in diameter at 15,20 and 25 C° after 4,3,& 3 weeks respectively. Strain 2 produced a few scattered aerial hyphae and extremely thin mycelium mat on 3% CMA (Fig.22).

6.3. Spruce sawdust/agar

Colonies grew to a diameter of 74 mm at 20 and 25 C° after 4 weeks (Fig.22). Growth was relatively uniform and moderately vigorous. Like other isolates it stained the medium red and the intensity of staining was directly related to the amount of growth i.e being most evident at optimum temperature and less evident at other temperatures.

CDA

Growth was generally characterised by dominant submerged mycelium and a poor development of aerial hyphae. Colony margin was rarely uniform and in most cases it was arachnoid. Patches of colour appeared at the 2nd week ranging from pale red to dark brown.

6.4. Utilization of different carbon sources in combination with

6.4.1. Inorganic nitrogen sources

[i] Sodium nitrate

Using sodium nitrate as sole nitrogen source,sucrose,lactose and maltose resulted in colonies of 74,62 and 70 mm diameter after 4 weeks at 25 C° respectively,whereas glucose,fructose and starch resulted in colonies of 60,74 and 60 mm in diameter after 4 weeks at only 20 C° respectively. Sucrose and fructose were most readily utilised compared with other carbon sources,but the

observed differences were not significant (Fig.23 & 25). Maltose occupied an intermediate position , whereas colonies on lactose,starch and glucose grew relatively slowly, but the observed differences were not significant.

[ii] Ammonium sulphate

If ammonium sulphate was used as sole carbon source,sucrose,glucose,maltose and lactose produced colonies of 74,40,52 and 40 mm in diameter after 4 weeks at 25 C° respectively, whereas fructose and starch resulted in colonies of 40 and 44 mm in diameter after 4 weeks at 20 C° respectively (Fig.24 & 25). Sucrose was more readily utilised compared with the other carbon sources. Maltose ranked next followed by starch,whereas fructose and lactose resulted in slower growth.

[iii] Comparison between sodium nitrate and ammonium sulphate

Where sucrose was the carbon source there was no significant difference in the amount of growth irrespective of whether the nitrogen source available was sodium nitrate or ammonium sulphate (Table 48). However,if the carbon source was fructose,glucose,lactose,maltose or starch the amount of growth was increased (Table 48) where sodium nitrate was the sole nitrogen source compared with ammonium sulphate. Maximum growth occurred on sucrose/sodium nitrate (plus basal medium) sucrose/ammonium sulphate and fructose/sodium nitrate,but the observed differences were not significant. Glucose/ammonium sulphate ,fructose/ammonium sulphate and lactose/ammonium sulphate produced slower growth,but the

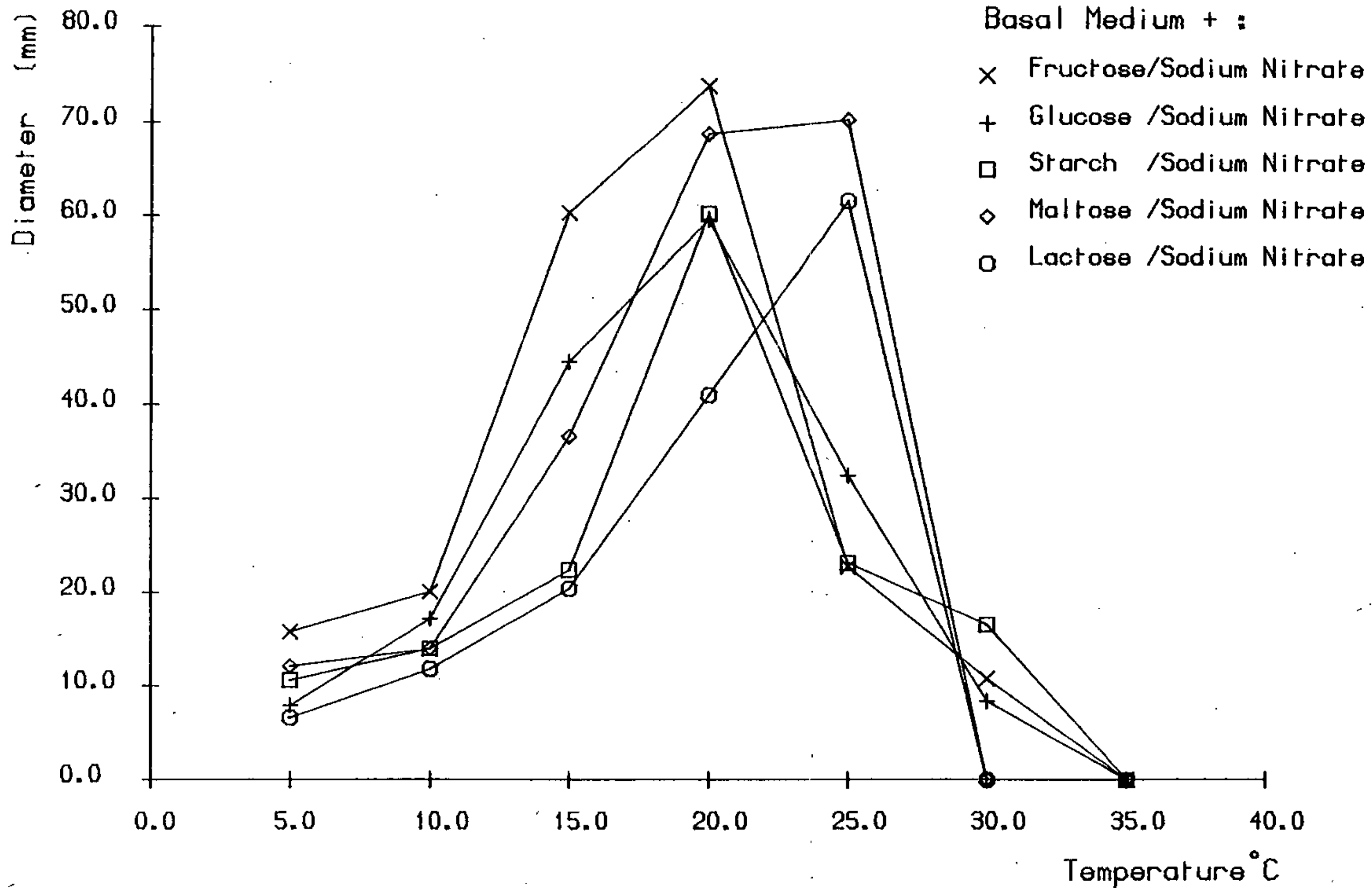

Fig:23 GROWTH STRAIN2

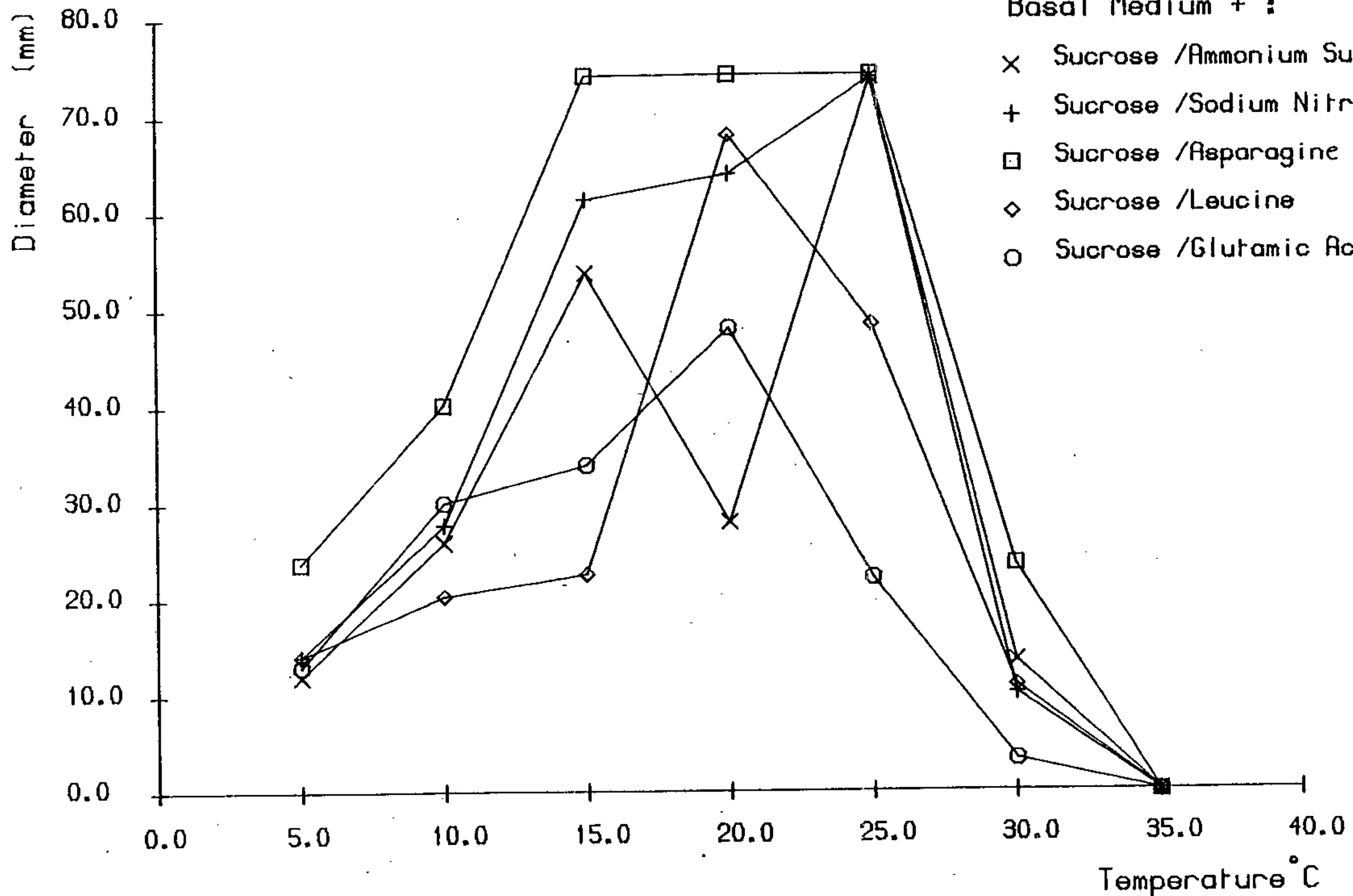

Fig:25 GROWTH / STRAIN2

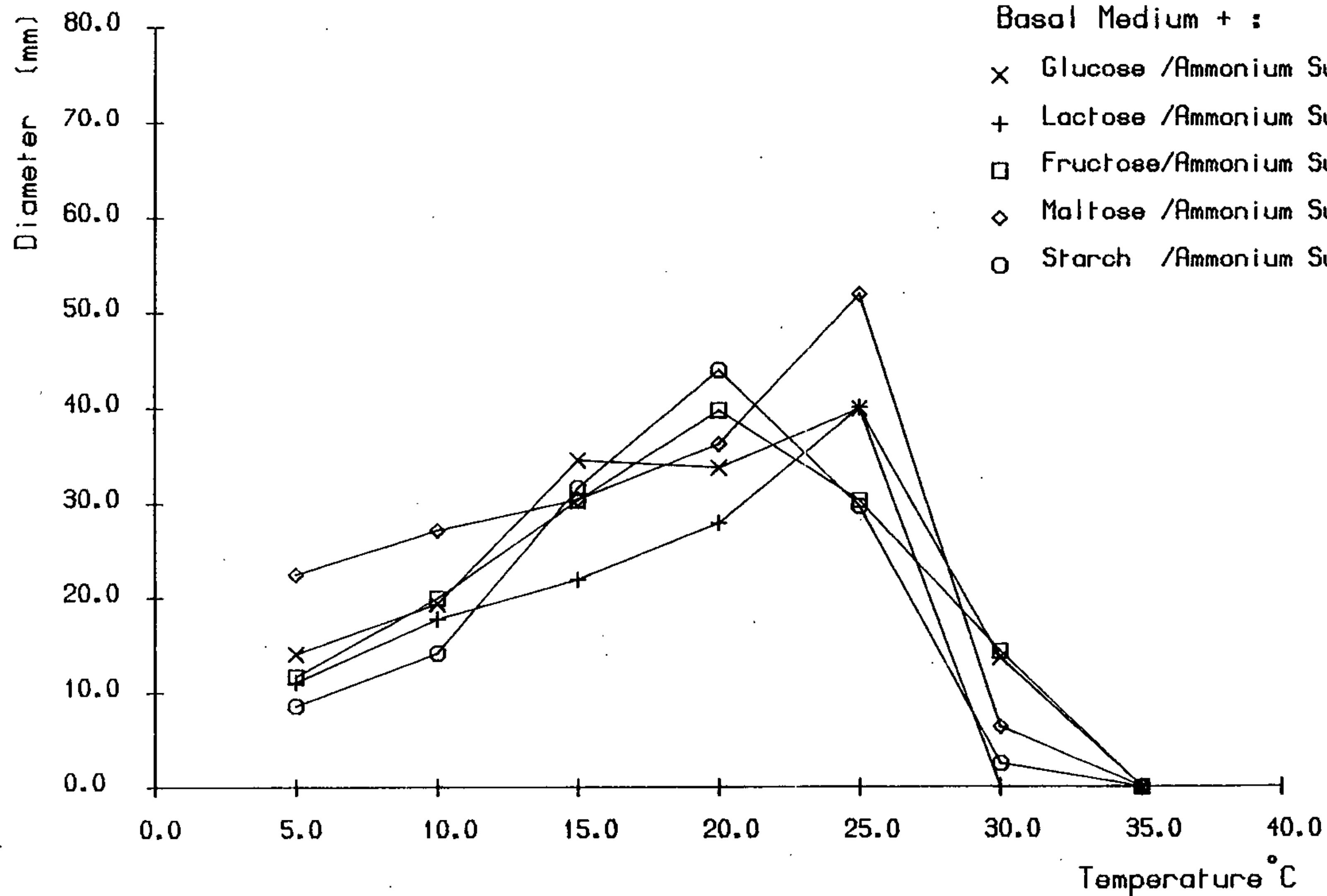

Fig:24 GROWTH / STRAIN2

Table 48: RESULTS OF PAIRED T-TESTS COMPARING GROWTH OF *S.sanguinolentum* STRAIN 2 AFTER 28 DAYS COMPARING DIFFERENT COMBINATIONS OF NITROGEN AND CARBON SOURCES
BASAL MEDIUM +

CARBON SOURCE	NITROGEN SOURCE	MEAN MAXIMUM DIAMETER (mm)	P
SUCROSE	SODIUM NITRATE	74.0	> 0.05
SUCROSE	AMMONIUM SULPHATE	74.0	
GLUCOSE	SODIUM NITRATE	59.7	< 0.001
GLUCOSE	AMMONIUM SULPHATE	40.0	
FRUCTOSE	SODIUM NITRATE	73.8	< 0.001
FRUCTOSE	AMMONIUM SULPHATE	39.8	
LACTOSE	SODIUM NITRATE	61.6	< 0.001
LACTOSE	AMMONIUM SULPHATE	40.1	
MALTOSE	SODIUM NITRATE	70.3	< 0.001
MALTOSE	AMMONIUM SULPHATE	52.0	
STARCH	SODIUM NITRATE	60.2	< 0.001
STARCH	AMMONIUM SULPHATE	44.0	

differences were not significant. Other carbon/nitrogen sources combinations(plus the basal medium)occupied intermediate positions.

[iv] Effect of carbon and nitrogen sources on optimum growth

The optimum temperature for growth remained the same (20 and 25 C°) if sucrose; fructose; lactose; maltose and starch were used as sole carbon sources irrespective of the nitrogen source. Where glucose was combined with sodium nitrate the optimum temperature was 20 C° but it increased to 25 C° if ammonium sulphate was used instead of sodium nitrate as a sole nitrogen source.

6.4.2. Organic nitrogen sources

Of all the organic nitrogen sources used in combination with sucrose and the basal medium (chapter 3),asparagine resulted in colonies of 74 mm diameter at 15,20 and 25 C° respectively (Fig.25). Leucine ranked next and glutamic acid produced comparatively smaller amounts of growth. Sucrose/asparagine;sucrose/sodium nitrate and sucrose/ammonium sulphate resulted in similar amounts of growth (Table 49). Asparagine supported maximum growth over a wider range of temperature i.e 15 20 & 25 C°,compared with sodium nitrate and ammonium sulphate (25 C°). Thus,asparagine was the most easily available nitrogen source compared with other organic and inorganic nitrogen sources.

6.4.3. Optimum temperature

Depending on the media used,the temperature optimum for growth fell between 10 and 25 C°.

Table 49: RESULTS OF PAIRED T-TESTS COMPARING GROWTH OF *S.sanguinolentum* STRAIN 2 AFTER 28 DAYS ON DIFFERENT NITROGEN SOURCES SUCROSE +BASAL MEDIUM +

NITROGEN SOURCE	MEAN MAXIMUM DIAMETER (mm)	PROBABILITY
ASPARAGINE	74.3	< 0.001
LEUCINE	68.1	
ASPARAGINE	74.3	< 0.001
GLUTAMIC ACID	48.1	
ASPARAGINE	74.3	> 0.05
SODIUM NITRATE	74.0	
ASPARAGINE	74.3	> 0.05
AMMONIUM SULPHATE	74.0	
LEUCINE	68.1	< 0.001
SODIUM NITRATE	74.0	
LEUCINE	68.1	< 0.001
AMMONIUM SULPHATE	74.0	
GLUTAMIC ACID	48.1	< 0.001
SODIUM NITRATE	74.0	
GLUTAMIC ACID	48.1	< 0.001
AMMONIUM SULPHATE	74.0	
GLUTAMIC ACID	48.1	< 0.001
LEUCINE	68.1	
SODIUM NITRATE	74.0	> 0.05
AMMONIUM SULPHATE	74.0	

Strain 3

6.1. 3% MEA

Strain 3 was one of the fastest growing strains (second only to strain 4 in vigour), and had a characteristic dense aerial mycelium. The colony margin was uniform and the colour was mainly dirty white throughout the colony (Plate 12). It produced honey-like brown droplets of liquid on 3% MEA after only 2-3 weeks. Colonies attained 74 mm in diameter at 10;15;20;25 and 30 C° after 3,2,2,2 and 4 weeks respectively (Fig.26). 3% MEA produced the most rich growth compared with all other media.

6.2. 3% CMA

After 3 weeks colonies of strain 3 attained 74 mm in diameter at 20 and 25 C° (Fig.26). Although the growth was relatively fast,the aerial hyphae were extremely scattered and the mycelial mat was very thin. Colonies were uniformly white.

6.3. Spruce sawdust/agar

Colonies attained 74 mm in diameter at 20 and 25 C° after 3 and 4 weeks respectively (Fig.26). The mycelium was mainly submerged and the medium was stained and became pale red within 2 weeks. The intensity of staining was directly related to the amount of growth being most evident at 25 C°.

CDA

Usually patches of colour appeared within 2 weeks on CDA. Generally, colour ranged from pale yellow through yellow to red or reddish brown. The mycelium was mainly

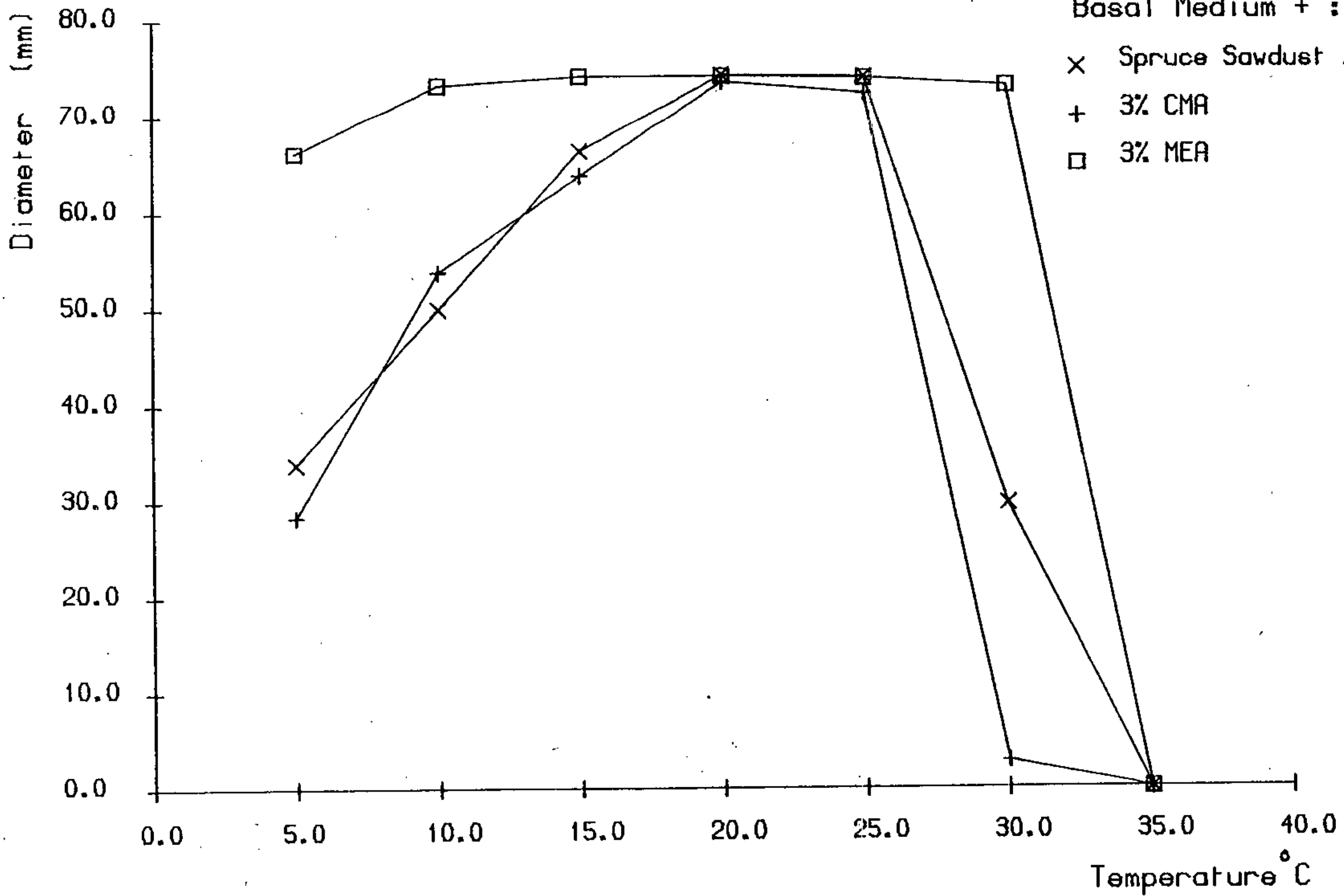

Fig:26 GROWTH / STRAIN3

submerged with a characteristic arachnoid margin.

6.4. Utilization of different carbon sources in combination with

6.4.1. Inorganic nitrogen sources

[i] Sodium nitrate

Using sodium nitrate as sole nitrogen source,glucose,lactose,maltose and starch produced colonies of 66,52,32 and 52 mm diameter after 4 weeks at 20 C° respectively,whereas sucrose and fructose produced colonies of 74 and 73 mm in diameter after 4 and 3 weeks respectively only if the temperature was raised to 25 C° (Fig. 27 & 29). Of all the carbon sources used sucrose and fructose produced maximum amounts of growth,but the observed differences were not significant. Maltose resulted in small amounts of growth compared with other carbon sources,whereas glucose,starch and lactose occupied intermediate positions.

[ii] Ammonium sulphate

If glucose; fructose; starch; maltose and lactose were used as sole carbon sources ,strain 3 produced colony diameters of 66,60,50,47 and 60 mm respectively at 20 C°. Sucrose and glucose were the carbon sources that resulted in maximum growth (66 mm diameter after 4 weeks at 25 and 20 C° respectively),but the differences were not significant (Fig.28 & 29). Maltose produced smaller amounts of growth (46.7 mm), whereas fructose; starch and lactose resulted in intermediate amounts of growth.

[iii] Comparison between sodium nitrate and ammonium sulphate

If sucrose; starch and fructose were used as carbon

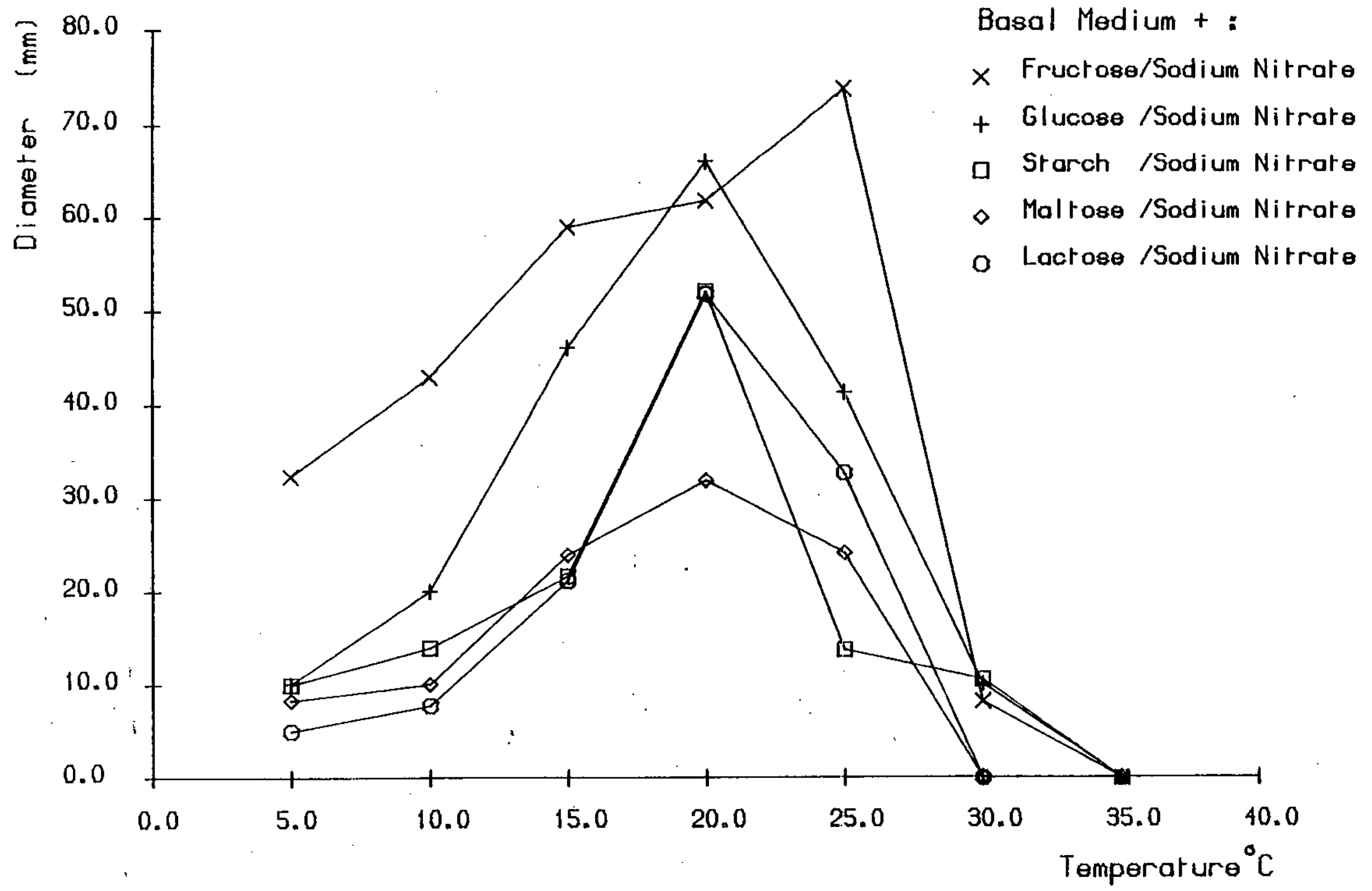

Fig:27 GROWTH / STRAIN3

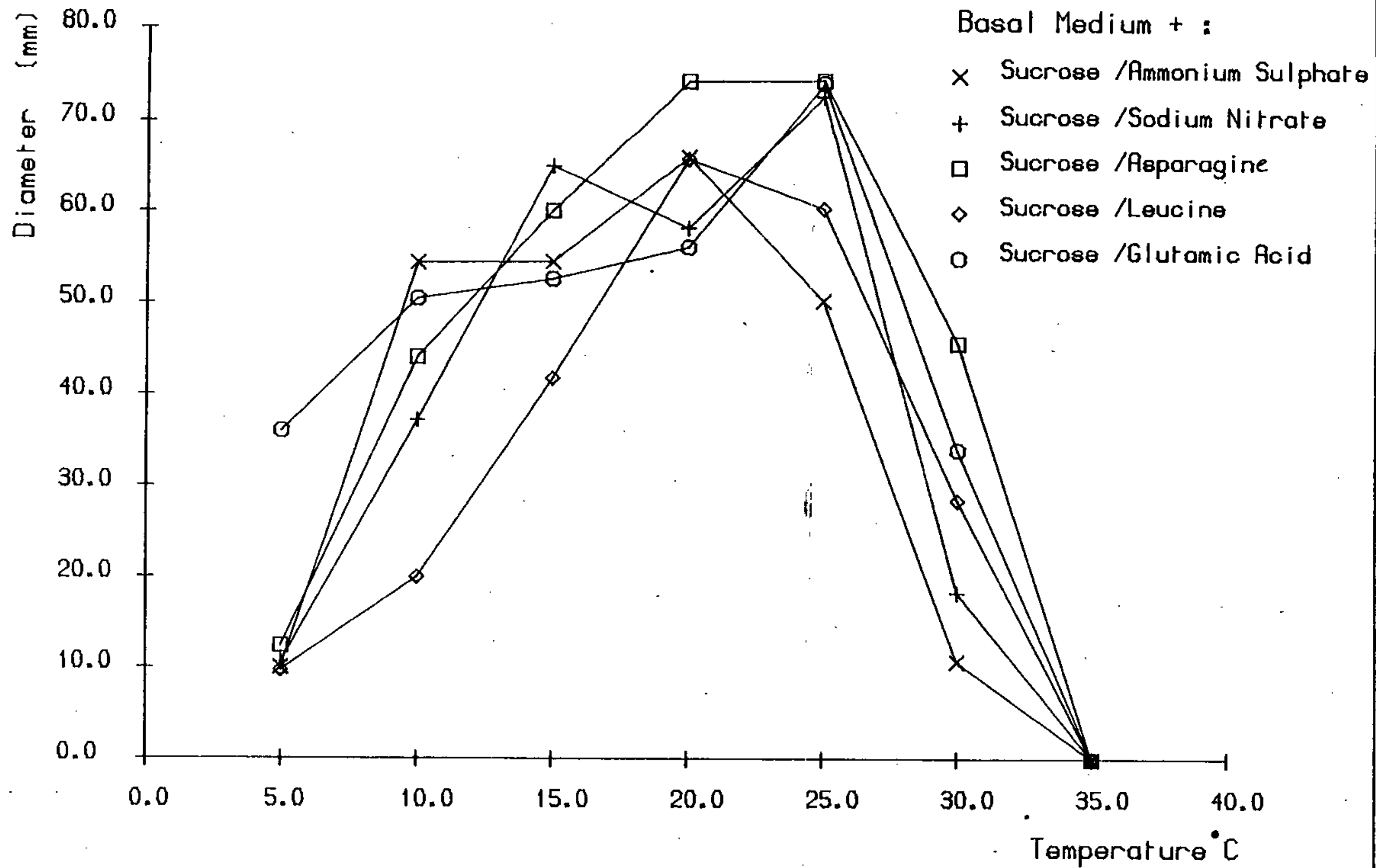

Fig:29 GROWTH / STRAIN3

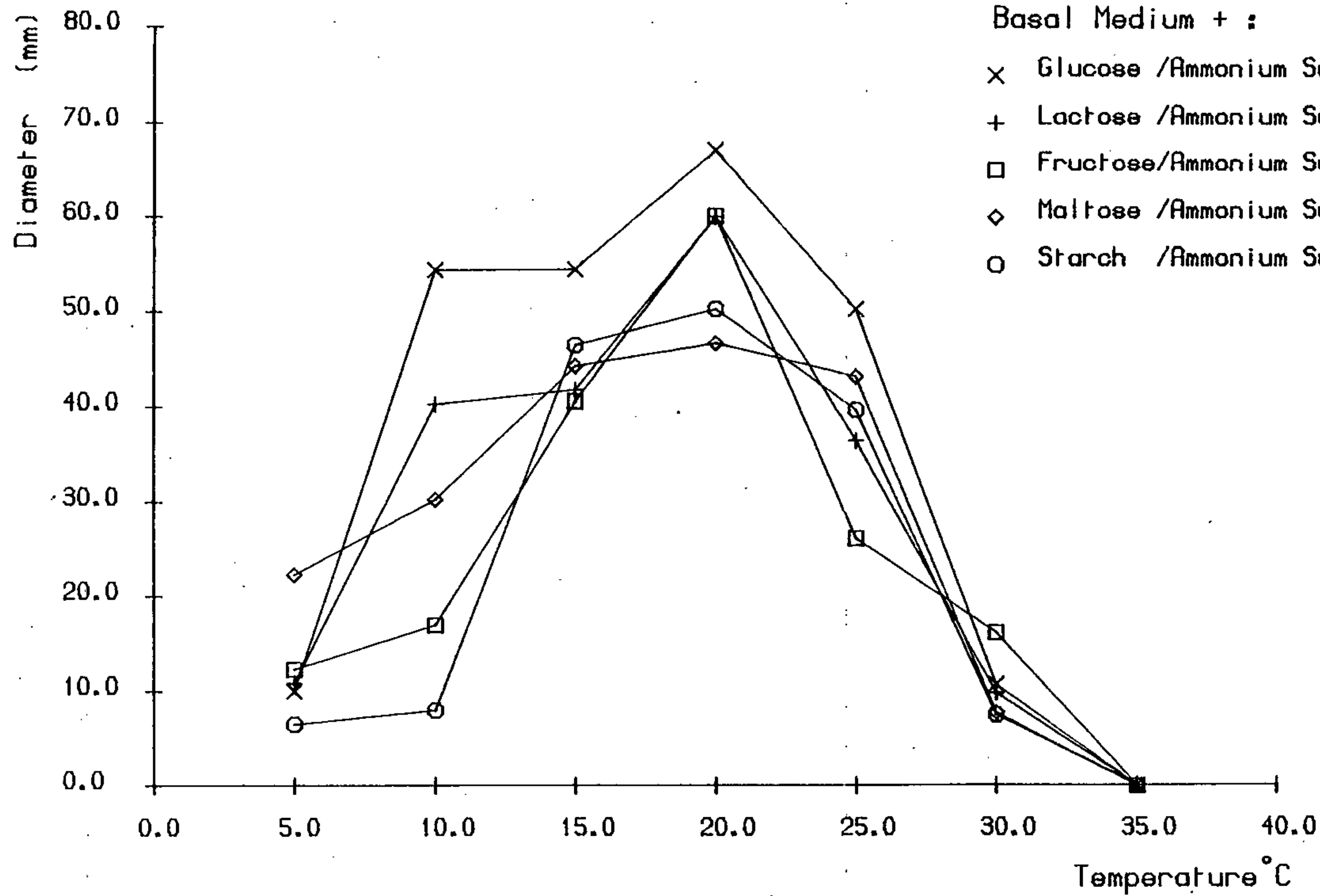

Fig:28 GROWTH / STRAIN3

sources it was evident that sodium nitrate supported far much better growth compared with ammonium sulphate,and the observed differences were significant (Table 50). However,if lactose and maltose were used as carbon sources, ammonium sulphate was a better nitrogen source compared with sodium nitrate (Table 50). If glucose was used as sole carbon source,strain 3 produced similar amounts of growth (66 mm in diameter) when grown on sodium nitrate or ammonium sulphate. Maximum growth was attained on sucrose/sodium nitrate (plus basal medium) (73 mm) and fructose/sodium nitrate (74 mm), but the differences were not significant. Small amounts of growth occurred on maltose irrespective of the nitrogen source used.

6.4.2. Organic nitrogen sources

Asparagine resulted in colony diameters of 74 mm at 20 and 25 C° after 4 weeks (Fig.29). Strain 3 also attained similar growth on glutamic acid and sodium nitrate at 25 C° after 4 weeks. Although the differences in the amount of growth were not significant (Table 51) comparing asparagine, glutamic acid and sodium nitrate ,it is possible that asparagine is a more easily available source of nitrogen compared with glutamic acid and sodium nitrate. Ammonium sulphate resulted in small amounts of growth (63 mm) and leucine (66 mm) resulted in intermediate growth.

6.4.3. Optimum temperature

Depending on the medium used,the temperature optimum for growth fell between 10 and 30 C°.

Table 50: RESULTS OF PAIRED T-TESTS COMPARING GROWTH OF S.sanguinolentum STRAIN 3 AFTER 28 DAYS ON DIFFERENT COMBINATIONS OF NITROGEN AND CARBON SOURCES
BASAL MEDIUM +

CARBON SOURCE	NITROGEN SOURCE	MAX.MEAN DIAMETER (mm)	P
SUCROSE	SODIUM NITRATE	72.7	< 0.001
SUCROSE	AMMONIUM SULPHATE	66.0	
FRUCTOSE	SODIUM NITRATE	73.8	< 0.001
FRUCTOSE	AMMONIUM SULPHATE	60.1	
GLUCOSE	SODIUM NITRATE	65.98	> 0.05
GLUCOSE	AMMONIUM SULPHATE	65.90	
LACTOSE	SODIUM NITRATE	51.8	< 0.001
LACTOSE	AMMONIUM SULPHATE	60.0	
MALTOSE	SODIUM NITRATE	32.0	< 0.001
MALTOSE	AMMONIUM SULPHATE	46.7	
STARCH	SODIUM NITRATE	52.1	< 0.05
STARCH	AMMONIUM SULPHATE	50.3	

Table 51: RESULTS OF PAIRED T-TESTS COMPARING GROWTH OF *S.sanguinolentum* STRAIN 3 AFTER 28 DAYS ON DIFFERENT NITROGEN SOURCES SUCROSE+BASAL MEDIUM +

NITROGEN SOURCE	MAXIMUM MEAN DIAMETER (mm)	PROBABILITY
ASPARAGINE	74.4	< 0.001
LEUCINE	65.8	
ASPARAGINE	74.4	> 0.05
GLUTAMIC ACID	74.1	
ASPARAGINE	74.4	> 0.01
SODIUM NITRATE	72.7	
ASPARAGINE	74.4	< 0.001
AMMONIUM SULPHATE	63.0	
LEUCINE	65.8	< 0.001
SODIUM NITRATE	72.7	
LEUCINE	65.8	> 0.001
AMMONIUM SULPHATE	63.0	
GLUTAMIC ACID	74.1	< 0.05
SODIUM NITRATE	72.7	
GLUTAMIC ACID	74.1	< 0.001
AMMONIUM SULPHATE	63.0	
GLUTAMIC ACID	74.1	< 0.001
LEUCINE	65.8	
SODIUM NITRATE	72.7	< 0.001
AMMONIUM SULPHATE	63.0	

Strain 4

6.1. 3% MEA

Strain 4 was one of the most vigorously growing strains and had a characteristic dense white aerial hyphae (Plate 12). Unlike other strains it rarely produces honey-like liquid drops on 3% MEA. Colonies attained 74 mm in diameter at 10,15 and 20 C° and 73 mm diameter at 25 C° after 4,2,2 & 2 weeks respectively (Fig. 30).

6.2. 3% CMA

The mycelium was mainly submerged with a few scattered aerial hyphae. Colonies remained white throughout the course of experiments. Colonies attained 74 mm diameter at 15 C° and 20 C° after 4 weeks (Fig.30)

6.3. Spruce sawdust/agar

The medium was stained red within 2 weeks. The intensity of staining was directly related to the amount of growth being pronounced where maximum growth occurred. Colonies attained 74 mm diameter at 20 and 25 C° after 3 weeks (Fig.30). Hyphae were highly scattered and the mycelium was basically submerged.

CDA

Colonies were often coloured after 4 to 5 weeks and the colour ranged from pale yellow through reddish brown to deep brown. Colony margin was rarely uniform and it was spider-web shaped. The mycelium was mainly submerged with a few aerial hyphae.

6.4. Utilization of different carbon sources in combination with

6.4.1. Inorganic nitrogen sources

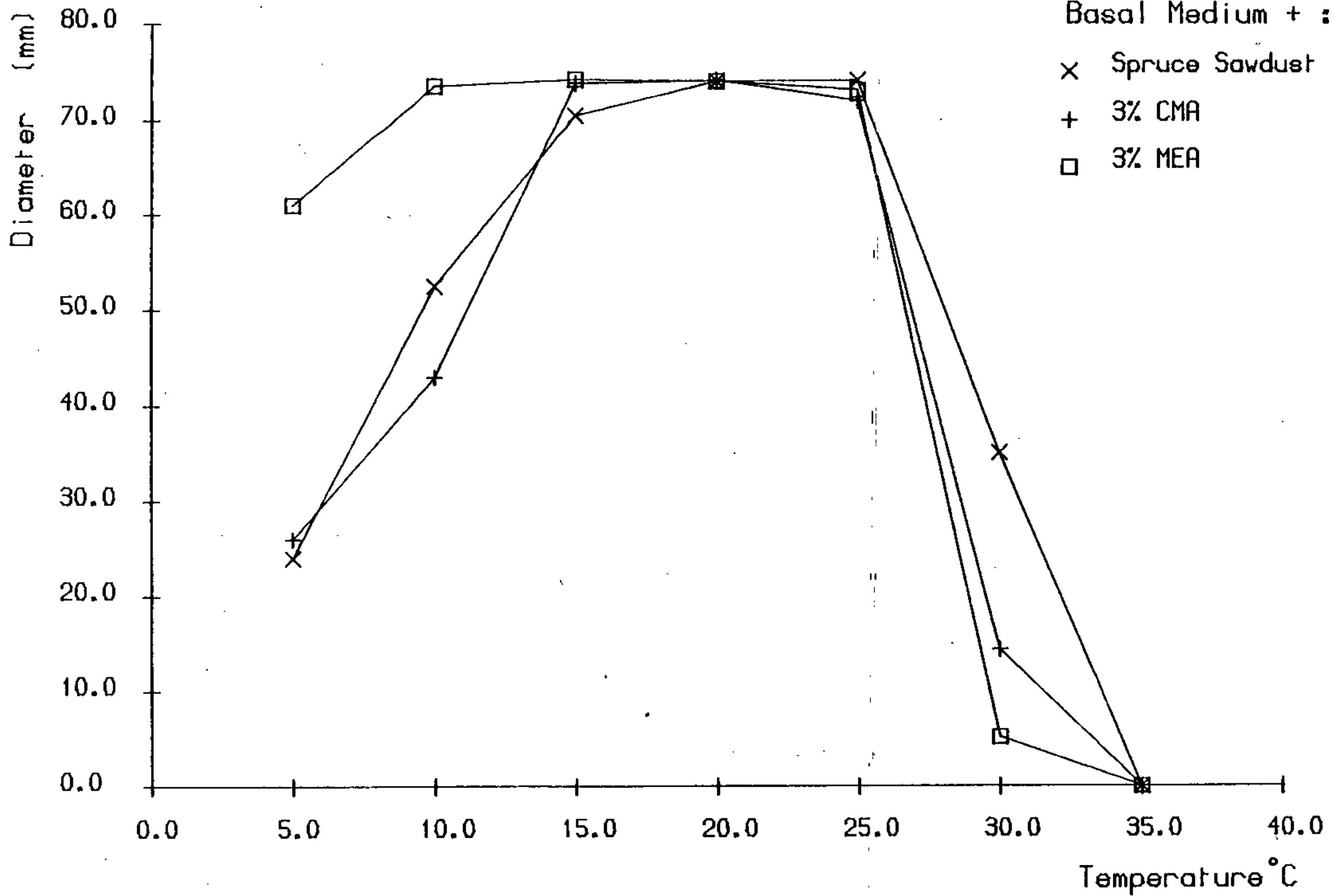

Fig:30 GROWTH / STRAIN4

[i] **Sodium nitrate**

Sucrose; glucose; lactose and maltose as sole carbon sources resulted in colonies of 73,60, 50 and 53 mm diameter at 25 C° after 4 weeks respectively (Fig.31 & 33). ,whereas fructose and starch produced colonies of only 36 mm at 15 and 20 C°; and 30 C° respectively after 4 weeks . Sucrose was the most readily available carbon source,and fructose and starch produced smaller amounts of growth,but the observed differences were not significant (Table 52).

[ii] **Ammonium sulphate**

Using ammonium sulphate as sole nitrogen source,lactose,starch and fructose resulted in colonies of 74,50 and 32 mm diameter after 4 weeks at 25 C° respectively,whereas glucose and sucrose produced colonies of 32 and 50 mm after 4 weeks at 15 and 30 C° respectively (Fig. 32 and 33). Lactose was the most easily utilised carbon source;glucose and fructose produced relatively smaller amounts of growth,but the observed differences were not significant. Starch,sucrose and maltose resulted in intermediate amounts of growth.

[iii] **Comparison between sodium nitrate and ammonium sulphate**

Most carbon sources supported much better growth if combined with sodium nitrate compared with ammonium sulphate,the exceptions being lactose and starch (Table 52). Both lactose and starch resulted in faster growth if the sole nitrogen source was ammonium sulphate compared with sodium nitrate. Table 52 shows that maximum growth occurred on lactose/ ammonium sulphate (plus the basal

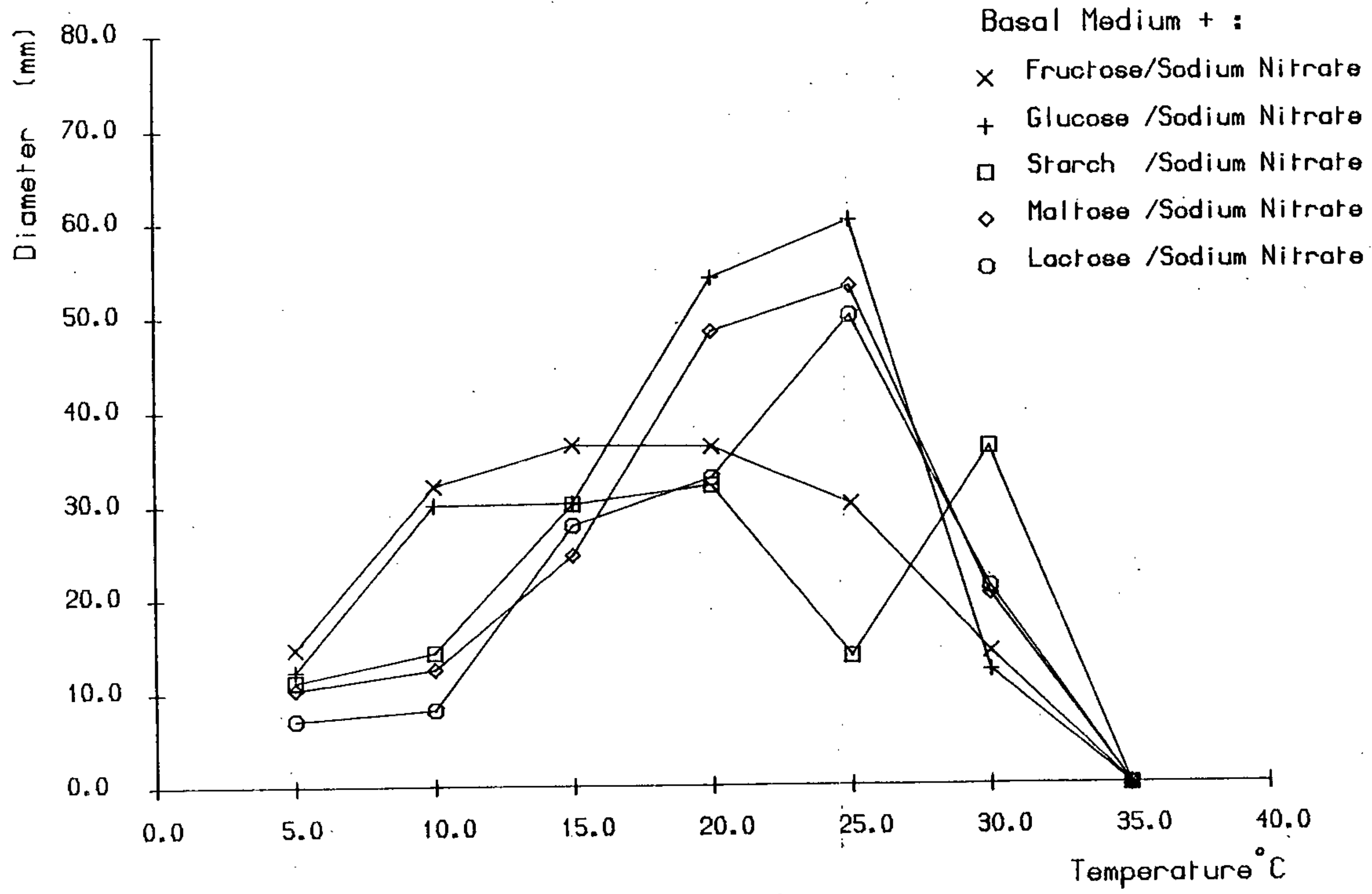

Fig:31 GROWTH / STRAIN4

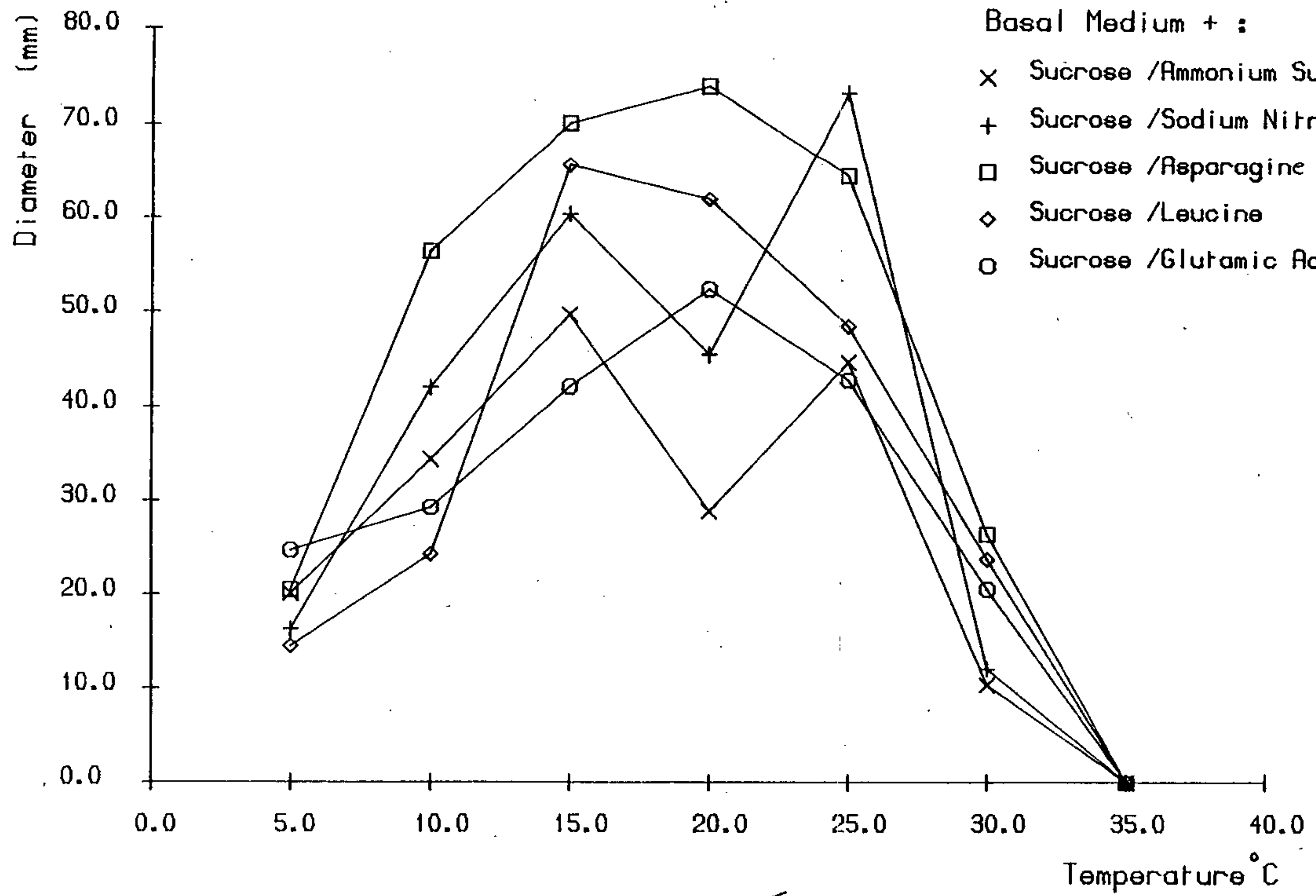

Fig:33 GROWTH / STRAIN4

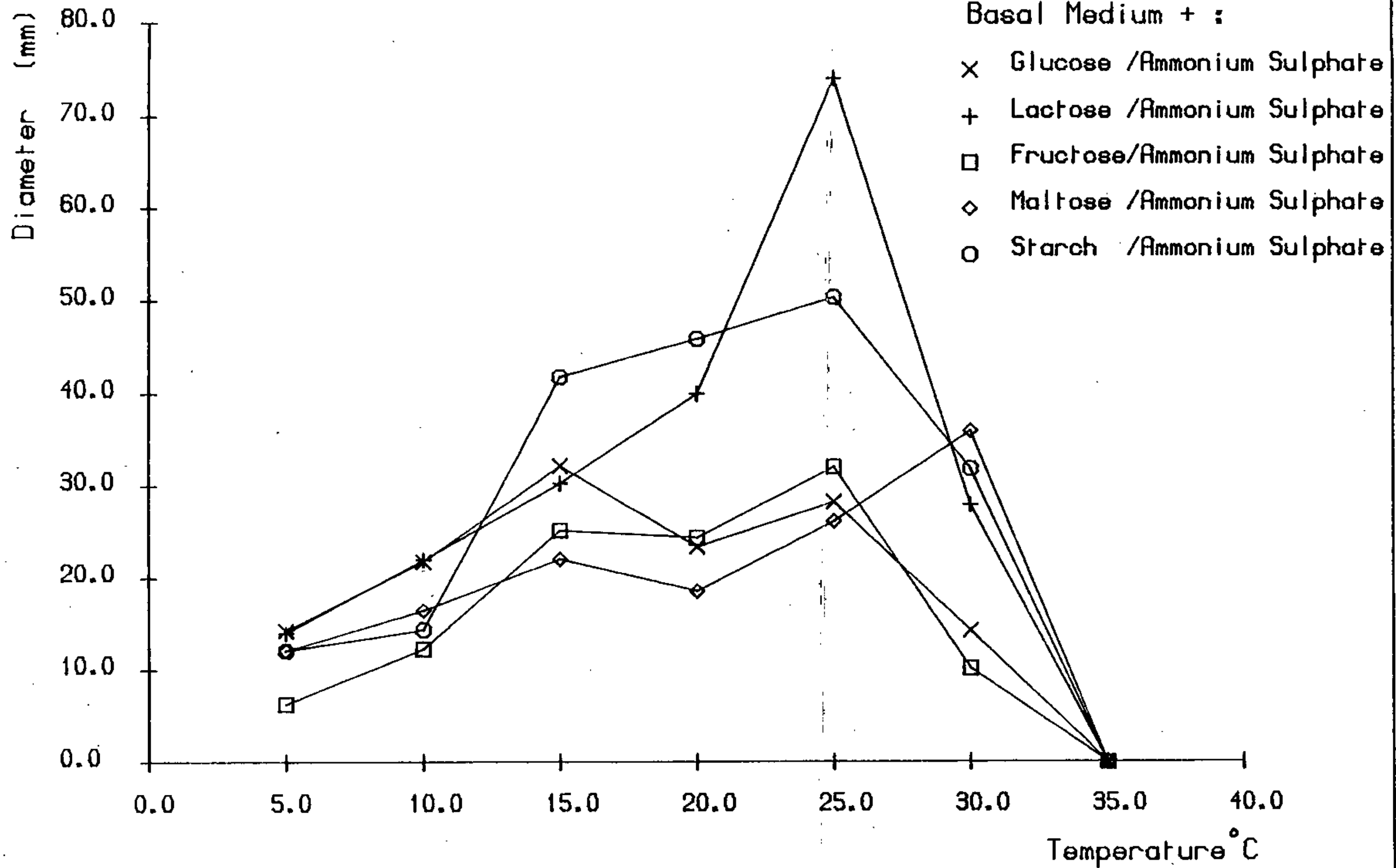

Fig:32 GROWTH / STRAIN4

Table 52: RESULTS OF PAIRED T-TESTS ON GROWTH OF S.sanguinolentum STRAIN 4 AFTER 28 DAYS ON DIFFERENT COMBINATIONS OF CARBON AND NITROGEN SOURCES
BASAL MEDIUM +

CARBON SOURCE	NITROGEN SOURCE	MAX.MEAN DIAMETER (mm)	PROBABILITY
SUCROSE	SODIUM NITRATE	73.3	< 0.001
SUCROSE	AMMONIUM SULPHATE	49.7	
GLUCOSE	SODIUM NITRATE	60.2	< 0.001
GLUCOSE	AMMONIUM SULPHATE	32.2	
FRUCTOSE	SODIUM NITRATE	36.4	> 0.001
FRUCTOSE	AMMONIUM SULPHATE	32.1	
LACTOSE	SODIUM NITRATE	50.1	< 0.001
LACTOSE	AMMONIUM SULPHATE	74.1	
MALTOSE	SODIUM NITRATE	53.2	< 0.001
MALTOSE	AMMONIUM SULPHATE	36.0	
STARCH	SODIUM NITRATE	36.1	< 0.001
STARCH	AMMONIUM SULPHATE	50.4	

medium) and sucrose/sodium nitrate,but the observed differences were not significant. Smaller amounts of growth occurred if glucose and fructose were used in individual combination with ammonium sulphate ,but differences were not significant. Other carbon/nitrogen sources supported intermediate growth.

[iv] Effect of carbon and nitrogen sources on optimum growth

Optimum temperature for growth differed with the different carbon and nitrogen sources available the exception being lactose where optimum temperature remained the same(25 C°) irrespective of the nitrogen source used. Optimum temperature dropped from 25 to 15 C° if sucrose and glucose were combined with sodium nitrate and ammonium sulphate respectively. Also optimum temperature dropped from 30 to 25 C° if starch was used in combination with sodium nitrate instead of ammonium sulphate respectively. On the other hand, optimum temperature increased from 15C° (sucrose/ammonium sulphate) to 25 C° (sucrose/sodium nitrate). Further the use of ammonium sulphate increased the optimum temperature from 25C° for maltose/sodium nitrate to 30 C°.

6.4.2. Organic nitrogen sources

Of the organic nitrogen sources studied asparagine resulted in best growth (74 mm diameter) after 3 weeks at 20 C°, compared with leucine and glutamic acid which produced colonies of 66 and 52 mm diameter after 4 weeks at 15 and 20 C° respectively (Fig. 33). The observed differences were significant (Table 53). Asparagine and

Table 53:RESULTS OF PAIRED T-TESTS ON GROWTH OF *S.sanguinolentum* STRAIN 4 AFTER 28 DAYS ON DIFFERENT NITROGEN SOURCES SUCROSE + BASAL MEDIUM +

NITROGEN SOURCE	MAX.MEAN DIAMETER (mm)	PROBABILITY
ASPARAGINE	74.0	< 0.001
LEUCINE	65.6	
ASPARAGINE	74.0	< 0.001
GLUTAMIC ACID	52.4	
ASPARAGINE	74.0	> 0.05
SODIUM NITRATE	73.3	
ASPARAGINE	74.0	< 0.001
AMMONIUM SULPHATE	49.7	
LEUCINE	65.6	< 0.001
SODIUM NITRATE	73.3	
LEUCINE	65.6	< 0.001
AMMONIUM SULPHATE	49.7	
GLUTAMIC ACID	52.4	< 0.001
SODIUM NITRATE	73.3	
GLUTAMIC ACID	52.4	< 0.01
AMMONIUM SULPHATE	49.7	
GLUTAMIC ACID	52.4	< 0.001
LEUCINE	65.6	
SODIUM NITRATE	73.3	< 0.001
AMMONIUM SULPHATE	49.7	

sodium nitrate produced similar amounts of growth ,but the difference was not significant (Table 53). However, cultures supplied with asparagine were most vigorously growing compared with sodium nitrate and further,strain 4 produced colonies 74 mm diameter after only 3 weeks where asparagine was the sole nitrogen source compared with sodium nitrate (4 weeks). Ammonium sulphate produced smaller amounts of growth (50 mm) after 4 weeks at 15 C° compared with other nitrogen sources.

6.4.3. Optimum temperature

Optimum temperature for growth was dependent on the medium used and fell between 10 and 30 C° .

6.4.4. Effect of pH *

All strains were grown on 3% MEA at pH's 4; 5; 6; 6.5; 7; 7.5 and 8 and incubated at 25 C° (chapter 3).

[i] Strain 1

The optimium pH's for growth using strain 1 were pH4 and pH5. At these hydrogen ion concentrations colonies of 51 mm diameter were produced after 4 weeks incubation at 25 C° (Fig. 34). Beyond pH6 the amount of growth declined gradually with the increasing pH and growth was minimum at pH 8 (32 mm after 4 weeks incubation at 25 C°).

[ii] Strain 2

Colonies of 74 mm diameter were produced at pH's 4-7 after 2-4 weeks (Fig. 35). Growth declined beyond pH7 being minimum at pH 8 (19 mm diameter after 4 weeks incubation at 25 C°).

[iii] Strain 3

* Note that the size of the petri dish was limited therefore it was not possible to determine precisely the limits of the optimum temperature and pH for growth in this chapter.

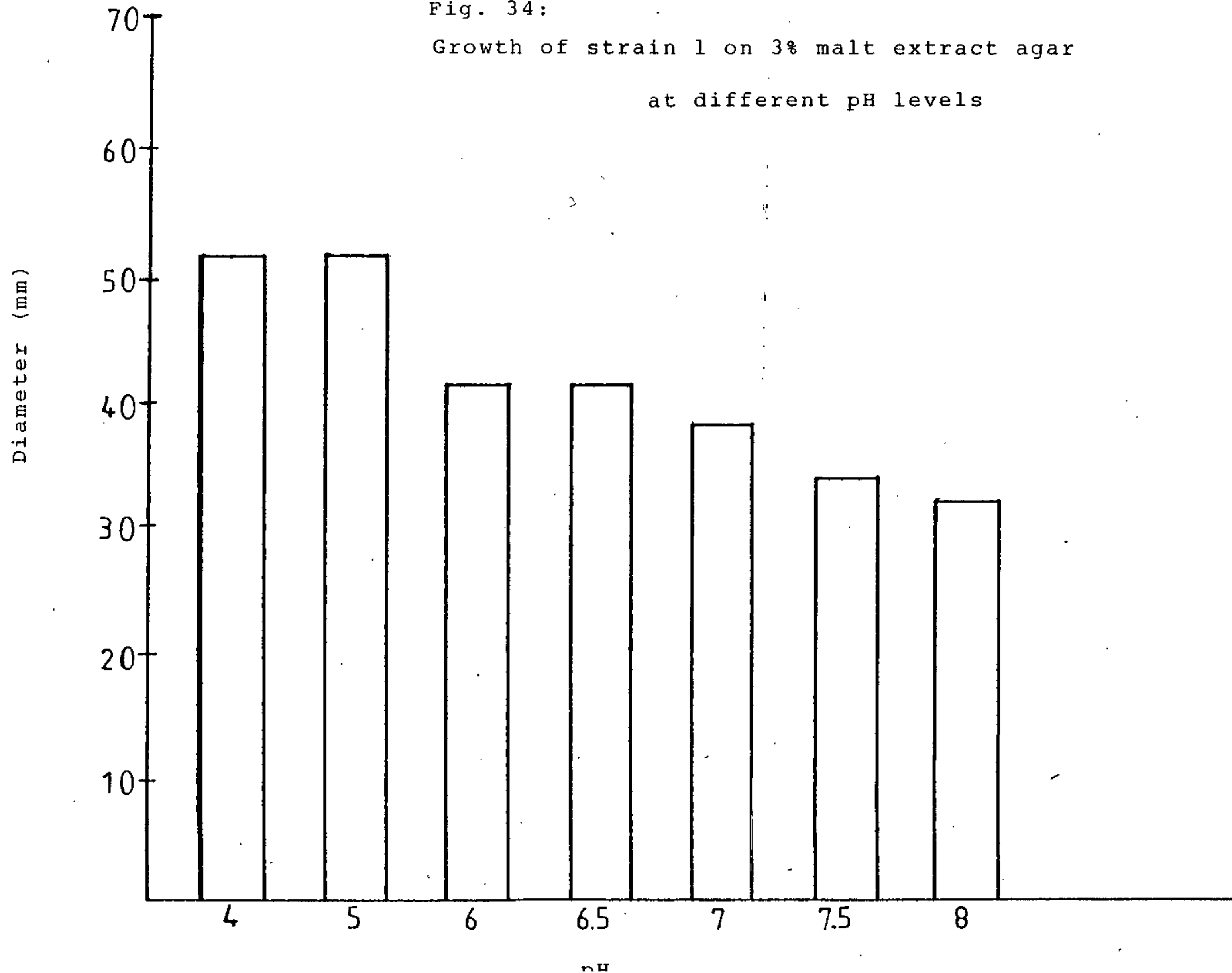

Fig. 34:
Growth of strain 1 on 3% malt extract agar
at different pH levels

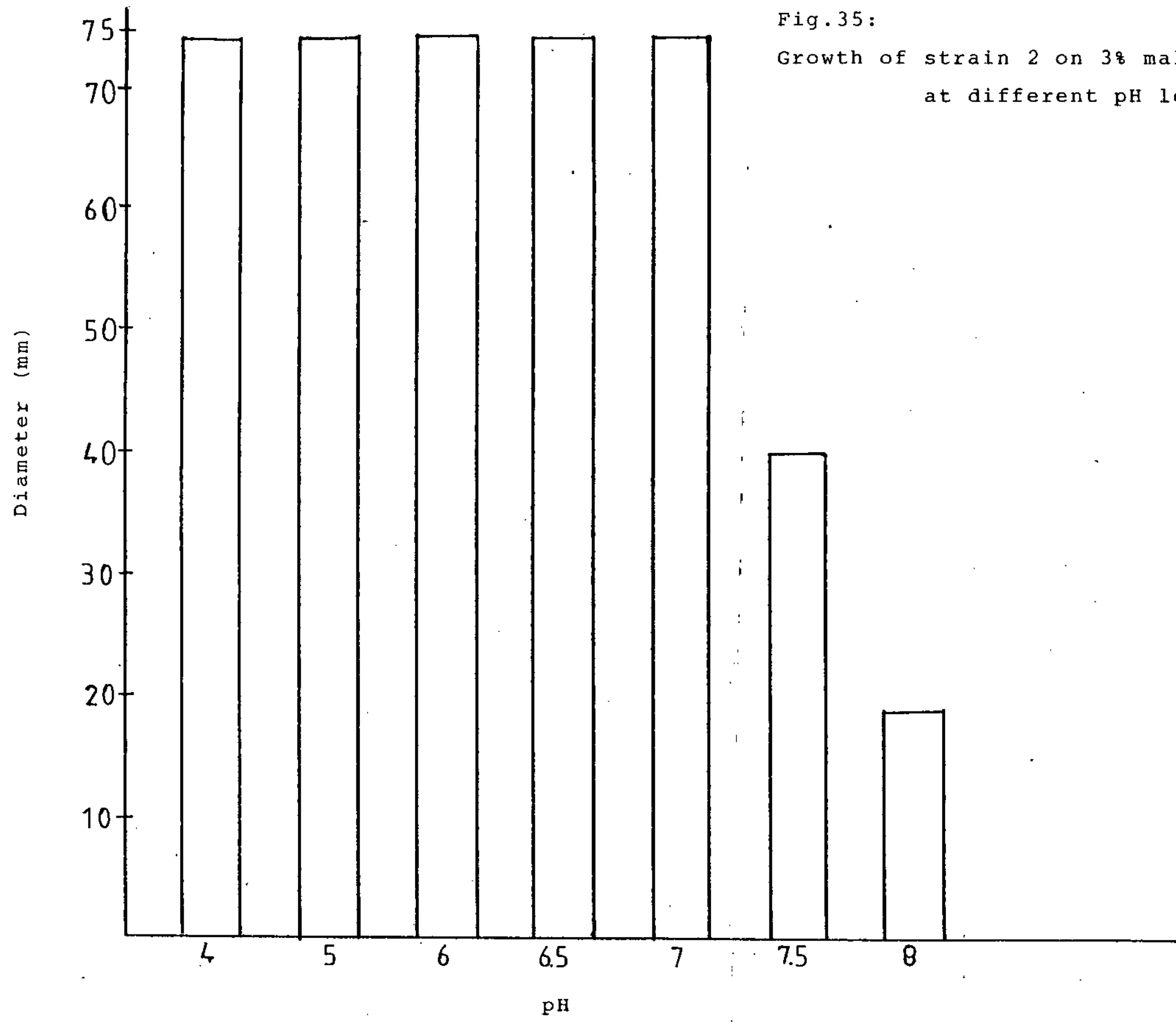

Fig.35:
Growth of strain 2 on 3% malt extract agar at different pH levels

Generally,growth was much better on acidic media and declined dramatically with increasing pH. Colonies of 74 mm diameter were produced at pH's from 4 to 6.5 after 2,2,2 and 3 weeks at 25 C° respectively. Beyond pH6.5 growth declined dramatically being minimum (21 mm diameter) at pH 8 (Fig.36).

[iv] **Strain 4**

Strain 4 showed much better growth over a a wider range of hydrogen ion concentrations. Strain 4 produced Colonies of 74 mm diameter after 2,2,2,2 and 4 weeks at 25 C° at pH's between 4 and 7 (Fig.37). Although the amount of growth had declined at pH's 7.5 and 8 , however,growth of strain4 was good compared with growth of the other strains at these hydrogen ion concentrations.

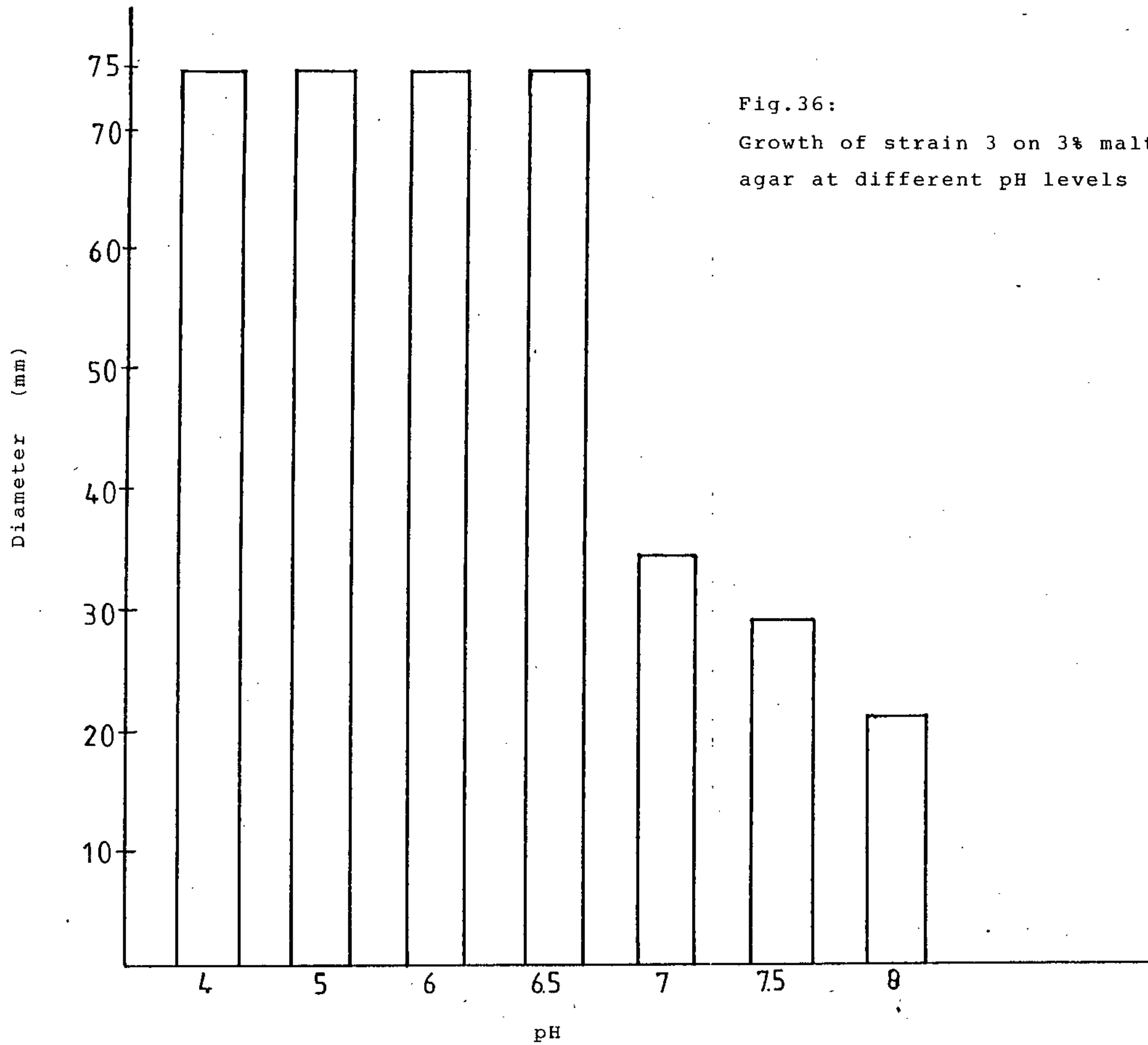

Fig.36:
Growth of strain 3 on 3% malt extract agar at different pH levels

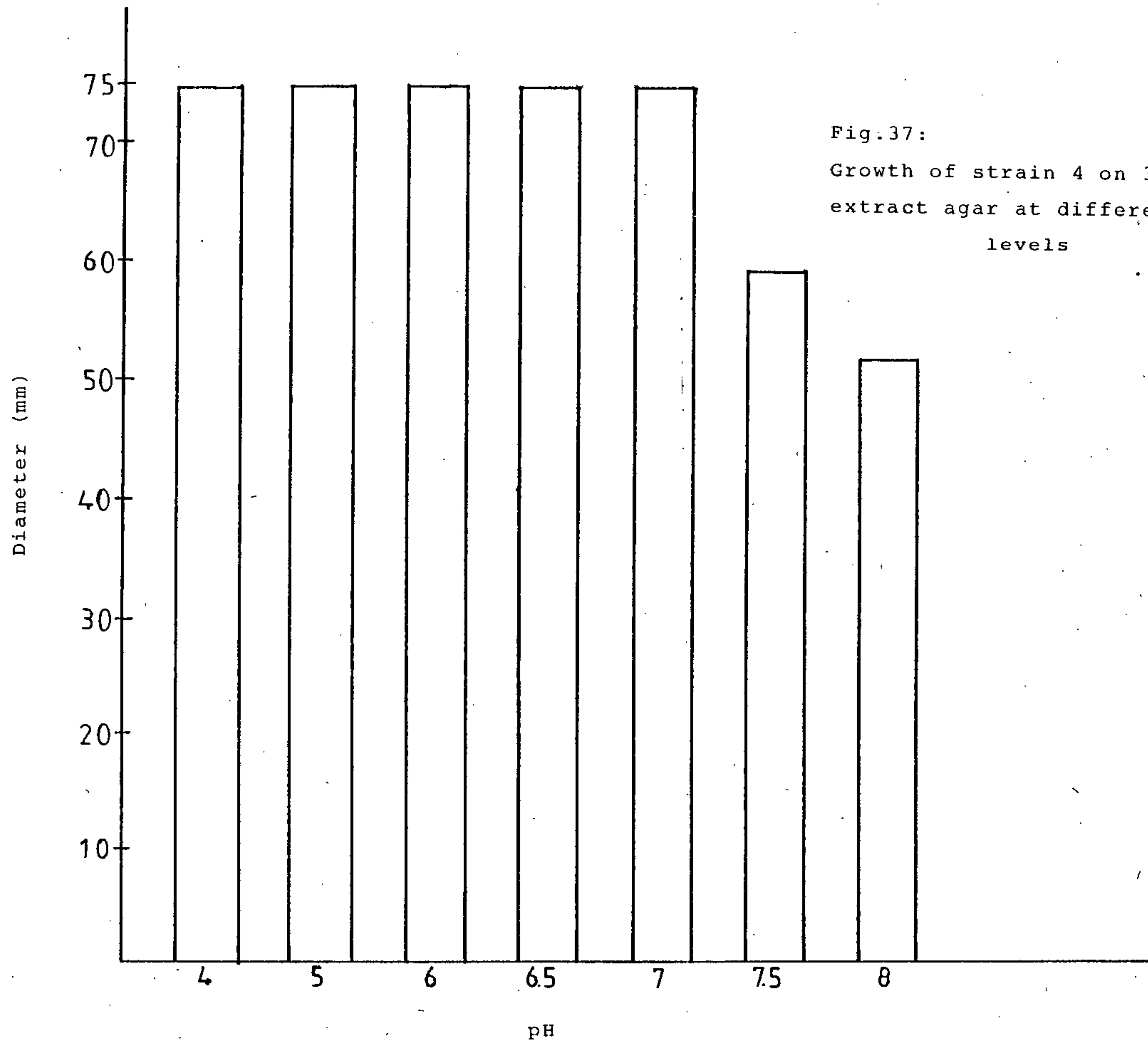

Fig.37:
Growth of strain 4 on 3% malt extract agar at different pH levels

CHAPTER 7

7. CELLULOSE AND LIGNIN DECOMPOSITION

7.1. Introduction

a. Chemical structure of wood

All timbers contain 3 major constituents namely cellulose ; hemicellulose and lignin (Anderson,1962). The first two are collectively known as holocellulose. These cell-wall substances are complicated polymers,the chemical constitution of which is still not completely known. Cellulose is a linear polymer of D-glucose,occurring in long chain molecules of condensed glucose residues. About 25% of dry wood comprises lignin which fills the intercellular spaces (Freudenberg,1965). Lignin has a high molecular weight and is structurally complicated (Freudenberg,1968, and Kirk,1971). The structure of lignin is not completely known but the basic unit that forms the polymer is phenylpropanoid. Lignin is found in the middle lamella,cell-walls of xylem vessels and fibres that strengthen the plant. Lignin content in mature woody tissues varies from 15 to 38% and is second only to cellulose. Other cell-wall constituents are cytoplasmic and reserve food materials,inorganic matter and a wide range of extractives deposited in the walls and lumens of cells during the formation of heartwood (Scheffer and Cowling ,1966).

b. Action of decay fungi on wood constituents

The ultimate effect of decay fungi on wood is a chemical disintegration of the various wood substances: 'It would thus appear to be feasible that (if) decay could be

considered in terms of its ultimate chemical effect on wood substance.'(Campbell,1932b). Wood polysaccharides e.g cellulose, hemicellulose and lignin have large molecules that can not pass the cell-walls of microorganisms. Therefore,these complex substances must be broken down into simpler soluble forms in order to be absorbed and assimilated. The breaking-down of these substances occurs by hydrolysis and is achieved by the action of enzymes (Campbell, 1930,1932a,Cartwright and Findlay,1946 and Brock,1974).

c. Differences between white and brown rot fungi

Boyce (1961) noted that either lignin or cellulose are preferentially attacked or both depending on the species of the fungus.

Two types of wood rot have been distinguished:

WHITE ROTS

These are caused by fungi that mainly decompose lignin at an early stage and later attack cellulose.

There is a general rule that the white rot fungi degrade lignin first and later attack cellulose as well (Campbell,1932A; Liese,1970; Highley and Kirk,1973 and 1979). As early as 1932,Campbell noted that white rot fungi could be further subdivided into:

1. White rots in which lignin and pentosans are attacked in the early stages, whereas cellulose is attacked at a late stage.

2. White rots that degrade cellulose and the associated pentosans first and the attack on lignin is

delayed.

3. White rots that attack both cellulose and lignin at the same time but in varying proportions.

This classification was on the basis of the primary attack,but at the advanced stages of decay a fungus in any of these groups might react in a way similar to another group. Thus,similarity was merely based on the early chemical actions on wood.

BROWN ROTS

This type is caused by those fungi that attack cellulose and hemicellulose at an early stage leaving lignin almost unaffected (Anderson,1962).

Scheffer and Cowling (1966) divided microorganisms that attack woody tissues into:

1. Wood destroying fungi that deteriorate cell-wall material and hence reduce wood strength.

2. Staining fungi and moulds that utilize soluble simple substances of the xylem and parenchyma cells.

3. Bacteria

Generally,very few studies have been made on the rate of degradation of cellulose,hemicellulose and lignin as a result of attack by white and brown rot fungi. This is particularly true in the case of conifers (Kirk,1973).

Results

In order to follow the progress of decay in naturally and artificially infected trees samples of timber were taken and analysed for lipid,cellulose and lignin content as described on pages69-74. The results are presented below.

7.2. Analysis of artificially inoculated standing trees

[i] Initial lipid,cellulose and lignin content

There was no significant variation in initial lipid content when comparing dominants,codominants and subdominants (Table 54). The initial cellulose content was significantly ($P < 0.05$) different in dominants,codominants and subdominants(Table 54). Mean % cellulose content was at a minimum in dominants (73.7%) and at a maximum in codominants (77.8%) and occupying an intermediate position in subdominants (76.7%). The difference in the initial cellulose content between codominants and subdominants was not significant (Table 55). On the other hand, initial lignin content was significantly different ($P < 0.001$) in different trees (Table 54). Mean % lignin content was maximum in dominants (23.2%) and minimum in codominants (18.9%) with subdominants (20.3%) again intermediate. It is important to note that the increase in percent cellulose was a relative one,being directly related to the corresponding decrease in lignin content. This situation applies to all further calculations of cellulose and lignin content in this chapter. The difference in the initial lignin content in codominants and subdominants was not significant (Table 55). Tables 56A,56B and 56C summarise data on the contents of lipid,cellulose and lignin in control and infected samples.

[ii] Effect of <u>S.sanguinolentum</u> six months after inoculation

6 months after inoculation no significant change had occurred in the lipid content. Prior to inoculation mean

Table 54: RESULTS OF ANALYSIS OF VARIANCE COMPARING INITIAL LIPID, CELLULOSE AND LIGNIN CONCENTRATIONS OF NORWAY SPRUCE BEFORE AND AFTER SIX MONTHS OF INOCULATION WITH <u>S.sanguinolentum</u> IN DIFFERENT TREE VIGOUR CLASSES

SOURCE OF VARIATION	DF	SS	MSS	F RATIO	PROBABILITY
%LIPID IN CONTROLS					
TREATMENT	2	2.8	1.4		
ERROR	51	134.4	2.6		
TOTAL	53	137.2		0.534	> 0.05
%CELLULOSE IN CONTROLS					
	2	153.5	76.8		
	51	782.3	15.3		
	53	935.8		5.0	< 0.05
%LIGNIN IN CONTROLS					
	2	175.3	87.6		
	51	492.9	9.7		
	53	668.2			
				9.0	< 0.001
MEAN % INCREASE IN CELLULOSE					
	2	203.7	101.9		
	33	540.8	16.4		
	35	744.5		6.2	> 0.01
MEAN % REDUCTION IN LIGNIN					
	2	290.7	145.3		
	33	420.1	12.7		
	35	710.8		11.4	< 0.001

Table 55:RESULTS OF T-TESTS COMPARING INITIAL CELLULOSE AND LIGNIN CONCENTRATIONS OF NORWAY SPRUCE TIMBER BEFORE AND AFTER INOCULATION WITH *S.sanguinolentum* FOR SIX MONTHS

GROUP 1	GROUP 2	MEAN 1	MEAN 2	T-VALUE	PROBABILITY
%CELLULOSE IN CONTROLS					
DOMINANT	CODOMINANT	73.7	77.8	3.1	< 0.01
DOMINANT	SUBDOMIN.	73.7	76.7	2.2	< 0.05
COD.	SUB.	77.8	76.7	0.9	> 0.05
%LIGNIN IN CONTROLS					
DOM.	COD.	23.2	18.9	4.2	< 0.001
DOM.	SUB.	23.2	20.3	2.8	< 0.01
COD.	SUB.	18.9	20.3	1.4	> 0.05
%REDUCTION IN LIGNIN					
DOM.	COD.	9.6	3.9	3.9	< 0.01
DOM.	SUB.	9.6	3.3	4.3	< 0.01
COD.	SUB.	3.9	3.3	0.5	> 0.05
%INCREASE IN CELLULOSE					
DOM.	COD.	8.8	4.5	2.7	< 0.05
DOM.	SUB.	8.8	3.4	3.3	< 0.01
COD.	SUB.	4.5	3.4	0.7	> 0.05

Table 56A:
DATA ON LIPID,CELLULOSE AND LIGNIN CONCENTRATIONS IN NORWAY SPRUCE BEFORE AND AFTER 6 MONTHS OF FIELD INOCULATION WITH S.sanguinolentum
SAMPLES 2;5;8;& 10 WERE INFECTED AND
THE REST WERE CONTROL SAMPLES

SAMPLE	LIPID		LIGNIN		CELLULOSE	
NO.	gm	%	gm	%	gm	%
			DOMINANT			
8G1	0.2	4	1.1	22	3.7	74
2	0.5	10	0.6	12	3.9	78
3	0.2	4	1.1	22	3.7	74
4	0.1	2	1.0	20	3.9	78
5	0.1	2	0.8	16	4.1	82
6	0.2	4	0.9	18	3.9	78
7	0.1	2	1.2	24	3.7	74
8	0.1	2	0.9	18	4.0	80
9	0.3	6	1.6	32	3.1	62
10	0.1	2	0.6	12	4.3	86
24G1	0.1	2	1.1	22	3.8	76
2	0.1	2	0.7	14	4.2	84
3	0.1	2	1.0	20	3.9	78
4	0.1	2	1.0	20	3.9	78
5	0.3	6	0.6	12	4.1	82
6	0.2	4	1.0	20	4.3	86
7	0.2	4	1.3	26	3.5	70
8	0.2	4	0.5	10	4.3	86
9	0.2	4	1.4	28	3.4	68
10	0.2	4	0.6	12	4.2	84
5G1	0.1	2	1.2	24	3.7	74
2	0.2	4	0.9	18	3.9	78
3	0.1	2	1.3	26	3.6	72
4	0.2	4	1.0	20	3.8	76
5	0.1	2	0.8	16	4.1	82
6	0.3	6	1.0	20	3.7	74
7	0.3	6	1.2	24	3.5	70
8	0.1	2	1.0	20	3.9	78
9	0.2	4	1.5	30	3.3	66
10	0.4	8	0.8	16	3.8	76

Table 56B: DATA ON LIPID,CELLULOSE AND LIGNIN CONCENTRATIONS IN NORWAY SPRUCE BEFORE AND AFTER 6 MONTHS OF FIELD INOCULATION WITH S.sanguinolentum ,SAMPLES 2;5;8 AND 10 WERE INFECTED AND THE REST WERE CONTROL SAMPLES

SAMPLE	LIPID		LIGNIN		CELLULOSE	
NO.	gm	%	gm	%	gm	%
			CODOMINANT			
3B1	0.1	2	0.8	16	4.1	82
2	0.2	4	0.7	14	4.1	82
3	0.1	2	1.0	20	3.9	78
4	0.1	2	1.0	20	3.9	78
5	0.1	2	0.9	18	4.0	80
6	0.1	2	0.9	18	4.0	80
7	0.1	2	1.1	22	3.8	76
8	0.1	2	0.7	14	4.2	84
9	0.4	8	1.1	22	3.5	70
10	0.1	2	0.8	16	4.1	82
17B1	0.1	2	0.9	18	4.0	80
2	0.1	2	0.9	18	4.0	80
3	0.3	6	0.7	14	4.0	80
4	0.2	4	0.9	18	3.9	78
5	0.1	2	0.9	18	4.0	80
6	0.2	4	0.7	14	4.1	82
7	0.2	4	0.7	14	4.1	82
8	0.1	2	0.8	16	4.1	82
9	0.1	2	0.9	18	4.0	80
10	0.2	4	0.5	10	4.3	86
20B1	0.2	4	1.1	22	3.7	74
2	0.1	2	0.8	16	4.1	82
3	0.1	2	1.0	20	3.9	78
4	0.1	2	1.0	20	3.9	78
5	0.2	4	0.6	12	4.2	84
6	0.3	6	1.1	22	3.6	72
7	0.1	2	0.9	18	4.0	80
8	0.2	4	0.7	14	4.1	82
9	0.2	4	1.2	24	3.6	72
10	0.2	4	0.8	16	4.0	80

Table 56C: DATA ON LIPID,CELLULOSE AND LIGNIN CONCENTRATIONS IN NORWAY SPRUCE BEFORE AND AFTER 6 MONTHS OF FIELD INOCULATION WITH S.sanguinolentum, SAMPLES 2;5;8 AND 10 WERE INFECTED AND THE REST WERE CONTROL SAMPLES

SAMPLE NO.	LIPID gm	LIPID %	LIGNIN gm	LIGNIN %	CELLULOSE gm	CELLULOSE %
			SUBDOMINANT			
10R1	0.3	6	0.9	18	3.8	76
2	0.1	2	0.9	18	4.0	80
3	0.1	2	1.0	20	3.9	78
4	0.1	2	1.0	20	3.9	78
5	0.3	6	1.0	20	3.7	74
6	0.1	2	1.1	22	3.8	76
7	0.1	2	1.1	22	3.8	76
8	0.1	2	0.9	18	4.0	80
9	0.2	4	0.8	16	4.0	80
10	0.2	4	0.8	16	4.0	80
5R1	0.2	4	1.0	20	3.8	76
2	0.2	4	0.8	16	4.0	80
3	0.1	2	0.9	18	4.0	80
4	0.1	2	1.0	20	3.9	78
5	0.1	2	0.9	18	4.0	80
6	0.1	2	1.1	22	3.8	76
7	0.3	6	1.1	22	3.6	72
8	0.2	4	0.9	18	3.9	78
9	0.1	2	1.1	22	3.8	76
10	0.1	2	0.9	18	4.0	80
20R1	0.3	6	0.8	16	3.9	78
2	0.3	6	0.6	12	4.1	82
3	0.2	4	1.0	20	3.8	76
4	0.1	2	1.1	22	3.8	76
5	0.2	4	0.9	18	3.9	78
6	0.1	2	1.0	20	3.9	78
7	0.1	2	1.2	24	3.7	74
8	0.1	2	1.0	20	3.9	78
9	0.1	2	1.1	22	3.8	76
10	0.1	2	0.8	16	4.1	82

lipid content was 3.6% ,but had changed in the 6 months period following inoculation to 4% ,but the difference was not significant (Table 57). There was a significant (P < 0.001) reduction in lignin content in dominants (Table 57). Mean % lignin fell from the initial level of 23.2% to 14.7% 6 months after inoculation. The same trend was observed in codominants where the mean % lignin fell from 18.9% to 15.2% (Table 58). In the subdominants there was also a significant (P < 0.01) (Table 57) change in percent lignin.This was reduced from an initial 20.3% to 17.3% (Table 58). A significant (P< 0.001) increase in percent cellulose occurred in dominants (Table 57). Mean % cellulose increased from 73.7% to 82.5% (Table 59). In codominants the increase in percent cellulose was significant (P < 0.001) (Table 57). Cellulose content increased from 77.8 to 82% (Table 59). A comparable situation occurred in subdominants where a significant (P < 0.001) (Table 57) increase in cellulose from 76.7% to 79.3% was noted (Table 59). There was a significant (P> 0.001) difference in the percentage of lignin lost when comparing dominants,codominants and subdominants (Table 54). The maximum lignin loss occurred in dominants (9.6%) and this difference was significant (P< 0.001) when compared with the loss in codominants (3.9%) and subdominants (3.3%) respectively. Differences between codominants and subdominants were not significant (Table 55). Differences in the increase of percent cellulose content comparing dominants,codominants and subdominants were significant (P< 0.01) (Table 54). The maximum increase

occurred in dominants (8.8%) and this was significantly ($P < 0.05$) greater than in codominants (4.5%) and in subdominants (3.4%, $P= 0.01$) respectively. Differences between codominantsand subdominants were not significant (Table 55).

If all tree classes are pooled there was a significant ($P <0.001$) reduction in lignin from 20.8% in controls to 15.7% in infected samples (Table 60). In contrast cellulose percent in controls and treatments increased significantly ($P <0.001$), the mean % increase being from 76.1% to 80.9% (Table 61).

7.3. Analysis of naturally infected trees

A. Initial lipid ,cellulose and lignin content

There was no significant variation in the initial lipid content of dominants,codominants and subdominants (Table 62). However, initial cellulose and lignin varied considerably. Initial cellulose was significantly ($P< 0.001$) different in dominants,codominants and subdominants. The initial cellulose content was maximum in dominants (81.9%) and minimum in subdominants (75.6%); codominants occupying an itermediate position (76.4%) . However,the difference in initial cellulose content was not significant when comparing codominants and subdominants (Table 63). Similar variation was observed in initial lignin content where a significant ($P< 0.001$) difference was recorded when all trees were compared i.e dominants ,codominants and subdominants (Table 62). The initial lignin content was maximum in subdominants (19.9%) ;intermediate in

Table 57: RESULTS OF T-TESTS COMPARING LIPID,CELLULOSE AND LIGNIN CONCENTRATIONS IN CONTROL WOOD SAMPLES AND IN SAMPLES INFECTED WITH *S.sanguinolentum* FOR SIX MONTHS IN THE DIFFERENT TREE VIGOUR CLASSES

SAMPLE	MEAN	STANDARD DEVIATION	STANDARD ERROR	T VALUE	PROBABILITY
		DOMINANT (%LIPID)			
CONTROLS	3.5556	1.464	0.345	-0.52	> 0.05
INFECTED	4.0000	2.697	0.778		
		DOMINANT (%CELLULOSE)			
CONTROLS	73.6667	5.456	1.286	-4.98	< 0.001
INFECTED	82.5000	3.425	0.989		
		DOMINANT (%LIGNIN)			
CONTROLS	23.2222	3.889	0.917	6.37	< 0.001
INFECTED	14.6667	3.114	0.899		
		CODOMINANT (%LIPID)			
CONTROLS	3.3333	1.815	0.428	0.86	> 0.05
INFECTED	2.8333	1.030	0.297		
		CODOMINANT (%CELLULOSE)			
CONTROLS	77.7778	3.623	0.854	-4.16	< 0.001
INFECTED	82.0000	1.907	0.550		
		CODOMINANT (%LIGNIN)			
CONTROLS	18.8889	3.008	0.709	3.55	> 0.001
INFECTED	15.1667	2.480	0.716		
		SUBDOMINANT (%LIPID)			
CONTROLS	3.0000	1.572	0.370	-0.57	> 0.05
INFECTED	3.3333	1.557	0.449		
		SUBDOMINANT (%CELLULOSE)			
CONTROLS	76.6667	1.940	0.457	-3.54	> 0.001
INFECTED	79.3333	2.164	0.620		
		SUBDOMINANT (%LIGNIN)			
CONTROLS	20.3333	2.196	0.518	3.70	< 0.001
INFECTED	17.3333	2.146	0.620		

Table 58: MEAN REDUCTION IN LIGNIN CONTENT SIX MONTHS AFTER FIELD INOCULATION WITH S.sanguinolentum

TREE CLASS	MEAN %LIGNIN		MEAN
	CONTROLS	INFECTED	%REDUCTION
DOMINANT	23.2	14.7	8.5
CODOMINANT	18.9	15.2	3.7
SUBDOMINANT	20.3	17.3	3.0

Table 59: MEAN CHANGE IN CELLULOSE CONTENT AFTER SIX MONTHS OF FIELD INOCULATION WITH S.sanguinolentum

TREE CLASS	MEAN %CELLULOSE		MEAN
	CONTROLS	INFECTED	%INCREASE
DOMINANT	73.7	82.5	8.8
CODOMINANT	77.8	82.0	4.2
SUBDOMINANT	76.7	79.3	2.6

Table 60: RESULTS OF T-TESTS POOLING DATA FOR ALL TREE VIGOUR CLASSES TO SHOW REDUCTION IN %LIGNIN 6 MONTHS AFTER FIELD INOCULATION WITH S.sanguinolentum

SAMPLES	MEAN	STANDARD DEVIATION	STANDARD ERROR	T VALUE	DF	PROBABILITY
CONTROLS	20.8148	3.551	0.483	7.24	88	< 0.001
INFECTED	15.7222	2.794	0.466			

Table 61: RESULTS OF T-TESTS POOLING DATA FOR ALL TREE VIGOUR CLASSES TO SHOW INCREASE IN %CELLULOSE 6 MONTHS AFTER FIELD INOCULATION WITH S.sanguinolentum

SAMPLES	MEAN	STANDARD DEVIATION	STANDARD ERROR	T VALUE	DF	PROBABILITY
CONTROLS	76.0741	4.202	0.572	-6.60	88	< 0.001
INFECTED	80.8889	2.723	0.454			

Table 62: RESULTS OF ANALYSIS OF VARIANCE TO COMPARE CHANGES IN LIPID,CELLULOSE AND LIGNIN CONTENT IN NORWAY SPRUCE 8 YEARS AFTER NATURAL INFECTION BY _S.sanguinolentum_ THROUGH EXTRACTION WOUNDS

SOURCE OF VARIATION	DF	SS	MSS	F RATIO	PROBABILITY
	%LIPID (CONTROLS)				
TREATMENT	2	7.70337	3.85168		
ERROR	51	257.55591	5.05012		
TOTAL	53	265.25928		0.76269	>0.05
	%CELLULOSE (CONTROLS)				
	2	423.18750	211.59375		
	51	540.81250	10.60417		
	53	964.00000		19.95383	< 0.001
	%LIGNIN (CONTROLS)				
	2	363.10547	181.55273		
	51	676.89453	13.27244		
	53	1040.00000		13.67893	< 0.001
	% LOSS IN CELLULOSE				
	2	6748.38672	3374.19336		
	33	947.50391	28.71223		
	35	7695.89062		117.51761	< 0.001
	INCREASE IN %LIGNIN				
	2	5619.5	2809.75		
	33	1195.25	36.2197		
	35	6814.75		77.5752	< 0.001

Table 63: RESULTS OF T-TESTS COMPARING CELLULOSE AND LIGNIN CONCENTRATIONS IN DIFFERENT TREE VIGOUR CLASSES IN TREES 8 YEARS AFTER NATURAL INFECTION WITH *S.sanguinolentum*

TREE CLASS	MEAN	T-VALUE	PROBABILITY
		%CELLULOSE (CONTROLS)	
DOMINANT	81.8889	5.0158	< 0.001
CODOMINANT	76.4444		
DOMINANT	81.8889	5.8347	< 0.001
SUBDOMINANT	75.5555		
CODOMINANT	76.4444	0.8189	> 0.05
SUBDOMINANT	75.5555		
		%LIGNIN (CONTROLS)	
DOMINANT	13.7778	3.7513	< 0.01
CODOMINANT	18.3333		
DOMINANT	13.7778	5.0323	< 0.001
SUBDOMINANT	19.8889		
CODOMINANT	18.3333	1.2809	> 0.05
SUBDOMINANT	19.8889		
		%LOSS IN CELLULOSE	
DOMINANT	49.4167	13.3711	< 0.001
CODOMINANT	20.1000		
DOMINANT	49.4167	13.1807	< 0.001
SUBDOMINANT	20.4000		
CODOMINANT	20.1667	0.1905	> 0.05
SUBDOMINANT	20.4000		
		INCREASE IN %LIGNIN	
DOMINANT	47.5000	10.8875	< 0.001
CODOMINANT	21.1000		
DOMINANT	47.5000	10.6840	< 0.001
SUBDOMINANT	21.1000		
CODOMINANT	21.0000	0.2035	> 0.05
SUBDOMINANT	21.1000		

codominants (18.3%) and minimum in dominants (13.8%). There was no significant difference in the initial lignin content between codominants and subdominants but the differences were significant ($P < 0.01$) when comparing dominants and codominants and dominants and subdominants ($P < 0.001$) (Table 63). Tables 64A,64B and 64C summarise data on chemical analysis of different trees.

B. Effect of S.sanguinolentum eight years after natural infection

B.1. Dominants

Lipid concentrations were greater in infected samples (6.2%) as compared with controls (4.3%) (Table 65). Cellulose was dramatically reduced from 81.9% in control samples to 32.5% in infected samples and the difference was significant ($P< 0.001$) (Table 65). The mean reduction in cellulose was 49.4% (Table 66). In contrast,lignin percent increased significantly ($P< 0.001$) from 13.8 to 61.3% in control and in infected samples respectively (Table 65). The mean increase in lignin percent amounted to 47.5% of the original lignin content. As noted above this increase represents a relative change being directly related to the decrease in cellulose content.

B.2. Codominants

A comparable situation occurred in codominants where lipid contents were 5.2 and 4.3% in control and infected samples respectively (Table 65). There was a considerable effect on both cellulose and lignin. Cellulose decreased significantly ($P< 0.001$) from 76.4% in control samples to 56.3% in infected samples (Table 65) or 20.1% (Table 66). The

lignin content increased significantly (P< 0.001) from 18.3 to 39.3% in controls and infected samples respectively (Table 65),or 21% (Table 67).

B.3. Subdominants

The change in lipid content was again not significant. The concentration of lipid was 4.6 and 3.8% in controls and in infected samples respectively (Table 65). In contrast,both cellulose and lignin were affected dramatically. There was a significant (P< 0.001) decrease in the amount of cellulose from 75.6% in controls to 55.2% in infected samples (Table 65),or 20% . This was accompanied with an increase in the percentage of lignin from 19.9% in controls to 41% in the infected samples. This rise was significant (P< 0.001) (Tables 65 & 67).

If the chemical composition of wood blocks derived from the different tree classes are now compared, 't' tests showed that the maximum loss of cellulose occurred in dominants (49.4%) and was significantly (P<0.001) different when compared with the loss in codominants (20.1%) and that in subdominants (20.4%) respectively (Table 63). The loss in cellulose was least in codominants and subdominants which were not significantly different when compared (Table 63). The dominants showed the maximum increase in percent lignin (47.5%) compared with codominants (21%) and subdominants (21.1%) respectively. The difference of that increase between dominants and codominants was significant (P< 0.001). Also a significant (P< 0.001) difference was demonstrated when comparing dominants and

Table 64A:
SUMMARY OF LIPID,CELLULOSE AND LIGNIN CONTENT OF CONTROLS AND NATURALLY INFECTED SAMPLES(8 YEARS)
(SAMPLES 2;5;8 and 10 WERE INFECTED AND THE REST WERE CONTROL SAMPLES

SAMPLE	LIPID		LIGNIN		CELLULOSE	
NO.	gm	%	gm	%	gm	%
			DOMINANT			
1G1	0.2	4	0.7	14	4.1	82
2	0.3	6	3.2	64	1.5	30
3	0.2	4	0.6	12	4.2	84
4	0.1	2	0.8	16	4.1	82
5	0.3	6	3.1	62	1.6	32
6	0.2	4	0.7	14	4.1	82
7	0.3	6	0.7	14	4.0	80
8	0.8	16	2.9	58	1.3	26
9	0.3	6	0.6	12	4.1	82
10	0.1	2	3.0	60	1.9	38
2G1	0.2	4	0.6	12	4.2	84
2	0.3	6	3.0	60	1.7	34
3	0.2	4	0.9	18	3.9	78
4	0.2	4	0.8	16	4.0	80
5	0.5	10	3.1	62	1.4	28
6	0.1	2	0.7	14	4.2	84
7	0.1	2	0.6	12	4.3	86
8	0.2	4	3.0	60	1.8	36
9	0.4	8	0.5	10	4.1	82
10	0.3	6	3.0	60	1.7	34
3G1	0.4	8	0.6	12	4.0	80
2	0.2	4	3.2	64	1.6	32
3	0.2	4	0.7	14	4.1	82
4	0.2	4	0.6	12	4.2	84
5	0.1	2	3.1	62	1.8	36
6	0.2	4	0.8	16	4.0	80
7	0.2	4	0.7	14	4.1	82
8	0.3	6	3.2	64	1.5	30
9	0.2	4	0.8	16	4.0	80
10.	0.3	6	3.0	60	1.7	34

Table 64B: SUMMARY OF LIPID,CELLULOSE AND LIGNIN CONTENT OF CONTROLS AND NATURALLY INFECTED SAMPLES (8 YEARS) SAMPLES 2;5;8 & 10 WERE INFECTED SAMPLES AND THE REST WERE CONTROL SAMPLES

SAMPLE NO.	LIPID gm	%	LIGNIN gm	%	CELLULOSE gm	%
			CODOMINANT			
1B1	0.1	2	1.0	20	3.9	78
2	0.2	4	2.2	44	2.6	52
3	0.1	2	0.9	18	4.0	80
4	0.1	2	1.1	22	3.8	76
5	0.1	2	2.4	48	2.5	50
6	0.3	6	1.0	20	3.7	74
7	0.1	2	0.9	18	4.0	80
8	0.3	6	2.0	40	2.7	54
9	0.3	6	0.8	16	3.9	78
10	0.1	2	2.1	42	2.8	56
2B1	0.3	6	1.0	20	3.7	74
2	0.3	6	2.0	40	2.7	54
3	0.4	8	0.7	14	3.9	78
4	0.6	12	0.6	12	3.8	76
5	0.1	2	2.1	42	2.8	56
6	0.1	2	0.9	18	4.0	80
7	0.3	6	1.0	20	3.7	74
8	0.2	4	1.8	36	3.0	60
9	0.1	2	1.2	24	3.7	74
10	0.2	4	1.9	38	2.9	58
3B1	0.3	6	1.0	20	3.7	74
2	0.3	6	2.2	44	2.5	50
3	0.4	8	0.6	12	4.0	80
4	0.2	4	1.2	24	3.6	72
5	0.1	2	1.9	38	3.0	60
6	0.3	6	1.0	20	3.7	74
7	0.4	8	0.7	14	3.9	78
8	0.3	6	1.5	30	3.2	64
9	0.3	6	0.9	18	3.8	76
10	0.4	8	1.5	30	3.1	62

Table 64C: SUMMARY OF LIPID,CELLULOSE AND LIGNIN CONTENT OF CONTROLS AND NATURALLY INFECTED SAMPLES (3YEARS),SAMPLES 2; 5;8 & 10 WERE INFECTED SAMPLES AND THE REST WERE CONTROL SAMPLES

SAMPLE NO.	LIPID gm	LIPID %	LIGNIN gm	LIGNIN %	CELLULOSE gm	CELLULOSE %
			SUBDOMINANT			
1R1	0.2	4	1.0	20	3.8	76
2	0.3	6	2.2	44	2.5	50
3	0.1	2	0.9	18	4.0	80
4	0.2	4	0.9	18	3.9	78
5	0.1	2	2.3	46	2.6	52
6	0.2	4	1.0	20	3.8	76
7	0.4	8	0.9	18	3.7	74
8	0.1	2	2.4	48	2.5	50
9	0.4	8	0.5	10	4.1	82
10	0.1	2	2.5	50	2.4	48
2R1	0.2	4	1.1	22	3.7	74
2	0.2	4	2.1	42	2.7	54
3	0.3	6	1.0	20	3.7	74
4	0.2	4	1.8	36	3.0	60
5	0.3	6	1.6	32	3.1	62
6	0.1	2	1.0	20	3.9	78
7	0.1	2	1.1	22	3.8	76
8	0.1	2	2.0	40	2.9	58
9	0.3	6	1.0	20	3.7	74
10	0.1	2	2.1	42	2.8	56
3R1	0.1	2	0.9	18	4.0	80
2	0.2	4	1.7	34	3.1	62
3	0.3	6	0.9	18	3.8	76
4	0.3	6	1.0	20	3.7	74
5	0.2	4	1.8	36	3.0	60
6	0.3	6	0.9	18	3.8	76
7	0.3	6	1.0	20	3.7	74
8	0.3	6	1.9	38	2.8	56
9	0.1	2	1.0	20	3.9	78
10	0.3	6	2.0	40	2.7	54

Table 65: RESULTS OF T-TESTS TO COMPARE THE EFFECT OF S.sanguinolentum 8 YEARS AFTER NATURAL INFECTION ON LIPID,CELLULOSE AND LIGIN CONCENTRATIONS IN TREES OF THE DIFFEREENT VIGOUR CLASSES

SAMPLE	MEAN	STANDARD DEVIATION	STANDARD ERROR	T VALUE	PROBABILITY
		DOMINANT(%LIPID)			
CONTROLS	4.3333	1.715	0.404	-1.58	> 0.05
INFECTED	6.1667	3.762	1.086		
		DOMINANT (%CELLULOSE)			
CONTROLS	81.8889	1.997	0.471	44.01	< 0.001
INFECTED	32.5000	3.529	1.019		
		DOMINANT (%LIGNIN)			
CONTROLS	13.7778	1.997	0.471	-63.3	< 0.001
INFECTED	61.3333	1.969	0.569		
		CODOMINANT (%LIPID)			
CONTROLS	5.2222	2.840	0.669	0.93	> 0.05
INFECTED	4.3333	2.060	0.595		
		CODOMINANT (%CELLULOSE)			
CONTROLS	76.4444	2.617	0.617	13.79	< 0.001
INFECTED	56.3333	4.579	1.322		
		CODOMINANT (%LIGNIN)			
CONTROLS	18.3333	3.581	0.844	-12.82	< 0.001
INFECTED	39.3333	5.416	1.563		
		SUBDOMINANT (%LIPID)			
CONTROLS	4.5556	2.036	0.480	1.00	>0.05
INFECTED	3.8333	1.801	0.520		
		SUBDOMINANT (%CELLULOSE)			
CONTROLS	75.5556	4.579	1.079	11.82	> 0.001
INFECTED	55.1667	4.707	1.359		
		SUBDOMINANT (%LIGNIN)			
CONTROLS	19.8889	4.776	1.126	-11.17	< 0.001
INFECTED	41.0000	5.494	1.586		

Table 66: %REDUCTION OF CELLULOSE 8 YEARS AFTER NATURAL INFECTION BY <u>S.sanguinolentum</u>

TREE CLASS	MEAN %CELLULOSE		MEAN %REDUCTION
	CONTROLS	INFECTED	
DOMINANT	81.9	32.5	49.4
CODOMINANT	76.4	56.3	20.1
SUBDOMINANT	75.6	55.2	20.4

Table 67: INCREASE IN %LIGNIN 8 YEARS AFTER NATUARAL INFECTION BY <u>S.sanguinolentum</u>

TREE CLASS	MEAN %LIGNIN		MEAN %INCREASE
	CONTROLS	INFECTED	
DOMINANT	13.8	61.3	47.5
CODOMINANT	18.3	39.3	21.0
SUBDOMINANT	19.9	41.0	21.1

subdominants (Table 63). However,the increase in percent lignin was not significantly different when codominants and subdominants were compared (Table 63).

If samples from all tree vigour classes (i.e dominant,codominant and subdominant) are pooled, there was a significant ($P < 0.001$) reduction in cellulose from 78% to 48% in control and in infected samples respectively (Table 69). On the other hand,a significant ($P < 0.001$) increase in percent lignin from 17.3 to 47.2% was also demonstrated (Table 70).

C. Comparison between younger and older crops

Trees from younger Norway spruce crops from compartment 11 (YAIR HILL FOREST) and trees from older Norway spruce crops from compartment 1016 (GLENTRESS FOREST) were used in these experiments. A series of t-tests were made to compare the amount of wood substance in healthy trees in the dominant,codominant and subdominant classes.

C.1. Dominants

Mean initial lipid concentrations in younger and in older crops were 3.6 and 4.3% respectively,but the observed differences were not significant (Table 68). Mean initial cellulose concentrations were 73.7 and 81.9% respectively and the observed differences were significant at $P < 0.001$. Finally,lignin concentrations were 23.2% and 13.8% respectively and the observed differences were significant at $P < 0.001$.

C.2. Codominants

Table 68: RESULTS OF T-TESTS COMPARING THE DIFFERENCES IN CONTENTS OF LIPID, CELLULOSE AND LIGNIN IN YOUNGER AND IN OLDER CROPS OF NORWAY SPRUCE IN THE THE DIFFERENT TREE VIGOUR CLASSES

SAMPLES	MEAN	STANDARD DEVIATION	STANDARD ERROR	T VALUE	PROBABILITY
		DOMINANT (%LIPID)			
YOUNGER	3.5556	1.464	0.345	-1.46	> 0.05
OLDER	4.3333	1.715	0.404		
		DOMINANT (%CELLULOSE)			
YOUNGER	73.6667	5.456	1.286	-6.00	< 0.001
OLDER	81.8889	1.997	0.471		
		DOMINANT (%LIGNIN)			
YOUNGER	23.2222	3.889	0.917	9.12	< 0.001
OLDER	13.7778	2.045	0.482		
		CODOMINANT (%LIPID)			
YOUNGER	3.3333	1.815	0.428	-2.26	> 0.01
OLDER	5.1765	2.921	0.708		
		CODOMINANT (%CELLULOSE)			
YOUNGER	77.7778	3.623	0.854	1.27	> 0.05
OLDER	76.4444	2.617	0.617		
		CODOMINANT (%LIGNIN)			
YOUNGER	18.8889	3.008	0.709	0.05	> 0.05
OLDER	18.3333	3.581	0.844		
		SUBDOMINANT (%LIPID)			
YOUNGER	3.0000	1.572	0.370	-2.57	> 0.01
OLDER	4.5556	2.036	0.480		
		SUBDOMINANT (%CELLULOSE)			
YOUNGER	76.6667	1.940	0.457	0.95	> 0.05
OLDER	75.5556	4.579	1.079		
		SUBDOMINANT (%LIGNIN)			
YOUNGER	20.3333	2.196	0.518	0.36	> 0.05
OLDER	19.8889	4.776	1.126		

Mean initial lipid concentrations in younger and in older crops were 3.3 and 5.2% respectively and the observed differences were significant at P < 0.01 (Table 68). Mean initial cellulose concentrations were 77.8 and 76.4% respectively,but the observed differences were not significant (Table 68). Finally,mean initial lignin concentrations were 18.9 and 18.3% in younger and in older crops respectively and the observed differences were not significant (Table 68).

C.3. Subdominants

Subdominants showed a similar pattern to that found in codominants. Mean initial lipid concentrations were 3 and 4.6% in younger and in older crops respectively and the observed differences were significant (P< 0.001) (Table 68). Mean initial cellulose concentrations were 76.7 and 75.6% respectively and the observed differences were not significant (Table 68). Finally,mean initial lignin concentrations were 20.3 and 19.9% respectively,but the observed differences were not significant (Table 68).

7.4. Analysis of artificially inoculated wood blocks

7.4.1. Analysis of control samples

There was no significant difference in initial lipid of wood blocks taken from dominant,codominant and subdominant trees,mean concentrations being 3.5,2.9 and 2.9% respectively (Table 71). The initial content of lipid,cellulose and lignin was summarised in table 20. The amount of cellulose was significantly different (P< 0.001) being (73.6%) ,(77.2%) and (77.4%) in dominant codominant and

Table 69: RESULTS OF T-TESTS COMPARING LOSS IN CELLULOSE 8 YEARS AFTER NATURAL INFECTION BY *S.sanguinolentum*

SAMPLES	MEAN	STANDARD DEVIATION	STANDARD ERROR	T VALUE	DF	PROBABILITY
CONTROLS	77.9630	4.265	0.580	14.52	41.07	< 0.001
INFECTED	48.0000	11.885	1.981			

Table 70: RESULTS OF T-TESTS COMPARING CHANGES IN LIGNIN CONCENTRATION 8 YEARS AFTER NATURAL INFECTION BY *S.sanguinolentum*

SAMPLES	MEAN	STANDARD DEVIATION	STANDARD ERROR	T VALUE	DF	PROBABILITY
CONTROLS	17.3333	4.430	0.603	-15.38	42.54	< 0.001
INFECTED	47.2222	11.082	1.847			

Table 71: RESULTS OF ANALYSIS OF VARIANCE COMPARING INITIAL CONTENTS OF LIPID CELLULOSE AND LIGNIN IN BLOCKS OF NORWAY SPRUCE WOOD

SOURCE OF VARIATION	DF	SS	MSS	F RATIO	PROBABILITY
		(%LIPID)			
TREATMENT	2	13.57031	6.78516		
ERROR	132	423.28906	3.20763		
TOTAL	134	436.85937		2.11591	> 0.05
		%CELLULOSE			
	2	400.06250	200.03125		
	132	611.25000	4.63068		
	134	1011.31250		43.19693	< 0.001
		%LIGNIN			
	2	347.90625	173.95312		
	132	543.82812	4.11991		
	134	891.73437		42.22255	< 0.001

subdominant trees respectively. The difference in the amount of cellulose was not significant comparing codominants and subdominants (Table 72). The percentage of lignin was significantly different (P< 0.001) in different trees (Table 71). Mean concentrations were maximum in dominants (23%) and minimum in subdominants (19.5%) and intermediate in codominants (19.8%). The difference in lignin content was not significant comparing codominants (19.5%) and subdominants (19.7%) (Table 72). Tables 73A,73B and 73C summarise data on chemical analysis of different wood blocks.

7.4.2. Effect of S.sanguinolentum

[i] One month after inoculation

Table 74 shows the percentages of lipid,cellulose and lignin in controls and in infected wood blocks taken from dominant,codominant and subdominant trees one month after inoculation. Statistical analysis on these data showed that none of the observed differences were significant (Table 75). It appears that the fungus has had little effect upon the major wood constituents.

[ii] Two months after inoculation

a. Wood blocks derived from dominant trees

Mean lipid concentration in the blocks was 3.3% in controls and 2.9% in inoculated blocks (Table 77). Table 76 summarises data on chemical analysis of wood blocks two months after inoculation with S.sanguinolentum . Cellulose was significantly (P< 0.001) reduced from 73.7% in controls to 70.5% in infected samples (Table 77) or 3.2% (Table 78).

Table 72: RESULTS OF T-TEST COMPARING INITIAL LIPID,CELLULOSE AND LIGNIN CONTENTS IN WOOD BLOCKS TAKEN FROM THE DIFFERENT TREE VIGOUR CLASSES

TREE CLASS	MEAN	T-VALUE	PROBABILITY
		%CELLULOSE (CONTROLS)	
DOMINANT	73.6444	7.7395	< 0.001
CODOMINANT	77.1555		
DOMINANT	73.6444	8.3273	< 0.001
SUBDOMINANT	77.4222		
CODOMINANT	77.1555	0.5878	> 0.05
SUBDOMINANT	77.4222		
		%LIGNIN (CONTROLS)	
DOMINANT	22.9778	8.2052	< 0.001
CODOMINANT	19.4667		
DOMINANT	22.9778	7.6859	< 0.001
SUBDOMINANT	19.6889		
CODOMINANT	19.4667	0.5193	> 0.05
SUBDOMINANT	19.6889		

Table 73A: SUMMARY OF ANALYSIS OF LIPID,CELLULOSE AND LIGNIN CONTENTS IN CONTROL WOOD BLOCKS (LABORATORY INOCULATION EXPERIMENT)

SAMPLE NO.	LIPID gm	LIPID %	LIGNIN gm	LIGNIN %	CELLULOSE gm	CELLULOSE %
			DOMINANT			
1G1	0.2	4	1.1	22	3.7	73
2	0.2	4	1.2	24	3.6	72
3	0.1	2	1.2	24	3.7	74
4	0.1	2	1.1	22	3.8	76
5	0.1	2	1.0	20	3.9	78
6	0.3	6	1.2	24	3.5	70
7	0.6	12	1.0	20	3.4	68
8	0.3	6	0.9	18	3.8	76
9	0.1	2	1.3	26	3.6	72
10	0.1	2	1.2	24	3.7	74
11	0.1	2	1.4	28	3.5	70
12	0.3	6	1.2	24	3.5	70
13	0.2	4	1.1	22	3.7	74
14	0.2	4	1.2	24	3.6	72
15	0.1	2	1.2	24	3.7	74
2G1	0.3	6	1.0	20	3.7	74
2	0.1	2	1.1	22	3.8	76
3	0.2	4	1.2	24	3.6	72
4	0.1	2	1.2	24	3.7	74
5	0.2	4	1.3	26	3.5	70
6	0.3	6	1.1	22	3.6	72
7	0.1	2	0.9	18	4.0	80
8	0.1	2	1.0	20	3.9	78
9	0.1	2	1.1	22	3.8	76
10	0.1	2	1.3	26	3.6	72
11	0.1	2	1.2	24	3.7	74
12	0.2	4	1.1	22	3.7	74
13	0.2	4	1.3	26	3.5	70
14	0.1	2	1.0	20	3.9	78
15	0.1	2	1.2	24	3.7	74
3G1	0.1	2	1.5	30	3.4	68
2	0.1	2	1.4	28	3.5	70
3	0.1	2	1.3	26	3.6	72
4	0.1	2	1.0	20	3.9	78
5	0.1	2	1.1	22	3.8	76
6	0.1	2	1.0	20	3.9	78
7	0.2	4	1.2	24	3.6	72
8	0.1	2	1.1	22	3.8	76
9	0.2	4	1.3	26	3.5	70
10	0.1	2	1.3	26	3.6	72
11	0.1	2	1.1	22	3.8	76
12	0.1	2	1.0	20	3.9	78
13	0.2	4	1.0	20	3.8	76
14	0.3	6	1.0	20	3.7	74
15	0.4	8	1.1	22	3.5	70

Table 73B: SUMMARY OF LIPID,CELLULOSE AND LIGNIN CONTENT IN CONTROL WOOD BLOCKS (LABORATORY INOCULATION EXPERIMENTS)

SAMPLE NO.	LIPID gm	LIPID %	LIGNIN gm	LIGNIN %	CELLULOSE gm	CELLULOSE %
			CODOMINANT			
1B1	0.1	2	0.9	18	4.0	80
2	0.1	2	1.0	20	3.9	78
3	0.2	4	1.0	20	3.8	76
4	0.2	4	1.0	20	3.8	76
5	0.1	2	1.1	22	3.8	76
6	0.1	2	1.0	20	3.9	78
7	0.4	8	0.9	18	3.8	76
8	0.4	8	0.9	18	3.7	74
9	0.1	2	1.0	20	3.9	78
10	0.3	6	1.0	20	3.7	74
11	0.1	2	1.0	20	3.9	78
12	0.1	2	0.9	18	4.0	80
13	0.3	6	0.9	18	3.8	76
14	0.3	6	0.8	16	3.9	78
15	0.1	2	1.0	20	3.9	78
2B1	0.3	6	0.9	18	3.8	76
2	0.1	2	0.9	18	4.0	80
3	0.2	4	1.0	20	3.8	76
4	0.4	8	0.9	18	3.7	74
5	0.1	2	1.0	20	3.9	78
6	0.2	4	1.0	20	3.8	76
7	0.1	2	1.0	20	3.9	78
8	0.1	2	1.0	20	3.9	78
9	0.1	2	0.9	18	4.0	80
10	0.2	4	0.9	18	3.9	78
11	0.2	4	1.0	20	3.8	76
12	0.1	2	1.1	22	3.8	76
13	0.1	2	1.1	22	3.8	76
14	0.2	4	0.9	18	3.9	78
15	0.2	4	1.0	20	3.8	76
3B1	0.1	2	1.0	20	3.9	78
2	0.1	2	0.9	18	4.0	80
3	0.3	6	0.9	18	3.8	76
4	0.1	2	1.0	20	3.9	78
5	0.2	2	1.0	20	3.9	78
6	0.2	4	1.0	20	3.8	76
7	0.1	2	1.0	20	3.9	78
8	0.1	2	1.1	22	3.8	76
9	0.1	2	0.9	18	4.0	80
10	0.1	2	1.0	20	3.9	78
11	0.2	4	1.0	20	3.8	76
12	0.2	4	0.9	18	3.9	78
13	0.2	4	1.0	20	3.8	76
14	0.1	2	1.1	22	3.8	76
15	0.2	4	1.0	20	3.8	76

Table 73C: SUMMARY OF ANALYSIS OF LIPID, CELLULOSE AND LIGNIN CONTENT IN CONTROL WOOD BLOCKS (LABORATORY INOCULATION EXPERIMENTS)

SAMPLE NO.	LIPID gm	LIPID %	LIGNIN gm	LIGNIN %	CELLULOSE gm	CELLULOSE %
			SUBDOMINANT			
1R1	0.1	2	0.9	18	4.0	80
2	0.1	2	1.0	20	3.9	78
3	0.1	2	1.1	22	3.8	76
4	0.1	2	0.9	18	4.0	80
5	0.2	4	0.9	18	3.9	78
6	0.2	4	0.9	18	3.9	78
7	0.2	4	1.0	20	3.8	76
8	0.1	2	1.1	22	3.8	76
9	0.1	2	1.0	20	3.9	78
10	0.1	2	1.0	20	3.9	78
11	0.1	2	0.9	18	4.0	80
12	0.1	2	1.0	20	3.9	78
13	0.1	2	1.0	20	3.9	78
14	0.1	2	1.1	22	3.8	76
15	0.1	2	1.0	20	3.9	78
2R1	0.1	2	1.0	20	3.9	78
2	0.2	4	1.0	20	3.8	76
3	0.1	2	1.1	22	3.8	76
4	0.1	2	1.1	22	3.8	76
5	0.3	6	0.8	16	3.9	78
6	0.1	2	1.1	22	3.8	76
7	0.1	2	1.0	20	3.9	78
8	0.2	4	1.0	20	3.8	76
9	0.1	2	0.9	18	4.0	80
10	0.2	4	0.9	18	3.9	78
11	0.2	4	0.9	18	3.9	78
12	0.2	4	1.0	20	3.8	76
13	0.1	2	1.1	22	3.8	76
14	0.1	2	1.2	24	3.7	74
15	0.2	4	1.0	20	3.8	76
3R1	0.1	2	1.0	20	3.9	78
2	0.2	4	1.0	20	3.8	76
3	0.2	4	0.9	18	3.9	78
4	0.2	4	0.9	18	3.9	78
5	0.2	4	1.0	20	3.8	76
6	0.1	2	1.0	20	3.9	78
7	0.1	2	0.9	18	4.0	80
8	0.1	2	1.1	22	3.8	76
9	0.1	2	1.0	20	3.9	78
10	0.1	2	0.9	18	4.0	80
11	0.0	2	0.9	18	3.9	78
12	0.1	2	0.9	18	4.0	80
13	0.1	2	1.1	22	3.8	76
14	0.2	4	1.0	20	3.8	76
15	0.4	8	0.8	16	3.8	76

Table 74: SUMMARY OF ANALYSIS OF LIPID, CELLULOSE AND LIGNIN CONCENTRATIONS IN WOOD BLOCKS BEFORE AND AFTER ONE MONTH INOCULATION WITH S.sanguinolentum

SAMPLE NO.	%LIPID CONTROL	%LIPID INFECTED	%LIGNIN CONTROL	%LIGNIN INFECTED	%CELLULOSE CONTROL	%CELLULOSE INFECTED
			DOMINANT			
1G1	2	6	22	22	76	72
13	4	2	22	22	74	76
14	4	8	24	22	72	70
6	6	4	24	26	70	70
9	2	2	26	24	72	74
2G14	2	2	20	20	78	78
11	2	2	24	22	74	76
1	6	4	20	22	74	74
6	6	6	22	24	72	70
5	4	4	26	24	70	72
3G13	4	8	20	22	76	70
3	2	2	26	26	72	72
4	2	8	20	20	78	72
5	2	2	22	20	76	78
7	4	2	26	24	70	74
			CODOMINANT			
1B9	2	2	20	22	78	76
1	2	2	18	18	80	80
8	8	6	18	16	74	78
3	4	2	20	20	76	78
15	2	2	20	22	78	76
2B15	4	2	20	20	76	78
7	2	2	20	18	78	80
11	4	4	20	22	76	74
12	2	2	22	22	76	76
9	2	2	18	20	80	78
3B4	2	6	20	20	78	74
12	4	6	18	16	78	78
8	2	4	22	20	76	76
14	2	2	22	24	76	74
1	2	2	20	20	78	78
			SUBDOMINANT			
1R6	4	6	18	20	78	74
15	2	4	20	20	78	76
1	2	0	18	20	80	80
8	2	0	22	24	76	76
7	4	2	20	18	76	80
2R1	2	4	20	22	78	74
13	2	2	22	22	76	76
14	2	0	24	26	74	74
4	2	2	22	20	76	78
7	2	8	20	18	78	74
3R5	4	2	20	18	76	80
15	8	8	16	18	76	74
6	2	6	20	18	78	76
3	4	2	18	20	78	78
8	2	2	22	24	76	74

Table 75: RESULTS OF T-TESTS COMPARING THE CONCENTRATION OF LIPID, CELLULOSE AND LIGNIN IN CONTROL AND ONE MONTH AFTER INOCULATION WITH *S.sanguinolentum*

SAMPLE	MEAN	STANDARD DEVIATION	STANDARD ERROR	T VALUE	PROBABILITY
		DOMINANT(%LIPID)			
CONTROL	3.4667	1.598	0.413	-1.00	> 0.05
INFECTED	4.1333	2.446	0.631		
		DOMINANT (%CELLULOSE)			
CONTROL	73.6000	2.746	0.709	0.51	> 0.05
INFECTED	73.2000	2.808	0.725		
		DOMINANT (%LIGNIN)			
CONTROL	22.9333	2.374	0.613	0.62	> 0.05
INFECTED	22.6667	1.952	0.504		
		CODOMINANT (%LIPID)			
CONTROL	2.9333	1.668	0.431	-0.32	> 0.05
INFECTED	3.0667	1.668	0.431		
		CODOMINANT (%CELLULOSE)			
CONTROL	77.2000	1.656	0.428	0.49	> 0.05
INFECTED	76.9333	1.981	0.511		
		CODOMINANT (%LIGNIN)			
CONTROL	19.8667	1.407	0.363	-0.32	> 0.05
INFECTED	20.0000	2.268	0.586		
		SUBDOMINANT (%LIPID)			
CONTROL	2.9333	1.668	0.431	-0.41	> 0.05
INFECTED	3.2000	2.704	0.698		
		SUBDOMINANT (%CELLULOSE)			
CONTROL	76.9333	1.486	0.384	1.00	> 0.05
INFECTED	76.2667	2.374	0.613		
		SUBDOMINANT (%LIGNIN)			
CONTROL	20.1333	2.066	0.533	-0.82	> 0.05
INFECTED	20.5333	2.560	0.661		

Table 76: SUMMARY OF ANALYSIS OF LIPID, CELLULOSE AND LIGNIN CONTENTS IN WOOD BLOCKS BEFORE AND 2 MONTHS AFTER INOCULATION WITH *S.sanguinolentum*

SAMPLE NO.	%LIPID CONTROL	%LIPID INFECTED	%LIGNIN CONTROL	%LIGNIN INFECTED	%CELLULOSE CONTROL	%CELLULOSE INFECTED
DOMINANT						
1G8	6	6	18	24	76	70
10	2	2	24	26	74	72
11	2	2	28	28	70	70
12	6	2	24	30	70	68
15	2	2	24	26	74	72
2G2	2	2	22	26	76	72
3	4	4	24	26	72	70
4	2	2	24	30	74	68
9	2	2	22	26	76	72
15	2	2	24	28	74	70
3G10	2	2	26	28	72	70
11	2	2	22	30	76	68
12	2	4	20	22	78	74
14	6	4	20	24	74	72
15	8	6	22	24	70	70
CODOMINANT						
1B10	6	4	20	24	74	72
11	2	2	20	20	78	78
12	2	2	18	20	80	78
13	6	4	18	22	76	74
14	6	4	16	20	78	76
2B2	2	2	18	22	80	76
5	2	2	20	24	78	74
10	4	4	18	20	78	76
13	2	2	22	24	76	74
14	4	2	18	22	78	76
3B15	4	2	20	26	76	72
9	2	4	18	20	80	76
10	2	2	20	24	78	74
11	2	2	20	22	76	76
3	2	2	20	24	76	74
SUBDOMINANT						
1R2	2	2	20	22	78	76
3	2	4	22	22	76	74
4	2	2	18	22	80	76
5	4	2	18	22	78	76
14	2	2	22	26	76	72
2R6	2	2	22	24	76	74
10	4	4	18	22	78	74
11	4	2	18	24	78	74
12	4	8	20	20	76	72
15	4	6	20	20	76	74
3R7	2	2	18	22	80	76
11	4	6	18	20	78	74
12	2	2	18	20	80	78
13	2	2	22	22	76	76
14	4	4	20	22	76	74

Table 77: RESULTS OF T TESTS COMPARING LIPID,CELLULOSE AND LIGNIN CONTENTS IN WOOD BLOCKS BEFORE AND AFTER 2 MONTHS OF INOCULATION WITH S.sanguinolentum IN THE DIFFERENT TREE VIGOUR CLASSES

SAMPLE	MEAN	STANDARD DEVIATION	STANDARD ERROR	T VALUE	DF	PROBABILITY
			DOMINANT (%LIPID)			
CONTROLS	3.3333	2.093	0.540	1.15	14	> 0.05
INFECTED	2.9333	1.486	0.384			
			DOMINANT (%CELLULOSE)			
CONTROLS	73.7333	2.492	0.643	5.53	14	< 0.001
INFECTED	70.5333	1.767	0.456			
			DOMINANT (%LIGNIN)			
CONTROLS	22.9333	2.492	0.643	-6.44	14	< 0.001
INFECTED	26.5333	2.446	0.631			
			CODOMINANT (%LIPID)			
CONTROLS	3.4667	1.598	0.413	2.45	14	< 0.01
INFECTED	2.6667	0.976	0.252			
			CODOMINANT (%CELLULOSE)			
CONTROLS	77.4667	1.767	0.456	6.87	14	< 0.001
INFECTED	75.0667	1.831	0.473			
			CODOMINANT (%LIGNIN)			
CONTROLS	19.0667	1.486	0.384	-8.41	14	< 0.001
INFECTED	22.2667	1.981	0.511			
			SUBDOMINANT (%LIPID)			
CONTROLS	2.8000	1.014	0.262	-1.17	14	> 0.05
INFECTED	3.3333	1.952	0.504			
			SUBDOMINANTS (%CELLULOSE)			
CONTROLS	77.4667	1.598	0.413	8.57	14	< 0.001
INFECTED	74.6667	1.633	0.422			
			SUBDOMINANTS (%LIGNIN)			
CONTROLS	19.6000	1.724	0.445	-4.94	14	< 0.001
INFECTED	22.000	1.690	0.436			

Table 78:%REDUCTION IN CELLULOSE IN WOOD BLOCKS 2 MONTHS AFTER INOCULATION WITH *S.sanguinolentum* IN THE DIFFERENT TREE VIGOUR CLASSES

TREE CLASS	MEAN %CELLULOSE		MEAN %REDUCTION
	CONTROLS	INFECTED	
DOMINANT	73.7	70.5	3.2
CODOMINANT	77.5	75.1	2.4
SUBDOMINANT	77.5	74.7	2.8

As a result there was a significant ($P < 0.001$) increase in the percentage of lignin from 22.9% to 26.5% or 3.6% (Table 79).

b. Wood blocks derived from codominant trees

Lipid concentration was significantly ($P < 0.05$) reduced in wood blocks inoculated for 2 months with S.sanguinolentum being 3.5 and 2.7% in controls and in inoculated samples respectively (Table 77). There was also a significant reduction ($P < 0.001$) in cellulose content and here mean cellulose content declined from 77.5 to 75.1% in controls and in infected samples respectively (Table 77) or 2.4% (Table 78). In contrast,lignin content increased significantly ($P < 0.001$); mean concentrations in control samples being 19.1% as compared with 22.3% in infected samples or 3.2% (Table 79).

c. Wood blocks derived from subdominant trees

There was no significant difference in lipid content comparing control and infected blocks. Lipid concentration was 2.8% in controls and 3.3% in infected samples (Table 77). Mean cellulose content dropped from 77.5% to 74.7% in controls and in infected samples respectively (Table 25) or 2.8% (Table 78). On the other hand,there was a significant ($P<0.001$) increase in percent lignin from 19.6% in controls to 22% in infected samples (Table 24) or 2.4% (Table 79).

Further, there was no significant difference in the amount of cellulose lost comparing blocks derived from dominants (3.6%) codominants (3.2%) and subdominants (2.4%) respectively (Table 80).

If data from blocks derived from dominant , codominant and subdominant trees are pooled a significant (P< 0.001) reduction in cellulose was noted. Mean cellulose concentration declined from 76.2% to 73.4% 2 months after inoculation (Table 81). At the same time a significant (P< 0.001) increase in percentage lignin was recorded. It increased from 20.5 to 23.6% 2 months after inoculation (Table 81).

[iii] Three months after inoculation

Results were summarised in table 82.

a. Wood blocks derived from dominant trees

No significant difference was observed in lipid concentration in controls (3.3%) compared with infected samples (2.7%) (Table 83). There was a significant reduction in cellulose concentration (P< 0.001). Mean % cellulose content was 73.7 in controls compared with 66.3 in inoculated samples (Table 83) or 7.4% mean reduction (Table 83A). Lignin % increased significantly (P< 0.001) from 23.1 to 31.1 in controls and in infected samples respectively (Table 83 and 83B).

b. Wood blocks derived from codominant trees

No significant difference was observed in lipid concentration in control blocks (3.9%) compared with inoculated blocks (3.7%) (Table 83). There was a significant reduction in cellulose (P< 0.001) . Mean % cellulose content was reduced from 76.8 to 68.8 in controls and in infected samples respectively (Tables 83 and 83A). Lignin % increased significantly (P< 0.001) from 19.5 to 27.5 (Tables

Table 79: INCREASE IN %LIGNIN IN WOOD BLOCKS 2 MONTHS AFTER INOCULATION WITH *S.sanguinolentum* IN THE DIFFERENT TREE VIGOUR CLASSES

TREE CLASS	%LIGNIN		MEAN %INCREASE
	CONTROLS	INFECTED	
DOMINANT	22.9	26.5	3.6
CODOMINANT	19.1	22.3	3.2
SUBDOMINANT	19.6	22.0	2.4

Table 80: RESULTS OF ANALYSIS OF VARIANCE COMPARING THE CHANGE IN CELLULOSE AND LIGNIN CONCENTRATIONS IN WOOD BLOCKS 2 MONTHS AFTER INOCULATION

SOURCE OF VARIATION	DF	SS	MSS	F RATIO	P
%REDUCTION IN CELLULOSE					
TREATMENT	2	6.57764	3.28882		
ERROR	42	117.33350	2.79365		
TOTAL	44	123.91113		1.17725	> 0.05
%INCREASE IN LIGNIN					
	2	11.19971	5.59985		
	42	145.60034	3.46667		
	44	156.80005		1.61534	> 0.05

Table 81: RESULTS OF ANALYSIS OF VARIANCE COMPARING %REDUCTION IN CELLULOSE IN WOOD BLOCKS 2 MONTHS AFTER INOCULATION FOR ALL TREE VIGOUR CLASSES

SAMPLE	MEAN	STANDARD DEVIATION	STANDARD ERROR	T VALUE	DF	PROBABILITY
CONTROLS	76.2222	2.636	0.393	11.22	44	< 0.001
INFECTED	73.4222	2.684	0.400			
INCREASE IN %LIGNIN						
CONTROLS	20.5333	2.573	0.383	-10.90	44	< 0.001
INFECTED	23.6000	2.911	0.434			

Table 82: SUMMARY OF ANALYSIS OF LIPID, CELLULOSE AND LIGNIN IN WOOD BLOCKS BEFORE AND AFTER 3 MONTHS OF INOCULATION WITH S.sanguinolentum

SAMPLE NO.	%LIPID CONTROL	%LIPID INFECTED	%LIGNIN CONTROL	%LIGNIN INFECTED	%CELLULOSE CONTROL	%CELLULOSE INFECTED
DOMINANT						
1G1	4	6	22	30	74	64
2	4	4	24	30	72	66
3	2	4	24	32	74	64
7	12	2	20	34	68	64
5	2	2	20	30	78	68
2G13	4	2	26	32	70	66
7	2	2	18	30	80	68
8	2	4	20	30	78	66
10	2	2	26	34	72	64
12	2	2	22	28	76	70
3G1	2	2	30	30	68	68
2	2	2	28	32	70	66
6	2	2	20	32	78	66
7	4	2	24	32	72	66
8	2	2	22	30	76	68
CODOMINANT						
1B6	2	6	20	28	78	66
2	2	6	20	28	78	66
7	6	6	18	28	76	66
4	4	4	20	28	76	68
5	2	6	22	22	76	72
2B1	6	4	18	24	76	72
8	2	4	20	28	78	68
3	4	2	20	28	76	70
4	8	2	18	28	74	70
6	4	2	20	26	76	72
3B6	4	4	20	28	76	68
2	2	4	18	26	80	70
3	6	2	18	30	76	68
7	2	2	20	30	78	68
3B5	2	2	20	30	78	68
SUBDOMINANT						
1R9	2	6	20	28	78	66
10	2	6	20	30	78	64
12	2	4	20	24	78	72
11	2	2	18	30	80	68
13	2	2	20	28	78	70
2R8	4	4	20	28	76	68
2	4	2	20	26	76	72
3	2	6	22	30	76	64
9	2	4	18	28	80	68
5	6	2	16	24	78	74
3R1	2	6	20	24	78	70
2	4	4	20	28	76	68
9	2	2	20	28	78	70
4	4	6	18	30	78	64
10	2	6	18	28	80	66

Table 83: RESULTS OF T TESTS COMPARING LIPID,CELLULOSE AND LIGNIN IN WOOD BLOCKS BEFORE AND AFTER 3 MONTHS OF INOCULATION WITH S.sanguinolentum IN ALL THE DIFFERENT TREE VIGOUR CLASSES

SAMPLES	MEAN	SRANDARD DEVIATION	STANDARD ERROR	T VALUE	DF	PROBABILITY
			DOMINANT (%LIPID)			
CONTROLS	3.3333	2.582	0.667	0.89	14	> 0.05
INFECTED	2.6667	1.234	0.319			
			DOMINANT (%CELLULOSE)			
CONTROLS	73.7333	3.845	0.993	8.07	14	< 0.001
INFECTED	66.2667	1.831	0.473			
			DOMINANT (%LIGNIN)			
CONTROLS	23.0667	3.369	0.870	-8.94	14	< 0.001
INFECTED	31.0667	1.668	0.431			
			CODOMINANT (%LIPID)			
CONTROLS	3.8667	2.200	0.568	0.17	14	> 0.05
INFECTED	3.7333	1.668	0.431			
			CODOMINANT (%CELLULOSE)			
CONTROLS	76.8000	1.474	0.380	10.58	14	< 0.001
INFECTED	68.8000	2.111	0.545			
			CODOMINANT (%LIGNIN)			
CONTROLS	19.4667	1.187	0.307	-11.37	14	< 0.001
INFECTED	27.4667	2.200	0.568			
			SUBDOMINANT (%LIPID)			
CONTROLS	2.8000	1.265	0.327	-2.09	14	> 0.05
INFECTED	4.1333	1.767	0.456			
			SUBDOMINANT (%CELLULOSE)			
CONTROLS	77.8667	1.407	0.363	10.68	14	< 0.001
INFECTED	68.2667	3.105	0.802			
			SUBDOMINANT (%LIGNIN)			
CONTROLS	19.3333	1.447	0.374	-13.48	14	< 0.001
INFECTED	27.6000	2.165	0.559			

Table 83A: %REDUCTION IN CELLULOSE IN WOOD BLOCKS 3 MONTHS AFTER INOCULATION

TREE CLASS	MEAN %CELLULOSE		MEAN %REDUCTION
	CONTROLS	INFECTED	
DOMINANT	73.7	66.3	7.4
CODOMINANT	76.8	68.8	8.0
SUBDOMINANT	77.9	68.3	9.6

Table 83B: INCREASE IN %LIGNIN IN WOOD BLOCKS 3 MONTHS AFTER INOCULATION

TREE CLASS	%LIGNIN		MEAN %INCREASE
	CONTROLS	INFECTED	
DOMINANT	23.1	31.1	8.0
CODOMINANT	19.5	27.5	8.0
SUBDOMINANT	19.3	27.6	8.3

83 and 83B).

c. **Wood blocks derived from subdominant trees**

A comparable situation occurred in wood blocks derived from subdominants where no significant differences were observed in mean % lipid content in control blocks (2.8%) compared with inoculated samples (4.1%) (Table 83). There was a significant reduction in cellulose content ($P< 0.001$) . Mean % cellulose was reduced from 77.9% to 68.3% in control and in infected wood blocks respectively or 9.6% mean reduction (Tables 83 and 83A). Lignin % increased significantly ($P< 0.001$) from 19.3 to 27.6% in inoculated blocks (8.3% mean increase,tables 83 and 83B).

Further analyses showed that differences in the amount of cellulose lost in dominants,codominants and subdominants were not significant. All wood blocks lost approximately equal amounts of cellulose (Table 84). A comparable situation also occurred in lignin (Table 84).

If data for all tree classes were pooled (dominants,codominants and subdominants) a significant reduction in cellulose content was noted (Table 85). Mean % cellulose fell from 76.1% to 67.85 in controls and in inoculated blocks respectively. In contrast there was a significant increase ($P< 0.001$) in lignin percent ; mean % lignin increased from 20.6 to 28.7% (Table 85).

7.4.3. Comparison between two and three months inoculation results

There was a significantly greater loss ($P< 0.001$) in cellulose 3 months after inoculation compared with 2 months in wood blocks derived from all 3 tree vigour

Table 84: RESULTS OF ANALYSIS OF VARIANCE COMPARING CHANGES IN CELLULOSE AND LIGNIN CONTENTS OF WOOD BLOCKS 3 MONTHS AFTER INOCULATION WITH *S.sanguinolentum*

SOURCE OF VARIATION	DF	SS	MSS	F RATIO	P
%REDUCTION IN CELLULOSE					
TREATMENT	2	36.97778	18.48889		
ERROR	42	469.33350	11.17461		
TOTAL	44	506.31128		1.65454	> 0.05
INCREASE IN % LIGNIN					
	2	1.24438	0.62219		
	42	362.66675	8.63492		
	44	363.91113		0.07206	> 0.05

Table 85: RESULTS OF T-TESTS COMPARING CHANGES IN CELLULOSE AND LIGNIN CONTENTS IN WOOD BLOCKS 3 MONTHS AFTER INOCULATION WITH *S.sanguinolentum* FOR ALL TREE VIGOUR CLASSES

SAMPLES	MEAN	STANDARD DEVIATION	STANDARD ERROR	T VALUE	PROBABILITY
%REDUCTION IN CELLULOSE					
CONTROLS	76.1333	3.027	0.451	16.52	< 0.001
INFECTED	67.7778	2.601	0.388		
INCREASE IN %LIGNIN 3 MONTHS AFTER INOCULATION (POOLED DATA)					
CONTROLS	20.6222	2.790	0.416	-19.19	< 0.001
INFECTED	28.7111	2.599	0.387		

classes (Table 86). Mean percentage losses of cellulose were 7.4% and 3.2% ;8% and 2.4% ;and 9.6% and 2.8% in dominants,codominants and subdominants at 3 and 2 months respectively (Table 87). There was a corresponding increase in percent lignin. The increase in percent lignin was much greater after 3 months as compared with 2 months after inoculation. The significance of differences in dominants ,codominants and subdominants were summarised in table 87.

T.viride contaminated 10 samples and these were replaced during the course of experiments. In these samples T.viride replaced S.sanguinolentum completely (Plate 13). It is of interest to note that 3 months later the examination of the contaminated samples showed no signs of decay or any abnormal discolouration indicating that T.viride succeeded in replacing and suppressing the activity of S.sanguinolentum (Plate 14).

7.5. Wood density analysis

Table 90 shows the density of wood blocks taken from dominant,codominant and subdominant trees. There was a significant ($P< 0.01$) difference in wood density between dominants ,codominants and subdominants (Table 88). The difference in wood density was not significant when dominants were compared with codominants and codominants were compared with subdominants (Table 89). Nevertheless,density was significantly ($P< 0.01$) different comparing dominants and subdominants. There was a progressive increase in mean density from dominants (0.2846 gm/cm^3) to codominants(0.3026 gm/cm^3) to subdominants

Plate 13: Wood plugs previously inoculated with *S.sanguinolentum* which was completely replaced by *T.viride* (green colour).

Plate 14: 1. Wood plug (contaminated with *T.viride*)

2. Wood plug infected with *S.sanguinolentum* (not contaminated with *T.viride*).

(0.3152 gm/ cm^3) (Table 90).

Table 86: RESULTS OF ANALYSIS OF VARIANCE COMPARING CHANGES IN CELLULOSE AND LIGNIN IN WOOD BLOCKS 2 AND 3 MONTHS AFTER INOCULATION WITH *S.sanguinolentum*

SOURCE OF VARIATION	DF	SS	MSS	F RATIO	P
	%REDUCTION IN CELLULOSE				
TREATMENT	5	726.93311	145.38661		
ERROR	84	586.66699	6.98413		
TOTAL	89	1313.60010		20.81670	< 0.001
	INCREASE IN %LIGNIN				
	5	569.95532	113.99106		
	84	508.26709	6.05080		
	89	1078.22241		18.83900	< 0.001

Table 87: RESULTS OF T-TESTS COMPARING CHANGES IN CELLULOSE AND LIGNIN CONTENTS IN WOOD BLOCKS 2 AND 3 MONTHS AFTER INOCULATION WITH *S.sanguinolentum*

SAMPLES	MEAN	T-VALUE	PROBABILITY
		%REDUCTION IN CELLULOSE	
DOM. 2MONTNS	3.3333	4.2833	< 0.001
DOM. 3MONTHS	7.4667		
COD. 2MONTHS	2.4000	5.8031	< 0.001
COD. 3MONTHS	8.0000		
SUB. 2MONTHS	2.8000	7.0467	< 0.001
SUB. 3MONTHS	9.6000		
		INCREASE IN %LIGNIN	
DOM. 2MONTHS	3.6000	4.7502	< 0.001
DOM. 3MONTHS	7.8667		
COD. 2MONTHS	3.2000	5.3440	< 0.001
COD. 3MONTHS	8.0000		
SUB. 2MONTHS	2.4000	6.5315	< 0.001
SUB. 3MONTHS	8.2667		

Table 88:RESULTS OF ANALYSIS OF VARIANCE COMPARING WOOD DENSITY IN THE DIFFERENT NORWAY SPRUCE TREE VIGOUR CLASSES

SOURCE OF VARIATION	DF	SS	MSS	F RATIO	P
TREATMENT	2	0.01232	0.00616		
ERROR	75	0.08482	0.00113		
TOTAL	77	0.09714		5.44544	< 0.01

Table 89:RESULTS OF T-TESTS COMPARING WOOD DENSITY IN NORWAY SPRUCE WOOD BLOCKS IN DIFFERENT TREE VIGOUR CLASSES

SAMPLE	MEAN	T VALUE	DF	PROBABILITY VALUE
SUBDOMINANT	0.3152	1.3525	25	> 0.05
CODOMINANT	0.3026			
SUBDOMINANT	0.3152	3.2823	25	< 0.01
DOMINANT	0.2846			
CODOMINANT	0.3026	1.9298	25	> 0.05
DOMINANT	0.2846			

Table 90: RESULTS OF WOOD DENSITY ANALYSIS COMPARING WOOD BLOCKS TAKEN FROM DIFFERENT TREE (NORWAY SPRUCE) VIGOUR CLASSES (gm/cm^3)

	SUBDOMINANT	CODOMINANT	DOMINANT
	0.364	0.276	0.260
	0.296	0.316	0.260
	0.324	0.272	0.244
	0.288	0.272	0.228
	0.284	0.232	0.256
	0.352	0.296	0.260
	0.304	0.316	0.256
	0.384	0.352	0.264
	0.332	0.284	0.268
	0.312	0.300	0.280
	0.300	0.304	0.260
	0.324	0.292	0.260
	0.304	0.300	0.348
	0.296	0.316	0.328
	0.344	0.348	0.256
	0.324	0.336	0.328
	0.284	0.284	0.288
	0.292	0.208	0.336
	0.288	0.268	0.296
	0.284	0.336	0.344
	0.280	0.292	0.308
	0.296	0.376	0.308
	0.368	0.340	0.288
	0.312	0.344	0.260
	0.344	0.280	0.304
	0.316	0.328	0.312
MEAN	0.3152	0.3026	0.2846

CHAPTER 8

8. DISCUSSION

The frequency of infection by S.sanguinolentum through injured tissues resulting from extraction operations differed in Norway spruce and in Japanese larch stands of comparable age. In addition ,the infection frequency by S.sanguinolentum also varied in extraction wounds of the same age (4 years) depending on tree species. The frequency of infection by S.sanguinolentum entering through extraction wounds was much greater in Norway spruce (47%) in compartment 11 than in Japanese larch trees either from compartment 3 (24%) or from compartment 21 (37%) (see page 76-80). Although the mean area of wounded tissues exposed to fungal invasion was much larger on damaged trees in both Japanese larch compartments (409 and 250 cm^2) as compared with Norway spruce (226 cm^2), the frequency of infection was much greater in Norway spruce trees. These results are in agreement with those of Pawsey and Gladman (1965) derived from their work in five Scottish forests. They found that Norway spruce was more susceptible to infection by decay fungi entering through extraction wounds compared with Sitka spruce and Japanese and European larch. Results obtained in the present study in isolating microorganisms from infected trees suggest that the higher susceptibility of Norway spruce and the relatively low susceptibility of Japanese larch to infection by S.sanguinolentum entering through extraction wounds was probably due to the activity of other microorganisms isolated together with S.sanguinolentum from the infected

trees. Support for this view is lent by the differences in the types and numbers of microbes isolated from the different tree species. S.sanguinolentum was the dominant species in Norway spruce,whereas C.cochliodes occurred more commonly than S.sanguinolentum in Japanese larch. The fact that S.sanguinolentum was isolated from Japanese larch only rarely suggested that C.cochliodes was an effective competitor with S.sanguinolentum in Japanese larch. Although C.cochliodes was also isolated from infected Norway spruce,there was no evidence that it is capable of replacing S.sanguinolentum in this situation . Hence,an interesting question emerged i.e why was it possible for C.cochliodes to dominate and suppress S.sanguinolentum in Japanese larch and not in Norway spruce?.

Some inherent characteristics in both Japanese larch and Norway spruce probably favour one microorganism and not the other e.g the nature and concentration of fungitoxic extractives in the different tree species. There is evidence that certain wood extractives are toxic to some fungi,the most common of which are phenolic compounds (see below).

Polyphenols are a broad class of wood extractives in many tree species (Hillis,1962). He noted that some plant tissues contain more polyphenols than others and also the amount of polyphenols varies from one species to the other. Polyphenols may play a significant role in the process of decay because of their toxic effect on many

pathogens. The amount of polyphenols also varies with different positions in the tree. Changes in air and water content in a tree might initiate certain chemical reactions that eventually produce heartwood. Further,Hillis was the first to report the occurrence of taxifolin in Japanese larch,noting that this compound is fungitoxic. Hence,taxifolin could have been responsible for the supression of S.sanguinolentum in Japanese larch in the present study. It is known that heartwood extractives are extremely important in decay resistance because of their toxicity to wood destroying fungi (Rudman,1964). Rudman noted that the most decay resistant wood in Eucalyptus spp was found in the butt outer heartwood,whereas the inner heartwood is most susceptible. The middle heartwood ranged from being susceptible to decay at its inner boundary to being resistant to decay at its outer heartwood boundary. Similar results had previously been found in Japanese and European larch (Pechmann and Schaile, 1950). Moving up the tree or towards the pith there is a reduction in tree resistance.

There is a general belief that durability of timber is genetically controlled (Scheffer and Hopp,1949; Scheffer and Englerth,1952 and Da Costa, Rudman and Gay, 1961). A study by Hubbes and Etheridge (1965) showed that the heartwood of balsam fir contains certain fungitoxic substances that act selectively for S.sanguinolentum and other primary invaders of this host against potential fungal competitors. They also noted these chemicals might be partially

destroyed by autoclaving. These results confirmed the previous work of Etheridge (1962) who found some fungitoxic substances in the heartwood of balsam fir that promoted the spread of S.sanguinolentum and not other competitors. The amount of decay in any given tree species is a function of inherent wood characteristics as well as external factors like the moisture content of the wood and the temperature (Scheffer and Cowling,1966). Scheffer and Cowling noted that the principal source of decay resistance in wood is the presence of toxic substances deposited during the formation of heartwood. Four major groups of wood extractives were found to have the toxic effect on microorganisms and these are carvacrol,thujaplicin,flavonoids and stilbenes. Thujaplicins are the most inhibitory group. Siegle (1967) extracted phenolic compounds (i.e pyrocatechol,pyrogallol and catechin) from healthy white birch,whereas stained (infected with microorganisms) wood of the same species produced only pyrocatechol. This led to the hypothesis that phenolic compounds in wood are substrates for the enzymatic oxidation initiated by decay fungi and that the products of oxidation were responsible for the discolouration in infected wood. Shain (1967) found that a phenol enriched,resin-soaked reaction zone was developed between the sound sapwood of loblolly pine and the sapwood previously infected with F.annosus . The reaction zone was formed in advance of the pathogen and acted as a host defense mechanism. Phenolic compounds were extracted from the reaction zone and were found to

be toxic to F.annosus and reduced its spread in loblolly pine trees. Among other fungitoxic substances extracted from the reaction zone were pinosylvin and pinosylvin monomethyl ether. Also,phenolic compounds were extracted from the reaction zone in Norway spruce by Shain and Hillis (1971). The most important of these was hydroxymatairesinol which was extremely toxic to F.annosus . There is evidence that certain fungi are capable of utilising phenolic compounds which are toxic to other decay fungi. Shortle,Tattar and Rich (1971) showed that Phialophora melinii was capable of detoxifying certain phenolic compounds found in sugar maple. By doing this Phialophora melinii paved the way for Fomes connatus to grow in sugar maple trees which otherwise would not exist in the presence of fungitoxic phenols. The selectivity of a host for certain microorganisms and the others was recently demonstrated by Lin and Hubbes (1978). They found that fresh P.resinosa stump surfaces act selectively for primary invaders such as F.annosus and not for competing microorganisms. Hart and Shrimpton (1978) concluded that decay resistance is a result of interactions of toxic and non-toxic extractives with each other and with fungi during the decay process. It may be impossible to confer on one substance out of the entire heartwood sole responsibility for decay resistance. More critical work is needed in this area of tree resistance to decay by various microorganisms.

Following successful infection by S.sanguinolentum in

both tree species it was evident that the fungus developed much more rapidly in Norway spruce than in Japanese larch. Mean radial and vertical expansion of decay and mean decay volume were considerably greater in Norway spruce than in Japanese larch. Again this might be explained by the fact that antagonism and competition of other microorganisms on S.sanguinolentum were less evident in Norway spruce than in Japanese larch or that Norway spruce is less resistant to decay. Mean vertical expansion of decay recorded in this study was 35 and 37.5 cm/year in younger and in older Norway spruce trees respectively which is in close agreement with Pawsey and Gladman's (1965) observed maximum value of 40 cm/year and Kallio's (1976) reported value of 39 cm/year. Pawsey and Stankovicova (1974a) reported that the upward expansion varied from 20 to 85 cm/year from 2-year-old scars. Previously,Pawsey and Stankovicova (1974b) found maximum extension of 83.8 cm in 3 months from a 5 cm deep,artificially inoculated increment borer hole in a Norway spruce stem. Recently,Hansen and Hansen (1980) reported maximum extension of 40 cm/year in artificially wounded Norway spruce trees. In the present study, the infection column in Norway spruce was found to be completely different from that in Japanese larch. The main difference was the discontinuity of the decay column and the absence of the grey zone surrounding the decay column in Japanese larch as compared with Norway spruce. . The term grey zone is probably analogous to several other terms which have been used before e.g pathological

heartwood (Busgen and Munch ,1929); protection wood (Jorgensen,1961); wound-initiated discolouration (Shigo,1967); reaction zone (Shain and Hillis,1971) and discoloured wood (Chen,Chang, Cowling,Hsu and Gates,1976). Shain and Hillis (1971) described the grey zone in Norway spruce as a non-specific response to several decay fungi and to mechanical injury. In the present study no attempt was made to analyse chemically the grey zone, hence it was not possible to say categorically whether this zone is effectively hindering the expansion of S.sanguinolentum or not. The most fungitoxic extractive isolated from the grey zone was hydroxymatairesinol in Norway spruce and was found to be inhibitory to F.annosus (Shain and Hillis,1971). Shain (1978) found that antifungal compounds accumulated in the reaction zone indicating that it is a host defense mechanism. This zone was described as necrotic tissues enriched with inhibitory extractives and produced in advance of the infection as a means of host response to infection by microorganisms.

The first 6 cm of wood measured from the outer bark of Norway spruce towards the pith were colonised by a variety of microorganisms other than S.sanguinolentum . Vertically these microorganisms were isolated from samples taken between 1 and 12 cm above and below the site of the wound. T.viride was the next most common species and moreover showed the next greatest radial expansion. Also T.viride expanded vertically as far as the distal parts of the decay columns. This frequency and pattern of distribution of T.viride suggested that this fungus had

potential capabilities of replacing S.sanguinolentum which was confirmed during artificial inoculation experiments (Page 166). The relatively low frequency of S.sanguinolentum isolated from wood closer to the wound was probably due to the frequent occurrence of a variety of other microorganisms which probably competed with S.sanguinolentum . This was confirmed by the dramatic reduction in the occurrence of other microorganisms with increasing distance towards the pith ,whereas the frequency of S.sanguinolentum increased progressively with distance from the outer bark towards the pith. The consistent isolation of T.viride from the decay column as the next most common species in Norway spruce and its antagonistic effect on S.sanguinolentum (discussed below) suggested a certain pattern of succession where T.viride followed S.sanguinolentum in the decay column and sometimes overgrew and replaced it. In addition,Bacteria seemed to be common in the later stages of decay and were the only microorganisms isolated from wet and extremely decayed samples. Gundersen (1967) found that Penicillium spp, Trichoderma spp and M.hiemalis inhabit naturally the outer bark of Norway spruce.

In the present study F.annosus was isolated from a very limited number of samples (0.1%) taken from infected Norway spruce and was completely absent from Japanese larch. This confirmed the results demonstrated by Pawsey and Gladman (1965) where F.annosus was isolated from only 0.25% of the scars on Norway spruce in their study (Page

6). The low frequency of *F.annosus* in wounded Norway spruce in compartment 1016 (0.1%) could not be attributed to any lack of inoculum as fresh *F.annosus* sporophores were abundant in this compartment. There are differences in the mode of infection of conifers by *F.annosus* in Britain,Europe and North America. In Britain, *F.annosus* was found to attack mainly freshly-cut stump surfaces,spreading through root contacts (Rishbeth,1950,1951,1957 and 1959) and rarely attacked through exposed wounds resulting from extraction operations (Pawsey and Gladman,1965). In North America,however *F.annosus* was a major wound pathogen attacking Western hemlock (Englerth,1942; Englerth and Isaac; 1944; Rhoads and Wright, 1946 and Wright and Isaac,1956). In Sweden,it was reported that *F.annosus* together with *S.sanguinolentum* were the most successful decay fungi that caused extensive rots in Norway spruce as a result of the infection of extraction wounds (Nilsson and Hyppel,1968). Further,Schonhar (1975) reported that *F.annosus* was isolated from only 1% of the samples taken from injured Norway spruce trees in Germany. Recently,Norokorpi (1979) found *F.annosus* causing butt rot but not occurring as a wound invader of Norway spruce in Northern Finland. The variations in climatic conditions from one country to another might have affected the biology of *F.annosus* and produced strains with different modes of infection. Another factor is possibly the antagonistic effect of of *S.sanguinolentum* on *F.annosus* which was previously reported by Rishbeth (1970).

The development of decay in Norway spruce was affected considerably by both tree and wound parameters. It is well-known that S.sanguinolentum attacks exposed woody tissues by means of air-borne spores which must alight on exposed (wounded) tissues for successful infection to occur. The results of the present study showed that the larger the area of exposed wood the larger is the vertical expansion of decay,thus confirming recent observations of Hansen and Hansen (1980). They found that the rate at which decay fungi attacked injured Norway spruce increased directly with increasing size of wound. This could be attributed to the possibility that the larger the area of exposed tissues the greater will be the chance for many air-borne spores of S.sanguinolentum to alight and establish successful infection. It has been well known that the inoculum potential (e.g air-borne spores of S.sanguinolentum) is an important factor in the process of infection.

Garrett (1960) noted that the success of a microorganism to colonise a substrate is determined by :

1. Saprophytic competitive ability of the particular microorganism
2. Inoculum potential of the microorganism
3. Environmental factors including the populations of other microorganisms.

Garrett defined the inoculum potential as follows: "Inoculum potential is defined as the energy of growth of a parasite available for infection of a host,at the surface of the host organ to be infected". To this Garrett added a

necessary corollary: "Implicit in this definition of inoculum potential as the energy of growth of a parasite is the qualification of energy of growth per unit area of host surface,without which any such definition would be meaningless". It follows that the concentration of inoculum potential per unit area of a host must be high enough to overcome the host resistance opposing the invasion. Baker,Maurer and Maurer (1967) provided a mathematical model showing the importance of the inoculum potential . The model showed that disease severity is dependent on the inoculum potential and disease potential,where disease potential is the susceptibility of the host to infection. A further explanation was demonstrated by Baker (1968) who found that with a few exceptions,increases in inoculum density should result in increases in increments of the disease. The author stated: "Each added increment of susceptible area on the root,each additional root tip and any increase in rate of root growth will increase the chance of infection court touching inoculum". Thus,it could be concluded that the inoculum potential can be increased either by the movement of a motile host towards the inocula e.g roots or by the accumulation of enough amounts of inocula on the host surface e.g air-borne spores of S.sanguinolentum . Hence,the direct relationship between the wound surface area and the vertical expansion of decay might be attributed to the inoculum potential i.e the larger the exposed area of woody tissues the more is the chance for more inocula to alight and cause successful

infection. Karkkainen (1971) reported that the increase in the amount of decay in Norway spruce was positively correlated with the scar area,but the correlation was not significant.

A direct relationship was demonstrated between dbh and radial penetration of decay. A negative correlation was demonstrated between dbh and and radial penetration of decay on the one hand,and the number of rings/5 cm from the outer bark on the other. This could be explained as follows:

Cell-walls of summer wood are much thicker compared with spring wood (Toumey,1947). It seems that these thick cell-walls present a physical barrier against the radial penetration of S.sanguinolentum . Trees with larger dbh or fast growing trees produce more wood during the growing season compared with small or slow growing trees of the same species,age and site quality. It follows that the more wood produced each year,the less is the number of annual rings per unit distance from the outer bole towards the pith. S.sanguinolentum or any other decay fungus has a certain capacity to penetrate the wood of Norway spruce.Hence if more physical obstacles (rings) were encountered the spread of radial penetration of the fungus is reduced. Thus,a fast growing tree has a considerably smaller number of rings per unit distance and this has resulted in more rapid radial expansion of S.sanguinolentum compared with a slow growing tree of the same species,age

and site quality.

Basham (1957) found a similar relationship between tree growth rate and the radial expansion of decay derived from his work on Jack pine (P.banksiana) ,red pine (P.densiflora) and white pines (P.strobus). He showed that the width of annual rings is directly related to the tree diameter. Larger or fast growing trees were found to have wider and fewer annual rings in the outer bole per unit distance compared with smaller or slow growing trees. The outer walls of the annual rings act as physical barriers to fungal penetration into the wood. Wider annual rings provide a wide layer of sapwood for decay fungi to attack. Slow growing trees have a large number of growth rings with relatively thicker cell-walls compared with a lesser number of rings with thinner cell-walls in fast growing or large trees. Thus,the radial penetration of decay was greater in fast growing than in slow growing trees. The direct relationship between the progress of decay and the frequency of growth rings was demonstrated by other investigators. Southam and Ehrlich (1943) believed that the differences in ring frequency might affect the progress of decay. Kimmey and Furniss (1943) found that the width of growth rings in Douglas fir in North America had a considerable effect on the rate of deterioration. They found that,other things being equal, the more growth rings per unit distance a tree contained,the slower was its rate of deterioration. Decay spread was found to be faster in fast growing trees than in slow growing trees. Foster and

Foster (1951) found that decay of Western hemlock in British Columbia had increased progressively with diameter,age and decreasing site quality. Although decay generally increased with increasing diameter, extensive variations in individual trees were noted. Parker and Johnson (1960) reported a direct relationship between tree volume and radial penetration of decay in white spruce and balsam fir in Prince George region of British Columbia. The importance of ring count was further noted by Da Costa,Rudman and Gay (1961) in Australia. They found that slow growing trees are more durable than fast growing trees e.g wider rings in teak (Tectona grandis L.f.) were associated with less durability. The growth of P.weirii Murr. in Douglas fir roots in the Pacific North-West was more rapid in roots of larger diameters than in smaller roots (Wallis and Reynolds,1962). Further,resistance of trees to decay was found to increase with the increase in the number of growth rings between the outer part of the tree and the pith in the genus Eucalyptus (Rudman,1964). Rudman found that the inner heartwood of Eucalyptus spp was most susceptible to decay. Recently, Karkkainen (1971) found a positive relationship between the number of growth rings/cm and the advance of decay upwards and downwards in Norway spruce,but this correlation was not significant. More recently,Norokorpi (1979) found that decay frequency in Norway spruce was strongly dependent on tree diameter and age.

In the present study it was found that the density of

wood is considerably greater in slow growing trees than in fast growing trees i.e slow growing trees had more weight of wood substance per unit volume compared with fast growing trees of the same species,age and site quality. Density or specific gravity of wood had a considerable effect on the progress of decay. In addition,a direct relationship between tree volume and dbh on the one hand,and decay volume on the other were also demonstrated in the present study. This confirmed the observations of Southam and Ehrlich (1943) :

1. Fast growing trees have a low specific gravity or light wood,hence more intracellular spaces are present in fast growing trees. These spaces are of considerable importance as they contain certain amounts of air and water. Both air and water are of great importance for fungal growth. There is a greater probability that the air to water ratio is within the requirements for fungal growth in fast growing than in slow growing trees.

2. Since decay is a chemical process,it is apparent that the rate of decay is directly related to the surface area of wood substance exposed to the action of fungal enzymes. A heavy wood has thicker cell-walls and this in turn would mean that vessels of heavy wood would have smaller lumina and hence a smaller surface area of wood substance exposed to the enzymatic activity of decay fungi. If this were true,the rate of decay would be smaller in slow growing trees compared with fast growing trees with light wood that contains relatively thinner cell-walls and

larger lumina,and hence a larger surface area of wood would be exposed to the enzymatic activity of decay fungi and consequently much greater decay would be expected. Thus,it could be concluded that the number of growth rings per unit distance and the density of wood interact to affect the progress of decay.

From the relationships between different tree and decay parameters demonstrated in this study, it was possible to demonstrate multiple correlations that facilitate the construction of other equations enabling the forest manager to estimate the damage by S.sanguinolentum with the knowledge of at least two variables. It was possible to estimate the decay volume provided that the wound surface area and the dbh are known as follows:

$$Y = 0.0001\ X1 + 0.00196\ X2 - 0.02177$$

Y = decay volume; X1 = wound surface area and X2 = dbh. The radial penetration of decay could also be estimated from the dbh and the number of growth rings/5 cm from the outer bark. Also the results allowed for the estimation of the vertical expansion of decay as a function of the wound surface area. Such models might be useful to the forest manager to estimate the progress of decay for different species on different site classes. It could be concluded that the wound surface area and tree growth rate were the most important factors that determine the progress of decay caused by S.sanguinolentum entering through extraction wounds on Norway spruce. It follows that damage to the residual crop should be avoided,in

particular the production of large wounds on fast growing trees,as these conditions result in the worst decay by S.sanguinolentum .

The correlations demonstrated for Norway spruce did not hold for Japanese larch. Most of the significant correlations between tree and wound parameters derived from the examination of infected Norway spruce,were not significant in Japanese larch. The only similarity is the direct relationship between the wound surface area and the vertical expansion of decay in Norway spruce and in Japanese larch in compartment 21. However,this relationship did not hold for Japanese larch in compartment 3 suggesting that other factors were involved in the relationship. On the other hand,there was a negative correlation between tree growth rate expressed as tree volume or dbh and the percentage of decay volume. This is obvious as a small percentage of decay in a larger tree would be equivalent to a higher percentage in a smaller tree. However,this relationship was not found in Norway spruce indicating the occurrence of some variation in the response of individual Norway spruce trees to infection. The many non-significant correlations recorded for Japanese larch further confirms that the activity of microorganisms especially C.cochliodes and some inherent characteristics (i.e the type and concentration of fungitoxic extractives) are two factors that might explain the limited progress of S.sanguinolentum in injured Japanese larch trees.

Re-isolation of fungi following artificial inoculations of

Norway spruce with S.sanguinolentum showed that few other fungi were able to colonise these sites. The results confirmed the increase in the frequency of S.sanguinolentum with increasing distance towards the pith. Other microorganisms were mainly concentrated in the superficial layers of the wood closer to the site of inoculation and their frequency declined dramatically from the outside towards the pith.

With the exception of S.sanguinolentum , C.cochliodes and T.viride ,all other microorganisms were isolated from samples taken between 1 and 7 cm above the site of inoculation and within a range of 20 cm below the site of inoculation. T.viride and C.cochliodes expanded as far as the distal parts of the decay columns. In three trees discolouration caused by S.sanguinolentum has already started,but did not develop further and was soon suppressed by the activity and antagonism of T.viride . In these trees T.viride has replaced S.sanguinolentum completely in the decay columns. In another instance C.cochliodes showed the same effect on S.sanguinolentum which was replaced by the former species. The fact that T.viride and C.cochliodes had replaced S.sanguinolentum in the decay columns suggested a form of microbial succession and it may provide a possible means of biological control of S.sanguinolentum by both T.viride and C.cochliodes . Further research is needed in this area as to whether T.viride and C.cochliodes are capable of effectively controlling the spread of S.sanguinolentum in Norway spruce

. The antagonistic effect of T.viride on several decay fungi is already known to many investigators. As early as 1934,Weindling reported that the antagonism of T.viride originated from the excretion of two distinctly different substances,gliotoxin active both as a bactericide and a fugicide and viridin exclusively a fungicide. It was noted that a single strain of T.viride produces either gliotoxin or viridin (Brian and Hemming,1945). Rishbeth (1951) reported antagonistic effect of T.viride on F.annosus and the former was capable of completely suppressing the latter only at high temperatures. Optimum temperature for growth of T.viride was reported to be between 25 and 30 C° and growth was slow at 10 C° and below (Ward and Henry,1961). Huppel (1963) confirmed the same results and found that T.viride was capable of replacing F.annosus only at temperatures between 25 and 30 C° . Webster and Lomas (1964) carried out experiments on various isolates of Trichoderma spp. They found that the antagonism of T.viride is due to the production of an antibiotic which may be neither gliotoxin nor viridin. However,both gliotoxin and viridin were produced by Gliocladium spp and this species,according to Webster and Lomas (1964) had been erroneously considered by Weindling (1934) as Trichoderma spp. Inhibitory effects of Trichoderma spp on F.annosus ; Rhizoctonia solani (Kuhn.) ; Pyronema domesticum (Sow ex Fr.) Sacc.; Fusarium oxysporum (Schlecht.) ex Fr.; Pythium ultimum (Trow.) and M.hiemalis , have been demonstrated in culture by Dennis and Webster (1971). However,they found

that the inhibitory effect was not due to the production of gliotoxin or viridin by Trichoderma spp but was due to other chloroform-soluble antibiotics secreted by Trichoderma spp,including trichodermin and peptide antibiotics. The inoculation of cubes of wood of Pinus spp with T.viride prevented the decay of these cubes by P.monticola (Toole,1971). Recently,the inoculation of pruning wounds on plum trees with spore suspensions of T.viride had effectively controlled the infection of these wounds by S.purpureum (Fr.) Fr. (Groscluade,Ricard and Dubos,1973). Further, Dubos and Ricard (1974) confirmed the effectiveness of T.viride propagules in preventing the infection of plum trees by S.purpureum .

In the artificial inoculation experiments, the treatment of the inoculation site (i.e left open or sealed with paraffin wax after the inoculation) had no effect on the the frequency of S.sanguinolentum above and below and at the distal parts of the decay columns and at all depths (measured from the outer surface towards the pith) comparing open with sealed inoculation sites.

The time of year at which the inoculations were made affected both the frequency of successful infection and the development of decay in some cases. No differences were found in the infection frequency in sealed sites inoculated in July compared with those inoculated in February. However,the infection frequency was much greater in open sites inoculated in July than in open sites inoculated in February. Trees inoculated in July showed much higher

infection frequency in dominant and codominant trees,whereas no significant differences were observed in different tree classes inoculated in February. Inoculation site treatment affected frequency of infection in July inoculations only and had no effect on February inoculations. Open sites inoculated in July showed a much greater frequency of infection than sealed inoculation sites. This might be attributed to the possible changes occurring when tissues are exposed to the air that might favour the development of S.sanguinolentum in open inoculation sites. Shigo (1965) suggested that when a tree is wounded a series of chemical changes takes place in the exposed tissues. These involve the excretion of certain chemicals by injured,exposed parenchyma cells. The exposure of these substances to the atmospheric air leads to their oxidation,and hence to a change in the original chemical composition of the injured tissues. Shigo reported changes in moisture content and pH of the exposed tissues. Sucoff,Ratsch and Hook (1967) confirmed the results of Shigo and added that changes in oxygen and carbon dioxide also take place in the exposed tissues. Thus,it could be concluded that leaving the inoculation site open would result in physical as well as chemical changes in the exposed tissues as a result of oxidation and changes pH and air to water ratio. These changes might have favoured the conditions for the growth of S.sanguinolentum in open compared with sealed inoculation sites. Some investigators found that the susceptibility of wood to infection by

S.sanquinolentum is increased with a reduction in the moisture content of the wood. Bier (1966) found that S.sanquinolentum successfuly colonised oven-sterilised samples of wood taken from the heartwood of Abies lasiocarpa at all moisture contents between 51 and 171% . However,greater inhibition of S.sanquinolentum occurred at higher moisture contents between 131 and 187%. This was confirmed by Nilsson and Hyppel (1968). Based on their results on the infection of wounded Norway spruce,with S.sanquinolentum and F.annosus in Sweden,they noted that there should be exposed woody tissues with a suitable moisture content for the growth of decay fungi. Such exposed zones occur at certain positions on the tree. The scar position on the root is of considerable importance e.g the infection frequency in root scars occurring at 0-50 cm from the stem base was 50-100% ; 10-25% in scars 50-100 cm and 0-5% in scars > one metre away from the stem base. These variations were related to the increase in the moisture content with distance from the tree base. More recently,Pawsey and Stankovicova (1974b) confirmed these results in artificially inoculated Norway spruce. The global mean infection frequency was much greater in trees inoculated in July than in those inoculated in February. If all values from the July and February investigations were pooled ,no significant difference in the infection frequency in all tree vigour classes was observed in sealed inoculation sites. However, infection frequency was greater in dominant trees compared with codominant and

subdominant trees with open inoculation sites. The higher frequency of successful infection in dominant trees,in some cases, could be attributed to the favourable conditions in fast growing trees that provide better conditions for fungal growth as discussed above (Pages 275-279).

Time of year also affects the rate of development of decay. Both radial and vertical expansion of decay was much greater in trees inoculated in July compared with those inoculated in February irrespective of tree vigour or inoculation site treatment. No differences in the global mean radial and vertical expansion of decay from open and sealed inoculation sites were observed irrespective of the time of inoculation. Thus,it could be concluded that time of year had influenced the radial and vertical expansion of decay. Inoculation site treatment also affected the frequency of successful infection in some cases. In trees inoculated in July there was a tendency to higher infection frequency in dominant trees compared with codominant and subdominant trees. These findings contrast with the observations of Pawsey and Stankovicova (1974b),who found no consistent correlation between the time of inoculation and the development of S.sanguinolentum in Norway spruce. These differences (compared with the results of this study) might be due to the different climatic conditions which existed on the two study sites i.e One in England and two in Scotland. However,more recently (Hansen and Hansen,1981) reported that the season of wounding had a marked influence on the development of S.sanguinolentum in

artificial wounds on Norway spruce e.g of the 84 wounds they found invaded with S.sanquinolentum , 6 were July wounds; 33 September wounds; 19 December wounds and 26 May wounds,but no correlation was found between the infection frequency and tree diameter classes. The correlations derived from artificial inoculation results further confirmed those obtained from naturally infected trees. Generally,fast growing trees favoured the development of decay fungi compared with slow growing trees. Two correlations recorded as significant from artificial inoculation experiments,were not significant in the case of naturally infected trees. These were the correlation between tree growth rate expressed as its dbh and the vertical expansion of decay and between the radial and the vertical expansion of decay. The relationship between the dbh and the vertical expansion of decay might be explained on the basis of variations in the wood density and specific gravity as previously discussed. The correlation between radial and vertical expansion of decay could be attributed to the possibility that the greater the radial expansion of decay,the more inocula might have penetrated the host and thus a greater vertical expansion of decay would be expected as well. Thus,it could be concluded that if the wound surface area is constant (artificial inoculation experiment) then the vertical expansion of decay is dependent on the radial penetration of decay ,but if the wound surface area is variable as in naturally injured and infected trees,the vertical expansion

of decay in this case is totally dependent on the surface area of exposed tissues as discussed previously (Pages 273-275).

Four strains of S.sanguinolentum were isolated from infected Norway spruce trees in this study. The differences between these strains were mainly macroscopic e.g shape,size of colonies,growth on different media and optimum temperature and pH. Generally,3% MEA (malt extract agar) supported the most extensive growth of all S.sanguinolentum strains. However,there were differences in the time required by each strain to attain a colony diameter of 74 mm and the temperature range at which this colony diameter was achieved.Colony diameters of 74 mm were achieved at 15 and 20 C° after 2 weeks;at 10,15,20 and 25 C° within 2-3 weeks; at 10,15,20,25 and 30 C° within 2-4 weeks and at 10,15,20 and 25 C° within 2-4 weeks for strains 1,2,3 and 4 respectively. It is clear that strain 3 attained a colony diameter of 74 mm over a comparatively wider range of temperature. In contrast,strain 1 achieved a diameter of 74 mm over the narrowest temperature range. Strains 1,2 and 3 often produce honey-like liquid drops on 3% MEA,whereas strain 4 rarely produced such exudates. Strain 4 was the most vigorous and produced very profuse aerial hyphae.

There were differences in the amount of growth supported by various carbon/nitrogen sources (plus basal medium) combinations. Strain 1 achieved 64 mm diameter after 4 weeks on fructose/sodium nitrate,whereas this combination of carbon and nitrogen sources resulted in

smaller amounts of growth of strain 4. Fructose/sodium nitrate and sucrose/sodium nitrate resulted in much better growth of strains 2 and 3. Maltose resulted in smaller amounts of growth of strains 1 and 3. Fructose/sodium nitrate and sucrose/ sodium nitrate resulted in faster growth of strains1,2 and 3; and strains 2,3 and 4 respectively. If ammonium sulphate was substituted as sole nitrogen source glucose/ammonium sulphate resulted in faster growth of strains 1 and 4,whereas strains 2 and 3 achieved the most extensive growth on sucrose/ammonium sulphate; and sucrose/ammonium sulphate and glucose/ammonium sulphate respectively. Maltose/ammonium sulphate resulted in smaller amounts of growth of both strain 1 and 3. Generally,sodium nitrate proved to be a more easily available nitrogen source for strains 2 and 4 compared with ammonium sulphate. In contrast,ammonium sulphate resulted in the most extensive growth of strain 1. Strain 3 was intermediate and it seems that both sodium nitrate and ammonium sulphate were almost equally available to this isolate. Asparagine was the most readily available nitrogen source compared with other organic and inorganic nitrogen sources. Generally, organic nitrogen sources were much better than inorganic nitrogen sources.

Strains 3 and 4 had a similar range of optimum temperature (10 to 30 C^{o}), whereas optimum temperature was between 10 and 25 and 5 and 25 C^{o} for strains 2 and 1 respectively. Visible growth ceased completely beyond 30 C^{o} for all strains. A similar range of optimum temperatures

was reported by other investigators. Cartwright and Findlay (1938) reported optimum temperatures between 10 and 29 C° for S.sanguinolentum ;15 to 26 C° (Robak,1942) and 7 to 26 C° (Yee,1974). The fact that no growth occurred beyond 30 C° , in this study,is in agreement with the results of Yee (1974) who found that no growth whatsoever occurred at or above 31 C° . This author found that S.sanguinolentum isolates produced tiny liquid drops only on liquid media and never on solid media,contrasting with the results of the present study as these exudates were produced on 3% MEA. The high variability of S.sanguinolentum strains was previously reported by Robak (1942); Yee (1974) and most recently by Hansen and Hansen (1981). Generally,acidic media promoted the total amount of growth of all strains and growth declined either gradually or dramatically towards alkalinity i.e being 74 mm in diameter at pH 4 and minimum at pH 8. Strain 1 attained 74 mm colony diameter at only two pH levels (4 & 5) ;strains 2 and 4 had a wider range of hydrogen ion concentrations at which they attained 74 mm diameter colonies (4 to 7) ,but the observed differences were not significant. Strain 3 occupied an intermediate position. It seems that strain 4 was the most tolerant to alkalinity as it produced relatively good growth at pH's 7.5 and 8 compared with other strains. The fact that S.sanguinolentum has a wide range of utilisable substrates ,a wide range of optimum temperature and pH coupled with its continuous presence on logs and litter on the forest

floor,makes it one of the most destructive decay fungi (Etheridge and Morin,1963).

Robak (1942) carried out a comprehensive study on the physiology of S.sanguinolentum and found that it varied considerably in culture. He observed a frequent occurrence of spherical swellings filled with dense contents. Robak did not consider these swellings as a diagonostic feature,as they were absent in some strains. More characteristic is the frequent occurrence of ladder-like anastomoses between parallelly running hyphae although this feature was also observed in other species. Growth of S.sanguinolentum on malt and potato dextrose agars was described as thin and arachnoid at first with no aerial hyphae. After some days the mat grew denser and takes on a conspicuous colour ranging from bright salmon red through srtong to rusty brown. Three types of further growth were observed:

1. The aerial mycelium was at first white and cottony,soon grew denser and after approximately ten days a thick and smooth,skin-like mat was developed. On malt agar the mat was thicker ,whereas the mat was depressed on potato dextrose agar. On both media the mycelial mat was woven and difficult to transplant. In two to four-week-old cultures tiny drops of liquid were exuded by the aerial mycelium. If these evaporated they leave pore-like pits. The colour of the colony remains white during the first ten days except the inoculum on which a yellow to orange colour appears after one week. Later the

colour spreads over smaller or greater parts of the colony.

2. Aerial mycelium is soft,cottony and presents a downy-wooly or wooly-clustered surface with a thin margin. The white colour of the colony remains the same for several weeks. Sometimes areas of yellow colour appear but restricted to the transplant only.

3. This growth is characterised by a very low,thin and scanty aerial mycelium. The submerged mycelium is red-orange or rusty brown dominating the whole mat. Colony margin is thin and arachnoid. The colour becomes molasseous brown with age. A denser aerial mycelium is developed only on the transplant. Growth on Czapek dox agar and on asparagine medium was extremely variable,not only from strain to strain,but also within the same strain. Growth was much thinner than on richer medium (e.g malt extract agar). Aerial mycelium was absent either totally or on a limited area of the colony. The colony margin was very thin,low and fringed. The medium is sometime stained a pale yellow changing with age into dark brown. In some isolates it was difficult to observe any clamp connections,whereas in others the clamp connections were too numerous to escape attention. Growth on different nitrogen sources was variable. Growth was slowly accelerated with increasing temperature. S.sanguinolentum had a wide range of optimum temperature between 15 and 26 C^{o} . Growth was inhibited at about 32 C^{o} . Robak (1942) and Yee (1974) described some strains of S.sanguinolentum that produced honey-like liquid drops in culture and this is

in agreement with the results of the present study.

A describtion of the growth characteristics of S.sanguinolentum in culture was also made by Nobles (1948):

1. Growth characters: Growth moderately rapid to slow,plates covered within three to five weeks. Hyphal advancing zone even or with broad shallow bays,with sparse cottony mycelium. Mat white at first and may become cream colour to chamois after four to six weeks and slightly raised. The newer growth is downy to cottony-floccose. The older part is felty and opaque. Reverse unchanged to honey-yellow. Odour of the culture is sweet. On gallic and tannic acid agars diffusion zones were moderately strong to strong.

2. Hyphal characters: Advancing zone: Hyphae are hyaline with simple septa and frequently branched. Cystidium-like structures are present probably analogous to conducting cells of fruit bodies. Contents are first hyaline and then dark brown. Submerged mycelium was also observed.

3. Type of rot:brown heart rot of conifers. A further description based on growth characters of S.sanguinolentum on malt agar (Difco) was also made by Nobles in a later paper (1965):

1. Production of extracellular oxidase:positive results
2. Septation of hyphae: thin-walled hyphae mainly simple septate. Single or multiple clamp connections occurred occasionally,being most frequent on the hyphae at the advancing zone. Hyphae hyaline and had resinous masses

clinging to the cell walls. Mat is white or pale coloured.

3. Occurrence of special structures formed by differentiation of hyphae: hyphae remains thin-walled and undifferentiated.

In the present study the degradation of cellulose and lignin was recorded in dominant,codominant and subdominant trees. 8 years after natural infection of Norway spruce with S.sanguinolentum a considerable reduction of cellulose had occurred in all tree classes examined. The greatest reduction of cellulose occurred in fast growing trees which lost about 50% of their initial cellulose content and this amounted to approximately twice the loss in slow growing trees. These results have further confirmed that fast growing trees were the worst affected by S.sanguinolentum . Several factors might explain the greater loss in wood substance in fast growing trees. These were wood density or specific gravity and the initial cellulose and lignin content of a tree. As early as 1917,Zeller investigated the effect of wood specific gravity and ring frequency on the rate of deterioration of wood expressed as the loss in weight of the material examined. The author concluded that the denser the wood,the more durable it is. Buckman (1934) confirmed the relationship between wood durability and its specific gravity ,but this relationship was found to hold good only for the sapwood and not the heartwood. Further,Garren (1939) confirmed the relationship between durability and specific gravity and added that this relationship is more evident during the early stages of of

infection. However,as infection develops,the higher specific gravity would mean more wood substance per unit volume and hence a decay fungus would cause more loss in wood with high specific gravity than in light wood. Spaulding and Hansbrough (1944) found that the development of decay was much greater in fast than in slow growing trees and they noted that the reason was due to the low specific gravity and to the availability of suitable amounts of air and water for fungal growth in fast as compared with slow growing trees. Recently,Liese (1970) found that the higher durability of dense wood is due to the fact that the denser the wood,the lesser is the surface area of wood substance exposed to the activity of the fungal enzymes as compared with light wood. Another factor that explains the greater loss of cellulose in fast compared with slow growing trees is probably the initial lignin content. Lignin encrusts other polysaccharides and hence protects them from exposure and accessibility to degrading enzymes secreted by decay fungi. Therefore an enzymatic system is required to depolymerise lignin so that other polysaccharides are no longer protected (Scheffer and Cowling,1966 and Liese,1970).

Some investigators found that the rate of decay is mainly governed by lignin modification rather than cellulose decomposition (Roelofsen,1956; Levi and Preston,1965 and Takahashi and Nishimoto,1976). Thus,the presence of much greater amounts of lignin in slow growing trees and the occurrence of lesser amounts of lignin in fast growing

trees might explain the greater loss of cellulose in fast growing trees compared with slow growing trees.

The results obtained from artificial inoculation of Norway spruce trees with S.sanguinolentum for 6 months contrasts with those from natural infections. In artificial inoculations,the loss was in lignin and it seems that cellulose was not affected. This is probably due to the fact that S.sanguinolentum as a white-rot fungus degrades lignin first and later attacks cellulose as well (Campbell, 1932a; Liese,1970; Highley and Kirk ., 1973 and 1979). However,it is also possible that a white-rot fungus might attack lignin in the early stages or both lignin and cellulose at the same time but in varying proportions (Campbell, 1932a). Again fast growing trees lost much greater amounts of lignin compared with slow growing trees (Page **217–224**).

Analysis of wood blocks,taken from dominant; codominant and subdominant trees,which were inoculated with S.sanguinolentum for one month showed that S.sanguinolentum had not yet begun to affect lignin or cellulose and probably the fungus was feeding on the media and on the soluble simple carbohydrates that might be available in the wood blocks. It has been shown that extracellular enzymes may not be effective if they diffuse outside the walls of the cells in which they are secreted. To be effective at least one hypha must be inside the cell (Scheffer and Cowling,1966). This might explain the negative results obtained one month after artificial inoculation of

wood blocks. However,analysis of artificially inoculated samples after 2 and 3 months showed a marked reduction in cellulose,although there were no significant differences in the amount of cellulose lost comparing tree classes (i.e dominant,codominant and subdominant).

. It is of interest to note that the analysis of decay in living trees, irrespective of natural or artificial infection showed a consistent maximum loss of wood substance in fast compared with slow growing trees,whereas this variation was not observed in artificially inoculated wood blocks. Three factors were probably responsible to these differences:

1. Taking wood samples from a living tree and surface sterilising them might have reduced the natural resistance of wood and have affected its ability to hinder the development of S.sanguinolentum .

2. The fungus was growing in a very rich medium (3% MEA) and was provided with reasonable humidity and optimum temperature for growth and all other competing microorganisms that naturally found with S.sanguinolentum in living trees were omitted from the substrate.

3. Orientation of decay blocks in vitro compared with in vivo .

Therefore,it seems that S.sanguinolentum was growing under favourable conditions and away from competing microorganisms and hence it consumed similar amounts of wood substance irrespective of the origin of the samples i.e from fast or slow growing trees. Roff and Whittaker (1966) recorded a 6% loss in weight of wood blocks taken

from lodgepole pine trees which were inoculated with S.sanguinolentum for 15 weeks. In this study,a 7.4% mean reduction in cellulose was recorded in wood blocks inoculated with S.sanguinolentum for 16 weeks.

Results of chemical analyses have further confirmed that fast growing trees are the worst affected with S.sanguinolentum compared with slow growing trees. In addition,T.viride succeeded in replacing and suppressing S.sanguinolentum in 10 samples. 3 months later the examination of these samples showed no signs of decay or any abnormal discolouration indicating and confirming the antagonistic effect of T.viride on S.sanguinolentum and its potential as a mean of possible biological control.

It is important to note that most of the decay occurred in the lower part of the trunks therefore the percent decay recorded in this study would be more serious as the rest of the tree is not usable as sawtimber. It follows that very serious commercial losses should be expected as a result of decay caused by S.sanguinolentum.

CHAPTER 9

9. CONCLUSIONS

1. The frequency of infection by S.sanquinolentum entering through similar-aged extraction wounds (4 years) varied with tree species. Infection frequency was much greater in Norway spruce compared with Japanese larch.

2. S.sanquinolentum was the most common species isolated from infected Norway spruce,whereas C.cochliodes was the dominant microorganism in Japanese larch.

3. The presence of different numbers and types of microorganisms in Norway spruce compared with Japanese larch, might be responsible for the differences in infection frequency in Norway spruce and as compared with Japanese larch.

4. Radial and vertical expansion of decay and decay volume was much greater in Norway spruce compared with Japanese larch. This might be explained by the fact that antagonism and competition by other microorganisms on S.sanquinolentum were less evident in Norway spruce than in Japanese larch..

5. The rate of decay expansion due to S.sanquinolentum in Norway spruce as recorded in the present study was in agreement with the results of other investigators.

6. The shape of the decay columns in Norway spruce was completely different from that in Japanese larch. In Japanese larch the decay columns were discontinuous and the grey zone was absent,whereas in Norway spruce decay columns were long and continuous and the grey zone occurred regularly.

7. No attempt was made in the present study to analyse chemically the grey zone,hence it was not possible to determine the possible effeciency of this zone in hindering the expansion of S.sanguinolentum .

8. The first six centimeters of wood measured from the outer bark of Norway spruce inwards were colonised by a variety of microorganisms other than S.sanguinolentum . These microorganisms were also isolated from samples taken between 1 and 20 cm vertically above and below the site of the wound.

9. T.viride was consistently the next most common species to S.sanguinolentum in Norway spruce and showed the next greatest radial expansion. It also occurred in the distal parts of the decay columns. This frequency and pattern of expansion suggest that T.viride may well antagonise and replace S.sanguinolentum in Norway spruce. Antagonism of S.sanguinolentum by T.viride was further confirmed during artificial inoculation of Norway spruce with S.sanguinolentum .

10. Although S.sanguinolentum was regularly isolated from samples of wood taken from the outer surface of the wound site through to the pith,most other microbes isolated were restricted to the superficial layers of the wood.

11. Bacteria seemed to be common in the later stages of decay and were the only microorganisms isolated from wet and extremely decayed wood samples.

12. F.annosus was isolated from a very limited number

of samples taken from Norway spruce confirming its previously observed rare occurrence as a wound-pathogen in Norway spruce.

13. The low frequency of F.annosus in wounded Norway spruce in compartment 1016 of Glentress forest could not be attributed to the lack of available inoculum as fresh sporophores of F.annosus were abundant in this compartment.

14. The development of decay caused by S.sanguinolentum in Norway spruce was influenced by wound and tree parameters as follows:

(i) Vertical extension of decay was directly related to wound surface area.

(ii) Radial penetration of decay was directly related to tree dbh.

(iii) Radial penetration of decay was inversely related to the number of growth rings per 5 cm from the outer bark inwards, Since the number of growth rings per 5 cm from the outer bark inwards was negatively correlated with tree dbh.

(iv) Decay volume was directly related to tree vigour expressed as tree volume or dbh.

(v) From individual correlations it was possible to demonstrate multiple correlations e.g between decay volume as a dependent variable and wound surface area and dbh as independent variables,and between radial expansion of decay (dependent variable) and dbh and frequency of growth rings per 5 cm from the outer bark towards the pith as

independent variables. Such correlations might be useful to the forest manager in estimating the development of decay.*

(xi) It was concluded that decay development is entirely governed by wound surface area and tree vigour. In turn tree vigour influences wood density and frequency of growth rings and both factors had a considerable effect on decay development.

15. Most of the correlations demonstrated for Norway spruce did not hold for Japanese larch. However,wound surface area was directly related with vertical expansion of decay ,but only in the case of Japanese larch trees in compartment 21 (Yair Hill forest) and not in compartment 3 (Yair Hill forest),suggesting that other factors may be involved in particular situations.

16. The many non-significant correlations recorded for Japanese larch suggest that the activity of competing microorganisms especially <u>C.cochliodes</u> might explain the limited progress of <u>S.sanquinolentum</u> in injured Japanese larch trees as compared with Norway spruce.

17. Generally,artificial inoculations of Norway spruce with <u>S.sanquinolentum</u> confirmed the natural infection observations. Again <u>S.sanquinolentum</u> was the dominant microorganism isolated and most of the correlations derived from natural infection of Norway spruce were also demonstrated for artificially inoculated trees.

18. In some cases treatment of the inoculation site (sealed or left open), time of year at which the inoculations were made and tree vigour influenced the

* The validity of these correlations was not tested on seperate sets of sample plots.

frequency of successful infections and development of decay caused by S.sanguinolentum . It seems that prolonged exposure of the inoculation site to the atmosphere favoured the development of S.sanguinolentum in open inoculation sites confirming previous investigations.

19. Frequency of infection with S.sanguinolentum was much greater in open sites inoculated in July compared with open sites inoculated in February.

20. Infection frequency was similar in sealed sites inoculated in July and February.

21. Open sites inoculated in July were more frequently infected than sealed sites inoculated at the same time.

22. There were no differences in the frequency of infection comparing open and sealed sites inoculated in February.

23. Global mean frequency of infection was much greater in trees inoculated in July compared with February inoculations.

24. Generally,fast growing trees were more frequently infected than slow growing trees.

25. Radial and vertical expansion of decay following inoculation with S.sanguinolentum were much greater in trees inoculated in July compared with those inoculated in February irrespective of tree vigour or inoculation site treatment (i.e sealed or left open).

26. Four strains of S.sanguinolentum were isolated during the course of experiments. Differences between these strains were mainly macroscopic.

27. Generally,sodium nitrate was a more readily available nitrogen source for strains 2 and 4 compared with ammonium sulphate. In contrast,ammonium sulphate was a better nitrogen source for strain 1 than sodium nitrate. Strain 3 was intermediate and both sodium nitrate and ammonium sulphate were equally available as nitrogen sources. Asparagine was the most readily available of all organic and inorganic nitrogen sources used.

28. Strains 3 and 4 had a similar range of optimum temperature (10 to 30 C°). The optimum temperature for growth for strains 2 and 1 was between 10 and 25 C° and between 5 and 25 C° respectively. Visible growth in all strains ceased completely above 30 C° .

29. Generally,acidic medium promoted the growth of all strains and growth declined gradually or dramatically with increasing alkalinity. Strain 4 was the most tolerant to alkalinity and produced relatively good growth at pH's 7.5 and 8.

30. Wood density was considerably lower in fast compared with slow-growing Norway spruce trees and the variation in wood density might partly explains differences in the amount of decay in dominant,codominant and subdominant trees.

31. A considerable loss of cellulose (%) occurred in Norway spruce 8 years after natural infection with S.sanguinolentum . Fast growing or dominant trees were the worst affected by the fungus and lost approximately 50% of their initial cellulose on average. The loss in

codominant trees and slow growing subdominant trees was much less and averaged about 20% of their initial cellulose content.

32. Six months after artificial inoculation of Norway spruce with *S.sanguinolentum* there were appreciable losses in initial lignin content and again fast growing trees were the worst affected compared with slow growing trees.

33. Fast growing trees were consistently the worst affected by *S.sanguinolentum* irrespective of the mode of infection (natural or artificial) compared with slow-growing trees.

34. Differences in the amount of wood substance lost as a result of infection with *S.sanguinolentum* in the three tree vigour classes (dominant, codominant and subdominant) examined might be attributed in part to differences in wood density or specific gravity and frequency of growth rings. Fast growing Norway spruce trees have light wood,* and a smaller number of growth rings per 5 cm from the outer bark inwards. In turn light wood has larger intracellular spaces and hence its air to moisture ratio is most probably within the required limits for fungal growth compared with the dense wood of slow growing trees. In addition a larger surface area of polysaccharides would be exposed and accessible to fungal enzymes in a light wood as compared with the smaller surface area exposed in a dense wood. The smaller number of growth rings per unit distance in fast growing trees favours rapid radial

* This was so because of the high proportion of spring wood in fast growing trees.

expansion of S.sanguinolentum ,since physical barriers are correspondingly fewer compared with the larger number of growth rings per unit distance in a slow growing tree. Here the larger number of physical barriers hinders radial expansion of S.sanguinolentum .

35. As S.sanguinolentum is capable of utilising a wide range of carbon and nitrogen sources,grows over a wide temperature and pH range and is also continuously present on dead organic matter on the forest floor,it is one of the most destructive wood-decay fungi

36. No changes in the major wood constituents i.e cellulose and lignin were detectable one month after artificial laboratory inoculation of wood blocks with S.sanguinolentum ,taken from Norway spruce. It is probable that the fungus was feeding on the medium (3% malt extract agar) and simple soluble carbohydrates present in the wood blocks.

37. In contrast, there were considerable losses in cellulose 2 and 3 months after artificial inoculation of similar wood blocks with S.sanguinolentum . Contrary to the results derived from natural and artificial infection of standing Norway spruce trees,losses in cellulose in wood blocks taken from dominant,codominant and subdominant trees were similar. This might be attributed to the fact that S.sanguinolentum was provided with optimal conditions for growth e.g temperature,humidity and the fact that other competing microorganisms were omitted and S.sanguinolentum was growing in pure culture. Another

factor was that the wood blocks were surface sterilised and this might have a considerable effect on the natural resistance of the wood to decay. Thus, S.sanguinolentum utilised similar amounts of cellulose irrespective of the origin of the wood blocks i.e taken from dominant,codominant or subdominant trees.

SUMMARY

A total of 103 Norway spruce (P 52) and (P29) in compartments 11 and 1016 of Yair Hill and Glentress forest (National grid reference: NT 442322 and NT 276415 respectively) and 93 Japanese larch trees (P 53) and (P54) in compartments 3 and 21 of Yair Hill forest (National grid reference: NT 455322 and NT 442328 respectively) were found to have extraction damage on stems and superficial roots. Extraction wounds were 4 and 8 years old and ranged from 52 to 1099 cm^2 surface area. Injured trees were felled and dissected and the radial and vertical expansion of decay and decay volume were recorded. Norway spruce was more frequently infected than was Japanese larch. The infection frequency was 47% in younger Norway spruce ;54% in older Norway spruce; 24% in Japanese larch in compartment 3 and 37% in Japanese larch in compartment 21. Stem wounds were more frequently infected compared with root injuries. *Stereum sanguinolentum* was the most common species isolated from infected Norway spruce trees,whereas *Chaetomium cochliodes* was the dominant species in Japanese larch. Other species isolated from Norway spruce were: *Trichoderma viride* ; *Penicillium* spp;*Fusarium sambucinum* ; *C.cochliodes* ;*Fomes annosus* ; *Mucor hiemalis* ; *Acremonium* spp;Bacteria and sterile unidentified mycelia. *Penicillium* spp; *T.viride* ; *C.cochliodes* and *Mortierella ramanniana* were isolated together with *S.sanguinolentum* from infected Japanese larch trees. The frequency of *S.sanguinolentum* increased progressively

towards the pith,whereas the frequency of all other microorganisms decreased with distance from the outer surface of the stem inwards in Norway spruce. T.viride showed the next greatest radial expansion in Norway spruce. S.sanguinolentum ; T.viride and C.cochliodes expanded as far as the distal parts of the decay columns,whereas all other microorganisms were isolated from samples taken between 1 and 12 cm above and 20 cm below the infection site. In some instances T.viride succeeded in overgrowing S.sanguinolentum in the decay columns in Norway spruce. F.annosus was rarely associated with infection through extraction wounds in Norway spruce and was isolated from only 0.1% of the samples,taken from the surface of the infection site inwards at 1 cm intervals. F.annosus was completely absent in Japanese larch. The shape and size of the decay columns were completely different in Norway spruce compared with Japanese larch.

Mean radial and vertical expansions of decay were much greater and the decay volumes much larger in Norway spruce than in Japanese larch of approximately the same age and having extraction damage that occurred at the same time (1980).

Wound and tree parameters had a considerable influence on the development of decay in Norway spruce. Positive correlations were demonstrated between wound surface area and vertical expansion of decay; tree dbh and radial penetration of decay; tree volume and decay volume and between the dbh and decay volume. Negative correlations

were demonstrated between the dbh and number of growth rings per 5cm from the outer bark towards the pith and between radial penetration of decay and number of rings per 5 cm from the outer bole inwards. Multiple correlations were also demonstrated between radial penetration of decay as a dependent variable and number of rings per 5 cm and dbh as independent variables. Also decay volume was dependent on both dbh and wound surface area. No significant correlations were found between decay volume and wound surface area; %decay volume and tree volume; radial penetration of decay and wound surface area; radial and vertical expansion of decay and between vertical expansion of decay and tree volume. Most of the significant correlations demonstrated for Norway spruce did not hold good for Japanese larch. In Japanese larch a negative correlation was demonstrated between tree vigour expressed as dbh or tree volume and %decay volume. Also a positive correlation between the wound surface area and vertical expansion of decay was demonstrated for Japanese larch in compartment 21 only and not in compartment 3. The rest of the correlations demonstrated for Norway spruce were not applicable to data from Japanese larch.

Four strains of S.sanguinolentum were isolated during the course of experiments,but the observed differences were mainly macroscopic. A detailed study on S.sanguinolentum strains was discussed. Generally,3% malt extract agar supported the most extensive growth of all S.sanguinolentum strains. Different strains produced

different colony diameters in different media with different carbon and nitrogen sources. Sodium nitrate proved to be much a better nitrogen source for strains 2 and 4 compared with ammonium sulphate. In contrast,ammonium sulphate resulted in the most extensive growth of strain 1; strain 3 occupying an intermediate position. Generally,organic nitrogen sources were far much better than inorganic nitrogen sources. Of all organic and inorganic nitrogen sources used,asparagine was the most readily available nitrogen source. Optimum temperature for growth for all strains was between 5 and 30 C° irrespective of the media used. Visible growth ceased completely at and above 30 C° for all strains. Generally,acidic media promoted the total amount of growth of all strains. Growth declined either gradually or dramatically towards alkalinity i.e being maximum at pH 4 and minimum at pH 8. Strain 1 had a comparatively limited range of optimum pH (4 and 5);strains 2 and 4 were tolerant of a wider range of hydrogen ion concentrations (pH 4-7) and strain 3 occupied an intermediate position. Strain 4 was the most tolerant to alkalinity and produced relatively good growth at pH 7.5 and 8 compared with the other strains. Generally, S.sanguinolentum has a wider range of available nutritional substances it can utilise and a wide range of optimum temperature and pH.

A total of 26 dominant; 26 codominant and 26 subdominant Norway spruce trees were inoculated with S.sanguinolentum by means of small wood plugs previously

inoculated with S.sanguinolentum . One series (78 trees) was inoculated in July (1982) and the other series (78 trees) was inoculated in February (1983). Half the inoculation sites were left open and the other half was sealed with paraffin wax. Trees were felled after 6 months and dissected and the development of decay was recorded as well as the microorganisms isolated from successfuly infected trees.

In some cases the frequency of infection and the development of decay varied with tree vigour,inoculation site treatment and time of year at which the inoculations were made. Pooling all values for inoculation treatments, there was no significant difference in the infection frequency in sealed sites inoculated in July compared with February inoculations. However, infection frequency was considerably greater in open sites inoculated in July than in open sites inoculated in February. Within the individual tree vigour classes and inoculation treatments it was evident that the frequency of infection was much greater in dominant and codominant trees compared with subdominant trees inoculated in July. In contrast, infection frequency was similar in all trees inoculated in February. Open inoculation sites had much greater frequency of infection compared with sealed sites all inoculated in July. In February inoculations there were no significant differences in infection frequency between open and sealed sites. The global mean infection frequency was much greater in trees inoculated in July than in trees inoculated

in February irrespective of tree class or inoculation site treatment. Pooling all July and February values,no significant differences in the infection frequency in all tree vigour classes were observed in sealed inoculation sites. However,infection frequency was much greater in dominant trees compared with codominant and subdominant trees in open inoculation sites.

Global mean radial and vertical expansion of decay was much greater in trees inoculated in July compared with February inoculations. Statistical analysis showed no significant differences in global mean radial and vertical expansion of decay from open inoculation sites compared with sealed inoculation sites irrespective of inoculation time ,except for the vertical expansion of decay in trees inoculated in July. Global mean vertical expansion of decay was much greater in open compared with sealed inoculation sites.

Isolations following artificial inoculations showed that S.sanguinolentum was the dominant microorganism. Other microorganisms isolated were T.viride , C.cochliodes ; Penicillium spp; F.sambucinum; Acremonium spp; members of Dematiaceae;sterile unidentified mycelia and Bacteria. Again the frequency of S.sanguinolentum increased progressively with increasing distance from the outer surface of the stem towards the pith and at the same time the frequency of all other microorganisms decreased dramatically. S.sanguinolentum ; T.viride and C.cochliodes expanded as far as the distal parts of the decay columns,whereas all other

microorganisms were isolated from samples taken between 1 and 20 cm above and below the inoculation site. There were no significant differences in the frequency of S.sanguinolentum in samples taken above and below the inoculation site and at the distal parts of the decay columns and in samples taken at the outer side of the stem inwards in 1 cm intervals up to > 10 cm comparing open and sealed inoculated sites.

In three trees T.viride had replaced and suppressed S.sanguinolentum and hence further expansion of decay was prevented in these trees. In one tree C.cochliodes showed the same effect on S.sanguinolentum . The development of decay in different trees was further affected by tree vigour and most of correlations derived from artificial inoculation results confirmed the results obtained from naturally infected trees. Significant positive correlations were demonstrated between dbh and radial penetration of decay;vertical expansion of decay and dbh and between radial and vertical expansion of decay. Also significant negative correlations were demonstrated between dbh and number of growth rings per 5 cm from the outer bole inwards and between radial expansion of decay and number of rings per 5 cm. Multiple correlation analysis showed that radial penetration of decay was dependent on both dbh and number of growth rings per 5 cm from the outer bark towards the pith.

8 years after natural infection with S.sanguinolentum , a 50% loss in cellulose (approximately) in dominant Norway

spruce trees had occurred. Mean loss in cellulose in codominants and subdominants was 20%. The analysis of Norway spruce previously inoculated with S.sanguinolentum for 6 months showed a reduction in the initial lignin content. In dominant trees mean reduction in lignin content amounting to 8.6% in dominant trees ; 3.7% in codominants and 3% in subdominants. No change was found in either cellulose or lignin in wood blocks taken from all tree vigour classes inoculated for one month with S.sanguinolentum . However,there was a reduction in cellulose content after 2 and 3 months. Wood blocks taken from all tree vigour classes lost similar amounts of cellulose. 2 months after artificial inoculation with S.sanguinolentum resulted in mean loss of 3.2% in cellulose in wood blocks derived from dominant trees; 2.4% in codominants and 2.8% in subdominants. These differences were not significant. 3 months after artificial inoculation with S.sanguinolentum resulted in mean loss of 7.4% in cellulose in wood blocks taken from dominant trees; 8% in codominants and 9.6% in subdominants,but these differences were again not significant. The reduction in cellulose content was much greater 3 months after inoculation compared with 2 months after inoculation.

Analysis of wood density in the three tree vigour classes (i.e dominant;codominant and subdominant) showed that fast growing or dominant trees had much lesser wood substance per unit volume compared with slow growing or subdominant trees. Codominant trees had intermediate

wood density.

Again T.viride replaced and suppressed S.sanguinolentum in 10 samples during the course of artificial inoculation experiments of wood blocks. 3 months later the analysis of the contaminated samples showed no signs of decay or any other abnormality indicating and confirming a possible antagonism of T.viride on S.sanguinolentum and its potentiality as a mean of biological control.

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APPENDICES

Appendix 1:TREE AND DECAY PARAMETERS (COMPARTMENT 11) NORWAY SPRUCE (P52)

	X1	X2	X3	X4	X5	X6	X7	X8
	200.00	1.70	0.020	22.00	0.35	10.00	05.70	09.00
	150.00	0.88	0.008	13.00	0.08	05.00	10.00	28.00
	215.00	1.45	0.010	15.00	0.13	12.20	07.70	27.00
	240.00	1.60	0.030	19.00	0.27	10.50	11.10	14.00
	244.00	1.60	0.009	17.00	0.22	04.60	04.10	21.00
	250.00	1.28	0.003	09.00	0.04	05.40	07.90	20.00
	250.00	1.75	0.010	19.00	0.25	15.60	04.00	15.00
	290.00	1.88	0.002	17.00	0.22	10.70	01.00	25.00
	400.00	2.50	0.050	20.00	0.26	15.50	19.20	12.00
	120.00	0.89	0.007	13.00	0.10	09.10	07.00	27.00
	080.00	0.60	0.050	20.00	0.35	15.00	14.30	09.00
	100.00	0.50	0.003	15.00	0.16	07.10	01.90	23.00
	150.00	0.83	0.002	08.00	0.03	05.60	06.70	26.00
	220.00	1.10	0.010	23.00	0.44	12.60	02.30	11.00
	110.00	0.84	0.003	16.00	0.17	11.20	01.80	22.00
	250.00	1.35	0.005	16.00	0.18	05.50	02.80	25.00
	076.00	1.20	0.020	19.00	0.26	11.20	07.70	15.00
	140.00	0.85	0.007	18.00	0.22	10.40	03.20	17.00
	200.00	1.30	0.006	17.00	0.18	06.23	03.30	23.00
	760.00	2.87	0.007	13.00	0.07	05.40	10.00	25.00
	240.00	1.55	0.004	16.00	0.09	08.20	04.40	26.00
	090.00	0.89	0.006	18.00	0.24	11.00	02.50	16.00
	420.00	2.00	0.008	14.00	0.08	07.00	10.00	22.00
MIN.	076.00	0.50	0.002	08.00	0.03	04.60	01.00	09.00
MAX.	760.00	2.90	0.050	23.00	0.44	15.60	19.20	28.00
MEAN	225.90	1.4	0.012	16.00	0.20	09.30	06.50	20.00

X1= WOUND SURFACE AREA (CM^2)
X2= VERTICAL EXPANSION OF DECAY (M)
X3= DECAY VOLUME (M^3)
X4= TREE DBH (CM)
X5= TREE VOLUME (M^3)
X6= MAXIMUM RADIAL PENETRATION OF DECAY (CM)
X7= %DECAY VOLUME
X8= NUMBER OF ANNUAL RINGS PER 5 CM FROM THE OUTER BARK INWARDS

Appendix 2:TREE AND DECAY PARAMETERS (COMPARTMENT 1016) NORWAY SPRUCE (P 29)

	X1	X2	X3	X4	X5	X6	X7	X8
	582.00	2.92	0.011	12.00	10.00	0.066	16.70	29.00
	360.00	2.00	0.008	28.00	16.60	0.871	00.48	21.00
	100.00	0.55	0.003	14.00	06.20	0.090	03.30	27.00
	160.00	1.10	0.001	09.00	02.30	0.030	03.30	35.00
	490.00	2.03	0.008	38.00	15.40	1.660	01.10	12.00
	415.00	0.90	0.007	25.00	16.30	0.628	01.10	17.00
	190.00	0.96	0.005	17.00	08.40	0.100	05.00	20.00
	325.00	1.82	0.007	28.00	16.40	0.850	00.80	17.00
	080.00	0.57	0.004	30.00	26.10	1.100	00.36	11.00
	541.20	0.37	0.001	23.00	04.30	0.540	00.19	16.00
	260.00	2.00	0.010	35.00	15.80	1.400	00.70	09.00
	220.00	2.10	0.004	18.00	08.00	0.340	01.20	22.00
	700.00	3.19	0.070	46.00	29.60	2.600	02.70	05.00
	320.00	1.67	0.004	19.00	07.80	0.360	01.10	23.00
	250.00	1.80	0.008	30.00	22.00	1.230	00.70	10.00
	147.40	1.90	0.020	26.00	16.50	0.790	02.50	18.00
	213.50	2.90	0.030	32.50	19.40	1.150	02.60	10.00
	105.00	0.52	0.030	26.00	17.40	0.880	03.40	19.00
	450.00	2.00	0.020	28.00	15.80	1.200	01.70	18.00
	314.95	1.73	0.004	18.00	05.60	0.260	01.54	20.00
	160.75	0.86	0.007	35.00	15.00	1.580	00.44	10.00
	350.00	2.40	0.004	15.00	05.60	0.170	02.40	27.00
	110.00	0.64	0.040	27.00	16.20	1.000	04.00	17.00
	400.64	1.30	0.004	15.00	04.50	0.110	03.64	25.00
	104.50	0.54	0.001	12.00	05.50	0.060	01.70	33.00
	440.45	1.27	0.010	36.00	16.20	1.640	00.61	08.00
	168.70	0.30	0.001	27.00	15.50	0.900	00.11	14.00
	446.01	2.80	0.020	21.00	12.50	0.420	04.80	21.00
	159.35	0.50	0.002	23.00	10.20	0.500	00.40	19.00
MIN.	080.00	0.30	0.001	09.00	02.30	0.030	00.11	05.00
MAX.	700.00	3.20	0.070	46.00	29.60	2.600	16.70	35.00
MEAN	295.33	1.50	0.012	24.6	13.10	0.777	02.40	18.00

X1= WOUND SURFACE AREA (CM^2)
X2= VERTICAL EXPANSION OF DECAY (M)
X3= DECAY VOLUME (M^3)
X4= TREE DBH (CM)
X5= MAXIMUM RADIAL PENETRATION OF DECAY (CM)
X6= TREE VOLUME (M^3)
X7= %DECAY VOLUME
X8= NUMBER OF ANNUAL RINGS PER 5 CM FROM THE OUTER BARK INWARDS

Appendix 3:TREE AND DECAY PARAMETERS (COMPARTMENT 3) JAPANESE LARCH (P53)

	X1	X2	X3	X4	X5	X6	X7	X8
	11.0	684.8	0.80	2.2	0.0009	0.11	19.0	0.82
	20.0	385.8	0.85	3.0	0.0020	0.18	09.0	1.10
	20.0	851.9	0.62	2.7	0.0020	0.22	08.0	0.91
	22.0	1099	0.61	2.9	0.0030	0.34	12.0	0.88
	21.0	644.3	0.95	2.3	0.0010	0.26	17.0	0.38
	10.0	229.8	0.60	1.6	0.0005	0.04	11.0	1.30
	18.0	126.7	0.50	1.5	0.0009	0.24	11.0	0.38
	13.0	104.7	0.68	2.1	0.0010	0.09	19.0	1.10
	20.0	252.0	0.76	3.0	0.0010	0.34	13.0	0.29
	13.0	278.4	0.59	2.2	0.0009	0.08	17.0	1.30
	22.0	206.0	0.50	1.5	0.0007	0.27	11.0	0.26
	11.0	051.6	0.66	2.7	0.0007	0.05	14.0	1.40
MIN.	10.0	051.6	0.50	1.5	0.0005	0.04	08.0	0.30
MAX.	22.0	1099.0	1.00	3.0	0.0030	0.34	19.0	1.40
MEAN	16.8	409.6	0.70	2.3	0.0012	0.20	13.0	0.84

X1= TREE DBH (CM)

X2= WOUND SURFACE AREA (CM^2)
X3= VERTICAL EXPANSION OF DECAY (M)
X4= MAXIMUM RADIAL EXPANSION OF DECAY (CM)

X5= DECAY VOLUME (M^3)

X6= TREE VOLUME (M^3)
X7= NUMBER OF ANNUAL RINGS PER 5 CM FROM THE OUTER BARKS INWARDS
X8= %DECAY VOLUME

Appendix 4: TREE AND DECAY PARAMETERS (COMPARTMENT 21) JAPANESE LARCH (P 54)

	X1	X2	X3	X4	X5	X6	X7	X8
	200.0	0.56	0.270	0.0007	2.4	16.0	19.0	0.26
	511.2	0.85	0.023	0.0020	3.5	09.0	10.0	8.70
	313.5	0.93	0.192	0.0010	1.1	11.0	16.0	0.50
	295.6	0.90	0.437	0.0020	2.4	12.0	22.0	0.46
	198.6	0.30	0.363	0.0003	1.6	15.0	20.0	0.08
	158.6	0.40	0.444	0.0003	1.0	13.0	22.0	0.07
	061.3	0.55	0.066	0.0003	1.5	10.0	15.0	0.45
	159.7	0.46	0.045	0.0002	0.8	16.0	11.0	0.44
	160.2	0.45	0.053	0.0005	2.2	10.0	11.0	0.94
	120.5	0.65	0.046	0.0004	1.3	13.0	11.0	0.87
	107.0	0.71	0.260	0.0004	1.1	14.0	18.0	0.15
	535.9	0.70	0.114	0.0004	0.7	14.0	15.0	0.35
	536.7	0.75	0.040	0.0006	2.0	14.0	13.0	1.50
	225.7	0.50	0.014	0.0007	2.5	17.0	08.0	5.00
	262.4	0.65	0.280	0.0009	2.2	14.0	17.0	0.32
	160.0	0.72	0.320	0.0030	1.7	18.0	18.0	0.94
MIN.	061.3	0.30	0.014	0.0002	0.7	09.0	08.0	0.07
MAX.	536.7	1.00	0.444	0.0030	3.5	18.0	22.0	8.70
MEAN	250.4	0.60	0.185	0.0010	1.8	14.0	15.4	1.31

X1= WOUND SURFACE AREA (CM^2)
X2= VERTICAL EXPANSION OF DECAY (M)
X3= TREE VOLUME (M^3)
X4= DECAY VOLUME (M^3)
X5= MAXIMUM RADIAL PENENTRATION OF DECAY (CM)
X6= NUMBER OF ANNUAL RINGS PER 5 CM FROM THE OUTER BARK INWARDS
X7= TREE DBH (CM)
X8= %DECAY VOLUME